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EVALUATION OF TUBEROSE GENOTYPES FOR DEVELOPMENT OF F_1 HYBRIDS FOR QUANTITATIVE, QUALITATIVE CHARACTERS AND NEMATODE RESISTANCE

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2013

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Thesis submitted in part fulfillment of the requirement for the degree of **Doctor of Philosophy** in **Horticulture (Floriculture and Landscape Gardening)** to Tamil Nadu Agricultural University, Coimbatore

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CERTIFICATE

This is to certify that the thesis entitled "**EVALUATION OF TUBEROSE GENOTYPES FOR DEVELOPMENT OF F_1 HYBRIDS FOR QUANTITATIVE, QUALITATIVE CHARACTERS AND NEMATODE RESISTANCE"** submitted in partial fulfilment of the requirements for the degree of **Doctor of Philosophy in Horticulture (Floriculture and Landscape Gardening)** to the Tamil Nadu Agricultural University, Coimbatore is a record of bonafide research work carried out by **Ms. P. RANCHANA** under my supervision and guidance and that no part of this thesis has been submitted for the award of any other degree, diploma, fellowship or other similar titles. However, part of the thesis work has been published in peer reviewed scientific journal of national/ international repute (enclosed).

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Dedicated to

My parents

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P. RANCHANA

ABSTRACT

EVALUATION OF TUBEROSE GENOTYPES FOR DEVELOPMENT OF F_1 HYBRIDS FOR QUANTITATIVE, QUALITATIVE CHARACTERS AND NEMATODE RESISTANCE

By

P. RANCHANA

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2013

Studies were undertaken to develop F_1 hybrids in tuberose for high yield, concrete recovery with resistance to root knot nematode (*Meloidogyne incognita*). The study was carried out with ten single and five double genotypes of tuberose (*Polianthes tuberosa* L.) to assess the performance, magnitude of variability and degree of association of characters, direct and indirect effects of twelve components for flower yield. The varieties were screened for their adaptability to tropical condition at Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore during 2011-13.

Fourteen crosses (*viz.,* Variegated Single x Calcutta Single, Variegated Single x Kahikuchi Single, Variegated Single x Mexican Single, Variegated Single x Navsari Local Single, Variegated Single x Pune Single, Variegated Single x Phule Rajani, Phule Rajani x Kahikuchi Single, Phule Rajani x Mexican Single, Phule Rajani x Hyderabad Single, Phule Rajani x Variegated Single, Shringar x Kahikuchi Single, Shringar x Variegated Single, Hyderabad Single x Variegated Single, Navsari Local x Variegated Single) were found to be compatible in which the pollen tube successfully reached the ovule and seed set observed in seven days after crossing.

The pollen tube length varied from 4.73 and 118.85 microns within one hour after incubation in single types. The same trend was observed with respect to 5, 10, 15, 20 and 25 hours after incubations with the range of 16.95 to 520.43; 26.68 to 839.33; 49.81 to 1020.63; 76.72 to 1234.95 and 78.25 to 1292.64 microns respectively. In general, the pollen tube was significantly longer in Variegated Single when compared to other genotypes.

Though the present seed germination in tuberose is low, seeds treated with GA_3 250 ppm for 8 hours (T_3) recorded improved highest germination (12.50 %) and it was followed by the treatment T_7 (KNO_3 @ 0.5 per cent for 8 hours) which registered a germination of 12.45 per cent. The minimum germination (7.96 %) was recorded in the treatments T_{17} and T_{18} (water soaking for 8 and 16 hours respectively). The results of the quick viability (tetrazolium) test expressed that the concentration of 0.25% 2,3,5-triphenyl tetrazolium chloride was found optimum to test the viability of seeds.

In the evalaution of genotypes, high heritability coupled with high genetic advance as per cent of mean were observed for flowering duration (99.07, 73.04), weight of florets per spike (99.02, 66.15), number of florets per spike (98.72, 65.08) and rachis length (98.47, 54.46) in single types while in double types it was observed for number of florets per spike (98.35, 69.81), number of spikes/m^2 (96.93, 65.78), rachis length (96.91, 45.18) and yield of florets per plot (95.78, 40.20), respectively.

High estimates of phenotypic and genotypic co-efficient of variation were observed for flowering duration (35.79, 35.62) followed by weight of florets per spike (32.43, 32.27) and number of florets per spike (32.01, 31.80) in single types while in double types it was observed for number of florets/ spike (24.59, 24.38) respectively indicating that these characters are under genetic control. Hence, these characters can be relied upon selection for further improvement.

In single types, positive and significant association was observed for number of florets/ spike with weight of florets per spike (0.256), yield of florets per plot (4 * 1 m) (0.178), number of spikes per m^2 (0.100) and length of the floret (0.094) at genotypic level. Similarly positive and significant association was observed for number of florets/ spike with yield of florets per plot (4 * 1 m) (0.996), weight of florets per spike (0.950), number of spikes per m^2 (0.500) and length of the floret (0.489) in double types.

Path analysis showed that the weight of florets per spike (0.6380), number of leaves per plant (0.5784), flowering duration (0.3746), rachis length (0.2776), days taken for sprouting of bulb (0.2460), length of the floret (0.2286) and days to spike emergence (0.0203) found to be the dominating contributors towards the flower yield in single types while in double types, the traits *viz.,* weight of florets per spike (0.6378), number of leaves per plant (0.5782), flowering duration (0.3756), rachis length (0.2779), days taken for sprouting of bulb (0.2580), length of the floret (0.2277) and days to spike emergence (0.0213) found to be the dominating contributors towards the number of spikes per m^2.

A wide genetic diversity have been observed through the D^2 analysis among the genotypes. The ten single genotypes were grouped into four clusters and five double genotypes into three clusters. The varieties from IIHR, Bangalore (Shringar, Prajwal) in single types and Suvasini and Vaibhav in double types have been grouped under different clusters.

Cultivars chosen from the same ecographic region (IIHR, Bangalore) were found scattered in different clusters in both single and double types indicating the presence of wide genetic variability among these types. The D^2 analysis revealed that the yield of florets per plot (4 * 1 m) contributed the maximum in both single and double types (36.25, 35.67 %) towards genetic divergence.

In screening studies against nematode, the genotype Kahikuchi Single was categorised under 'moderately resistant' (MR), while Calcutta Single, Hyderabad Single and Mexican Single were categorised under 'susceptible' (S). Whereas, Navsari Local, Phule Rajani, Prajwal, Pune Single, Shringar and Variegated Single were brought under 'highly susceptible' group.

To conclude, based on the mean performance and genetic divergence the genotypes Prajwal (Single), Suvasini (Double) have been identified for commercial cultivation. In hybridization, among the ninety crosses made between ten single types, only fourteen crosses found to be successful as evidenced through seed set. These fourteen F_1 hybrid progenies are slow growing and takes longer duration for attaining maturity. These progenies are now in initial vegetative stage. They have to be evaluated in future to assess the growth, yield, quality parameters and also its resistance to nematodes. For remaining 76 unsuccessful crosses, the techniques *viz.,* shortening of the

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Abbrevaiations

@	:	at the rate of		kg	:	Kilogram
cc	:	cubic centimeter		g	:	Gram
conc	:	Concentration		GA_3	:	Gibberellic acid
Cv	:	Co-efficient of variation		IBA	:	Indole Butyric acid
D	:	Day		cm	:	centimeter
DAP	:	Days after pollination		mg/l	:	Milligram per litre
DAA	:	Days after anthesis		PAL	:	Phenyl ammonia lyase
Dept	:	Department		%	:	Percentage
et al.,	:	co workers		RBD	:	Randomized Block Design
min	:	Minute		Sed	:	Standard error of mean deviation
Mg	:	Milligram		*viz*	:	Namely
µg	:	Microgram		i.e.	:	That is
NO	:	Number		HAP	:	Hours After Pollination
OD	:	Optical density value		NaOH	:	Sodium Hydroxide
pH	:	Negative logarithm of hydrogen ion concentration		K_3PO_4	:	Potassium phosphate (tribasic)
/ plant	:	Per plant		H_3BO_3	:	Boric acid
Ppm	:	Parts per million		$ZnSO_4$	:	Zinc Sulphate
/ lit	:	Per litre		$FeSO_4$	:	Ferrous Sulphate
PO	:	Peroxidase		TNAU	:	Tamil Nadu Agricultural University
RDF	:	Recommended dose of fertilizers		FCRD	:	Factorial Completely Randomized Design
FAA	:	Formalin: Acetic acid: Alcohol		IAA	:	Indole acetic acid
FYM	:	farm yard manure		RKI	:	Root knot index
NaOCl	:	sodiumhypochlorite		RKN	:	Root knot nematode
GC-MS	:	Gas chromatography-Mass Spectrometry		PKM	:	Periyakulam
etc.,	:	Excetra		hrs	:	hours
Fig.	:	Figure				

CHAPTER I

INTRODUCTION

Floriculture is now recognized as an important agribusiness sector capable of gaining higher returns per unit area and providing employment opportunity in many developing countries like India, Pakistan, Brazil, Egypt and Kenya. Although the value of exports of floriculture products from India is Rs.23,066 lakhs in 2010-11, it is still a marginal player in the world trade. The annual growth rate of floriculture in India is 24 % (DGCIS Annual Export).

In India the area under loose flowers such as marigold, jasmine, aster, rose, chrysanthemum and tuberose during the year 2011 was 1,91,000 ha under open field conditions. Nearly 20 per cent of area under floricultural crops is mainly concentrated in Tamil Nadu (32,000 ha), followed by Karnataka (27,000 ha), West Bengal (23,100 ha) and Andhra Pradesh (21,800 ha) (Indian Horticulture Database, 2011).

Tuberose (*Polianthes tuberosa* Linn.), is an important commercial flower crop and is extensively cultivated in many sub-tropical and tropical parts of the world including India (Benschop, 1993). It is native of Mexico (Bailey, 1919) and belongs to the family Amaryllidaceae (Bailey, 1930). Tuberose is a bulbous perennial plant with tuberous roots producing long spikes, which bear waxy white and fragrant flowers profusely throughout the year. In India, tuberose flowers is harvested and used as loose flowers to a large extent. In Tamil Nadu, it is commercially cultivated in over 1667 ha area with an annual production of 20,004 tonnes and it is followed by Karnataka, West Bengal, Andhra Pradesh, Maharashtra, Gujarat, Uttar Pradesh and Delhi (Indian Horticulture Database, 2011).

The spikes can be used as vase flowers or in bouquets whereas the loose flowers are used for making garlands, veni's, gajra, bangles, etc. and essential oil extraction. Colouring of floral spikes systematically through the vase solution can also be done for use in vases (Sambandhamurthi and Appavu, 1980). The flowers yield a very valuable floral concrete (0.08 – 0.11 per cent) upon solvent extraction (Singh, 1995). The absolute of tuberose (essential oil) extracted from floral concrete is used in the preparation of various high value perfumes and cosmetics.

Apart from domestic consumption, tuberose cut spikes has got a very good export potential to other countries (Singh and Uma, 1995). The average flower yield of tuberose in India is 6300 kg/ha (Belorkar *et al*., 1993). Tuberose floral concrete and essential oil are fetching higher price than the other floral concretes and essential oils. However inspite of its importance, adaptability and export potential the research priority given for this crop was less in Tamil Nadu especially in crop improvement aspects.

Among the constraints on tuberose production, pests are in the top rank. Plant parasitic nematodes are no exceptions as they also play a major role in bringing down the production of this crop. Tuberose plants have been reported to be severely affected by several plant parasitic nematodes, especially by the root knot nematodes, *Meloidogyne spp*. It has more than 3000 host species and is of polyphagous nature. According to Trugill and Block (2001), *Meloidogyne incognita* is one of the most important apomictic species found in temperate and tropical countries and it is the possible single most damaging crop pathogen in the world. They have a wider occurance in the tuberose growing subtropical and tropical regions and reported to cause 10 and 14 per cent reduction in flower number and spike weight, respectively (Khan and Parvatha Reddy, 1992). *M. incognita* has become a major threat for tuberose cultivation. Chemical methods of control of the nematodes such as fumigation and soil application of nematicides pose numerous health hazards both to human being and beneficial fauna of soil. Moreover these practices also boost the cost of cultivation. In endemic areas of nematodes, such practices may make the tuberose cultivation itself an uneconomical. Hence, the use of resistant varieties is the most economical and easy practice. Identification of nematode resistant donors and utilization in development of F_1 hybrids by hybridization with high yielding lines coupled with quality parameters will be a boon to tuberose growers for maximising production of quality tuberose without much residue of chemicals through the use of nematicides.

Tuberose being asexually propagated crop differs from sexual ones in its response to different breeding methodologies. Hence, systematic efforts on introduction and evaluation of improved varieties have not been undertaken consistently. The varietal improvement work so far attempted in Tamil Nadu was only mere selection and to a limited extent through mutation. The released varieties are maintained easily by asexual

means. This provides an ample scope in the crop improvement through characterization of genotypes for variability available among the population. The best performing lines/ genotypes can be selected for further genetic advancement.

Systematic work on tuberose breeding has been taken up at various research institutions mainly at Indian Institute of Horticultural Research (IIHR), Bangalore and Mahatma Phule Krishi Vidyapeeth (M.P.K.V.) Rahuri, Maharastra. Apart from these institutions, few other State Agricultural Universities were also involved in research in this crop. Presently, the research work in crop improvement and scientific cultivation is meagre in Tamil Nadu. This supports the importance of conducting such research to meet the ever growing demand for tuberose flowers in domestic and international markets.

The simple approach to create new genotypes is to take advantage of the available genetic diversity and document clear- cut information on the extent of genetic diversity among or between the genotypes. Till date, genetic improvement of the crop have been confirmed conventionally and pivotal to these improvement efforts, is the collection and evaluation of its germplasm. The sex of the tuberose though governed by genetic factors is highly influenced by soil and environmental factors. Therefore the precise knowledge of combining ability is responsible for yield and yield components. Hence, it is a basic pre requisite for launching a successful crop improvement programme.

The exploitation of hybrid vigour in tuberose will provide a quantum jump in boosting the yield. If the genetic architecture of the crop is explained, a hybrid with high yield and quality can be developed successfully. The commercialization of developed hybrids will be made easy in tuberose through multiplication of bulbs. Hence, it is important to develop F_1 hybrids with high yield and more concrete recovery. With this in view, attempts were made to evaluate the parents and hybrids under open condition for further breeding programme.

In order to increase the yield and productivity of this flower crop, breeding is necessary to evolve high yielding genotypes. Moreover, the information on the extent of variability, association of component characters on yield are limited. Association of characters would help in minimizing the pressure of time on breeders, by providing correct information on the characters, which have to be considered for formulating a selection index. Correlation studies helps to understand the mutual association between two variables.

In tuberose, dichogamy and self-incompatibility causes problem in seed setting. Seed setting in tuberose is quite erratic in the single flowered cultivars while in double type no seed set was observed. Yadav and Maity (1989) reported that seed set was higher in variegated single types. As tuberose is a vegetatively propagated crop, it is possible to create genetic variability by allowing genes to express in different combinations. This can be achieved by selecting seedlings raised from seeds of open pollinated and cross pollinated types. Joshi and Pantulu (1941) stated that there is no defect or deformation in the development of pollen grains or embryo sac. The exact cause of sterility or poor seed set is not known. Further, detailed studies on floral biology, pollination time, stigma receptivity, pollen viability, pollen germination, self and cross compatibility in the open pollinated genotypes and on the existing cultivars are lacking.

In the light of above, an attempt was therefore made to develop F_1 hybrids for high yield coupled with good qualities and resistance to root knot nematode (*Meloidogyne incognita*) with the following objectives.

- To study the quantitative and qualitative traits of the genotypes in the germplasm pool and to select the best performing ones.
- To study the stigma receptivity, pollen viability, pollen germination, seed setting behaviour and self- incompatibility in tuberose genotypes
- To identify the compatible parents through crossing programme
- To screen the genotypes for resistance against root knot nematode and to characterise the biochemical basis for its resistance

CHAPTER II

REVIEW OF LITERATURE

Tuberose (*Polianthes tuberosa* L.) is an important flower crop grown on commercial scale in India. In any crop breeding programme, the main basis of selection is the variability that exists in a plant population. Likewise, a thorough knowledge on the intensity of association of various characters that attributes to the complex character (yield) will improve the efficiency of selection programme. Genetic amelioration of this crop is associated with problems of basic self- incompatibility, erratic seed setting and narrow genetic variability. The basic information regarding varietal evaluation, pollen viability, pollen germination, stigma receptivity, self and cross-compatibility behaviour, hybridization, incompatibility, post fertilization event, seed set and seed germination are lacking in this crop. The available information on these aspects in tuberose and other related crops are reviewed below.

2.1. Varietal evaluation and hybrid development

2.1.1 Tuberose

Existence of four cultivars *viz.,* single, double, semi double and variegated types in tuberose was reported by Sandhu and Bose (1973). Single tuberose produced more number of florets per spike and longer duration of flowering. Commercially 'single' types appear to be much better but aesthetically 'double' types are preferred (Banker and Mukhopadhyay, 1980).

Desai (1975) obtained a desirable mutant with gamma irradiation treatment. The flowers of the mutant were bolder. The treatments with higher doses resulted abnormalities like bifurcated stalks, colouring in midrib, etc. As many as 13 mutants were isolated from different gamma irradiation treatments by Abraham and Desai (1976) and they grouped them into four types based on variations in leaf morphology. Inter-specific hybrids were produced by Brudrant (1985) and Howard (1977, 1978). However, they could not get desirable mutants as commercial hybrids.

Irulappan *et al.,* (1980) studied 11 tuberose types and recorded Calcutta Single (456 flowers per clump) as the highest yielder followed by Mexican Single (447 florets),

while ‘Calcutta Double’ was found to be a very poor (43 flowers) yielder. Sambandamurthi (1983) obtained a desirable mutant with gamma irradiation which are having variegated leaves, branched flower stalks and three florets per axil.

Irradiation treatments using gamma rays were carried out by Gupta *et al.* (1984) who reported two desirable mutants. They christened the mutants as ‘Rajat Rekha’ and Swarna Rekha. Leaves of Rajat Rekha have silvery white streaks along the midrib of the blade, which make it look very ornamental even when not in flowering. Leaves of Swarna Rekha has golden streaks along their margins, which makes it very pretty even at vegetative stage. Shen *et al*. (1987) attempted reciprocal crosses between single and double types and obtained more single progenies than double progenies. The seeds obtained from a cross between single x double resulted in 85 per cent single type of plants, whereas it was as high as 85.40 per cent in a cross between double x single. It was further observed that cross between single x double and double x single produced 15 and 14 per cent double type of plants respectively.

Patil *et al*. (1990) assessed four cultivars *viz*., single, double, semi double and variegated for number of flower stalks, flowers produced per hectare, average floret weight and stalk height. Among all the four cultivars, single type scored the best and was recommended for commercial production of loose flowers and for garland making. Meenakshi Srinivas *et al.* (1995) developed two hybrids ‘Shringar (Single) - Mexican Single x Pearl Double’ and ‘Suvasini (Double) - Mexican Single x Pearl Double’ at the Indian Institute of Horticultural Research, Hessaraghatta, Bangalore. ‘Shringar’ has pinkish buds, bigger flowers, more florets per spike, higher yield (36%) compared to the existing local single variety. ‘Suvasini’ (Double) has longer spike, pure white florets, bold flower opening, bigger florets, more florets per spike and higher spike yield (26%) compared to existing ‘Pearl Double’.

Niranjan Murthy and Meenaksi Srinivas (1997) evaluated four single and five double types of tuberose genotypes for flower yield and floral traits and reported that among single types, the hybrid ‘Shringar’ recorded the highest flower yield and the hybrid ‘Suvasini’ recorded the highest spike yield among double types. Gurav *et al.* (2005) developed a hybrid ‘Phule Rajani’ by crossing Local Single x Shringar (Single) at

Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharastra. It bears single type of greenish white flower bud, white flowers, flower spikes remain straight in field and vase. It has an appropriate spike length with well distributed 46 – 58 florets on the spike. The flower buds are greenish white which is very much acceptable to the consumers for both loose flower as well as cut flower production. The rachis length is more than 50% of the spike length.

The performance of five cultivars of tuberose under the agro-climatic conditions of Kalyani, West Bengal was studied and the findings revealed that 'Calcutta Single' performed better than other cultivars in respect of number of leaves per plant (124.58) and number of spikes per m^2 (101.45). The highest plant height (32.66 cm), longest spike (96.98 cm) and increased weight of spike (143.33 g) were recorded in Calcutta Double as compared to the other cultivars (Anon., 2000b). Evaluation of four varieties *viz*., 'Shringar', 'Suvasini', Single and Double under Vellanikkara, Kerala conditions revealed that the cultivar Single was comparatively better than others with regard to days taken for flowering (150.3) and days taken for first floret opening (18.4 days). The maximum length of spike was recorded in cultivar Double (82.5 cm) (Anon., 2000c).

Vijayalaxmi *et al*. (2010) evaluated seven single cultivars of tuberose for vegetative and floral traits and reported that among single types, the hybrid Prajwal excelled others in respect of both vegetative and floral traits.

2.1.2 Varietal evaluation and hybrid development in related genera

Rai *et al*. (2000) evaluated 16 varieties of gladiolus under sodic wasteland. Based on different characters such as plant height and number of tillers per plant, the varieties like White Prosperity, White Goddess, Red Beauty, Friendship, Venetei, Aldebran, First Lady were found superior in comparison to others. Sanjai and Brahma Singh (2000) evaluated 20 gladiolus varieties for cut flower and corm production in Ladakh by considering different characters and found that variety Princess Margaret Rose showed maximum plant height (136.6 cm). Kamble (2001) studied the performance of gladiolus cultivar in Arabhavi (Karnataka) and reported that cultivar Trust recorded maximum plant height (81.06 cm) and was significantly superior over other cultivars. He also found that cultivar Vedanapoli (21.27) showed maximum number of leaves at 60 DAP.

Sidhu and Arora (2000) evaluated the gladiolus varieties for summer flower production in Ludhiana and reported that variety White Prosperity produced the longest spikes (102.27 cm) and size of florets was maximum in variety Rose Supreme (8.92 cm). Rao *et al*. (2000) developed the hybrids of gladiolus and they were evaluated for three years in IIHR, Bangalore. Among them hybrid numbered 84-6-13 (Poonam x Vinks Glory) and hybrid numbered 85-1-13 (Geliber Herald x Vinks Glory) were found to be promising. Out of these two hybrids, 85-1-13 was selected and it was named and released as Arka Suvarna for commercial cultivation.

Roy and Sharga (2000) studied the performance of exotic gladiolus cultivars in Lucknow and reported that cultivars namely Jester, White Prosperity and Rose Supreme were ideal for commercial cultivation by considering various parameters. Neeraj *et al*. (2000) evaluated 26 genotypes of gladiolus under North Bihar conditions. Among these PG-17, Moralo and Her Majesty were promising genotypes for more number of florets per spike, while Darshan, PG-9 and V-Tasant.

Singh *et al*. (2000) studied the effect of cultivar response on keeping quality of gladiolus spikes and found that the cultivars Applause, Hunting Song, Jacksonville Gold, Mayur, Melody and White Prosperity increased the vase life by 10 days when the spike was harvested at 5-7 florets showing colour. Sidhu and Arora (2000) tested the vase life of six gladiolus varieties and found that the variety Eurovision recorded the longest vase life (5.2 days). Gupta *et al*. (2001) evaluated 11 cultivars of gladiolus. Maximum spike length was recorded in cultivar Pacific White (72.5 cm). Number of spikes per plant ranged from 1 to 2.8 (Aldeburan). The highest number of florets per spike observed in cultivar Pacific White (15.2) followed by Day Dream (14.0). The floret length ranged from 8.3 cm (Interpit Bicolor) to 11.2 cm (Red Sparkle). The maximum increases in spike length was noticed in the cultivar Red Sparkle (14.5%).

Kamble (2001) studied the performance of gladiolus cultivars and reported that maximum spike length (93.90 cm), spike weight (127.26 g), diameter of florets (11.91 cm) and number of florets per spike were noticed in cultivar Summer Sunshine and Vadanapali showed maximum spike girth and spike yield per ha. Jhon and Khan (2002) evaluated 23 genotypes of Tulip for their floral and bulb quality characters under

Kashmir valley conditions and found that cultivar Barcelona, Golden Apeldoorn and Apeldoorns Elite were taller as compared to the remaining genotypes. The genotypes West Point, Candel, China Pink, Apeldoorn's Elite and Golden Apeldoorn were superior in respect of diameter and cup depth of flowers. Golden Oxford, Golden Apeldoorn and Apeldoorn's Elite, Mrs. Jhon T. Scheepers, Golden Apeldoorn and Blenda proved superior in respect of weight of bulbs per plant.

Gupta *et al*. (2002) studied the performance of gladiolus cultivars in Malwa region of Madhya Pradesh. They found that American Beauty and Spring Green recorded the maximum spike per corm (1.3) followed by Propeticious (1.25) and White Prosperity recorded maximum spike length (83.20 cm) followed by Thumbiliana (72.00 cm) and they tested the duration of spike with eight cultivars of gladiolus and reported that the variety White Prosperity showed the highest durability. Studies conducted on performance of various cultivars of gladiolus under valley conditions of Uttaranchal by Jagadish *et al*. (2003) revealed that Oscar cultivar showed best performance as far as spike length and number of florets per spike is concerned.

Evaluation of gladiolus cultivars under Mahabaleshwar conditions by Patil (2003) revealed that the variety Sancerre produced larger spikes, more number of florets per spike. Varieties Yellow Stone and Tropic Sea were also found to be superior in respect of spike length and number of florets per spike compared to rest of varieties. Seetharamu *et al*. (2003) evaluated six varieties of gladiolus under polyhouse conditions. American Beauty preformed well with respect to plant height (Stalk length), number of florets per spike and uniform distribution of florets on the spike, corm and cormel production, followed by Her Majesty and Cheaper White for hill zone.

Nair and Shiva (2003) evaluated several gladiolus cultivars for cut flower production. The cultivar Darshan produced the maximum number of spikes per plant (3.0) and Dhiraj had the maximum number of florets per spike (12.94) with 5.32 florets opening at a time. Pusa Suhagin had the largest vase life (9.20). Basavaraddy (2004) evaluated elite hybrids of gladiolus for cut flower production under Transition tract of Karnataka and reported that the hybrids AB x MC, M-HVG and MC x AB were found

to be good for cut flower production with respect to their number of days taken for spike initiation, number of days taken for first floret to open, length of spike, rachis length, size of the floret and number of marketable spikes.

Kamble *et al*. (2004) showed that among the nine gladiolus cultivars studied, the maximum plant height was observed in the cultivar Trust (81.1 cm) followed by Vedanapoli (69.9 cm), American Beauty (65.7 cm) and Melody (65.2 cm), whereas the cultivar Sylvia recorded only 61.30 cm and the maximum vase life was resulted in the cultivars Summer Sunshine (8.3 days) and Snow White (8.0 days), whereas, the minimum was observed in the cultivar Sylvia (5.3 days). Rajiv Kumar and Yadav (2005) reported that among the 31 gladiolus cultivars, Pusa Dhanvantri produced the tallest plant (115.8 cm) followed by Pusa Sagun (111.2 cm) and Pusa Gunjan (106.2 cm).

Kishan *et al.* (2005) studied on the performance of gladiolus under Delhi conditions and found that variety Dhanvantari produced tallest plants (130.83 cm), followed by Anjali (124.00 cm) whereas sylvia was the smallest (74.33 cm) in height. Anjali produced maximum number of leaves per plant (11.33) followed by Dhanavantari (10.66). Lepcha *et al*. (2007) studied 13 gladiolus genotypes under mid hill conditions of Uttarakhand and the maximum vase life was recorded in White Prosperity (11.25 days) and was on par with cvs. Her Majesty, Red Majesty and Candyman whereas, minimum was recorded in cv. Thumbeliana (4.25 days).

Rupa Rani *et al.* (2007) evaluated 15 cultivars of gladiolus to find out their suitability of commercial cultivation under Ranchi conditions. Performance of the cultivar American Beauty was the best in respect of plant height, length of spike, weight of spike, number of floret, size of floret and duration of flowering in field conditions and corm diameter. Performance of varieties such as White Prosperity and Pink Friendship were next to American Beauty for the above characters and the keeping quality was found to be best in American Beauty and Pink Friendship.

In gladiolus, Anon (2008) found that the maximum days to sprout was taken by Eurovision (12.8 days) followed by Snow Princess (12.6 days), whereas minimum days to sprout (7.6 days) was taken by Rose Supreme. Maximum plant height (75.8 cm) at 60^{th} DAP was recorded in Rose Supreme, which was on par with Subhangini (75.4 cm),

whereas the minimum plant height (44.7 cm) was recorded in the American Beauty under Pantnagar conditions and the the longest shelf life (13.5 days) was resulted in Legend followed by Pricilla, Tropic Sea and Peter Pears with the vase life of 12.5 days..

Swain *et al.* (2008) evaluated twelve exotic gladiolus cultivars for cut flower and corm production purposes under Orissa conditions. Among the 12 cultivars, American Beauty, Congo Seal, Charmi Flower, Eight Wonder and Jubily recorded 100 per cent corm sprouting whereas the minimum (80%) was recorded in White Prosperity. The cultivar Pacifica was found to be superior to the above cultivars with respect to plant height (178.0 cm), number of leaves per plant (11.3), size of leaf (246.0 cm^2), length of spike (98.3 cm), length of floret (11.10 cm) and diameter of floret (12.5 cm).

Tul Bahadur Poon *et al*. (2009) evaluated fifteen gladiolus cultivars for cut flower production in Bangalore conditions and found that the varieties Arka Kesar, H.S.84-7-11 and Shobha were early in respect of days to spike emergence, full spike emergence, the first bud showing color and flowering.

Kandpal and Chandra (2010) conducted a trial to evaluate eight gladiolus varieties for two growing conditions, one in winter and other in summer seasons. The performance of various gladiolus cultivars and seasons showed that the significant effect on days to emergence of spike (47.2 days), days to first floret showing colour (58.8 days), days to 50 per cent of florets to open (65.4 days) and days to complete flowering (70.8 days) were observed in the cv. Royal Jublee, whereas total number of florets per spike (20.4), length of spike (106.0 cm) and length of rachis (65.9 cm) were maximum in the cv. Shabnam from among all the cultivars during winter season.

Rahul Kumar *et al.* (2010) evaluated twelve genotypes of gladiolus and reported that Snow Princess performed well with respect to important characters like early flowering, length of spike, vase life *etc*. as compared to the other genotypes.

Sankari *et al.* (2010) evaluated 42 gladiolus varieties under Shevaroy hills conditions and reported that the variety Pusa Swarnima recorded the longest length of spike (155.6 cm) and weight of spike (73.1 g). Number of florets per spike (23.0) and number of florets remaining open at a time (9.0) were the maximum in the variety Legand.

The varieties Pusa Swarnima, Legand, Pricilla and Shobha were found suitable for cultivation in Shevaroy hills and the maximum vase life in plain water ranged from 8 to 14 days in Legand and the minimum in Jester.

Shanmugasundaram *et al.* (2010) evaluated 12 gladiolus varieties under costal conditions of Karaikal and reported that the maximum plant height was recorded in Pacifica (154.2 cm) followed by White Prosperity (144.2 cm) and Eight Wonder (141.7 cm).

In gladiolus, the variety Arka Kesar was identified as the best variety with quality spike characters and suitable for cut flower production under Shevaroys condition (Vetrivel, 2010). Syed Atif Shaukat *et al*. (2012) evaluated eight cultivars of gladiolus under kashmir condition. Among this, the cultivars White Prosperity, High Style and Fidelio performed well with respect to both vegetative and floral characteristics.

2.2 Variability, Heritability and Genetic Advance

The success of any breeding programme depends on the extent of genetic variability available in the population. Genotypic co-efficient of variation indicates the relative magnitude of genetic diversity present in the material and helps to compare the genetic diversity associated with different characters. The amount of heritable phenotypic variability is more relevant than the total amount of phenotypic variability for the character. Hence, this can be estimated by heritability.

Heritability estimates provide information on the degree of inheritance of characters from parent to progeny. Knowledge on the heritability of different characters in relation to their contribution towards yield is a pre-requisite for an efficient breeding programme.

Heritability value alone may not provide clear predictability of the breeding value. Its estimate along with genetic advance is usually more useful than simple heritability values in predicting the resultant effect for selecting the best individuals (Johnson *et al*., 1955). This is due to the fact that a character may have high heritability but very less phenotypic variation thus giving low values of genetic advance.

Genetic advance is another breeding parameter for determining the amount of expected change that could occur due to selection. Heritability with genetic gain is more useful in predicting the resultant effects for selecting the best individual (Johnson *et al.,* 1955).

The expected genetic gain as proposed by Burton and Devane (1953) is the product of heritability, phenotypic standard deviation and selection differential. Genetic advance is more useful in predicting the actual selection value than heritability, though the latter indicates the relative effectiveness of selection based on phenotypic expression of the character.

2.2.1. Tuberose

Banker and Mukhopadhyay (1980) reported significant differences among the four cultivars of tubersoe (*Polianthes tuberosa* L.) for morphological characters which include number of leaves, number of spikes per plant, length of spike, number of florets per spike, number of days required for flowering and number of bulbs produced per plant.

Gurav *et al.* (2005) recorded very high estimates of heritability coupled with moderate to high genetic advance as per cent of mean for 100 floret weight, spike weight, weight of bulb/ plant, length of flower stalk, plant height and rachis in tuberose.

Vijayalaxmi *et al.* (2010) registered high phenotypic and genotypic coefficient of variation, high heritability coupled with high genetic advance as percent of mean for the variety Prajwal among seven cultivars.

2.2.2 Other related genera

Bichoo *et al.* (2002) recorded high genotypic coefficient of variation for number of cormels plant^{-1}and ten cormels weight in gladiolus indicated the presence of sufficient genetic variability for selection in these traits whereas high heritability and high genetic advance were registered in days to basal floret opening, plant height, spike length and number of florets spike^{-1}.

Balamurugan *et al*. (2002) noticed high GCV in gladiolus for number of side shoots whereas, low for longevity of individual florets and duration of first floret. Further they reported that there was a considerable difference in GCV and PCV for number of side shoots plant^{-1}, duration of flowering, longevity of individual florets and number of florets which indicated that these characters are highly influenced by environment.

In dahlia, Syamal and Kumar (2002) concluded that, traits like plant height, diameter of flower and number of days taken to flower registered high genetic advance combined with high heritability.

Singh (2003) studied the variability of 18 dahlia cultivars and stated that, high phenotypic and genotypic variations were found for number of flowers plant^{-1} (55.42-35.48). The characters flower diameter, dry flower weight and fresh flower weight exhibited high heritability coupled with high genetic advance indicating additive gene action. Number of seeds flower^{-1}, disc diameter, leaf width and number of branches plant^{-1} showed moderate heritability with low genetic advance thus showing the non-additive gene action.

Neeraj *et al*. (2005) recorded the variability studies in 26 diverse genotypes of gladiolus. High heritability with high genetic advance (as per cent of mean) were recorded for number and weight of cormels plant^{-1} and average weight of corm. High heritability with comparatively moderate genetic advance was obtained for number of corms plant^{-1}, length of spike and days to spike initiation. Heritability in these characters attributed to additive gene effect.

Sheela *et al*. (2005) concluded that, among the different heliconia varieties evaluated to ascertain genetic parameters of variability, PCV were higher than GCV for all the characters studied. However, higher PCV and GCV estimates were found for flower yield, bract size and leaf area. High heritability with high genetic advance was observed for flower yield, leaf area and bract size. Pratap and Rao (2006) registered that, among the 10 genotypes of gladiolus high genetic advance as per cent of mean coupled with high variability was noticed for the traits *viz*., plant height, number of florets spike^{-1} and days taken for flowering. Sheela *et al*. (2006) conducted variability studies in heliconia varieties and reported that the PCV were higher than GCV for all the characters indicating the role of environment in the expression of genotype.

In gladiolus, high heritability with genetic advance as per cent of mean was observed in the characters such as mother corm weight and diameter, number of florets per spike, number of daughter corms per plant, weight of daughter corm and vase life. But, least genetic advance as per cent of mean was observed in the characters such as number of days taken for first floret opening and diameter of daughter corm and rachis length was positive and significantly correlated with mother corm weight, plant height at 60th DAP, number of leaves per plant at 60th DAP, length of spike, weight of daughter

corm and vase life. But, it is negative and significantly correlated with number of days taken for first floret opening, number of daughter corms per plant and marketable spikes per plant at both genotypic and phenotypic levels (Vetrivel, 2010).

2.3. Correlations and path analysis

2.3.1. Correlation studies

The ultimate aim of any breeding programme is to develop a variety with desirable characters like high yield, resistance to diseases or pests or drought. The desirable character is controlled by many factors. Hence, it is necessary to know how these factors are related in terms of magnitude and direction with yield and among themselves. Such information on correlation will help plant breeders in planning a more efficient breeding programme.

The idea of correlation was first presented by Galton (1889) and was later elaborated by Fisher (1918) and Wright (1921). They observed phenotypic correlation represents the true picture of relationship among the genes governing the characters. Jain (1962) described that genotypic correlation was mainly due to two causes *viz.,* Pleiotrophy and linkage. Pleiotrophy (a single gene affecting the phenotypic expression of more than one trait) is probably the major cause for genetic correlation and becomes important when the correlation is high. If the correlation is low then it might be said that the two characters are inherited independently or they are governed by different sets of genes.

In any crop improvement programme for increasing the yield the information on the association of these characters will facilitate the selection of high yielding genotypes. The phenotypic, genotypic and environmental correlation coefficient existing between yield and its components have been widely studied in flower crops and brief review is presented below.

2.3.1.1. Tuberose

Sambandamurthi (1983) found that there was a very high association between number of flower stalks, number of flowers and number of suckers and the positive correlation was observed between yield and plant height, spike length, number of flower stalk, number of flowers and number of suckers.

Niranjan Murthy and Meenakshi Srinivas (1997) in a study with single and double types of tuberose reported positive association of flower yield with number of flowers per spike, flower diameter and 100 flower weight in single types. While in double types, the spike yield was positively associated with rachis length, flower length and flower diameter.

Positive and significant correlation was found for length of spike, diameter of flower, number of spikes per plant and number of flowers per spike with yield in tuberose (Kannan *et al*., 1998).

Nagaraja *et al.* (1999) reported that in tuberose a positive association of plant height, number of leaves with vase life and flower yield with number of florets per spike. Whereas the number of leaves had a negative and significant correlation with number of spike per plant and number of florets per spike.

In tuberose, Nagaraja and Gowda (2002) found the positive association of plant height and number of leaves with vase life and the flower yield with flower spike. The shoot length had negative and significant correlation with number of spikes per plant and number of florets per spike.

Correlation analysis carried out among eight tuberose single genotypes and six tuberose double genotypes by Radhakrishna, 2002 and the results revealed that, in single genotypes, number of flowers spike^{-1} showed significant positive correlation with loose flower yield and spike length. In double genotypes, spike yield showed positive and significant correlation with number of leaves plant^{-1}, rachis length, flower length and flower diameter.

2.3.1.2. Other related genera

Misra *et al*. (1987) documented that number of flowers per plant had positively significant correlation with spread of plant, which indicated that with the increase in number of flowers per plant, larger spread might be obtained. Days to first flowering had negative correlation with spread plant of in dahlia.

In dahlia, Srinivas (1993) found a significant positive correlation between number of inflorescences per plant, spread of plant, number of ray florets, duration of flowering, thickness of peduncle, peak flowering and negative significant association with number of disc florets.

In gladiolus, John *et al*. (1996) showed that length of spike exhibited significant positive correlation with plant height, number of florets per spike and size of florets, whereas strong negative correlation was found to be associated with days to floret opening at both phenotypic and genotypic levels.

Character association studies were conducted on 25 genotypes of gladiolus grown at three locations. A significant association of number of florets per spike with durability of whole spike, length of rachis and plant height indicated that single plant selections would be more effective for gladiolus improvement (Deshraj *et al*., 1997).

In gladiolus, Sakkeer Hussain *et al*. (2001) reported that, the characters *viz*., durability of whole spike, number of florets, rachis length, plant height and number of cormels plant^{-1} showed a significant positive correlation with number of florets spike^{-1}.

Correlation study indicated that, number of branches, days taken to flower, number of flowers, flower diameter and durability of flower are primary characters for economic yield in dahlia (Syamal and Kumar, 2002).

The correlation studies in gladiolus over different environments conducted by Nazir *et al.* (2003) indicated that significant and positive correlation of number of florets per spike was observed with rachis length, durability of whole spike and plant height indicating that an increase in these characters can be helpful in increasing rachis length which is one of the characters for export purposes.

In heliconia, number of flowering shoots produced per year had a strong positive correlation with vase life, number of flowers per bract and size both at genotypic and phenotypic levels (Sheela *et al*., 2006).

In heliconia, correlation revealed that, number of flowering shoots year^{-1} showed a strong positive correlation with vase life, number of flowers bract^{-1} and flower size both at genotypic and phenotypic levels (Sheela *et al*., 2006).

Prabhat Kumar *et al.* (2011) revealed that, the correlation analysis carried out on 29 diverse genotypes of gladiolus for 15 characters related to growth and flowering. In this, plant height exhibited highly significant and positive correlation with weight of corm, corm diameter, rachis length and number of leaves per plant.

2.3.2. Path co-efficient analysis

Study on path co-efficient developed by Wright (1921) provides an effective means of partitioning the total correlation into direct and indirect effects of various characters on yield. The available literature on the path analysis in different flower crops is reviewed here.

Anuradha and Gowda (2000) studied the path coefficient analysis in 25 genotypes of gladiolus, which revealed that high magnitude of positive direct effect of plant height, length of rachis, length of floret and length of spike. Number of florets per spike and weight of daughter corm showed positive direct effects. The indirect effect of plant height exhibited a high magnitude of direct effect on diameter of floret. Length of spike, length of rachis, durability of spike and days to emergence showed positive direct effects on number of florets per spike.

Sakkeer Hussain *et al*. (2001) stated that days to 50 per cent flowering recorded maximum positive direct effect towards number of florets per spike while days to first floret showing colour showed maximum indirect positive effect, in gladiolus.

Katwate *et al*. (2002) studied path analysis in gladiolus hybrids and revealed that character rachis length exhibited the highest direct effect (1.487) followed by weight of cormels per plant (0.571), length of spike (0.46) and number of leaves per plant (0.425) on the number of florets per spike.

In gladiolus, path analysis projected mother corm diameter, weight of daughter corm, number of florets per spike and marketable spikes per plant as the dominating contributors towards the rachis length under Shevaroys conditions (Vetrivel*,* 2010).

2.4. Bulb parameters

2.4.1. Tuberose

Sadhu and Das (1978) reported that the bulb yield increased with the increasing size of bulbs and depth of planting but decreased with lower planting density.

Bhattacharjee *et al*. (1979) reported that planting of large size bulbs (3.0-3.5 cm) significantly increased the production of bulb and bulblets.

Mukhopadhyay and Bankar (1981) found that wider spacing and large sized bulbs used at planting time produced heavier bulbs and bulblets per original bulb compared to closer spacing and smaller bulbs.

Sathyanarayana Reddy and Kartar Singh (1997) reported that the bulb production in tuberose increased with the increase in bulb size used for planting. Saleable bulbs (> 1.5 cm) per plant were maximum from the plant raised with bulbs measuring 2.1 to 3.0 cm in diameter.

Singh and Manoj Kumar (1999) studied the bulb production of tuberose cv. Double and found that the number of bulbs per plant was higher at planting density of 25 x 20 cm.

2.4.2 Related genera

Misra and Choudhary (1976) reported that gladiolus cv. Vink's Glory was the best multiplier of the corms and cormels and it is followed by Gold Dust, Sylvia and Winter Gladioli.

Lal and Singh (1978) studied the performance of seven gladioli cultivars and found that cultivar House of Orange produced the largest number of cormels (146) per plant.

Jana and Bose (1981) observed that in Hippeastrum large sized bulbs (5.1 to 5.5 cm) produced large number of bulblets and flowers whereas shallow planting (<5 cm depth) increased the number of bulblets.

Negi *et al*. (1982) studied the performance of four new gladiolus cultivars *viz*., Sapna, Meera, Nazarana and Poonam for different characters. They found that all the four new cultivars are good for corms and cormel production.

Misra *et al*. (1987) in their two year experiment under Katrain condition with 12 cultivars of gladiolus found that the miniature cultivars produce more number of corms than the late bloomers, which produce less number of corms. But late bloomers produce bigger cormels as these get ample time for development.

Saini *et al*. (1991) studied the performance of six gladiolus cultivars and found that 'Melody' was good multiplier of corms and cormels.

John *et al*. (1996) evaluated 41 gladiolus cultivars for cormel production and reported that cultivars 'Buff Beauty', 'King Lear' and 'White Prosperity' were adjudged best for production of cormels.

Sidhu and Arora (2000) conducted a trial to evaluate six gladiolus varieties for summer flower production and found that corm and cormel weight was significantly higher in the variety Nava Lux. However, number of corms per plant was the highest in the variety White Prosperity (1.3), while number of cormels per plant was the highest in the variety Summer Sunshine (12.0).

Rai *et al*. (2000) evaluated 16 gladiolus varieties under sodic waste land and found that number of corms per plant was the maximum in the variety First Lady (1.9 corms) followed by White Prosperity (1.6 corms). The size of corm was the maximum in Friendship (10.9 cm2), followed by White Prosperity (10.2 cm^2).

Sanjai and Brahma (2000) evaluated 20 gladiolus varieties for corm production in Ladakh and concluded that the variety Victor Borge showed higher number of cormels per plant (55.7), followed by White Prosperity (50.2), Eurovision (48.8) and Novelty (48.8).

Roy and Sharga (2000) studied 10 gladiolus cultivars under Lucknow conditions and found that mother: daughter corm ratio was the highest (1:1.8) in case of cultivar Priscilla. Diameter of corm was the maximum in case of the cultivars Ice Gold (6.6 cm) and Rose Supreme (6.6 cm).

Kamble (2001) evaluated gladiolus varieties in Arabhavi (Karnataka) conditions and reported that size (6.8 cm) and weight (143.9 g) of daughter corm were maximum in the variety Summer Sunshine.

Nair and Shiva (2003) evaluated several gladiolus cultivars for cut flower production. While the maximum number of corms per plant was produced by Green Willow (1.6), Pusa Subagini produced the maximum number of cormels per plant (45.9).

Kishan *et al*. (2005) studied the performance of gladiolus under Delhi conditions and found that the maximum weight of single corm was recorded by Gold Dust (124.7 g) and Dhanavantari (120.0 g), while minimum by Vinks Glory (66.7 g). The highest weight of cormels (16.0 g) was recorded by Chandani and the lowest (3.7 g) in Melody. African Star produced the maximum number of cormels per plant and the minimum by Gold Dust.

Anon (2008) revealed that the highest number of corms per plant was produced by Jester (4.1), followed by Punjab Morning (2.6), while the minimum number of corms per plant was produced in Bannos Memory (1.1) under Pantnagar conditions.

Swain *et al.* (2008) evaluated twelve exotic gladiolus cultivars for cut flower and corm production purposes under Orissa conditions. Among the cultivars, American Beauty recorded the maximum weight of corm (49.5 g), diameter of corm (6.3 cm) and number of cormels per plant (41.0).

Sankari *et al.* (2010) evaluated 42 gladiolus varieties under Shevaroy hills conditions and reported that weight of corm was the maximum in Pricilla (70.9 g) and number of cormels per plant ranged from 0 to 91. Mahawer *et al*. 2010 studied the performance of nine dahlia cultivars under Aravalli hillps of Udaipur and reported that the cv. NT Pompon found best to increase the number of tubers.

2.5 Floral biology

In *Polianthes tuberosa*, anthesis starts around 5 p.m. and ends around 7 p.m. and maximum anthesis was observed between 5.30 p.m. to 6.30 p.m. Anther dehiscence starts along with anthesis at 5 p.m. and ends within 30 minutes. Stigma becomes receptive from the day of anthesis and continues up to 72 hours after flower opening. Stigmatic receptivity reaches a peak on second and third day. If stigmas are pollinated when flowers are in bud stage, the day before flowering, or one and two days after opening of flowers, there will be no pollen tube production. Considerable pollen tube growth is expected if the flowers are pollinated three days after opening (Shen *et al*., 1987). The rate of pollen tube growth inside the style of cross- pollinated flowers is higher at 10, 24, 28 and 48 hours after pollination, when compared to that in self- pollinated flowers (Uma, 1990).

2.5.1 Pollen viability

As early as in 1951, Calvino conducted series of studies in rose with respect to the pollen fertility where he described various aspects of pollen fertility such as morphological and biochemical characters of pollen grains, percentage of aborted pollen, the relationship between pollen, number of chromosomes and certain somatic characters, the reserve substance in pollen germ inability under different conditions, viability and methods of prolonging viability of pollen grains.

Tuberose

Sitharamu (1993) observed the percentage of pollen viability or stainability by acetocarmine test in nine tuberose genotypes. The maximum percentage of pollen viability was recorded in IIHR- 6 and is followed by cv. Single and cv. Variegated Single.

Other genera

Pollen fertility in 96 varieties of garden roses was studied by Sahare and Shastry (1963). Of these varieties of Hybrid Tea class in 'Kaiserin August','Victoria' showed the highest pollen sterility percentage (98 %), the cultivatrs 'Ena Harkness' exhibited the lowest pollen sterility of 10 per cent only. It was also observed that three varieties of each of triploids and an aneuploid 'Hybrid Tea' had very high pollen sterility status.

Shrivelled, unstained and empty pollen grains were considered as sterile ones. The average pollen fertility in rose varieties varied from 19 per cent ('Gruss' and 'Goburg') to 36 percent ('Crimson Glory') during winter season (Lata, 1971). Swarup *et al*. (1973) determined pollen fertility in different cultivars in a total of 184 cross combinations, while attempting hybridization between 'Hybrid Teas' and 'Floribunda' or among themselves. Lata and Gupta (1975) studied the effect of ionizing radiation on roses and determined pollen fertility after staining the pollen grains for two hours with acetocarmine in the cultivar 'Montezuma' and its pink and reddish orange flowered mutants. Unstained and shrivelled pollen grains were treated as sterile. The pollen fertility decreased in pink flowered mutants as compared with control, whereas it was increased in reddish flowered mutants.

Gowda *et al*. (1977) observed the percentage of pollen viability or stainability by acetocarmine test and also seed number per hip in eight rose cultivars. The percentage of pollen fertility varied from 12.29 ('Super Star') to 56.76 ('White Christmas'). It was also shown that percentage of pollen fertility in controlled pollination and pollen stainability percentage by acetocarmine test were almost the same. Semeniuk (1979) found out the pollen fertility percentage in three new cultivars namely 'Spotless Gold', 'Spotless Yellow' and 'Spotless Pink' which were the selections from F_3 populations.

Lata (1980) while studying the effect of ionizing radiation on seven rose cultivars, observed that irradiation brought about a loss in pollen fertility which was confirmed on the basis of acetocarmine stainability test.

Shevchenko and Feofilova (1981) studied the number of viable, non-viable and mean diameter of pollen grains in Canna hybrids and cultivars. They reported that pollen viability ranging from zero to 93 per cent.

Dadlani and More (1988), while studying 34 *Jasminum* cultivars belonging to eight species indicated that lack of seed setting was due to the absence of anthers/ pollen or pollen sterility, non-viability or failure to germinate depending upon the genotype. They reported that pollen fertility and viability were highest in *Jasminum sambac*, *J. flexile* and *J. auriculatum.*

Pollen fertility status of 48 rose cultivars was estimated by using 0.5 per cent acetocarmine stain and versatile stain by Sandhya (1987). She recorded that maximum pollen fertility (79.9 %) was observed in Hybrid Tea cv. 'Happiness'. Among the floribundas, cv. 'Chandrama' showed the maximum pollen fertility (79.9 %), cvs. 'Aguarius' (19.3 %) and 'Roi des Rois' (20.47 %) were found to have minimum percentage of fertile pollen grains among Hybrid Teas and Floribundas respectively.

Aswath *et al.* (1989) reported that failure of seed setting in climber *Pyrostegia venusta* was due to the development of non- viable pollen grains and also due to occurance of degenerating ovules at megaspore tetrad stage.

Gurumurthy (1990) estimated the pollen fertility status of 10 rose cultivars by using versatile stain. The data revealed that the percentage of pollen fertility in 10 cultivars varied from 24.71 per cent in cv. 'Lilli Mariene' to a maximum of 71.40 per cent in cv. 'Proud Land'.

2.5.2 Pollen germination

Tuberose

Sitharamu (1993) examined the percentage of pollen germination by hanging drop technique in nine tuberose genotypes. The maximum percentage of pollen germination was recorded in IIHR- 6 and is followed by cv. Single and cv. Variegated.

Other genera

Kancalova (1977) reported that maximum pollen germination and pollen tube growth occurred in 30, 35 and 40 per cent sucrose with 1.5 per cent agar at 28 to 35^0C. He also observed branched pollen tube occasionally in the germinating pollen of individual *Rosa spp.*

Visser *et al.* (1977) reported that the pollen grains of Hybrid Tea rose germinated well in a 15 percent sucrose solution with 40 ppm boric acid. *In vitro* pollen germination was studied by Henny (1977) in Spathiphyllum and observed that pollen germinated well in media containing 5- 30 per cent sucrose.

Gilissen (1978) reported that in petunia, the pollen germination in a medium containing 10 per cent sucrose and 0.01 per cent H_3BO_3 was largely related to relative humidity. Germination was minimum at zero relative humidity, but when pollen was transferred from zero to 100 per cent relative humidity, they germinated immediately. Further, he observed that the decreased pollen germination was resulted when it is exposed to 400 KR x rays.

Pollen germination in 43 Iris cultivars and two wild types were studied by Yabuya *et al.* (1982). They reported that pollen germination ranged from 27.7 (cv. Syakakonishiki) to 88.5 per cent (wild type). Average pollen germination of 68.5 per cent was observed in all the cultivars and varieties. Liu *et al.* (1983) reported that in Litchi the anthers were cultutred on a 10 per cent sucrose medium with four growth regulators i.e., 2,4,D- 200 ppm, NAA- 2 ppm, Triacontanol- 20 to 30 ppm, B-9 - 70 ppm and boric acid- 5 ppm. The germination rates were maximum with 2,4-D.

Ostrolucka and Bencat (1987) investigated pollen germination in culture with 0, 15 or 25 per cent sucrose. The percentage germination and pollen tube growth of some woody ornamentals were found to be high in 20 per cent sucrose.

Markose and Aravindakshan (1987) indicated that a medium containing 20 per cent sucrose, one per cent agar and 100 ppm boric acid was best in hibiscus.

In pansy, John *et al*. (1988) reported that maximum pollen germination (12.6 %) after 48 hours in five per cent sucrose solution followed by four per cent (8.87 %). They also stated that the pollens did not germinate in sucrose solution below 0.5 and above 7.0 per cent concentration.

Luzmy and Burivalova (1989) observed pollen germination capacity of 27 rose cultivars and four rose root stocks in laboratory for two years. Highly significant differences were found between the cultivars (4.0 to 37.5 %) and between the root stock pollens (21 to 70 %).

Aswath *et al.* (1990) reported that five per cent sugar solution was found best for optimum pollen germination of Bignoniaceae family ornamental climbers.

Gudian *et al.* (1991) studied *in vitro* pollen germination of freshly collected pollens of rose cv. Meitakilar at different environmental temperature and relative humidity. Higher temperature (23^0C and 30^0C) improved pollen germination and increased pollen tube growth compared with lower temperature (13^0C and 17^0C). A RH of 63 per cent resulted in the longest pollen tubes.

2.5.3 *In vivo* studies on Pollen- Pistil interaction

In general, the difficulty in obtaining distant hybrids increases with increase in the phylogenetic distance between the species involved. Pre and post fertilization barriers may not be equally strong in all the combinations as the genes responsible for these barriers act at different stages of development. Hence detailed studies on pollen stigma interaction, embryo development upon direct hybridization would be necessary to overcome the interspecific barriers for better exploitation of the different genomes for crop improvement programmes.

2.5.4 Pollen- pistil interaction

The different stages in the pollen stigma interaction in distant crosses is the failure of pollen grain to germinate on a foreign stigma, reduction in pollen tube growth and failure of pollen tubes to discharge male gametes into the embryo sac. Hence it is worthwhile to study the pollen germination and pollen tube growth in *in vitro* and *in vivo* to assess the strength of incompatibility manifested in distant hybridization programmes.

2.5.5 Pollen germination and pollen tube growth

Rosen (1961) studied the mechanism of pollen tube growth in *Lilium spp*. The polar organization of the cytoplasm reflects on the unipolar growth of pollen tubes. The dictyosomes produce secondary vesicles that display a net flow in the direction of the plasma membrane at the tip of the tube where growth takes place. The vesicles produced a new membrane and cell wall components necessary for extension by fusing with plasma membrane. The growth of the pollen tubes could be regarded as an example for the high secretory activity.

Brewbaker and Kwack (1963) demonstrated the importance of calcium ions for pollen germination and pollen tube growth in Oenothera. Voyiatzi (1995) studied the effect of sucrose, boric acid and calcium on pollen germination of rose cultivars. The optimum level of sucrose and boric acid enhanced the germination whereas calcium exhibited inhibitory effect. They noticed negative correlation between calcium concentration and germination percentage.

Heslop- Harrison (1983) reported the function of pectin on the pollen tube. The pectic sheath formed on the surface which is in direct contact with the secretions of the stigma and transmitting tract and it may well be the site of recognition reaction. As in a somatic cell, the microfibrils cellulose provides tensile strength and elasticity, while compact callose inner lining reinforces the tube in the radial direction. The callose structure propably showed a further important function in regulating the movement of materials across the wall of the older parts of the pollen tube.

2.5.6 Pollen tube attrition

Pollen tube attrition is an important phenomenon in determining reproductive success of plants. Arrest of pollen tube growth is more common in incompatible crosses as to maintain genetic identity of a species. So studies on the pollen tube attrition in interspecific crosses will reflect the strength of relationship among the species.

Yono *et al.* (1975) studied the behaviour of pollen tubes and the developmental process of ovules in Pelargonium (Geranium). They noticed that though many pollen grains germinated on the stigma, most of the pollen tubes are conceived to stop half way in various part of the pistil. Some tubes in the stigma, some in the style and other in the ovary. Only one to three pollen tubes were found to reach the ovary and fertilize the egg.

Williams *et al.* (1982) detected 10 different abnormalities of arrested pollen tube tips including burst, tapped, swollen, coated, spiralling, spiky and variable diameter of syndromes of incompatible inter specific crosses in Rhododendron. They also noticed the site of pollen tube arrest within the pistil and error syndrome of tip growth and callose deposition anomalies.

2.5.7 Embryology

Endosperm is the most common nutritive tissue for the developing embryos in angiosperms. Functionally it is comparable to the female gametophyte in gymnosperms but has a unique origin, whereas the female gametophyte in gymnosperms differentiates before fertilization and is haploid, the endosperm is the product of fertilization and is usually triploid. After double fertilization the egg is called zygote and the fusion of products of polar and the secondary male gamete is termed primary endosperm nucleus. The former develops into an organised embryo whereas the latter give rise to an almost formless tissue, the endosperm. The endosperm may either be consumed by the developing embryo or it may persist in the mature seed and continued to support the growth of embryo during seed germination. Hence the endosperm plays a significant role in the evolution of angiosperms because of its physiological and genetic relationships to the embryo. The evolutionary significance of the endosperm is certainly due to its role in the development of the seed (Stebbins, 1976). Differences or failure of seed development in distant hybridization is due to its harmony in the development of endosperm or embryo.

2.6 Seed set

Tuberose

Shen *et al.* (1987) reported that in tuberose crosses between single and double cvs. produced fruits and seeds when female parent was fertilized on 2- 3 days after anthesis (DAA). Further, they stated that reciprocal crosses between single and double cultivars produced many single and double plants.

Breeding behaviour and pollination efficiency of *Polianthes tuberosa* cvs. Single and Variegated Single was studied by Uma (1990). She recorded zero fruit set when cv. Single was selfed or open pollinated. But 63.78 per cent of fruit set was observed

when cv. Single was cross pollinated with the cv. Variegated Single as male parent. The highest fruit set of 12.13 per cent and 28. 84 per cent were obtained when cv. Variegated Single was self -pollinated and cross- pollinated with cv. Single, respectively.

Seed setting, pollen viability and pollen germination behaviour of nine tuberose genotypes was studied by Sitharamu (1993) after open pollination, selfing and cross pollinations. He recorded maximum fruit set in the genotype IIHR-1 under open pollination.

In tuberose, crosses were made in nine different combinations and the successful fruit set was noticed in four crosses *i.e*. IIHR-6 x Shringar, Mexican Single x Shringar, Shringar x Mexican Single, IIHR- 6 x Mexican Single (Krishnamurthy, 2000).

Other related genera

The capsules obtained from the crosses made six days after anthesis in *Lilium hybridum* cv. Enchantment produced 46 mature seeds, whereas the others yielded only 13- 27 seeds per capsule (Nimi *et al.,* 1997).

Higher per cent of fruit set was observed in inter varietal crosses of Charis Malleria and Aroma (63.6 %) as compared with the different selfing. Self incompatibility resulted in poor fruit set in garden roses as compared with the crosses. Good seed set was observed in all intervarietal crosses involving six tetraploid cultivars of roses though they were found to be generally incompatible. However, negative result was obtained in Grass and Loburg which was highly female sterile cultivar (Lata, 1971).

The percentage of successful crosses in rose varied from 24.4 to 96 in different cross combinations. The total number of hips also showed larger variations in different crosses. There were as many as 50 seeds per hip (Pink Parfait X Swathi) and two seeds in a hip (Delhi Princess x Crimson Glory) (Swarup *et al.,* 1973).

The hip setting and behaviour of rose cultivars through selfing and crossing was greatly influenced by temperature. The period between August- December was found to be favourable for carrying out hybridization programme. The hip set was improved by artificial crossings. The hips matured early by artificial crossing by taking less number of days. The number of seeds per hip was observed better during August- December with artificial crossing (Gurumurthy, 1990; Sandhya, 1987) with rose cvs. ‘Happiness’, ‘Paradise’ and ‘Queen Elizabeth’.

2.6.1 Seed germination

Tuberose

The fruits obtained from crosses between Single x Double resulted in 15- 23 fertile seeds, whereas the fruits obtained by cross between Double x Single recorded 19 to 31 fertile seeds per capsule (Shen *et al.,* 1987) in tuberose.

The germination of F_1 seeds obtained from crosses Single x Variegated was 36.38 per cent in Variegated cultivar and it took 53.64 days to germinate. Germination of 21.26 per cent was recorded in F_1 seeds obtained from cross between 'Variegated' x 'Single' and the seeds took only 35.64 days for germination which seems to be earlier (Uma, 1990).

The maximum percentage of germination was obtained in a cross between IIHR-6 x cv. Double (85.4 %) and IIHR-6 x IIHR-5 (75 %), whereas it was slightly low in case of cross between IIHR-6 x IIHR-3 (69.71 %). Seeds obtained from the cross between IIHR-6 x IIHR-2, cv. Single x IIHR-4 germinated slightly earlier (13 days) compared to rest of the cross combinations (Sitharamu, 1993).

Seed viability studies indicated that the seeds obtained from the reciprocal crosses involving single and variegated cultivars of tuberose were more fertile with higher seedling vigour than self and open-pollinated seeds of variegated cultivar (Uma and Gowda, 1990).

The maximum percentage of germination (16.5 %) obtained in cross between Shringar x Mexican Single when compared to seeds obtained from rest of the crosses made in tuberose (Krishnamurthy, 2000).

Other genera

Seed germination was studied in the open pollinated, selfed and crossed seeds of 10 gladiolus varieties. Germination percent in general was quite high (70- 100 %) in most of the samples (Dhaduk *et al*., 1989).

There was 75 per cent germination in the seeds of *Lilium hybrida* cv. Enchantment obtained from the cross made at zero day of anthesis (Nimi *et al*., 1997).

2.6.2 Seed testing procedures

2.6.2.1 Pre-treatments for breaking dormancy

Seed dormancy may be defined as a state in which growth is temporarily suspended. Presence of dormancy poses problem in the field by showing variations in germination resulting in abnormal field stand. A number of concepts and theories of seed dormancy have emerged through the years by several researchers. The dormancy may also be due to immature embryo and after ripening requirements and the presence of the inhibitors (Kohli and Kumari, 1986).

Sharga *et al.* (1970) reported that improved germination of African Marigold seeds were noticed when soaking in GA_3 for 30 min at 25 ppm. Bhat and Dhar (1971) investigated on the nature of dormancy in *Belladona* seeds and concluded that the dormancy is due to the presence of inhibitor and suggested the leaching of seeds with H_2SO_4, ethylalcohol and petroleum ether to overcome dormancy.

Denial Sundaraj *et al*. (1971) reported that for *Tephrosia purpuria* seeds, scarification with sand followed by presoaking in hot water at 50°C for 5minutes was the best for improving germination whereas, seed treatment with concentrated sulphuric acid for 5 minutes alone was suggested by Dharmalingam *et al*. (1973) for improving germination.

Uniform germination of balsam seeds was resulted by soaking them in GA @ 500 ppm (Jouret, 1977). Increased germination over control was resulted when balsam and lavendula seeds were soaked in GA_3 @ 100 – 200 ppm (Renard and Clere, 1978).

Improved germination percentage of 56 – 64% was resulted by soaking the seeds of chrysanthemum in GA_3 for 24 h @ 20, 40, 60 and 80 ppm (Barkar, 1980). Miller and Holcomb (1982) reported that increased primula seed germination of 88 % was resulted when it was soaked in 250 ppm GA_3 for 24 h. Soaking the seeds of pansy in GA_3 @ 100 - 200 ppm improved its germination. Selvaraju (1985) observed that among the different chemicals used as presowing treatment, the maximum augmentation on seed quality attributes was obtained with potassium nitrate. Seed soaking in 0.75 per cent potassium nitrate for 16 hours was found to enhance germination and vigour.The scarification did not hasten germination in species of Heliconia (Criley, 1988).

Drewes and Staden (1990) reported that GA_3 and GA_{4+7} accelerated germination at 25^0C in marigold. Singh and Srivastava (1990) discerned that soaking of lavender seeds in one per cent HCl for 12 h in case of pre-soaked seeds in water or 24 h incase of dry seeds, washing in running water followed by soaking in water for 24h and then sowing gives reasonably good germination. Mehta and Sen (1991) reported that seeds of *Cassia italia* exhibited seed dormancy and pre-treatment with concentrated H_2SO_4 and mechanical scarification improved the germination.

Increased germination percentage and reduced mean germination time of petunia seeds was resulted by soaking the seeds in GA $_{4+7}$ (Finch Savage *et al.*, 1991). Dry seeds infused with 10 mM GA_3 resulted improved germination in balsam and coreopsis (Persson, 1993). Seed soaking in 0.75 % KNO_3 improved the germination percentage of Afrrican Marigold to 89.5 % (Selvaraju and Selvaraj, 1994).

Soaking the seeds of zinnia for 24 h with GA_3, GA_{4+7} and NAA improved its germination (Blamowski and Borowski, 1995). Accelerated seed germination was resulted in *Primula vulgaris* after soaking in GA 4+7 (10^{-4} m) + BA (10^{-5} m) and seed soaking in GA $_{4+7}$ resulted improved germination in petunia (Finchsavage *et al*., 1991). Percentage germination was highest after mechanical scarification (90-100 %) in the seeds of *Cassia biflora*, *C. dalbergia*, *C. fistula*, *C. javanica*, *C. nodosa*, *C. renigera*, *Senna siamea and S. spectabilis* (Geetha *et al*., 1997).

Improved germination of 90 % was resulted by soaking the phlox seeds in GA_3 1000 ppm for 8 h (Sathyanarayanan, 2000). Seed soaking of gaillardia, zinnia in GA_3 @ 200 ppm for 16 h resulted improved germination over control (Vijayan, 2002). Osman Karaguzel *et al.* (2004) tried pre- sowing treatment with *Lupinus* seeds and best response was observed in seeds scarified with concentrated H_2SO_4 for 12 or 16 hours.

Takami *et al.* (2009) investigated the effect of seed wing removal on the recovery of germination in *Lilium formolongi* Hort. Wing removal acclerated seed germination at tempertares of 18, 22 and 24^0C. Abscisic acid (ABA) denied the effect of wing removal through gibberellin did not. These results suggested that wing removal improved seed germination rate by accelerating water absorption and decreasing germination inhibitors.

Paric *et al.* (2008) reported that the seeds of Lilium species generally have deep dormancy. Removal of seed coats and cutting seeds allowed germination. Ramzan *et al*. (2010) tested the dehusked seed of *Gladiolus alatus*. Seeds without husk gave the promising outcome of 74% germination while seeds with husk merely acquired 63% germination after 30 days.

2.6.3 Standardization of quick viability test (TZ test)

Weber and Wiesner (1980) standardized that staining was optimum after 4 h soaking with 0.1 per cent tetrazolium (TZ) and kept in the dark at room temperature for the seeds in *Achillea millefolium*, *Lewis flax*, *Amelanchier alnifolia*¸ *Ceretoides lanata*, *Rhus trilobata* and *Symphoricarpos albus*.

Tetrazolium staining is a dependable and accepted biochemical method for testing the inherent germination capacity of a seed lot. This is especially important in crop seeds in which quick results are needed (Pasha and Das, 1982).

The seed lots of *Largerstroemia parviflora* bisected longitudinally and stained with 0.1 per cent triphenyl tetrazolium chloride for 5 h is done to determine the viability of seeds rapidly (Babeley and Kandya, 1986).

Natarajan (2000) reported that the soaking of marigold seeds in 0.25 per cent solution of tetrazolium chloride for one hour was found to be optimum to obtain a desirable staining pattern to assess the viability status.

The soaking of zinnia and gaillardia seeds in 0.25 per cent solution of tetrazolium chloride for one hour was found to be optimum to obtain a desirable staining pattern (Vijayan, 2002).

Natarajan (2003) observed that soaking of petunia seeds in 0.25 per cent solution of tetrazolium chloride for 2½ h was found to be optimum to obtain a desirable staining pattern.

Sriram (2004) found that the TZ seed-staining pattern was better in one per cent TZ solution at 40^0C treated for 4 h in case of *Ocimum sanctum* and *Solanum nigrum* but for *Hibiscus sabdariffa* and *Solanum viarum*, the treatment durations were 6 and 8 h, respectively. Seeds of senna showed better staining in 0.5 per cent at 40^0C soaked for 8 hours.

Selvakumari (2005) revealed that soaking with 0.1 (or) 0.2 per cent tetrazolium chloride solution for two hours and/or one hour respectively was found to be optimum for assessing china aster seed viability within a short time.

2.7 Post fertilization events

2.7.1 Incompatibility

Tuberose

Tuberose possess the characters of self-incompatibility and dichogamy, thereby it prevent the fruit set upon selfing (Shen *et al*., 1987; Sitharamu,1993; Uma and Gowda, 1990).

Other genera

The prevalence of pollination barrier in the form of self-incompatibility in *Amaryllis* belladonna was reported for the first time by Darwin (1876) in *Zephyranthes carinata* and *Hippeastrum aulicum* (Darwin, 1877).

Luckwill (1949) reported that the development of an ovary into a fruit is dependent on hormonal stimuli derived chiefly from developing seeds and collapse of the selfed ovule. It may therefore, be assumed to have had an adverse effect on the growth of the ovaries.

Lewis (1949) suggested that, the reaction of self-incompatibility results from the identical genes in pollen and styles which polymerize to form the incompatibility complex. This may act as growth inhibitor to further reduction and growth of pollen tube.

A late acting pollen inhibition is observed in three species of Lilium by Brock (1954) where self pollen tubes were seen to reach the base of the style in all three but in two species of *L. candidum* and *L. szovitsianum*, the ovary enlarged following self- pollination. This was not the case with *L. candidum* and no evidence of endosperm development was found out of 312 ovules examined and in *L. szovitsianum*, 11 of 468 possessed developing endosperm. It appears more likely that incompatible pollen tubes were routinely rejected before reaching the ovule, but the rare occurrence of developing ovules in *L. candidum* may be an indication that rejection response is not absolute.

Ovary as the site of inhibition was reported in Narcissus, Hemerocallis, Lilium, Gasteria, Ribes and Annona (Bateman, 1954; Arasu, 1968) and in Freesia (Pandey, 1970). Brewbaker (1957) opined that in these species, styles being hollow, pollen tubes did not come in contavt with the stylar tissue which was necessary for growth inhibition to occur.

Plant species with hallow styles where insufficient contact between pollen/ tubes and stylar tissue could prevent inhibition to occur at an early stage (Brewbaker, 1957). Lewis and Crowe (1958) reported that interspecific and intergeneric incompatibility usually occurred unilaterally in which zygote formation was prevented when self- incompatible species were pollinated by self compatible species.

Brewbaker (1959) summarising the distribution of self incompatibility among crop plants reported the occurrence of homomorphic, gametophytic self incompatibility in Iridaceae, Amaryllidaceae and Liliaceae families.

Pandey (1959) stated that the incompatibility substances are concentrated in and limited to the first layer of stigma which contains the pollen incompatibility substance and its contact through the pollen pore with the stigmatic surface, being sufficient to produce the incompatibility response.

Linskens (1964) demonstrated that the inhibition of incompatibility pollen tube growth in self- pollinated buds was directly proportional to the age of the bud. Higher percentage of fruit set in intervarietal crosses of rose cvs. ‘Charles Mallerin’ and ‘Aroma’ (63.60 %) was observed as compared with selfing. Self- incompatibility resulted in poor fruit set in garden roses as compared with other crosses (Lata, 1971).

Unilateral self-incompatibility was reported among *Lilium spp.* by Ascher and Peloquin (1968). Fett *et al.* (1976) and Ascher (1978) reported the localization of incompatibility barrier in the ovary and demonstrated that it was neither in the stigma nor in the style of *Lilium longiflorum*, whereas Hiratsuka *et al.* (1983) opined that, the self-incompatibility reaction in *Lilium longiflorum* occurred in all portions of the inner surface of the stylar canal and was not restricted to any portion.

Occurance of gametophytic self- incompatibility among six cvs. of *Hemerocallis* was reported by Arisumi (1975). Linskens (1975) and Gilissen and Brantjes (1978)

reported that, petunia had monofactorial gametophytic self- incompatibility and that the site of pollen tube rejection after incompatible pollination was the ovule. In the genus *Gasteria*, Brandham and Owens (1978) reported that the self- incompatibility reaction was controlled at least by two genes and confirmed that the site of pollen tube inhibition was in the ovule.

Kawase and Tsukamoto (1977) determined the percentage of self-fertility following self pollination in chrysanthemum. The plants were generally self-incompatible, but this varied from year to year and even between individual flower on the plant. In self pollinated progenies the degree of self- incompatibility was generally similar to that of parents but progeny of cv. 'Katanazakura' (KB line) displayed a comparatively high degree of compatibility. With nine progenies of KB line self- fertility was higher in tubular florets than ray florets.

Aswath (1985) studied three climbers namely, *Pyrostegia venusta*, *Tecoma jasminoides* and *Arrabaedia magnifica*, observed that degeneration of ovules occurred at different stages of development commencing from degeneration of archesporium and megaspore mother cell.

The flowers from three genotypes of Hydrangea were collected 1, 2, 4, 8, 24, 48 and 72 hours after self pollination, stained with aniline blue and the results revealed the persistence of self incompatibility from 1 to 72 hours after pollination (Sandra, 2004)

In petunia, self-incompatibility prevents the growth of a pollen tube through the style which arrested the seed set upon selfing (Thomas and Timothy, 2009).

2.8 Root knot nematode

One of the production constraints of tuberose in India as well as other tuberose producing countries is the incidence of root knot nematode caused by *Meloidogyne incognita*. This root knot nematode is wide spread and the host range is also very wide with more than 3000 plant species, representing virtually all plant families (Jenkins and Taylor, 1967). *Meloidogyne incognita* is one of the most commonly occurring plant parasitic nematodes which enjoys the wide distribution in Tamil Nadu and has been observed to attack large number of economic crops including tuberose (Sunderababu and

Vadivelu, 1988). Among the causes of low yield, occurrence of root knot nematode is one of the main constraints in the production of tuberose (Khan and Parvatha Reddy, 1992). The root knot nematode *Meloidogyne incognita* is one of the most prevalent species associated with tuberose in India (Nagesh *et al.*1997).

2.8.1 Epidemiology of root knot nematode

Among the various species of *Meloidogyne,* three important species *viz., Meloidogyne incognita, Meloidogyne arenaria* and *M. javanica* are widely prevalent in tropical region between 35^0S and 35^0N latitudes and *Meloidogyne hapla* is predominant in temperate regions (Taylor and Sasser, 1978).

2.8.2 Pathogen

Meloidogyne incognita causes a yield loss up to 15 percent in tuberose (Khan and Parvatha Reddy, 1992). Powell (1971) proved that root knot nematode facilitates entry and establishment of pathogenic fungi and bacteria in the root system.

Dhawan and Sethi (1976) reported that *Meloidogyne incognita* completed its lifecycle on tuberose within 36 days. The penetration started 24 h after inoculation and continued up to seventh day. Guar and Prasad (1980) observed 80.7 per cent reduction at 4000 larvae per plant and reported that the growth and yield were negatively correlated with initial and final population of nematode.

2.8.3 Symptomatology

Varma *et al.* (1979) in brinjal reported that the plants infected with *Meloidogyne incognita* were poor in growth and in same infestation, plants die prematurely. Foliar symptoms include stunting, a general unthrifty appearance and leaf chlorosis (yellowing). General symptoms of nutrient deficiency were also reported. Leaves wilted, particularly during hot sun period and recovered slowly when irrigated (Parvathareddy, 1986).

Swain *et al*. (1987) noticed typical symptoms of nematode injury on both above ground and below ground plant parts. Nematode infestation of roots generally involved stunting, general unthriftiness, premature wilting and slow recovery by improved soil moisture conditions, leaf chlorosis (yellowing) and other symptoms characteristic of

nutrient deficiency in brinjal. Sharma *et al.* (1994) stated that plant symptoms occur because of root disfunction, reducing root volume and foraging and poor utilization efficiency of roots for water and nutrients.

Hazarika (1990) in brinjal noticed stunted or declining of plants occurred in patches rather tahn uniformly throughout entire field. Positive diagnostic confirmation is provided by symptoms of root galling, where gall size may range from a few spherical swellings to extensive areas of elongated, convoluted, timorous swellings along the entire surface of roots. Reddy *et al.* (1997) also observed the same symptoms. Borah and Phukan (2000) reported that the affected plants have an unthrifty appearance and often showed symptoms of stunting, wilting or chlorosis (yellowing). Symptoms are particularly severe when plants are infected soon after planting.

Hassan *et al.* (2000) in tomato noticed that below ground, the roots had obvious galls or knot like swelling, these swellings prevented the movement of water and nutrients to rest of the plant resulting in stunted plant growth. Below ground, the symptoms caused by root knot nematode were quite disinfective. Chakrabarty (2001) recorded lumps or galls, ranging in size from one to ten mm in diameter envelop all over the roots. In severe infestations, heavily galled root was rotted, leaving a poor root system with a few large galls. Umamaheswari and Rajeswari Sundarababu (2001) observed reduced root system with galling. There was poor top growth and foliage is frequently chlorotic (yellow) and severe infections cause wilting of the foliage.

Rahman *et al.* (2002, 2002a) reported that in *Solanum sp.* the disease was expressed by gall formation in the root system and ultimately the plant became weak due to interruption in nutrient uptake from the soil and at severe infection the plants may die. Above the ground, stunting of plants, yellowing of leaves, galls deformed and stunted roots were reported by Koike *et al.* (2003) in vegetables.

Symptoms of nematode infection are the formation of root galls which resulted in growth reduction, nutrient and water uptake reduction, increased wilting, mineral deficiency and poor yielding plants (Abad *et al.*, 2003). Nematode symptoms consisted of stunting, yellowing and a general unthrifty appearance of plants. Infected plants may

wilt or die in hot, dry weather. Below ground, the roots had obvious galls or knot- like swellings. These swellings prevented the movement of water and nutrients to the rest of the plant resulting in stunted plant growth (Serfoji *et al.*, 2010).

2.8.4 Screening techniques

An ideal and effective screening technique is essential to distinguish between resistant and susceptible genotypes with high yield potential.

Screening tests on tomato cultivars and hybrids, banana and cowpea cultivars were conducted to find the resistant or susceptible varieties or cultivars to *Meloidogyne incognita* by Devi and Thakur (2007), Kumar and Jain (2008) and Olowe (2009).

Rajendran *et al.,* 1975 screened the crossandra cultivars against root-knot nematode, *Meloidogyne incognita* and noticed that the cultivars red and orange were susceptible to *Meloidogyne incognita.*

In carnation, Cho *et al.* 1996 screened 33 cultivars against root-knot nematode, *Meloidogyne incognita* and reported that the cultivars Desio, Castelaro, Kappa, Rara, Izu Pink, Target and Antalia were highly resistant to *Meloidogyne incognita* and 12 cultivars were moderately resistant and remaining 14 cultivars were susceptible.

Among the five varieties screened in gladiolus, cultivars viz., Summer Shine and White prosperity were found to be moderately resistant. These varieties had less number of females with egg masses and also less number of eggs and seven asiatic lily varieties were screened for their reaction to root-knot nematode, *Meloidogyne incognita.* Among the varieties tried, the variety Cordelia was moderately resistant and this had less number of female with egg masses and also less number of eggs (Nirmal Johnson*,* 2000).

2.9 Mechanism of disease resistance

Identification of resistance is possible through quantifying the biochemical components present in the genotype. The biochemical constituents like phenol, orthidihydroxy phenols, cholorogenic acid, IAA oxidase, peroxidase (PO) and polyphenol oxidase (PPO) are available in tuberose and these biochemical constituents possess resistance properties. It is obvious in many cases that the biochemical factors in conferring non- preference and antibiosis. Many researchers documented the change in

PO, PPO, AAO, and PAL activity in relation to nematode resistant plants (Ganguly and Dasgupta, 1979, 1984; Zacheo and Zacheo, 1988; Sirohi and Dasgupta, 1993; Zacheo *et al*., 1995).

2.9.1 Total phenol

Plants have a wide range of phytochemicals which impart protective action against nematodes. Among these an aromatic ring bearing a hydroxyl substituent called phenolic substituent has antifungal, antibacterial and antiviral activities. Accumulation of phenolic compounds in host parasite reaction is the general phenomenon of resistance and breakdown of these compounds determined the degree of resistance (Farkas and Kiraly, 1962 and Sindhan and Parashar, 1984). Post infectional increase of phenols might be due to the tendency of phenols to accumulate at the site of infection which are involved in the defence mechanisms of plants through the interference in the metabolic activities of pathogens (Farkas and Kiraly, 1962). Several researchers have emphasized the role of phenols as an expression of defence mechanism by the host plants (Bajaj *et al.,* 1983; Zacheo *et al.*, 1990). Resistance to herbivores is manifested by biosynthesis of a family of plant defence chemicals by either constitutive and/or induced mechanisms (Harborne, 1988; Gatehouse, 2002). Phenolic compounds enhance the mechanical strength of host cell wall and also inhibit the invading pathogenic organisms. The total phenol in root were found to be in higher quantities in the tolerant varieties of gladiolus and asiatic lily (Nirmal Johnson*,* 2000).

Association of phenolics with resistant reaction of plants to the nematode infestation seems to be rather inevitable. The more necrotic tissue is resulted in more phenolics to the host which ultimately results in the extermination of the invading parasite (Roy, 1981). *Lycopersicum esculentum* and *Lycopersicum pimpinellifolium* resistant to *Hederodera rostochinensis* shown to have higher total phenol content than the susceptible hosts (Giebel, 1970). Singh and Choudhury (1973) observed distinct categories of phenolic content. Susceptible cultivars showed 40-44, highly tolerant 79, resistant 97- 102 and immune 107-117 g total phenol/ g of root. Increase in phenol content appears to be associated with increase in the degree of resistance to root knot nematodes.

Masood and Husain (1976) analysed the phenol content in roots and shoots of three tomato varieties *viz.,* Nemared (resistant to *Meloidogyne incognita*), Chicogrande (moderately resistant) and Marglobe (highly susceptible). Highest concentration of phenols was recorded in Nemared and lowest in Marglobe. Sethi and Sharma (1976) estimated the total phenol content of roots of resistant and susceptible varieties of cowpea infected with *Meloidogyne incognita*. The result showed higher amount of total phenol in Barsati Mutant which is resistant and very low concentration in susceptible Pusa Barsati variety.

Som and Choudhary (1976) also reported that the resistant cultivars exhibited higher amount of phenols than the susceptible ones. Tomato varieties resistant to root knot nematodes (Narayana and Reddy, 1980) were also found to have higher phenol content than susceptible varieties. The phenolic compounds are the best known factors involved in susceptible- resistance response. Increased in concentration of phenols following infection with nematodes were reported by Ganguly and Dasgupta (1984) in tomato.

Sharma *et al*. (1990) studied the alteration in phenol and proline content of *Meloidogyna incognita* infected brinjal cultivar. Higher concentration of proline and phenol were detected in 30, 60 and 90 days old infected roots of resistant cultivar (RHR-51) cultivar of brinjal as compared to susceptible and uninfected roots. An increase in phenolic content of 559.0 percent was observed in 60 days old infected roots of resistant cultivar.

Gopinath and Madalageri (1986) and Sadhankumar (1995) observed a significant correlation of phenol with resistance and suggested a possible role of phenol in the mechanism of wilt resistance in brinjal and tomato respectively. *Lycopersicum hirsutum f.sp. glabratum,* a resistant wild species registered a high total phenol content when compared to the susceptible commercial varieties and the difference in total phenol content was highly significant (Kalloo, 1986).

Singh and Abidi (1988) also reported high phenol content in cultivars of tomato which showed high disease resistance with phenol content ranging from 514 mg/ 100 g to 933 mg/ 100 g. Sudha (1991) observed that these lines showed resistance at field level and had remarkably higher amounts of phenols than the commercial varieties. Malhotra (1991) also reported higher total and OD phenols in resistant cultivars than susceptible

cultivar following infection with *Fusarium oxysporum*. Markose (1996) reported that the bacterial wilt resistant chilli variety Ujwala exhibited significant increase in total phenol content in roots.

Indu Rani *et al.* (2008) studied on biochemical basis of resistance to root knot nematode and reported that among the parents, CLN 2026C and SL 120 recorded the highest level of phenol, orthodihydroxy phenol. Similar results were also reported by Sundharaiya (2008) in tomato and Janani (2009) in pepper.

2.9.2 Orthodihydroxy phenols

Orthodihydroxy phenols are known to be highly toxic and play a major role in plant disease resistance (Mahadevan, 1966). They get easily oxidized by polyphenol oxidase and peroxidase to highly reactive quinones which are effective inhibitors of sulphydryl enzymes thereby preventing the metabolic activity of host and parasitic cells (Mahadevan, 1970).

The ortho-dihydroxy phenols in root were found to be in higher quantities in the tolerant varieties of gladiolus and asiatic lily (Nirmal Johnson., 2000). Higher amount of orthodihydroxy phenol content was observed in egg plant roots infected with *Meloidogyne incognita* (Balasubramanian and Purushothaman, 1972). Masood and Husain (1976) also observed the highest concentration of orthidihydroxy phenol in *Meloidogyne incognita* resistant cultivar Nemared than the susceptible cultivar Marglobe in tomato. Orthodihydroxy phenolic compounds such as caffeic acid, chlorogenic acid, ortho quinones and tannins were shown to strongly inhibit the activities of cellular enzymes produced by microorganisms in addition to growth inhibition (Hunter, 1978).

A positive association between orthodihydroxy phenol content in roots and bacterial wilt has been established. Resistant lines have been reported to have higher orthodihydroxy phenol content compared to susceptible lines in tomato, brinjal and chilli (Rajan, 1985; Markose, 1996 and Paul, 1998). Sankari (2000) also reported higher amount of total and orthodihydroxy phenols in the leaves of hybrids and its parents in tomato, resistant to leaf curl virus.

2.9.3 Host enzymes

Defence reaction occurs due to accumulation of PR proteins (chitinase, β 1,3-glucanase), chalcone synthesis, phenylalanine ammonia lyase, peroxidase, phenolics, callose, lignin and phytoalexins. Studies on the involvement of enzymes in host pathogen relationship are important in infestation/ infection by any plant pest and changes in plant as affected by nematode (Owens and Specht, 1966)

2.9.3.1 Peroxidase (PO)

Peroxidase have been implicated in the regulation of plant cell elongation, phenol oxidation, polysaccharide cross- linking, IAA oxidation, cross linking of extension monomers, oxidation of hydroxyl- cinnamyl alcohols into free radical intermediates and wound healing (Vidhyasekaran *et al.,* 1997). Extensive research have been carried out by several authors on the induction of peroxidase activity in response to *Meloidogyne incognita* in plants and are reviewed hereunder.

Huang *et al.* (1971) reported that *Meloidogyne incognita* infection in tomato resulted in increased peroxidase activity in the host tissue during the development of second stage larvae and adult female but not third and fourth larval stages. Infection of some plants by parasitic nematodes correlated with an increase in peroxidase activity (Gregory and McClure, 1978).

Bajaj *et al.* (1985) reported that *Meloidogyne incognita* induced an increase in peroxidase activity in resistant tomato cultivars and decrease in peroxidase activity in susceptible cultivars. Shukla and Chakraborty (1988) reported that *Meloidogyne incognita* resistant variety of tobacco and tomato plants had significantly higher peroxidase enzyme specific activity than susceptible varieties. Inoculated seedlings of the susceptible cultivar alone showed an increase of peroxidase activity in the first phase, but both cultivars presented higher peroxidase in the second phase. The resistant cultivar always exhibited higher peroxidase activity than susceptible one.

The peroxidase activity continuously increased in both resistant (SL-120) and susceptible (Pusa Ruby) cultivars of tomato after inoculation with *Meloidogyne incognita* (Ganguly and Dasgupta, 1979). Ibrahim (1991) reported that increased peroxiadse activity

constituted defence mechanism of plants against invasion or damage caused by nematodes. Molinari (1991) detected isoperoxidase in resistant and susceptible tomato roots uninfected and infected by *Meloidogyne incognita* but the peroxidase activity and cellular locations were induced differently in resistant and susceptible cultivars by nematodes.

Bradley *et al.* (1992) reported that the increased peroxidase activity correlated with resistance in many species including barley, cucurbits, cotton, tobacco, wheat and rice and that could prevent pathogen penetration. Increased activity of peroxidase in tomato was positively correlated with nematode resistance (Rajasekar *et al.,* 1997; Sirohi and Dasgupta, 1993).

Chakrabarti and Mishra (2002) reported that among 10 cultivars of chick pea, BG 1067 showed increased peroxidase activity (1.97 units/min/g of roots) with the highest phenolic activity (9.7 mg/g) in *Meloidogyne incognita* infected roots and thus categorized as tolerant to root knot nematode.

Jothi and Rajeswari Sundarababu (2002) observed increase in peroxidase activity in brinjal cv. CO2 inoculated with the root knot nematode, *Meloidogyne incognita.* Similar trend of increased activity was noticed in tomato by Indu Rani *et al*. (2008) and Sundharaiya (2008).

2.9.3.2 Polyphenol oxidase (PPO)

Polyphenol oxidasse usually accumulates upon wounding in plants. Biochemical approaches to understand polyphenol oxidase function and regulation are difficult because the quinonoid reaction products of polyphenol oxidase covalently modify and cross-link the enzyme. Polyphenol oxidase can be induced *via.,* octadecanoid defense signal pathway (Constabel *et al.,* 1995). Incraese in polyphenol oxidase after the entry of nematode was attributed to a triggered phenol oxidation process induced by pathogen (Maraite, 1973).

Ganguly and Dasgupta (1984) observed sequential development of polyphenol oxidase (PPO) activity in resistant and susceptible tomato varieties after inoculation with *Meloidogyne incognita.* Nematode infestation did not show any interference of enzymatic synthesis in shoot region. In nematode infested roots an initial decrease in enzyme activity was observed in both varieties. During the post infection period, the increase in enzymatic change in resistant varieties was higher than the susceptible varieties.

Rajagopalraman *et al.* (1992) reported higher polyphenol oxidase (PPO) activity in cowpea cv. Pusa Dofalsi resistant to *Meloidogyne incognita.* Devarajan and Seenivasan (2002) observed that inoculation of *Meloidogyne incognita* increased the polyphenol oxidase (catechol oxidase) activity in all four tested cultivars of banana.

Increased activity of polyphenol oxidase was observed in tomato in response to infection against root knot nematode by Indu Rani *et al*. (2008) and Sundharaiya (2008).

2.9.3.3 Phenylalanin ammonia lyase (PAL)

PAL catalyzes the determination of L- phenylalanine to trans-cinnamic acid, which is the first step in the biosynthesis of large class of plant natural products based on the phenylpropane skeleton, including lignin monomers as well as certain classes of phytoalexins. Induction of enzymes such as phenylalanine ammonia lyase and peroxidase leading to the accumulation of phenolics and lignin can occur in response to insect and pathogen attack, exposure to oxidizing pollutants, mechanical stimulation and are thought to function in the resistance of plants to damage by these stresses. Phenylalanine ammonia lyase is the key enzyme in inducing synthesis of salicylic acid (SA) which induces systemic reistance in many plants. Phenylalanin ammonia lyase is a product of cinnamic acid; it is directly linked with lignifications (Podile and Laxmi, 1998; Silva *et al.,* 2004).

Edens *et al.* (1995) reported that transcription of genes encoding phenyl alanine ammonia lyase (PAL) and activity of this enzyme was found to be increased in resistant cultivars of nematode infected soyabean. Loganathan (2002) reported that cabbage and cauliflower plants exhibited a higher induction of phenylalanine ammonia lyase from seven days after challenge inoculation and reached a maximum of 14 days with *Meloidogyne incognita.*

Nithya Devi *et al.* (2007) reported that nematode inoculated plants recorded the highest phenylalanine ammonia lyase activity (17.9 unit/ min/ g) than control in banana. The nematode infestation increased the activity of PAL in all the banana accessions tested and differences were significant.

2.9.4 Other enzymes

2.9.4.1 IAA oxidase

The root knot nematode infection in tomato can affect the synthesis and/ or activity of a number of enzymes, especially IAA oxidase. Viglierchio and Yu (1965) while examining the response of different plants to *Meloidogyne incognita* found that small galls appeared on roots with higher IAA oxidase activity and that large galls were characteristic of roots with low IAA oxidase activity.

2.9.4.2 Acid phosphatase

Valeri and Greggory (1991) noticed that acid phosphatise belongs to a broad group of enzymes that catalyze the hydrolysis of inorganic phosphate from phosphor mono esters at low pH. Acid phosphatise is ubiquitous in nature and activity has been reported in a wide range of plants. APS-1 encodes acid phosphatise-1, one of the important enzymes present in tomato. APS-1 is closely linked to *Mi* gene, a gene conferring resistance to nematodes.

Pankaj *et al.* (1998) observed the increased activity of Ascorbic Acid Oxidase (AAO) in roots of resistant tomato (SL-120, Hisar Lalit and NR-7) than susceptible varieties (Pusa Ruby and Pusa Gaurav). The importance of acid phosphatise in resistance to*Meloidogyne incognita* infesting tomato was emphasised by Ganguly *et al.* (2000). The activity of acid phosphatase increased significantly in resistant vartiety (Nemamukt) than susceptible variety (Pusa Ruby).

CHAPTER III

MATERIALS AND METHODS

The present investigation on "Evaluation of tuberose genotypes for development of F_1 hybrids for quantitative, qualitative characters and nematode resistance" was carried out at the Department of Floriculture & Landscaping, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore during the period June 2011 to March 2013. The details of materials used and experimental methods adopted are presented in this chapter.

3.1. MATERIALS

3.1.1 Location of the experimental field

The field experiment was conducted at field number 10 C of the Botanical Gardens, Department of Floriculture and Landscaping, Tamil Nadu Agricultural University, Coimbatore. The geographical details of the experimental location is as follows

Latitude	: 11^0 02" N
Longitude	: 76^0 57" E
Altitude	: 426.76 m above MSL

3.1.2 Weather and Climate

The meteorological data recorded during the study period (June 2011 to March 2013) is presented in Annexure I.

3.1.3 Soil characters

The soil in the experimental field is sandy loam in texture. The soil properties of experimental field are furnished in Annexure II.

3.1.4 Planting material and crop details

Medium sized bulbs (3.0 - 3.5 cm diameter) weighing about 25 grams of 15 tuberose cultivars obtained from various centres were utilized for the study. The details of the cultivars are given in following table.

S.No	Genotypes	Source
Single types		
1.	Calcutta Single	Bidhan Chandra Krishi Viswavidyalaya (BCKV), Kalyani, West Bengal
2.	Hyderabad Single	Hyderabad
3.	Kahikuchi Single	Assam
4.	Mexican Single	Mexico
5.	Navsari Local	Navsari Agricultural University (NAU), Gujarat
6.	Phule Rajani (Mexican Single x Shringar)	Mahatma Phule Krishi Vidyapeeth (MPKV), Rahuri, Maharastra
7.	Prajwal (Shringar x Mexican Single)	Indian Institute of Horticultural Research (IIHR), Bangalore
8.	Pune Single	Pune
9.	Shringar (Mexican Single x Pearl Double)	Indian Institute of Horticultural Research (IIHR), Bangalore
10.	Variegated Single	-
Double types		
11.	Calcutta Double	Bidhan Chandra Krishi Viswavidyalaya (BCKV), Kalyani, West Bengal
12.	Hyderabad Double	Hyderabad
13.	Pearl Double	Mexico
14.	Suvasini (Mexican Single x Pearl Double)	Indian Institute of Horticultural Research (IIHR), Bangalore
15.	Vaibhav (Mexican Single x IIHR - 2)	

3.2. Experiment I: EVALUATION OF TUBEROSE GENOTYPES FOR HIGHER FLOWER AND CONCRETE YIELD

The experimental design and layout adopted are as follows:

Design	- Randomized Block Design (RBD)
Number of replications	- 3
Number of genotypes	- 15
Spacing	- 45× 20 cm
Plot size	- 4 m x 1 m
Date of planting	- 08.06.2011

3.2.1 Lay out and planting

Ridges and furrows were formed with a spacing 45 cm between two ridges and the bulbs were planted 20 cm apart half way down the ridge at a depth of 5 cm. The gross size of an individual plot was 4 x 1 m in each replication. Medium sized bulbs of 3.0 - 3.5 cm diameter weighing about 25 grams were selected and treated with growth regulator (GA_3 200 ppm) for 12 hours. The treated bulbs were planted in double rows at 45 x 20 cm spacing accommodating 44 plants per plot. Nine plants in the centre of the bed were chosen for observation (Plate 1).

3.2.2 Cultural practices

Recommended dosage of 200: 200: 200 NPK kg ha^{-1} were applied as per the recommendation of the Crop Production Manual, TNAU, 2012. Half of the RDF was applied as basal and remaining half the dose was applied in two splits i.e., 30 and 45 days after planting respectively.

Micronutrients (H_3BO_3 @ 0.1 % + $ZnSO_4$ @ 0.5 % + $FeSO_4$ @ 0.2 %) sprayed four times at 60, 120, 180 and 240 days after planting and other cultural practices (Ganesh, 2010).

3.2.3 Observations

3.2.3.1 Observation on growth parameters

Nine plants were selected replication wise in each genotype and tagged for recording the observations on various growth and flower characters.

3.2.3.1.1 Days taken for sprouting of bulb (days)

Sprouting of bulbs was recorded till all the bulbs sprouted in each treatment and the number of days taken for completion of sprouting of all the bulbs in each treatment was counted.

3.2.3.1.2 Plant height (cm)

The plant height was measured from the ground level to the terminal end of the plant and the mean was worked out.

3.2.3.1.3 Number of leaves per plant (nos.)

The number of leaves per plant was counted and mean was worked out.

3.2.3.1.4 Days to spike emergence (days)

Number of days taken for emergence of spike in each genotype was counted from the date of planting of bulb in a plot.

3.2.3.1.5 Duration of Flowering (days)

Duration of flowering of a single spike was counted from the date of opening of first pair of florets in a spike to the date of opening of last pair of florets in a spike.

3.2.3.2 Observations on Spike characters

3.2.3.2.1 Spike length (cm)

The spike length was measured from the base to tip of the spike and mean values were calculated.

3.2.3.2.2 Rachis length (cm)

Rachis length was measured from the point of the appearance of basal floret in the spike to the tip of the spike. The mean was worked out.

3.2.3.2.3 Number of florets per spike (nos.)

The number of florets produced per spike was counted.

3.2.3.2.4 Length of the floret (cm)

The length of the floret was measured in a fully developed floret from the basal to the distal end of the corolla tube in a spike and mean values were calculated.

3.2.3.2.5 Weight of florets per spike (g)

The weight of florets per spike was measured by weighing the individual florets in a spike and mean values were calculated.

3.2.3.2.6 Number of spikes per m^2 (nos.)

The total number of spikes harvested during the period of observation was counted for every square meter.

3.2.3.2.7 Yield of florets per plot (Kg)

Harvesting was done by picking the pair of fully developed unopened florets and the sum of recorded weight across all the harvests made.

3.2.3.3 Observations on quality characters

3.2.3.3.1 Vase life (days)

The spikes were harvested when one or two basal florets opened. After cleaning the stem and recutting under water, the harvested spikes were placed in ordinary tap water and its longevity was recorded in number of days. The stage at which florets started drooping was taken as index for end of vase life.

3.2.3.3.2 Concrete recovery (%)

The fully opened flowers of all single and double types of tuberose were harvested before 9.30 AM. Concrete content of flowers was analyzed by solvent extraction method (ASTA, 1960) with food grade hexane, averaged and expressed in per cent of concrete recovery. A sample of 50 gram was taken in the glass column of Soxlet apparatus and concrete content was estimated using food grade hexane as solvent. Soluble extract was then drained off into a pre weighed 100 ml beaker (W_1).

The extract was then evaporated on a steam bath and heated for 30 minutes in an oven at 60^{o}C, cooled and weighed (W_2). The concrete content was calculated using the following formula and expressed in percentage.

$$\text{Concrete recovery (\%)} = \frac{W_2 - W_1}{50} \times 100$$

3.2.3.3.3 Identification of chemical compounds

The chemical compounds present in the concrete are identified by Gas Chromatography- Mass Spectrometry (GC-MS) (Leela *et al.,* 2002). The concrete obtained should be dried over anhydrous sodium sulphate. The component identification was achieved by the GC-MS analysis using Clarus 500 (Perkin –Elmer, USA). Helium was used as a carrier gas and the samples were injected in split mode (30:1). Mass spectra were acquired over a 50-450 atomic mass unit range. Compounds were identified by comparing the mass spectral data with those in the in-house library *viz.,* NIST, WILEY.

3.2.4 Floral biology of different tuberose genotypes

The studies on floral biology and pollination were carried out in all tuberose genotypes. The details of the observations recorded are presented below.

3.2.4.1. Flower morphology

3.2.4.1.1 Days to first floret opening (days)

Number of days taken for first floret opening in each plant was counted from the date of planting of bulb to the opening of first pair of florets in a spike.

3.2.4.1.2 Floret diameter (cm)

The floret diameter was measured only after opening with scale and and mean values were calculated.

3.2.4.1.3 Bud length (cm)

Bud length was measured from base to the tip of the bud and and mean values were calculated.

3.2.4.1.4 Hundred flower weight (g)

Weight was measured from randomly selected hundred fully opened florets.

3.2.4.1.5 Bud colour

The colour of the matured buds was recorded a day prior to opening.

3.2.4.1.6 Floret colour

The petal colour of freshly opened florets was recorded.

3.2.4.1.7 Row of petals

Row of petals was measured by counting the number of whorls of petal arrangement in each floret.

3.2.4.1.8 Number of petals per floret

Number of petals per floret was measured by counting the number of petals.

3.2.4.1.9 Number of pistils

Number of pistils was recorded by counting from 10 florets in each genotype and expressed as mean number of pistils.

3.2.4.1.10 Length of the stamen (cm)

The length of stamen was recorded in 10 florets in each genotype.

3.2.4.1.11 Length of the pistil (cm)

The length of pistil was recorded in 10 florets in each genotype.

3.2.4.1.12 Filament length (cm)

The length of filament was recorded in 10 florets in each genotype.

3.2.4.2 Anthesis time

Anthesis time was recorded by observing the time of opening of matured floret from 10 different tagged florets.

3.2.4.3 Stigma receptivity

To assess the duration of the stigma receptivity, artificial pollination of flowers was carried out under controlled condition during two days, three days and four days after anthesis. Flowers were emasculated at pre-anthesis stage and covered with butter paper cover. Ten florets were pollinated at the above mentioned three stages starting from 6.00 am to 9.00 am at one hour interval. Pollen grains were dusted over the receptive stigma. Flowers were bagged with butter paper cover after pollination. The bags were removed 10 days after pollination and those that showed fruit set were counted to assess the receptivity of stigma.

3.2.4.4 Anther dehiscence

Ten florets were tagged for a day before anthesis. Observations on dehiscence were recorded on next day *i.e.*, the anthesis stage at one hour interval from 3.30 to 6.30 pm. Appearance of longitudinal split in the pollen sac indicated the commencement of anther dehiscence. The per cent of anther dehiscence at different time intervals were worked out and the time at which maximum per cent of anther dehisced was recorded as the time of anther dehiscence.

3.2.5 Palynology

The palynological study which includes pollen output, pollen stainability and pollen germinability were carried out as done by Sathiamoorthy (1973) in banana.

3.2.5.1 Studies on geometry of the pollen grains

Pollen grains of the 10 genotypes (single type) were used for the study. Pollen grains were collected from freshly dehisced anthers by gently tapping the anthers on glass slides containing a drop of glycerol. Then cover slips were placed over the pollen and slides were observed under microscope connected with a computer in ordinary light. The observations were recorded from at least 25 pollen grains for each genotype.

The following observations were recorded using the Biowizard software.

3.2.5.1.1 Size of pollen grain (μm^2)

It refers to feature area, the total number of detected pixels with the feature.

3.2.5.1.2 Equivalent diameter of the pollen grain (microns)

It refers to equivalent circle diameter i.e., the diameter of the circle having the same area as the feature.

3.2.5.1.3 Radius of the pollen grain (microns)

It refers to circle radius i.e., the radius of the circle.

3.2.5.1.4 Perimeter of the pollen grain (microns)

It refers to the total length of the boundary of the feature. This is calculated from the horizontal and vertical projection with an allowance for the number of corners.

3.2.5.2 Pollen output

Pollen production per floret was estimated using a Haemocytometer method as suggested by Sathiamoorthy (1973). Three samples of five anthers from each genotypes were collected just prior to dehiscence. Anthers were crushed with a small glass rod in a vial containing 2.5 ml of distilled water and a drop of teepol for obtaining a good suspension of pollen grains in water.

The contents were thoroughly shaken and two drops were pipetted out and placed on each of the two "counting chambers" of a "Spencer bright line Haemocytometer". The number of pollen grains in each of the eight "corner squares" was recorded. This was repeated five times for each sample and was designated as sub samples. The average number of pollen grains per square multiplied by 2500 gave the quantity of pollens per anther.

3.2.5.3 Pollen viability

The pollen viability and fertility were studied by the following methods.

3.2.5.3.1 Acetocarmine test

The freshly dehisced pollen grains were collected from all the single tuberose genotypes in sterilized petri dish. The pollen grains were dusted on the cavity slide followed by a drop of Acetocarmine stain on the slides. Deeply stained, normal and plumped pollen grains were considered viable while shrivelled, deformed and weakly

stained pollen grains were recorded as sterile ones. Pollen fertility was assessed for three days *viz*., first day, second day and third day of anther dehiscence and expressed in percentage.

3.2.5.4 *In vitro* studies on pollen grain

The following medium (Brewbaker and Kwack, 1963) was used for pollen germination.

Sucrose	15 % W/V
Boric acid	100 ppm
Calcium Nitrate	200 ppm
Magnesium Sulphate	200 ppm
Potassium Nitrate	100 ppm

Since the medium did not yield desirable results, a preliminary experiment was conducted to standardize the medium involving different combinations of boric acid and sucrose. The standardized medium was a modification of the earlier composition for *in vitro* pollen germination. Hence, sucrose 15 percent and boric acid 100 ppm were substituted with 20 per cent sucrose (W/V) and 200 ppm boric acid respectively. The details of standardisation are presented in chapter IV.

Procedure

Pollen grains from freshly dehisced anthers and those from anthers after 15, 30 and 45 minutes of dehiscence were collected and used for pollen germination. Pollen grains from different genotypes were dusted on a drop of medium placed on a cavity glass slide and observed under the microscope. The materials were replicated three times with five slides per replication and the following observations were recorded.

3.2.5.4.1 Percentage of pollen germination

Number of pollen grains germinated in medium after five minutes of incubation was recorded and expressed as percentage on the total number of pollen grains placed.

3.2.5.4.2 Length of pollen tube (microns)

Measurement was made from the base (point of emergence) of the pollen tube emerged from pollen to the tip of the pollen tube after 1, 5, 10, 15, 20 and 25 minutes after anther dehiscence.

3.2.5.5 *In vivo* studies on pollen grain

3.2.5.5.1 Fixing of pistils

Self pollinated pistils of 10 single genotypes (Calcutta Single, Hyderabad Single, Kahikuchi Single, Mexican Single, Navsari Local, Phule Rajani, Prajwal, Pune Single, Shringar and Variegated Single), cross pollinated pistils of different combinations of above parents were collected at 2, 4, 6, 8, 10, 12, 14, 24 and 26 hours after pollination, fixed in 18 ethanol: 1formalin: 1glacial acetic acid (FAA) fixative and stored at 4- 10^0C for 24 hours. The pistils were then transferred to 70 per cent ethanol and stored in refrigerator till further use.

The *in vivo* studies with pistils were carried out by aniline blue fluorescence technique described by Sitch (1990). However, the procedures had to be modified in respect of the softening period, strength of NaOH and concentration of K_3PO_4, aniline blue by conducting a preliminary experiment. The standardized procedure is outlined below

1. The stigma and stylar pollinated pistils along with the ovary were fixed in 18:1:1 (FAA) fixative at different periods of interval after pollination.
2. The pistils were gently washed with distilled water for three to four times
3. Pistils were macerated in 8 N NaOH for 3 hours
4. Pistils were thoroughly washed in distilled water and stained for 4 hours to 12 hours in 0.1 percent aniline blue prepared in 0.3N K_3PO_4.
5. The stained pistils were placed on a glass slide containing a drop of glycerol and covered with 22 x 60 mm cover slip and pressed gently.
6. The slides were observed under Nikon- microphot- FX microscope with fluorescence attachment, illuminated with 200 W high pressure ultraviolet lamp. The observations were taken with B (380- 490 nm) and/ or BG (650 nm) excitation filters in combination with BA 520 or BA 530 barrier filters. Colour

photographs were taken in Kodak Gold 400 ASA film either with barrier filter (greenish yellow background) or without barrier filter (blue background with bright white fluorescing pollen tube).

The observations recorded were:

3.2.5.5.2 Abundance of germinated pollen on a scale from 1 to 4

The number of pollen grains sticking on to the stigmatic or stylar surface showing pollen tube growth even after washing were counted (Plate 3 and 4). Different grades were assigned as given below.

Grade	Description	Number of pollen grains
1	High	Above 150
2	Medium	76- 150
3	Low	1-75
4	Nil	0

3.2.5.5.3 Pollen tube length (microns)

It was measured from the base to the tip of longest pollen tube. The data were collected from10 pistils and the mean value was calculated.

3.2.5.5.4 Number of pollen tubes at stigmatic surface

Number of pollen tubes germinated on the stigmatic surface was counted from 10 pistils and the mean and its percentage were worked out.

3.2.5.5.5 Number of pollen tubes at the middle of the style

Number of pollen tubes observed in the middle of stylar region was counted from 10 pistils and the mean and its percentage were worked out.

3.2.5.5.6 Number of pollen tubes at the entry of ovary

Number of pollen tubes entered into ovary was counted from 10 pistils and the mean and its percentage were worked out.

3.2.5.5.7 Number of ovules with pollen tube at micropylar end (%)

The number of ovules with pollen tube at micropylar end was recorded from 10 ovaries.

The above observations were recorded from 10 pistils per cross combination per replication and observations were replicated thrice. The data of pollen tube length, number of pollen tubes at stigmatic surface, middle of the style and entry of ovary were analysed in CRD.

3.3. EXPERIMENT II

The tuberose genotypes were subjected to screening for resistance to *Meloidogyne incognita* under pot culture condition

3.3.1 Maintaining pure culture of root knot nematode (*Meloidogyne incognita*)

Highly susceptible tomato cultivar PKM-1 was used for developing pure culture of root knot nematode. Plants of tomato were raised in the pots filled with steam sterilized loamy soil mixed with fine river sand. The potted plants were inoculated with J_2 stage of *Meloidogyne incognita* @ 2-3 numbers per pot and maintained for further studies (Plate 5).

3.3.2 Testing materials under pot culture condition

Pot culture experiment was conducted under glasshouse condition at the Department of Nematology, TNAU, Coimbatore (Plate 6). The genotypes were raised in pots for artificial inoculation. The tuberose bulbs were planted in earthernware containing five kilogramme of sterilized pot mixture (Red soil: Sand: FYM in 2:2:1 ratio). A total number of 10 bulbs in each genotype was maintained in each replication. The plants involved in the study were inoculated with *Meliodogyne incognita* two second stage juveniles per one gram of soil.

3.3.3 Inoculation

The method of Sasser *et al.* (1957) was followed for inoculating nematodes. Infected roots from pure culture were cut into small pieces of about 2 cm long and placed in sodium hypochlorite (NaoCl) 0.5% solution. The container was shaken for about 3 minutes to dissolve the gelatinous matrix and freeing the eggs from the egg mass and

incubated for 48 hours under laboratory condition. The inoculum concentration was adjusted to a known number by addition of water. The eggs were kept in petridishes and frequently aerated with the use of aerator to enable hatching (Plate 7). The nematode inoculums (J_2) were placed at a depth of 2 cm near to the rhizosphere and covered with sterile sand. Each pot was inoculated with J_2 of *Meloidogyne incognita* at the rate of two juvenile (J_2)/g of soil 15 days after planting (Plate 8).

3.3.4 Assessment of nematode population in soil, roots and root knot index

The roots of test plants were harvested 45 days after inoculation and washed free of soil. The root knot index (RKI) was calculated by using the method developed by Heald *et al.* (1989). The population of *Meloidogyne incognita* in soil and roots were assessed by using modified Baermann Funnel Technique (Cobb, 1918; Schindler, 1961).

3.2.3. Assessment of disease resistance (Root gall indexing)

The degree of resistance of tuberose genotypes to root knot nematode was assessed based on the root knot index as per Heald *et al.* (1989).

Percentage of galls	Grade	Degree of resistance
0	1	Highly Resistant (HR)
1-25	2	Resistant (R)
26-50	3	Moderately Resistant (MR)
51-75	4	Susceptible (S)
76-100	5	Highly Susceptible (HS)

3.3.5 Estimation of nematode population in soil

200 cubic centimeter (cc) of soil was taken and the soil sample was processed by Cobb's sieving and modified Baermann funnel method (Cobb, 1918; Schindler, 1961). The soil suspension was passed through a set of series. The catch in 320 mesh sieve was carefully washed down into a glass beaker. The nematode suspension was poured onto a modified Baermann funnel. After 48 hours, the larval population drawn in the petriplate

was assessed under stereozoom microscope. The soil nematode population was assessed 45 days after inoculation.

3.3.6 Plant characters under root knot nematode infection

3.3.6.1 Root length (cm)

The length of root was measured from the base of the plant to the tip of the roots in five randomly selected plants and mean was calculated.

3.3.6.2 Root weight (g)

Five randomly selected plants were uprooted and the roots were washed in running tap water. The root weight was recorded and mean was calculated.

3.3.6.3 Number of galls per 10 gram root

Randomly selected plants were uprooted and the root galls were counted

3.3.6.4 Number of egg masses per gram of root

Exactly one gram of root was weighed and stained. The number of egg masses was counted under microscope after staining.

3.3.6.5 Number of eggs per egg mass

Egg masses collected from the galled roots were dispersed by adding sodium hypochloride solution and dispersed eggs per egg mass were counted.

3.3.6.6 Number of females per gram of root

Exactly one gram of root was weighed and stained. The number of females was counted under microscope after staining.

3.3.6.7 Root knot index

Randomly selected plants were uprooted and the root gall indexing was done by assigning 0-5 scale suggested by Heald *et al.* (1989).

3.3.7 Biochemical basis of root knot nematode resistance

All the genotypes included in the field trail were used for biochemical studies to assess and determine the defence mechanism. The following biochemical factors were studied.

3.3.7.1 Total phenol (roots)

Folin ciocalteau reagent method was used for estimating the total phenol (Bray and Thrope, 1954). One ml of ethanol root extract was taken in a boiling tube to which one ml of Folin ciocalteau reagent and 2 ml of 20 per cent sodium carbonate were added. This mixture was heated exactly for one minute on a water bath. After cooling 2 ml distilled water was added and the blue colour development was read at 660 nm.

3.3.7.2 Orthodihydroxy phenol (mg g^{-1})

Arnow's method was followed for the estimation of Ortho-dihydroxy phenol (Malik and Singh, 1980). One gram of plant tissue was homogenized in a mortar and pestle with 10 ml methanol. The homogenized material was centrifuged at 3000 rpm for 10 minutes. The supernatant was collected in a separate test tube. The sediments were reground in a mortar and pestle with 5 ml methanol, centrifuged as above and pooled together to form a total volume of 15 ml. One ml of alcohol extract was pipetted out and added with 0.5 N HCl, 1 ml Arnow's reagent (10 g $NaNO_2$ + 10 g Sodium molybdate in 100 ml of distilled water), 10 ml distilled water and 2 ml of 1N NaOH. Soon after the addition of alkali, pink color appeared. The absorbance of the pink colour was read at 515 nm.

3.3.8 Enzymes present in plants and their resistance

The biochemical constituents *viz.,* peroxidase, polyphenol oxidase, phenylalanine ammonia-lyase, IAA oxidase and acid phosphatase were estimated in all the parents involved in this study. Young physiologically active leaves of five randomly selected plants after inoculation were taken. Leaf obtained from different time interval was homogenized in chilled pestle and mortar with 1 ml cold 0.1M phosphate buffer (pH 6.5). The extract was centrifuged at 6000 rpm for 10 minutes at 4^0C in a refrigerated centrifuge and supernatant was used as enzyme extract.

3.3.8.1 Peroxidase activity

Peroxidase activity was assayed, following the method described by Srivastava (1987). Reaction mixture consisted of 1.5 ml of Guaicol solution, 100 µl of enzyme preparation and 100 µl of 1 percent H_2O_2. At the start of the enzyme reaction, the

absorbance of the mixture was set to zero at 420 nm and change in the absorbance were recorded at 30 seconds intervals. Boiled enzyme preparation served as control. Peroxidase activity was expressed as changes in absorbance/ minute/ gram fresh weight.

3.3.8.2 Polyphenol oxidase activity

Polyphenol oxidase activity was assayed using the method described by Srivastava (1987). Standard reaction mixture contained 1.5 ml of 0.1 M phosphate buffer (pH 6.5), 0.5 ml of enzyme preparation and 0.5 ml of 0.01 M catechol. At the start of the enzyme reaction the absorbance was set to zero at 495 nm. The changes in the absorbance were recorded at 30 seconds intervals and polphenol oxidase activity was expressed as changes in the OD of the reaction mixture per minute per 200 mg of fresh weight of tissue.

3.3.8.3 Phenylalanine ammonia lyase (PAL) activity (n mol transcinnamic acid min^{-1} g^{-1} tissue)

Plant samples (500 mg) were homogenized in 2 ml of ice cold 0.1 M sodium borate buffer at pH 7.0 containing 1.4 mM of 2-mercaptoethanol and 0.1 g of insoluble polyvinylpyrrolidone. The extract was filtered through cheese cloth and the filtrate was centrifuged at 16,000 g for 15 min. The supernatant was used as enzyme source. PAL activity was determined as the rate of conversion of L- phenylalanine to transcinnamic acid at 290 nm (Dikerson *et al.,* 1984). Samples containing 0.4 ml of enzyme extract were incubated with 0.5 ml of 0.1 M borate buffer, pH 8.8 and 0.5 ml of 12 mM L- phenylalanine in the same buffer for 30 min at 30^0C. The amount of transcinnamic acid synthesized was calculated (Dikerson *et al*., 1984).

3.3.8.4 Acid phosphatase activity

Powdered root sample was homogenized with 0.1 M sodium acetate buffer (pH 5.0) in a pre chilled pestle and mortar. The homogenate was centrifuged at 10000 rpm for 20 minutes in a refrigerated centrifuge. The reaction mixture, in a final volume of 2 ml contained 100 mM sodium acetate buffer (pH 5.0), 5 mM pNPP as substrate and enzyme. After 10 minutes of incubation at 37^0C, the reaction was stopped by the addition of 1 ml of 1 N NaOH and the p- nitrophenol released. The released

p-nitrophenol was yellow in colour in alkaline medium and was monitored at 405 nm and specific activity expressed as *m*moles of p-nitrophenol released minute^{-1} mg^{-1} protein.

3.3.9 Histopathology

Infected roots of tuberose (10 single + 5 double types) after 40 days of inoculation were washed free of soil, fixed in FAA (Formaline: acetic acid: alcohol) for at least 48 hours. The infected roots were stained in boiling 0.1 per cent acid fuschin and after cooling excess stain were washed in water. A small bit of root piece was then placed on slide in a drop of lactophenol, smeared, covered with a cover slip and pressed gently. Few infected root portions were also dehydrated, stained and cut at 12- 15µ thickness and examined microscopically (Johansen, 1940).

3.3.10 EXPERIMENT III: SEED SET STUDIES IN TUBEROSE GENOTYPES

All the 10 single genotypes were studied for their seed setting behavior by open pollination, selfing and artificial crossing under natural conditions. Whereas the double types were not included for the study as they generally do not set seeds.

3.3.10.1 Open pollination

The fully developed flower buds in all the single genotypes were left for open pollination. Fruit set data were recorded after a week and percentage of fruit set was estimated.

3.3.10.2 Selfing

The selfing was carried out on fully developed flower buds of hybrids and cultivars of single types. The fully developed flower buds were bagged to prevent the entry of foreign pollen grains. The fresh pollen from bagged flowers was dusted on to the stigmatic surface after two, three and four days of flower opening. After pollination, flowers were bagged with butter paper cover. Seed set was observed a week after pollination and percentage of fruit set was estimated.

3.3.10.3 Artificial crossing

Four to six mature flower buds of a spike distained to open in next two days were selected, emasculated and bagged as female parent. Two days after emasculation the flowers were pollinated using pollen from bagged spikes of desirable male parent. Pollination was carried out during morning hours between 6 am to 9 am. After crossing, the flowers were bagged and labeled for recording the following observations which were replicated four times. Hybridization was attempted in all the single types in following cross combinations and seed setting behavior was studied (Plate 2 and 9).

1. Calcutta Single x other Single genotypes
2. Hyderabad Single x other Single genotypes
3. Kahikuchi Single x other Single genotypes
4. Mexican Single x other Single genotypes
5. Navsari Local x other Single genotypes
6. Phule Rajani x other Single genotypes
7. Prajwal x other Single genotypes
8. Pune Single x other Single genotypes
9. Shringar x other Single genotypes
10. Variegated Single x all single genotypes

The various parameters studied during the investigation are detailed below.

3.4 Number of days taken for fruit set

Period required for fruit set was recorded from the day of pollination to the day of fruit set. The data were recorded from 13 successful crosses in four replications and the mean number of days taken for fruit set in each parental combination was calculated.

3.5 Days taken for fruit maturity

Number of days taken for fruit maturity was recorded from the day of pollination to the day of fruit maturity. It was recorded in each parental combination for different months.

3.6 Harvesting of capsules

Matured capsules of 13 successful crosses were harvested separately for different months.

3.7 Per cent fruit set

The number of fruits set in different crosses was recorded and percent fruit set was assessed against the number of flowers pollinated.

3.8 Seeds per capsule

It was calculated by taking mean number of seeds per 10 capsules in each replication.

3.9 Standardization of seed treatment for enhancing germination

In general, all the genotypes have dormancy mechanism in seeds. To break the dormancy and to enhance the germination,open pollinated seeds of Prajwal (best genotype) was selected and were treated with different chemicals as per the treatments listed below.

Design : FCRD

No. of factors : 2 (Growth regulators and time)

No. of treatment combinations: 16

No. of replications : 3

3.9.1 Details of seed treatments for enhancing germination

Treatment	Treatment details
T_1	Treating of seeds with GA_3 (250 ppm) for 8 hours
T_2	Treating of seeds with GA_3 (250 ppm) for 16 hours
T_3	Treating of seeds with GA_3 (500 ppm) for 8 hours
T_4	Treating of seeds with GA_3 (500 ppm) for 16 hours
T_5	Treating of seeds with KNO_3 (0.25 %) for 8 hours
T_6	Treating of seeds with KNO_3 (0.25 %) for 16 hours

T_7	Treating of seeds with KNO_3 (0.5 %) for 8 hours
T_8	Treating of seeds with KNO_3 (0.5 %) for 16 hours
T_9	Treating of seeds with Thiourea (0.5 %) for 8 hours
T_{10}	Treating of seeds with Thiourea (0.5 %) for 16 hours
T_{11}	Treating of seeds with Thiourea (1 %) for 8 hours
T_{12}	Treating of seeds with Thiourea (1 %) for 16 hours
T_{13}	Treating of seeds with IBA 100 ppm 8 hours
T_{14}	Treating of seeds with IBA 100 ppm 16 hours
T_{15}	Treating of seeds with IBA 200 ppm 8 hours
T_{16}	Treating of seeds with IBA 200 ppm 16 hours
T_{17}	Water soaking 8 hours
T_{18}	Water soaking 16 hours

3.9.1.1 Germination

Four replicates of 100 seeds each were germinated in pots filled with sand (ISTA, 1993) and kept in the germination room maintained at 25 ± 2^0C and 90 ± 5% RH. Count was made on the number of normal seedlings at 28 days after sowing and the germination was calculated and expressed as percentage.

3.9.1.2 Root length

The root length of the 10 normal seedlings was measured from the collar region to the tip of the primary root and expressed in cm.

3.9.1.3 Shoot length

10 normal seedlings were selected at random and length of the shoot was measured from collar region to the growing tip. The mean shoot length was expressed in centimeter.

3.9.1.4 Vigour index

The vigour index was calculated by using the formula suggested by Abdul- Baki and Anderson (1973) and expressed as whole number.

Vigour index= Germination percentage x (Root length + Shoot length)

3.9.1.5 Dry matter production

The 10 normal seedlings utilized for seedling measurements were first dried under shade and then dried in a hot air oven maintained at 85 ± 1^0C for 24 hours. After drying, they were cooled in a desiccator for 30 minutes, weighed and expressed in gram.

3.9.2. Standardization of quick viability (tetrazolium) test

The seeds were preconditioned by soaking the seeds in water for 12 h or by keeping them in between the moistened blotter paper for 16-18 h. The embryos were separated from the seeds by pressing the seed coat with hand or with the help of needle.

The separated embryos were soaked in 2,3,5 - triphenyl tetrazouim chloride solution of different concentrations (first prepare the phosphate buffer by mixing 39 ml of 0.2 M solution of monobasic sodium phosphate and 61 ml of 0.2 M solution of dibasic sodium phosphate to a total of 100 ml. Then dissolve 0.25, 0.50 and 1g 2,3,5 - triphenyl tetrazouim chloride in phosphate to make 100 ml) *viz.,* 0.25 and 0.50 per cent and incubated for different durations *viz.,* 2 and 2½ h in a hot air oven maintained at 40°C and were also kept in dark for overnight (16 h) and then observed for the staining pattern based on red colour formazon and the viability percent was estimated by adopting the following formula.

$$\text{Viability (\%)} = \frac{\text{Number of seeds fully stained}}{\text{Total number of seeds placed}} \times 100$$

3.10 Study of post- fertilization development

In the present research programme, an attempt has been made to understand the histological changes that occur after fertilization and the development of the embryo to assess the causes of non- seed setting in tuberose upon selfing and in some of the crosses. The details regarding the procedure followed for histological studies are as follows.

3.10.1 Material

The flowers of cvs. Calcutta Single, Hyderabad Single, Kahikuchi Single, Mexican Single, Navsari Local, Phule Rajani, Prajwal, Pune Single, Shringar and Variegated Single were used in this study. They were selfed/ crossed as mentioned below and the various developmental stages of the ovule were studied.

3.10.2 Method for histological study

3.10.2.1 Sampling

The flowers after crossing/ selfing were grouped based on the successive stages of their development starting from pollination till maturity. The developing ovary was fixed at 10 days intervals from the day of anthesis to 40 days after anthesis.

3.10.2.2 Fixation

Fixation was done using carnoys B fixative (Ethyl alcohol: Chloroform: Acetic acid in 6:3:1 by volume) for a period of three hours.

3.10.2.3 Dehydration

Fixed materials were washed in 70 per cent alcohol and dehydrated using ethanol and n-butanol grades leaving the material in each grade for a period of three hours in the following order

S.NO	Water (%)	Ethanol (%)	n-butanol (%)
1.	30	70	-
2.	20	80	-
3.	10	90	-
4.	05	95	-
5.	05	95	-
6.	-	75	25
7.	-	50	50
8.	-	25	75
9.	-	-	100
10.	-	-	100

3.10.2.4 Infiltration and embedding

Paraffin wax of 58- 60^{0}C melting point was used for infiltration and embedding. Small chips of paraffin wax were added succesfully to the medium of pure n-butanol containing dehydrated sample until the medium reached the saturation at room temperature. The material in wax- butanol mixture was kept in an oven maintained at 60^{0}C. After every 4 hours, the molten paraffin was poured off and replaced with fresh molten paraffin. The procedure of replacing with fresh molten paraffin was repeated till the material lost the traces of butanol. Thus for complete infiltration 6 - 7 changes of molten paraffin were given. The materials were subsequently embedded in paraffin wax (58- 60^{0}C MP) by employing paper- boat technique (Jensen, 1962).

3.10.2.5 Microtoming and affixing the sections to slides

Uniform sections of 8µm thickness were taken with the help of Erma Rotary Microtome. An adhesive was prepared using gelatin (0.4 %) and a little quantity of potassium dichromate.

Paraffin sections were cut into shorter units of convenient size with the help of a blade. A few drops of the adhesive were poured on the cleaned slides and the sections were arranged compactly on the surface of the adhesive. The slides were then warmed over a slide warmer maintained at about 45^{0}C to facilitate expansion and stetching of the sections to their original size. The adhesives was drained off after the stretching was complete. The slides were kept in slanting position for 24 hours to dry in a dust free place.

3.10.2.6 Deparaffinishing and hydrating the sections

Deparaffinising was done using Xylol. N-butanol and different grades of ethanol were used for hydration. The steps followed are listed below:

Xylol (1) - 5min.

Xylol (2) - 5 min.

n- Butanol (1) - 5 min.

n- Butanol (2) - 5 min.

100% Ethanol- 5min.

90% Ethanol - 5 min.

70% Ethanol - 5min.

50% Ethanol - 5 min.

Water - 5 min.

3.11 Statistical analysis

The experimental data were statistically analyzed as per the method suggested by Panse and Sukhatme (1985). The critical differences were worked out for 1 per cent (0.01) and 5 per cent (0.05) probability.

3.11.1 per *se* performance

The mean performance of the different varieties for quantitative and biochemical characters were studied.

3.11.2 Unit analysis

The parameters like mean, standard error and coefficient of variation were calculated for the characters by the standard method of analysis (Panse and Sukhatme, 1967).

1. Grand mean = $\frac{\text{Total of all values}}{n}$

Where, n = number of observations

2. Standard deviation (SD) = $\sqrt{\text{Variance}}$

3. Standard error (SE) = $\frac{SD}{\sqrt{n}}$

3.11.3 Heritability and variability studies

3.11.3.1 Genotypic and Phenotypic variances

The genotypic and phenotypic variances are calculated as suggested by Johnson *et al*. (1955).

$$\text{Genotypic variance (g)} = \frac{M_1 - M_2}{r} \times 100$$

Where, M_1 = mean sum of squares of genotypes

M_2 = mean sum of squares of error

r = number of replications

Phenotypic variance (p) = g + e

e = environment variance

3.11.3.2 Phenotypic and genotypic coefficient of variations

The phenotypic coefficient of variation and genotypic coefficient of variation were calculated as suggested by Burton (1952).

$$\text{Phenotypic Coefficient of Variation (PCV)} = \frac{\sqrt{\text{Phenotypic variance}}}{\text{Grand mean}} \times 100$$

$$\text{Genotypic Coefficient of Variation (GCV)} = \frac{\sqrt{\text{Genotypic variance}}}{\text{Grand mean}} \times 100$$

The range of heritability was categorized as suggested by Burton (1952).

i.e.,

0 – 10 = low

10 – 30 = Moderate

Above 30 = High

3.11.4 Heritability (%)

The heritability (h^2) in broad sense was calculated according to Lush (1940).

$$\text{Heritability (Broad sense)} = \frac{\text{Genotypic variance}}{\text{Phenotypic variance}} \times 100$$

Range of heritability was categorized in to low (5 – 10%), moderate (10 – 30%) and high (30 – 60%).

3.11.5 Genetic advance as per cent mean

$$\text{Genetic advance as per cent mean} = \frac{GA}{GM} \times 100$$

Where, GA is the Genetic Advance

Genetic advance for each character was computed according to the method suggested by Johnson *et al.* (1955).

Genetic advance (GA) = σ p x h^2 x k

Where,

σp = phenotypic variance

h^2 = heritability

K = 2.06 selection differential at 5% selection intensity

GM is the Genetic mean

Range of GM is categorized as follows

Less than 10 percent = Low

10 – 20 percent = Moderate

More than 20 percent = High

3.11.6. Correlations

Phenotypic, Genotypic and Environmental coefficients were worked out according to Panse and Sukhatme (1967).

$$\text{Phenotypic correlation coefficient } r_{xy}(p) = \frac{COV\ .\ XY\ (P)}{\sqrt{VX(P)\ .\ VY(P)}} \times 100$$

Where, COV.XY (P) = Phenotypic variance between character X and Y and V_X (P) and Vy (P) are the respective Phenotypic Variance

$$\text{Genotypic correlation coefficient } r_{xy}(G) = \frac{COV\ .\ XY\ (G)}{\sqrt{VX(G)\ .\ VY(G)}} \times 100$$

Where, COV.XY (G) = Genotypic variance between character X and Y and V_X (G) and Vy (G) are the respective Phenotypic Variance

$$\text{Environmental correlation coefficient } r_{xy}(E) = \frac{\text{COV. XY (E)}}{\sqrt{VX(E) \,.\, VY(E)}} \times 100$$

Where, COV.XY (P) = Phenotypic variance between character X and Y and V_X (E) and Vy (E) are the respective Phenotypic variance

The significance of correlation was tested by referring to the table given by Snedeer and Cochran (1967). It was tested at 5% and 1% levels.

3.11.2. Path coefficient analysis

Path coefficient analysis was estimated as suggested by Dewey and Lu (1959) to study the direct and indirect effects.

3.11.3. Mahalanobis D^2 statistics

Mahalanobis's generalized distance (1936) estimated by D^2 statistic has been used as an efficient tool in the quantitative estimation of genetic diversity for a rational choice of potential parent for breeding programme. The D^2 values were used to quantify genetic diversity among the genotypes and classify the entire germplasm into distinct clusters, which was done following to Tocher's method (Rao, 1960).

Steps in D^2 statistics

Testing of significance

The difference between mean values of the quantitative characters under study were tested for their significance before taking up the computation of D^2 values.For this purpose, the error and error+genotype variance and covariances matrices were formed using the ANOVA tables presented by the computer. By employing pivotal condensation method as suggested by Rao (1952), of the determinant of error matrix and error of variety matrix were calculated. Then the values of 'V' statistic was worked out using Wilk's Lamda (ƛ) criteria.

$$X = \frac{\text{Determinant of error matrix}}{\text{Determinant of error + variety matrix}}$$

'V' statistic = $-m\log_e x$

Where

$$m = n - \frac{p+q+1}{2}$$

p = number of characters

n = degrees of freedom of error + accessions

e = 2.7183

The value of 'V'statistic was compared with the tabulated chi-square values for pq degrees of freedom at five percent level and the significance was tested.

Computation of D^2 values

1. For this the original values were transformed into normalised variables (X's). Then the correlated normalised variables (X's) were converted into uncorrelated variables (Y's). This transformation was done by pivotal condensation method as described by Rao (1952).
2. The statistical distance square between genotypes i & j, D2ij is simply the sum of squares of the differences for the uncorrelated variables of the characters under study (Y_1 to Yp). Thus all possible distance square values n(n-1)/2 were worked out by taking the sum of the difference between pairs of corresponding Y values considering two gneotypes at a time.
3. Determination of group constellations or clusters

   The grouping or clustering was done using the simple criterion suggetsed by Rao (1952). The criterion of grouping was that any populations belonging to the same cluster should atleast on the average show a smaller D^2 than those belonging to different clusters.

To start with,the populations were arranged in order of their relative distances from each other, were chosen and a third type having smallest averages D^2 values from the first two populations was added. Similarly the fourth one was chosen and the process was continued upto a stage where there was disruptive increase in the average D^2 by the addition of a particular population. This population excluded from that cluster and the

grouping was continued omitting populations which had already appeared in other clusters. The process was continued until all the populations were grouped in one or other cluster.

4. Intra or Inter- cluster difference

After establishing the group constellation or clusters, the average inter and intra-cluster divergence were worked out taking into consideration all the componenet D^2 values possible among the members of the two clusters. The square root of the D^2 values gave the distance (D) between the clusters. The intra - cluster distance was arrived by taking average of the component types in that cluster.

CHAPTER IV

EXPERIMENTAL RESULTS

Investigations on the performance, pollen studies, seed setting behaviour, post fertilization events and nematode resistance studies of ten genotypes of single and five double types of tuberose were carried out at the Department of Floriculture and Landscaping, Tamil Nadu Agricultural University, Coimbatore. The data obtained on different characters of tuberose genotypes were subjected to statistical analysis and the results are presented in this chapter.

4.1. Evaluation of tuberose genotypes on growth and flowering parameters

Observations were recorded on the growth, flowering and quality parameters *viz*., days taken for sprouting of bulb, plant height, number of leaves per plant, days to spike emergence, duration of flowering, spike length, rachis length, number of florets per spike, floret length, weight of florets per spike, number of spikes per m^2, yield of florets per plot, vase life and concrete recovery in 10 single genotypes *viz*., Calcutta Single, Hyderabad Single, Kahikuchi Single, Mexican Single, Navsari Local, Phule Rajani, Pune Single, Prajwal, Shringar, Variegated Single and five double genotypes *viz.,* Calcutta Double,Hyderabad Double, Pearl Double, Suvasini and Vaibhav.The results were statistically analyzed and presented in table 1.

4.1.1. Growth parameters

4.1.1.1. Days taken for sprouting of bulb

Days taken for sprouting of bulb of different genotypes are presented in the Table 1& 3 . The genotypes differed significantly with respect to days taken for sprouting of bulb. Among the single types, the genotype ‘Prajwal’ recorded least number of days for sproting of bulb (12.12 days) while the genotype ‘Hyderabad Single’ recorded more number of days (16.48 days).

The genotype ‘Suvasini’ recorded minimum number of days for sprouting of bulb (12.32 days), which was on par with ‘Vaibhav’ (12.67 days)while it was maximum in ‘Hyderabad Double’ (16.15 days) among the double types.

4.1.1.2. Plant height

The plant height varied significantly among genotypes at three months after planting (Table 1 & 3). Among the single types, 'Variegated Single' recorded increased plant height (117.50 cm) which was on par with 'Prajwal' (113.05 cm), while 'Phule Rajani' recorded the least plant height (72.50 cm) (Table 1).

Among the double types, the genotype 'Suvasini' recorded the highest plant height (86.25 cm), while 'Hyderabad Double' recorded the lowest plant height (68.87 cm) (Tbale 3).

4.1.1.3. Number of leaves per plant

The number of leaves varied significantly at three months after planting (Table 1 & 3). Among the single types, the genotype 'Prajwal' recorded the highest number of leaves (260). Whereas it was least (220) in Hyderabad Single (Table 1).

The genotype 'Suvasini' recorded maximum number of leaves (270). Whereas, it was (235) in 'Hyderabad Double', which was on par with 'Pearl Double' (238) (Table 3).

4.1.1.4. Days to spike emergence

Days to spike emergence of different genotypes are presented in the Table 1& 3. The genotypes differed significantly for this character. Among the single types, 'Prajwal' recorded less number of days to spike emergence (78 days) while 'Calcutta Single' recorded more number of days (94 days).

The genotype 'Suvasini' recorded minimum number of days to spike emergence (84 days), which was on par with 'Vaibhav' (85 days), while it was maximum in 'Hyderabad Double' (89 days) among the double types.

4.1.1.5. Duration of Flowering

The duration of flowering varied significantly among different genotypes of tuberose. Among the single types 'Prajwal' showed maximum duration of flowering (17 days) while it was minumum (6.37 days) recorded in 'Hyderabad Single' (Table 1).

Among double types, the maximum duration of flowering was recorded in 'Suvasini' (12.40 days), which was on par with 'Vaibhav' (11.43 days), while it was minimum in 'Hyderabad Double' (9.12 days) (Table 3).

4.1.2. Spike parameters

4.1.2.1. Spike length

The tuberose genotypes showed significant differences with respect to length of spike (Table 2 & 4). Among the single types, increased spike length (102.50 cm) was recorded in 'Variegated Single' which was on par with 'Prajwal' (98.05 cm),while in 'Phule Rajani' it was the least (52.50 cm).

Among the double types, the maximum spike length was recorded in the genotype 'Suvasini' (71.25 cm), which was on par with 'Vaibhav' (66.38 cm), while in 'Hyderabad Double' it was minimum (53.87 cm).

4.1.2.2. Rachis length

Significant differences were observed among different genotypes for rachis length (Table 2 & 4). Among the single types, the maximum rachis length (cm) was observed in the genotype 'Pune Single'(35.75 cm), which was on par with 'Prajwal' (28.52 cm), while it was minimum (15.30 cm) in 'Hyderabad Single'.

Among the double types, increased rachis length (54.00 cm) was observed in 'Vaibhav' while it was least in 'Hyderabad Double'(33.95cm).

4.1.2.3. Number of florets per spike

There was significant difference with respect to number of florets per spike. Among the single types, the genotype 'Prajwal' registered the highest number of florets per spike (47) followed by 'Navsari Local' (45), while in 'Mexican Single' it was the least (17) which was closely followed by 'Calcutta Single' (25) (Table 2).

The maximum number of florets per spike (54) was recorded in 'Suvasini', while it was minimum in 'Pearl Double' (30), which was on par with 'Hyderabad Double' (34) and 'Calcutta Double' (35) among the double types (Table 4).

4.1.2.4. Floret length

The genotypes showed significant differences for floret length (Table 2 & 4). Among the single types the genotype 'Prajwal' (6.40 cm) recorded maximum floret length. The floret length was minimum (6.10 cm) in 'Pune Single'.

The maximum floret length (7.50 cm) among double types was recorded in 'Suvasini', while it was minimum (6.70 cm) in 'Hyderabad Double' which was on par with 'Calcutta Double' (7.10 cm).

4.1.2.5. Weight of the florets per spike

The genotypes differed significantly for weight of the florets per spike (Table 2 & 4). The genotype 'Prajwal' produced increased weight of the florets per spike (74.80 g) among single types, while it was minimum in 'Mexican Single' (28.32 g), which was on par with 'Calcutta Single' (29.25 g).

Among the double types, the genotype 'Suvasini' registered maximum weight of the florets per spike (146.88 g) which was significantly higher compared to all the genotypes**,** Whereas, it was minimum in 'Hyderabad Double' (108.56 g).

4.1.2.6. Number of spikes per m^2

There was significant difference with respect to number of spikes per m^2. Among the single types, 'Prajwal' registered the highest number of spikes per m^2 (47) followed by 'Shringar (40), while 'Navasri Local' registered the lowest number of spikes per m^2 (25.75) which was closely followed by the genotypes 'Calcutta Single' (31), 'Mexican Single' (32.20) and 'Kahikuchi Single' (33.00) respectively (Table 2).

The maximum number of spikes per m^2 (34.10 nos.) among double types was recorded in 'Suvasini', while it was minimum in 'Pearl Double' (21.01 nos.) (Table 4).

4.1.2.7. Yield of florets per plot (4 * 1 m)

The tuberose genotypes showed significant differences with respect to yield of florets per plot. Among the single types, the yield of florets per plot (4.40 kg/ plot) was the highest in 'Prajwal' which was on par with 'Shrinagr' (4.26 kg/ plot), while it was the lowest in 'Mexican Single' (2.51 kg/ plot) (Table 2).

Among the double types, the highest florets yield was recorded in 'Suvasini' (3.42 kg), which was on par with 'Vaibhav' (3.26 kg/ plot), while the genotype 'Peral Double' recorded the lowest (2.42 kg/ plot), which was closely followed by the genotypes Hyderabad Double (2.48 kg/ plot) and Calcutta Double (2.57 kg/ plot) (Table 4).

4.2. Quality parameters

4.2.1. Vase life

The data on vase life and concrete recovery in different genotypes are presented in the Table 5 & 6.

The genotypes differed significantly with respect to vase life. Among the single types, the genotype 'Prajwal' showed maximum vase life (12.97 days) which was on par with 'Variegated Single' (12.80 days) and 'Phule Rajani' (12.07 days), while it was minimum (8.91 days) in the genotype 'Mexican Single' which was on par with 'Pune Single' (9.12 days).

Among the double types, maximum vase life (12.40 days) was noticed in the genotype 'Suvasini', while it was minimum (8.62 days) in 'Pearl Double' which was on par with 'Hyderabad Double' (9.00 days) and Calcutta Double (9.10 days).

4.2.2. Concrete recovery

The genotypes differed significantly with respect to concrete recovery (Table 5 & 6). Among the single types, the genotype 'Prajwal' showed maximum concrete recovery (0.16 %) while the genotypes 'Hyderabad Single' and 'Pune Single' recorded minimum concrete recovery (0.11 %).

The genotype 'Suvasini' among double types recorded maximum concrete recovery (0.09 %), which was on par with 'Vaibhav' (0.07 %) while it was minimum in 'Calcutta Double' (0.06 %).

4.2.3. Identification of chemical components of tuberose concrete

The identification of chemical compounds in two promising genotypes *viz.,* Prajwal (Single) and Suvasini (double) was carried out using GC-MS analysis. The results obtained are furnished in Table 7 & 8. The study revealed that the major components of the concrete observed were Octacosone, 5- Isopropyl- 4- (trifluoromethyl)- 1H- Pyrimidin-2-one, 1-cyclopropyl-3,4-dimethyloxy-eugenol, Hentriacontane and 2,3-Dihydroxyhex-2-enylbenzoate in Prajwal. Whereas, in Suvasini, the components observed were Dotriacontane, 5- Isopropyl-4- (trifluoromethyl)-1 H- pyrimidine-2-one, Benzene-1,2-dimethoxy-4-(1-propenyl), Hexadecane-2,6,10,14-tetramethyl and 5-Benzoxy-2-methylhexanenitrile.

4.3. Flower morphology

4.3.1. Days to first floret opening

Days to first floret opening of different genotypes are presented in the Table 9 & 10. The genotypes differed significantly with respect to days to first floret opening. Among the single types, the genotype 'Prajwal' showed the least number of days to spike emergence (98.01 days) while in 'Calcutta Single' it was more (101.23 days).

The genotype 'Suvasini' among double types recorded minimum number of days to spike emergence (104.56 days), which was on par with 'Vaibhav' (105.63 days), while it was maximum in 'Hyderabad Double' (108.21 days).

4.3.2. Floret diameter

Significant differences were observed among different genotypes for floret diameter. Among the single types, the maximum floret diameter was observed in 'Prajwal' (4.89 cm), while it was minimum (4.63 cm) in 'Navsari Local' (Table 9).

Among the double types, 'Suvasini' showed maximum floret diameter (5.78 cm) while it was minumum (5.08 cm) in 'Hyderabad Double'(Table 10).

4.3.3. Bud length

The tuberose genotypes showed significant differences with respect to bud length (Table 9 & 10). Among the single types, the bud length (6.45 cm) was maximum in 'Prajwal' (98.05 cm), while 'Pune Single' recorded minumum bud length (6.12 cm).

Among the double types, it was maximum in 'Suvasini' (7.56 cm), which was on par with 'Vaibhav' (7.41 cm), while the genotype 'Calcutta Double' recorded minimum bud length (7.06 cm).

4.3.4. Hundred flower weight

The genotypes differed significantly for hundred flower weight (Table 9 & 10). The genotype 'Prajwal' showed maximum hundred flower weight (170.31 g) among single types, while it was minimum in 'Calcutta Single' (115.18 g).

Among the double types 'Suvasini' registered maximum hundred flower weight (272.20 g) which was significantly higher compared to all the genotypes**,** Whereas, it was minimum in 'Hyderabad Double' (265.42 g).

4.3.5. Bud colour

The colour of the matured buds of all single types are green in colour (Table 9 & 10). But in 'Phule Rajani', along with green colour a slight pink tinge at the tip of the floret. Similarly in 'Prajwal' and 'Hyderabad Single', dark pink tinge observed at the tip of the florets .

In double types, the bud colour was green in 'Calcutta Double', 'Hyderabad Double' and 'Pearl Double'. But in 'Suvasini' the green bud colour was tinged with pink at the tip of the floret, While in 'Vaibhav' it was tinged with dark green at the tip.

4.3.6. Floret colour

The floret colour noticed was uniformly white in all single types. But in Prajwal it was white with slight pink tinge at the tip of the floret (Table 9).

In all the double types, the floret colour was white except Suvasini, where the white colour is tinged with slight pink at the tip of the floret (Table 10).

4.3.7. Row of petals

In all single types, number of row of the petals is only one (Table 9). But in all double types, it varied from three to four rows (Table 10).

4.3.8. Number of petals per floret

The number of petals per floret is six in all single types (Table 9), while in all double types, it varied from 13 to 14 (Table 10).

4.3.9. Number of pistils

In all single types, number of pistil is only one (Table 9), while in all double types, it was absent (Table 10).

4.3.10. Length of the stamen

Among the single types, the lengthiest stamen (1.22 cm) was found in the genotype 'Mexican Single' (Table 9).

4.3.11. Length of the pistil

The pistil length was measured from the length of style, stigma and ovary. The lengthiest pistil was noticed in the genotype 'Shringar' (3.67 cm) among the single types (Table 9).

4.3.12. Filament length

The maximum length of filament (3.15 cm) was observed in the genotype 'Navsari Local' among the single types (Table 9).

4.3.13. Anthesis time

Anthesis time noticed in all single types varied from 3.45 to 4.50 p.m and in double types, it varied from 3.45 to 5.30 p.m. (Table 9).

4.3.14. Stigma receptivity

The results showed that the maximum stigma receptivity was observed three days after anthesis in all single types (Table 9).

4.3.15. Anther dehiscence

The microscopical observation revealed the extrose, longitudinal splitting in tuberose. The colour of the anther lobe was yellow bearing yellow pollen grains in all single types (Table 9).

With respect to the time of anther dehiscence in both the types, the dehiscence started from 5.45 pm and continued upto 7.30 pm (Table 10).

4.4. Pollen morphology

The fresh pollen collected from five flowers in each of single genotypes was observed under the compound microscope. Based on the appearance of exine of the pollen grain, the shape of the pollen was found to be round in all the pollen samples.

4.4.1. Pollen size and equivalent diameter

Measurements were made to find out the pollen diameter and size of different single genotypes. They varied widely among the genotypes. Mean pollen diameter ranged from 125.16 to 185.81 microns. Among them, Shringar recorded the maximum equivalent diameter of 185.81microns followed by Navsari Local (161.94 microns). Hence the mean pollen size was the highest in Shringar (Table 11).

4.4.2. Pollen radius and perimeter

The trend was more or less same in the case of pollen radius and perimeter.Variation was observed for pollen radius and perimeter among the genotypes

which ranged from 59.84 to 90.10 microns, 459.04 to 645.10 microns. Among the genotypes, Shringar recorded the maximum pollen radius and diameter (90.10 and 645.10 microns) followed by Navsari Local with 80.06 and 566.11 microns. However it was minimum (59.84 and 459.04 microns) in Phule Rajani (Table 11).

4.4.3. Pollen output

Wide variation was observed for pollen production among the genotypes which ranged from 5000 to 10,000 pollen grains per anther. Among the genotypes, Variegated Single produced the maximum of 10,000 pollen grains per anther, followed by Phule Rajani (9375) and Shringar (8750) (Table 12).

4.4.4. Pollen viability

Variation was observed for pollen stainability among the genotypes which ranged from 39.83 to 96.73 per cent. Among the genotypes, Variegated Single recorded the maximum pollen stainability of 96.73 per cent followed by Phule Rajani (90.52%). However, the minimum of 39.83 per cent was recorded by the genotype Prajwal (Table 12).

4.4.5. Pollen germinability

There were significant differences in the pollen germination among the genotypes evaluated.The mean pollen germinability of the different genotypes ranged from 22.12 to 99.21 per cent. The genotype Variegated Single registered the maximum germinability of 99.21 per cent followed by Phule Rajani (90.96 %). However, it was least (22.12 %) in Prajwal (Table 12).

4.4.6. *In vitro* studies

4.4.6.1. Standardization of pollen germination medium

Attempts were made to standardize the *in vitro* medium by altering sucrose and boric acid content of Brewbaker and Kwacks (1963) medium so as to make it suitable for germination of tuberose pollen grains. All the fourteen combinations recorded mean germination percentages exceeding that of the original medium excepting two combinations which had 30 per cent sucrose and 50 and 100 ppm boric acid. Among the three levels of sucrose, 20 per cent concentration resulted in the highest perc ent

germination. Among the five different levels of boric acid, 200 ppm was found to be best. The medium which contained 20 per cent sucrose and 200 ppm boric acid resulted in the highest per cent germination (97.3) against 52.3 per cent recorded in the original (Table 13).

4.4.6.2. Pollen tube length

There were significant differences in pollen tube length among various species. The pollen tube length ranged from 4.73 and 118.85 microns in one hour after incubation. The same trend was observed in 5 hours, 10 hours, 15 hours, 20 hours and 25 hours after incubations with the range of 16.95 to 520.43 microns; 26.68 to 839.33; 49.81 to 1020.63; 76.72 to 1234.95; 78.25 to 1292.64 microns respectively. The pollen tube was significantly longer in Variegated Single when compared to others. Among the different genotypes there was a significant increase in pollen tube growth in 1 hour than the forty five minutes duration after it was placed in the media (Table 14).

4.4.7. *In vivo* studies

4.4.7.1. Preparation of material for microscopic observations

4.4.7.1.1. Standardization of NaOH and softening period

Studies were made to standardize the strength of NaOH and duration to soften the pistils of tuberose genotypes for microscopic observations. The softening of the pistils of tuberose genotypes was optimum, when the pistils were kept in 8N NaOH for 3 hours (Table 15).

4.4.7.1.2. Standardization of K_3PO_4 and aniline blue

The optimum staining and better fluorescence were obtained when 0.1 per cent aniline blue was dissolved in 0.3 N K_3PO_4 (Table 16).

4.4.7.1.3. Abundance of pollen germination on stigma upon crossing

Studies were made on the abundance of pollen grain germination on the stigmatic surface to find out the pollen stigma compatibility among the different genotypes and the results are expressed in the scale of 1 to 4. All the genotypes recorded a scale of 3 (1 to 75 pollen grains germinated on the stigma) upon selfing (Table 17). The pollen grains of Calcutta Single was highly compatible with the stigma of Variegated Single;

Hyderabad Single was highly compatible with Phule Rajani; Kahikuchi Single was highly compatible with Phule Rajani, Shringar and Variegated Single; Mexican Single was highly compatible with Phule Rajani and Variegated Single; Navsari Local was highly compatible with Variegated Single, Phule Rajani was highly compatible with Variegated Single; Pune Single was highly compatible with Variegated Single and Variegated Single was highly compatible with Hyderabad Single, Navsari Local, Phule Rajani and Shringar exhibiting more than 150 germinated pollen grains per stigma. High level of incompatibility at stigmatic level was noticed when Prajwal was used as pollen parent against all the single genotypes and vice versa.

4.4.7.1.4. Pollen tube length

4.4.7.1.4.1. Selfing of parents

Studies were conducted to measure the pollen tube length of the tuberose genotypes at different time intervals under *in vivo* conditions. None of the pollen tubes reached ovule upon selfing.

4.4.7.1.4.2. Crossing

Experiments were conducted to find out the crossability in all single genotypes using the same genotypes in all combinations. Out of the 90 crosses made, only 14 combinations were found to be compatible in which the pollen tube successfully reached the ovule. The combinations were

1. Variegated Single x Calcutta Single
2. Variegated Single x Kahikuchi Single
3. Variegated Single x Mexican Single
4. Variegated Single x Navsari Local Single
5. Variegated Single x Pune Single
6. Variegated Single x Phule Rajani
7. Phule Rajani x Kahikuchi Single
8. Phule Rajani x Mexican Single
9. Phule Rajani x Hyderabad Single
10. Phule Rajani x Variegated Single

11. Shringar x Kahikuchi Single
12. Shringar x Variegated Single
13. Hyderabad Single x Variegated Single
14. Navsari Local x Variegated Single

Among the incompatible combinations, the growth of pollen tube stopped after 4 hours in the crosses (Calcutta Single x all Single genotypes, Kahikuchi Single x all Single genotypes, Mexican Single x all Single genotypes, Pune Single x all single genotypes) , 6 hours in the crosses Shringar with Calcutta Single, Hyderabad Single, Mexican Single, Navsari Local, Phule Rajani, Prajwal and Pune Single; Hyderabad Single x Variegated Single, Navsari Local x Variegated Single, 8 hours in the crosses of Phule Rajani x Calcutta Single, Navsari Local, Pune Single, Shringar and 12 hours in crosses of Variegated Single with Hyderabad Single, Prajwal and Shringar.

Among the compatible crosses resulted by using Variegated Single as seed parent, the pollen tube length ranged between 724. 64 to 1234.95 microns but there were significant differences in pollen tube length among the different combinations (Table 18).

4.4.7.1.5. Number of pollen tubes at stigmatic, stylar and at the entry of ovary

Crossing

In the crosses, out of 90 combinations, the pollen tube reached the stylar regions in thirteen crosses. Out of this, the pollen tube entered the ovary in four crosses involving Variegated Single; three crosses using Kahikuchi Single; two crosses using Mexican Single; one crosses using Calcutta Single, Hyderabad Single, Navsari Local as pollinators. In the stigmatic regions, the number of tubes ranged from 180 – 250 (Variegated Single x Calcutta Single; Shringar x Kahikuchi Single) to 296 – 318 (Phule Rajani x Variegated Single). The per cent pollen tubes in the stylar regions varied from 102 – 157 (Phule Rajani x Kahikuchi Single) to 175 – 212 (Phule Rajani x Variegated Single) and only 25 per cent of the pollen tubes seen at the stigma, entered the ovary in different crosses (Table 19).

4.4.7.1.6. Number and percentage of ovules with pollen tube at micropylar end

The total number of ovules in the ovary and the number of ovules with pollen tube was counted and the percentage worked out. The data indicated that the number of

ovules in the ovary ranged from 14 to 17 (Table 20). There were wide variations in respect of per cent ovules that received pollen tubes on different combinations.In the case of only two crosses (Phule Rajani x Variegated Single; Phule Rajani X Mexican Single) 94 per cent of the ovules received the pollen tubes. A low percentage of 50 was recorded in the cross between Variegated Single x Navsari Local.

4.5. Plant characters under root knot nematode infection (single types)

4.5.1. Root length

The mean data for root length varied from 45.32 to 54.89 cm (Table 21). When the tuberose genotypes were analyzed for root length, Kahikuchi Single recorded the highest root length of 54.89 cm followed by Calcutta Single, Hyderabad Single and Mexican Single (53.21, 52.98, 51.76 cm), whereas the lowest root length was recorded by Shringar (45.32 cm).

4.5.2. Root weight

The evaluation of tuberose genotypes for root weight ranged from 61.23 g to 63.75 g (Table 21). Among the tuberose genotypes compared, Kahikuchi Single recorded the highest root weight of 63.75 g followed by Calcutta Single, Hyderabad Single and Mexican Single with 62.98, 61.53 and 60.99 g, whereas Shringar had the lowest root weight of 11.23 g.

4.5.3. Soil nematode population per 200 cc of soil

The performance of fifteen tuberose genotypes for soil nematode population per 200 cc of soil ranged from 122.61 to 291.98 (Table 21). The data indicated that significantly lower number of nematode population was noticed in Kahikuchi Single (122.61 per 200 cc of soil), followed by Calcutta Single, Hyderabad Single and Mexican Single with 160.32, 162.93 and 165.84 per 200 cc of soil, whereas it was found higher in Prajwal (291.98 per 200 cc of soil).

4.5.4. Root knot index

Parameters like root knot index and soil nematode poplation were used to evaluate the resistance or susceptibility of tuberose genotypes against *Meloidogyne incognita* (Table 21). The tuberose genotypes were inoculated with *Meloidogyne incognita* under

pot culture condition. The results revealed that Kahikuchi Single had the least count of gall index of 3, brought under the category of 'moderately resistant' (MR), whereas Calcutta Single, Hyderabad Single and Mexican Single exhibited higher number of galls with the gall index of 4 and categorised under 'susceptible' (S). The genotypes Navsari Local, Phule Rajani, Prajwal, Pune Single, Shringar and Variegated Single recorded the higher gall index of 5 and thus categorised under 'highly susceptible'.

4.5.5. Number of galls per 10 g root

The number of galls per 10 g root sample ranged from 30.61 to 127.96 (Table 22). Significantly lower number of galls was observed in Kahikuchi Single with 30.61 followed by Calcutta Single, Hyderabad Single and Mexican Single with 56.89. The highest number of galls was recorded in Shringar (127.96 galls/ 10 g root).

4.5.6. Number of egg masses per gram of root

The performance of fifteen tuberose genotypes for number of egg masses per gram of root ranged from 5.21 to 33.86 (Table 22). Among the genotypes Kahikuchi Single recorded the lowest number of egg masses per g of root (5.21) followed by Calcutta Single, Hyderabad Single and Mexican Single (8.72) and the highest number of egg masses per gram of root was observed in Shringar with 33.86.

4.5.7. Number of eggs per egg mass

The mean data of fifteen tuberose genotypes ranged from 94.55 to 220.78 for number of eggs per egg mass (Table 22). The results indicated that significantly lower number of eggs per egg mass in Kahikuchi Single (94.55) followed by Calcutta Single, Hyderabad Single and Mexican Single (8.72) and the highest number of eggs per egg mass was recorded in Shringar (220.78).

4.5.8. Number of root knot nematode females per gram of root

Values for number of root knot nematode females per gram of root in tuberose genotypes ranged from 25.80 to 88.22 (Table 22). Kahikuchi Single was observed to have lower number of root knot nematode females (25.80/ g) followed by Calcutta Single, Hyderabad Single and Mexican Single (41.82/ g), whereas higher number observed in Shringar (88.22/ g).

4.5.9. Nematode resistance characters

4.5.9.1. Root phenol content

The total phenol in roots of different single genotypes was analysed. The results revealed that, there was an increased level of phenol upto 96 hours after inoculation and thereafter there was a slight decrease in root phenol content. Among the ten single genotypes of tuberose, Kahikuchi Single registered the highest phenol content in roots (17.05 mg g^{-1}) of root followed by Mexican Single, Hyderabad Single and Calcutta Single (9.99, 9.95 and 9.93 mg g^{-1} of root). The lowest amount of phenol content in the roots was noticed in Prajwal (8.98 mg g^{-1}) (Table 23).

4.5.9.2. Root ortho-dihydroxy (OD) phenol content

Estimation of ortho- dihydroxy phenol content in the roots of Kahikuchi Single showed that the highest ortho- dihydroxy phenol content was recorded by the genotype Kahikuchi Single (12.95 mg g^{-1}). The lowest value was recorded in Pune Single (4.21 mg g^{-1}). The ortho- dihydroxy phenol content was induced after nematode inoculation and increased upto 96 hours, after that there was a slight decline (Table 24).

4.5.9.3. Root peroxidase activity

The results of peroxidase activity in the genotypes of tuberose showed an induction after the inoculation and it increased upto 96 hours, after which a slight decrease in the activity of peroxidase was noticed. Among the ten tested genotypes, Kahikuchi Single recorded the highest value of 3.19 OD $min^{-1}g^{-1}$. It was followed by Hyderabad Single, Calcutta Single and Mexican Single (1.38, 1.37 and 1.36 OD $min^{-1}g^{-1}$) and the genotype Navsari Local recorded the lowest peroxidase activity of OD 0.79 $min^{-1}g^{-1}$ (Table 25).

4.5.9.4. Root polyphenol oxidase activity

Polyphenol oxidase activity was estimated in the ten tuberose genotypes and the results indicated that the genotype Kahikuchi Single recorded the highest polyphenol oxidase activity of 3.18 OD mg g^{-1} followed by Hyderabad Single,Mexican Single and Calcutta Single (1.55, 1.53 and 1.52 OD mg g^{-1}). After inoculation, the polyphenol oxidase activity incresaed upto 96 hours and later there was a significant decrease in this activity. The lowest polyphenol oxidase activity of 0.85 OD mg g^{-1} was recorded in Prajwal (Table 26).

4.5.9.5. Phenylalanine ammonia lyase

Increased activity of enzyme PAL was observed after inoculation with root knot nematodes. The enzyme activity reached the highest level at 96 hours after inoculation and thereafter it declined with a decreasing rate. Among the tuberose genotypes Kahikuchi Single recorded the highest PAL activity of 15.39 nmol of trans cinnamic acid min^{-1} g^{-1} followed by Hyderabad Single, Calcutta Single and Mexican Single (9.64, 9.63 and 9.61 nmol of trans cinnamic acid min-1 g-1). The lowest activity was recorded in Navsari Local (5.58 nmol of trans cinnamic acid min^{-1} g^{-1}) (Table 27).

4.5.9.6. Root acid phosphatase activity

Similar trend of increasing activity was also noticed for this enzyme. The estimation of acid phosphatase activity in the roots of tuberose genotypes showed an increased activity after inoculation. There was a gradual increase in acid phosphatase activity in Kahikuchi Single (117.15 *m*moles p-nitrophenol min^{-1} mg^{-1} protein) followed by Mexican Single, Calcutta Single and Hyderabad Single (112.94, 112.92 and 112.36 *m*moles p-nitrophenol min^{-1} mg^{-1} protein). Whereas the lowest activity of acid phosphatase was observed in Shringar (56.87 *m*moles p-nitrophenol min^{-1} mg^{-1} protein) (Table 28).

4.6. Plant growth parameters (Double types)

4.6.1. Root length

The mean data for root length among five double types varied from 53.52 to 56.89 cm (Table 29). When the tuberose genotypes were analyzed for root length, all the genotypes recorded the high root length. Among them, the genotype Vaibhav exhibited the lowest root length of 53.52 cm.

4.6.2. Root weight

The evaluation of tuberose genotypes for root weight ranged from 62.34 g to 64. 85 g (Table 29). Among the tuberose genotypes evaluated, Vaibhav recorded the highest root weight of 64.85 g.

4.6.3. Soil nematode population per 200 cc of soil

The performance of five tuberose genotypes for soil nematode population per 200 cc of soil ranged from 280.44 to 286.42 (Table 29). The data indicated that all the genotypes

showed higher soil nematode population per 200 cc of soil. Among them, however less nematode population of 280.22 was observed in Calcutta Double.

4.6.4. Root knot index

Parameters like root knot index and soil nematode poplation were used to evaluate the resistance or susceptibility of tuberose genotypes against *Meloidogyne incognita* (Table 29). The tuberose (double) genotypes were inoculated with *Meloidogyne incognita* under pot culture condition. The results revealed that all the genotypes recorded a gall index of 5, attaining a reaction category of 'highly susceptible' (HS).

4.6.5. Number of galls per 10 g root

The number of galls per 10 g root ranged from 123.55 to 126.76 (Table 30). Higher number of galls was recorded in all double genotypes (Pearl Double, Vaibhav, Calcutta Double, Suvasini and Hyderabad Double with 123.55, 123.56, 124.62, 124.68 and 126.76).

4.6.6. Number of egg masses per gram of root

The performance of five double tuberose genotypes for number of egg masses per g of root ranged from 30.68 to 34.32 (Table 30). All genotypes showed higher number of egg masses per g of root (Pearl Double, Suvasini, Calcutta Double, Vaibhav and Hyderabad Double) was observed with 30.68, 31.72, 32.81, 32.92 and 34.32 egg masses per gram of root.

4.6.7. Number of eggs per egg mass

The mean data of number of eggs/ egg mass in tuberose genotypes ranged from 202.49 to 222.78 (Table 30). The results indicated that all the genotypes showed higher number of eggs per egg mass. Among them, however, Vaibhav registered the lowest number of eggs per egg mass (202.49).

4.6.8. Number of root knot nematode females per gram of root

Values for number of root knot nematode females per gram of root in tuberose genotypes ranged from 82.10 to 88.31 (Table 30). All the genotypes showed higher number of root knot nematode females per gram of root. Among them, however, Vaibhav showed the lowest number of root knot nematode females per gram of root (82.10).

4.6.9. Nematode resistance characters

4.6.9.1. Root phenol content

The total phenol content in roots of five double genotypes was analysed. The results revealed that, there was an increased level of phenol upto 96 hours after inoculation and thereafter there was a slight decrease in root phenol content. All the double genotypes recorded the low root phenol content. Among the genotypes, however, Vaibhav exhibited the highest root phenol content of 9.22 mg g^{-1} (Table 31).

4.6.9.2. Root ortho-dihydroxy (OD) phenol content

Estimation of ortho- dihydroxy phenol content was done in the roots of tuberose genotypes. The results showed that all of them recorded the lower ortho- dihydroxy phenol content. However, Calcutta Double exhibited higher ortho- dihydroxy phenol content of 4.29 mg g^{-1} among the genotypes. Ortho- dihydroxy phenol was induced after nematode inoculation and increased upto 96 hours, after which there was a slight decline (Table 32).

4.6.9.3. Root peroxidase activity

The results of peroxidase activity in the genotypes of tuberose showed an induction after nematode inoculation and it increased upto 96 hours and later decrease in the activity of peroxidase. Among the five tested double genotypes, Vaibhav recorded the highest value of 0.82 OD $min^{-1}g^{-1}$ (Table 33).

4.6.9.4. Root polyphenol oxidase activity

Polyphenol oxidase activity was estimated in the five tuberose genotypes. The results indicated that the genotype Vaibhav recorded the highest polyphenol oxidase activity of 0.89 OD mg g^{-1}. After nematode inoculation, the polyphenol oxidase activity incresaed upto 96 hours and later there was a significant decrease in this activity (Table 34).

4.6.9.5. Phenylalanine ammonia lyase

Increased activity of enzyme PAL was observed in roots after nematode inoculation. The enzyme activity reached the highest level at 96 hours after inoculation and thereafter it declined with a decreasing rate. All the double genotypes recorded less phenylalanine ammonia lyase content. Among the tuberose genotypes Vaibhav recorded the highest PAL activity of 5.67 nmol of trans cinnamic acid min^{-1} g^{-1} (Table 35).

4.6.9.6. Root acid phosphatase activity

Similar trend of increasing enzyme activity was also noticed in nematode infected roots. The estimation of acid phosphatase activity in the roots of tuberose genotypes showed an increased activity after inoculation. A gradual increase in acid phosphatase activity was observed in Pearl Double (58.38 *m*moles p-nitrophenol min^{-1} mg^{-1} protein (Table 36).

4.7. Artificial selfing

4.7.1. Fruit set as influenced by pollination on second, third and fourth day after anthesis

Highest percentage of fruit set by artificial selfing (Table 37) was recorded in Variegated Single (23.62 %), which was followed by Shringar (21.74 %), whereas the lowest fruit set was recorded in Mexican Single (5.98 %) at seven days after pollination where flowers were pollinated second day after anthesis. The maximum percentage of fruit set was observed in Shringar (33.62 %) followed by Pune Single (32.24 %), while it was minimum in Mexican Single (10.98 %) at seven DAP where flowers were pollinated third day after anthesis (Table 38).

The results of fourth day after anthesis showed that no fruit set was obtained in any of the genotypes at seven DAP where flowers were pollinated fourth day after anthesis (Table 39).

However, it was observed that fruits dropped in all genotypes after seven days of pollination when flowers were pollinated second, third DAA.

4.8. Artificial crossing (Single type x Single type)

4.8.1. Fruit set as influenced by pollination on fourth day after anthesis

It was observed from the table (40 & 41) that, the highest percentage of fruit set was recorded in the cross using between Phule Rajani X Variegated Single (50.40 %) followed by cross between Pune Single X Variegated Single (28.07 %). Further the crosses in Calcutta Single, Kahikuchi Single, Mexican Single and Navsari Local using Variegated Single as pollen parent was also found successful (24.00 %, 25.00 %, 21.42 % and 25.02 %). But other single types did not set fruits.

On fourth day after anthesis, the maximum percentage of fruit set was noticed in the reciprocal cross between Variegated Single X Phule Rajani (78.40 %) followed by cross between Mexican Single X Phule Rajani (70 %). However, it was minimum in crosses between Hyderabad Single X Phule Rajani (6.66 %) and Kahikuchi Single X Phule Rajani (10 %) at seven DAP. But the crosses in other single types using the same Phule Rajani as pollen parent did not set fruits.

A fruit set of 29.10 percentage was recorded in the cross between Variegated Single X Shringar followed by cross Kahikuchi Single X Shringar (19.71). Other crosses using Shringar as pollen parent did not set fruits.

The fruit set noticed in cross between Variegated Single X Hyderabad Single and Variegated Single X Navsari Local was 13.84 and 50.98 % respectively. The crosses in other single types using Hyderabad Single and Navasari Local as pollen parents did not set fruits.

4.9. Standardization of tetrazolium test

At 40^0C, the embryos of tuberose seed treated with 0.25 per cent tetrazolium solution and incubated for 2 h recorded 62 per cent complete staining and 38 per cent partial staining seed. However 86 per cent of complete staining and 14 per cent partial staining was recorded after 2½ h incubation period. Whereas the embryos treated with 0.5 per cent tetrazolium solution and incubated for 2 h registered 85, 14 and 1 per cent completely, partially and over stained seed, respectively. While at 2½ h incubation period 94 per cent completely stained, 1 per cent partially stained and 5 per cent over stained seeds obtained.

On overnight treatment of embryos at dark with 0.25 per cent solution recorded 90 per cent completely stained, 1 per cent partially stained and 9 per cent over stained seeds. But with 0.5 per cent solution, 85 per cent completely stained and 15 per cent over stained seeds respectively (Table 42).

4.10. Effect of seed treatments for enhancing germination

4.10.1. Germination (%)

Among the pre-sowing treatments, seeds treated with GA_3 250 ppm for 8 hours (T_3) recorded the highest germination (12.50 %) and it was followed by the treatment T_7

(KNO_3 0.5 per cent for 8 hours) which registered 12. 45 % germination. The minimum germination (7.96 %) was recorded in the treatments T_{17} and T_{18} (water soaking for 8 and 16 hours) respectively. The interaction between G x D registered the superiority of seed treatment with GA_3 100 ppm for 8 h for obtaining maximum germination (Table 43).

4.10.2. Root length (cm)

Among the seed treatments, treatment T_3 (GA_3 250 ppm for 8 hours) recorded the longest root (13.24 cm) followed by 13.00 cm in T_7 (KNO_3 0.5 per cent for 8 hours). The shortest root length of 7.46 cm was registered in treatments T_{17} and T_{18} (water soaking for 8 and 16 hours) respectively. However the interaction effect was non significant (Table 43).

4.10.3. Shoot length (cm)

The shoot length of the seedling was the maximum with seeds treated with GA_3 250 ppm for 8 hours (T_3) (14.32 cm) followed by 14.23 cm in T_7 (KNO_3 0.5 per cent for 8 hours). It was minimum (7.21 cm) in treatments T_{17} and T_{18} (water soaking for 8 and 16 hours). The interaction between G x D was non significant (Table 43).

4.10.4. Vigour index

Among the treatments, treatment T_3 (GA_3 250 ppm for 8 hours) recorded the the maximum (978.38) followed by 965.304 in T_7 (KNO_3 0.5 per cent for 8 hours). It was minimum (263.473) in the treatments T_{17} and T_{18} (water soaking for 8 and 16 hours). However the interaction effect was non significant (Table 43).

4.10.5. Drymatter production (g/ seedlings^5)

Seeds treated with GA_3 250 ppm for 8 hours (T_3) recorded the maximum dry weight (0.24 g) while it was the minimum in the treatments T_{17} and T_{18} (water soaking for 8 and 16 hours) (0.04 g) among the treatments. The interaction between G x D was non significant (Table 43).

4.10.6. Mean number of days taken for fruit set

The period required for seed setting from the day of pollination to the day of seed set for different crosses made are presented in Table 44.

It was observed from the data the minimum number of days taken for fruit set was recorded in the cross between Mexican Single X Variegated Single (7.45) followed by Calcutta Single X Variegated Single (7.50), while the maximum number of days taken for fruit set (8.25) was recorded in Navsari Local X Variegated Single.

The minimum number of days taken for fruit set was noticed in the cross between Kahikuchi Single x Phule Rajani (7.50) followed by Variegated Single X Phule Rajani (7.75) and maximum number of days taken for fruit set in the cross between Mexican Single X Phule Rajani (8.45).

The number of days taken for fruit set was also low in the cross between Variegated Single X Shringar (8.50) followed by the cross between Kahikuchi Single X Shringar (8.76). Similarly, the mean number of days taken for fruit set (8.95 and 8.76) was also recorded in the crosses between Variegated Single X Hyderabad Single and Variegated Single X Navsari Local.

4.10.7. Mean number of days taken for fruit maturity

The period required for seed maturation from the day of pollination to the day of harvesting of matured capsules for different crosses made are depicted in Table 44.

It was observed from the data that, the minimum number of days taken for fruit maturity was recorded in cross between Navsari Local X Variegated Single (76.75) followed by cross between Kahikuchi Single X Variegated Single (78.97), while the maximum number of days taken for fruit maturity (81.86) was recorded in Pune Single X Variegated Single. It was also minimum in cross between Kahikuchi Single x Phule Rajani (80.00) followed by cross between Variegated Single X Phule Rajani (81.00), while the cross between Mexican Single X Phule Rajani showed minimum (85.08).

Other crosses *viz.,* Variegated Single X Shringar (81.50) followed by crosses between Kahikuchi Single X Shringar (83.74) alos showed less number of days for fruit maturity.

Similarly, it was minimum (85.98 and 85.43) in the crosses between Variegated Single X Hyderabad Single and Variegated Single X Navsari Local.

4.10.8. Mean number of seeds per capsule in different crosses

The mean number of seeds per capsule for different crosses made are presented in the Table 44.

It was observed from the data that, the more number of seeds per capsule was recorded in the cross between Phule Rajani X Variegated Single (38.00) followed by cross between Pune Single X Variegated Single (32.96), while the minimum number of seeds per capsule (20.00) was recorded in Kahikuchi Single X Variegated Single.

The number of seeds per capsule was also more in cross between Variegated Single x Phule Rajani (41.00) followed by Hyderabad Single X Phule Rajani (28.97) and it was less in the cross between Mexican Single X Phule Rajani (16.23). Next to this, the cross between Kahikuchi Single X Shringar (25.00) followed by crosses between Variegated Single X Shringar (24.00) showed higher number of seeds per capsule.

The more number of seeds per capsule (26.00 and 32.00) was also recorded in the crosses between Variegated Single X Hyderabad Single and Variegated Single X Navsari Local.

4.10.9 Seed germination in different crosses

Seeds obtained from different crosses were sown seperately after imposing the treatment T_3 (GA_3 250 ppm for 8 hours) to study the germination behaviour. The results of this experiment are given below

It was observed from the table (45) that, the range of germination varied between 12.23 to 12.56 per cent. In general, it showed low percentage of germination. Among them, the highest percentage of germination was recorded in the cross between Calcutta Single X Variegated Single (12.50 %) followed by the cross Mexican Single X Variegated Single (12.45 %), while the lowest germination of 12.23 % was recorded in Pune Single X Variegated Single cross.

The maximum percentage of germination was also noticed in cross between Variegated Single x Phule Rajani (12.56 %) followed by cross Mexican Single X Phule Rajani (12.52 %) while it was minimum in the cross between Hyderabad Single X Phule Rajani (12.45 %).

The percentage of germination was also higher in the cross between Kahikuchi Single X Shringar (12.51 %) followed by cross between Variegated Single X Shringar (12.50 %). Similar results obtained in other crosses *viz.,* Variegated Single X Hyderabad Single and Variegated Single X Navsari Local (12.54 amd 12.43 %) respectively.

4.10.10. Mean number of days taken for germination

The number of days taken for germination was recorded from the day of sowing to day of sprouting in different crosses and the results are furnished in Table 45.

It was observed from the data that, the number of days taken for germination was less between Phule Rajani X Variegated Single cross (28.96) followed by Pune Single X Variegated Single (30.21), while it was more (38.25) in Kahikuchi Single X Variegated Single cross.

Similar observations were also recorded between Variegated Single x Phule Rajani (28.96) followed by Kahikuchi Single X Phule Rajani (31.67) crosses while maximum number of days taken for germination observed in the cross between Mexican Single X Phule Rajani (36.82).

When Shringar was used as pollen parent, the results showed that the days taken for germination was less between Kahikuchi Single X Shringar (32.98) followed by Variegated Single X Shringar (34.72).

The number of days taken for seed germination between the crosses Variegated Single X Hyderabad Single and Variegated Single X Navsari Local were 35.98 and 32.87 respectively.

4.11. Post fertilization events

4.11.1. Histological studies

The histological changes in tuberose upon selfing and crossing were studied at various stages after fertilization and are described below. The observations at various stages of fruit development are presented in table 46 & 47.

4.11.1.1. Selfing

On the day of anthesis (DOA) normal development of fruit wall, ovule, embryosac, nucellus, hypostase, funiculus and integument were observed. At this stage

ovule had a length of 245 µm and width of 150 µm. The length of embryosac was 65 µm and width 40 µm. Nucellus had a length of 150 µm with a width of 120 µm. Hypostase had a length of 40 µm with a width of 30 µm. The thickness of funiculus was 60 µm. The integument had a thickness of 30 µm, while the thickness of outer epidermis was 4 µm. Inner epidermis and mesocarp had a thickness of 3 µm and 160 µm respectively at zero day after anthesis. At this stage fusion nucleus did not undergo division (Plate). At fourth DAA (days after anthesis) the symptoms of degeneration of egg apparatus and antipodal cells was noticed in selfed material. The fusion nucleus did not show any signs of division. At this stage ovule had increased to a length of 280 µm and had a width of 160 µm. The length of embryosac was 70 µm and width was 40 µm. Nucellus measured 200 µm length wise and 10 µm breadth wise. Hypostase had a length of 50 µm and width of 45 µm. Funiculus and integument had a thickness of 80 µm and 50 µm respectively. Outer epidermis, inner epidermis and mesocarp had a thickness of 5 µm, 4 µm and 260 µm respectively.

Selfed ovary showed complete degeneration of ovule, embryosac, egg apparatus and fruit wall on seventh DAA. By tenth DAA, degeneration appeared to be complete and the fruits got aborted. At this stage ovule had a length of 180 µm and a width of 100 µm. Embryosac measured 40 µm lengthwise and 35 µm breadth wise. Nucellus, hypostase, funiculus, integument were not differentiated at this stage. However outer epidermis, inner epidermis, mesocarp measured 4.5, 3.5 and 170 µm respectively.

No observations could be recorded during subsequent stages as the fruits aborted and fallen off by tenth day upon selfing and also in all failure crosses.

4.11.1.2. Crossing

On the day of anthesis there was normal development of fruit wall, embryosac, nucellus, hypostase, funiculus and integument. At this stage ovule had a length of 290 µm and width of 135 µm. The length of embryosac was 60 µm with a width of 45 µm. Nucellus had a length of 140 µm and width of 100 µm. Hypostase had a length of 45 µm and width of 35 µm. The thickness of funiculus and integument were 60 and 20 µm respectively. The thickness of outer e[idermis, inner epidermis and mesocarp was 4, 3 and 150 µm respectively.

On fourth DAA, there was normal development of all the tissues. At this stage fusion nucleus did not show any sign of division. Ovule had a length of 300 µm and width of 220 µm. The length of embryosac was 65 µm with a width of 60 µm. Nucellus had a length of 190 µm and width of 165 µm. Hypostase measured 65 µm lengthwise and 50 µm widthwise. Funiculus, integument, outer epidermis, inner epidermis and mesocarp had the thickness of 75, 30, 6, 5 and 250 µm respectively.

Even on tenth DAA, the fusion nucleus did not exhibit any sign of division though there was normal development of the fruit. At this stage seed had a length of 460 µm and width of 280 µm. The length of embryosac was 70 µm and width was 50 µm. Nucellus had a length of 340 µm and width of 280 µm. Hypostase measured 125 µm lengthwise and 80 µm widthwise. The funiculus and integument had a thickness of 120 µm and 40 µm respectively. Outer epidermis, inner epidermis and mesocarp had a thickness of 12, 8 and 280 µm respectively at this stage.

On twenty DAA, fusion nucleus remained as such without any division even though there was normal development of the seed upon crossing. At this stage the seed had increased to a length of 580 µm and width of 440 µm. The length of embryosac was 175 µm with a width of 100 µm. Nucellus had increased to a length of 400 µm and width of 350 µm. Hypostase measured 150 µm lengthwise and 110 µm breadthwise. Funiculus, integument, outer epidermis, inner epidermis, mesocarp had thickness of 150, 40, 14, 10 and 300 µm respectively.

At thirtieth DAA, fully developed torpedo shaped embryo was observed. Between twentieth and thirtieth DAA all the developmental stages were observed to take place. At this stage ovule had a length of 700 µm and width of 60 µm. Embryosac measured 280 µm lengthwise and 150 µm widthwise. Embryo had a length of 200 µm with a width of 130 µm. Tegma and testa had a thickness of 30 µm and 70 µm respectively.

At fortieth DAA, the torpedo shaped embryo enlarged to its maximum length i.e., 480 µm with a width of 170 µm. Seed had a length of 750 µm and width of 550 µm. Embryosac measured 500 µm lengthwise and 190 µm widthwise. The thickness of tegma and testa was 60 and 20 µm respectively at fortieth DAA.

4.12. Genetic variability, heritability and genetic advance in single types

The varieties exhibited significant amount of variability for all the twelve characters studied. Range, mean, phenotypic and genotypic co-efficients of variation, heritability and genetic advance as per cent of mean are presented in Table 48.

4.12.1. Days taken for sprouting of bulb

The phenotypic coefficient of variation was 9.74 % and genotypic coefficient of variation was 9.08 % for days taken for sprouting of bulb. The days taken for sprouting of bulb recorded a high heritability value of 88.83 %. The genetic advance as per cent of mean was 17.43.

4.12.2. Plant height

The phenotypic co-efficient of variation and genotypic co-efficient of variation (17.50, 17.16 % respectively) was moderate. However high heritability (96.24 %) coupled with low genetic advance as per cent of mean (34.69) were noticed.

4.12.3. Number of leaves per plant

The number of leaves per plant showed minimum coefficient of variation at phenotypic and genotypic levels (5.50 % and 4.34 %, respectively). High heritability estimate (62.16 %) and low genetic advance as per cent mean (17.05) were recorded for this character.

4.12.4. Days to spike emergence

The phenotypic coefficient of variation and genotypic coefficient of variation (6.60 and 5.60 % respectively) was low. Similarly, low heritability estimate (71.99 %) and low genetic advance as per cent mean (19.78) were recorded for this character.

4.12.5. Duration of Flowering

High phenotypic coefficient of variation and genotypic coefficient of variation were recorded for the duration of flowering (35.79 % and 35.62 % respectively). It also showed a high heritability (99.07 %) coupled with high genetic advance as per cent mean (73.04).

4.12.6. Spike length

The phenotypic co-efficient of variation (21.91 %) and genotypic co-efficient of variation (21.64 %) was moderate for spike length. However, high heritability (97.58 %) and moderate genetic advance as per cent mean (44.04) were obtained.

4.12.7. Rachis length

The rachis length showed the phenotypic co-efficient of variation of 26.85 per cent and genotypic co-efficient of variation of 26.64 per cent. High heritability estimate (98.47 %) coupled with moderate genetic advance as per cent mean (54.46) were recorded.

4.12.8. Number of florets per spike

Moderate phenotypic and genotypic co-efficient of variation were recorded for the number of florets per spike (32.01 % and 31.80 % respectively). It also showed a high heritability (98.72%) coupled with high genetic advance as per cent mean (65.08).

4.12.9. Floret length

Moderate phenotypic and genotypic co-efficient of variation (3.11% and 1.45 % , respectively) were obtained. High heritability estimate (78.23 %) and low genetic advance as per cent mean (15.39) were recorded.

4.12.10. Weight of the florets per spike

High phenotypic and genotypic coefficient of variation were recorded for the weight of florets per spike (32.43 % and 32.27 % respectively). It also showed a high heritability (99.02 %) coupled with high genetic advance as per cent mean (66.15).

4.12.11. Number of spikes per m^2

Moderate phenotypic and genotypic co-efficient of variation (16.68 % and 16.34 % respectively) were obtained. High heritability of 95.00 per cent and moderate genetic advance as per cent mean (42.96) was obtained.

4.12.12. Yield of florets per plot

Moderate phenotypic and genotypic co-efficient of variation (21.96 % and 21.71 % respectively) were obtained. High heritability of 98.00 per cent and moderate genetic advance as per cent mean (44.22) was obtained.

4.13. Genetic variability, heritability and genetic advance in double types

The varieties exhibited significant amount of variability for all the twelve characters studied. Range, mean, phenotypic and genotypic co-efficients of variation, heritability and genetic advance as per cent of mean are presented in Table 49.

4.13.1. Days taken for sprouting of bulb

The phenotypic coefficient of variation was 11.76 % and genotypic coefficient of variation was 11.27 % for days taken for sprouting of bulb. High heritability (91.91 %) coupled with low genetic advance as per cent of mean (22.26) were noticed.

4.13.2. Plant height

The phenotypic co-efficient of variation and genotypic co-efficient of variation (10.28 % and 9.78 % respectively) was moderate. However high heritability (90.52 %) coupled with low genetic advance as per cent of mean (19.17) were noticed.

4.13.3. Number of leaves per plant

The number of leaves per plant showed moderate coefficient of variation at phenotypic and genotypic levels (6.14 % and 5.25 %, respectively). High heritability estimate (73.06 %) and low genetic advance as per cent mean (9.25) were recorded for this character.

4.13.4. Days to spike emergence

The phenotypic coefficient of variation and genotypic coefficient of variation (3.58 and 1.49 % respectively) was low. Similarly high heritability estimate (77.38 %) and low genetic advance as per cent mean (1.28) were recorded for this character.

4.13.5. Duration of Flowering

Moderate phenotypic coefficient of variation and genotypic coefficient of variation were recorded for the duration of flowering (11.71 % and 11.28 % respectively). It also showed a high heritability (92.83 %) coupled with moderate genetic advance as per cent mean (42.39).

4.13.6. Spike length

The phenotypic co-efficient of variation was 12.66 per cent and genotypic co-efficient of variation (12.26 %) was moderate for spike length. However, high heritability (93.79 %) and moderate genetic advance as per cent mean (44.46) were obtained.

4.13.7. Rachis length

The rachis length showed the phenotypic co-efficient of variation of 17.62 per cent and genotypic co-efficient of variation of 17.35 per cent. High heritability estimate (96.91 %) coupled with high genetic advance as per cent mean (45.18) were recorded.

4.13.8. Number of florets per spike

High phenotypic and genotypic co-efficient of variation were recorded for the number of florets per spike (24.59 % and 24.38 % respectively). It also showed a high heritability (98.35%) coupled with high genetic advance as per cent mean (69.81).

4.13.9. Floret length

Moderate phenotypic and genotypic co-efficient of variation (5.04 % and 3.93 % respectively) were obtained. High heritability estimate (60.68 %) and low genetic advance as per cent mean (6.30) were recorded for floret length.

4.13.10. Weight of the florets per spike

Moderate phenotypic and genotypic coefficient of variation were recorded for the weight of florets per spike (13.66 % and 13.29 % respectively). It also showed a high heritability (94.67 %) coupled with moderate genetic advance as per cent mean (46.64).

4.13.11. Number of spikes per m^2

Moderate phenotypic and genotypic co-efficient of variation (17.92 % and 17.64 % respectively) were obtained. High heritability of 96.93 per cent and high genetic advance as per cent mean 65.78 was obtained.

4.13.12. Yield of florets per plot

Moderate phenotypic and genotypic co-efficient of variation (15.31 % and 14.98 % respectively) were obtained. High heritability of 95.78 per cent and moderate genetic advance as per cent mean 40.20 was obtained.

4.14. Genotypic and phenotypic correlations

The correlation co-efficient at both genotypic and phenotypic levels was computed for twelve characters of single and double types and is presented in tables 50, 51, 52 & 53 respectively.

In general genotypic correlation co-efficients were found to be higher than phenotypic correlation co-efficients in both the types.

4.14.1. Genotypic correlation in single types

4.14.1.1. Days taken for sprouting of bulb

Days taken for sprouting of bulb showed highly significant positive correlation with days to spike emergence (0.559) and number of florets/ spike (0.108). This trait however showed negative correlation with weight of florets per spike (-0.725), yield of florets per plot (4 * 1 m) (-0.549), number of spikes per m^2 (-0.401), flowering duration (-0.341), plant height (-0.292), spike length (-0.258), number of leaves per plant (-1.180), length of the floret (-1.014) and rachis length (-0.076) (Table 50).

4.14.1.2. Plant height

Highly significant and positive correlations for plant height was observed with spike length (0.997), yield of florets per plot (4 * 1 m) (0.978), rachis length (0.593), number of spikes per m^2 (0.457), weight of florets per spike (0.402) and number of leaves per plant (0.197) (Table 50).

4.14.1.3. Number of leaves per plant

The number of leaves per plant had highly significant and positive correlation with length of the floret (0.705), weight of the florets per spike (0.652), yield of florets per plot (4 * 1 m) (0.577), flowering duration (0.368), number of spikes per m^2 (0.326) and spike length (0.229). It also had positive significant correlation with rachis length (0.149). However, the days to spike emergence (-0.738) had highly significant but negative correlation with this character (Table 50).

4.14.1.4. Days to spike emergence

Days to spike emergence showed highly significant positive correlation with number of florets per spike (0.068). This trait however showed negative correlation with

number of spikes per m^2 (-0.910), weight of florets per spike (-0.871), yield of florets per plot (4 * 1 m) (-0.737), spike length (-0.707), rachis length (-0.558), length of the floret (-0.526) and flowering duration (-0.307) (Table 50).

4.14.1.5. Duration of Flowering

Duration of flowering showed positive correlation with yield of florets per plot (4 * 1 m) (0.602), weight of florets per spike (0.430), number of spikes per m^2 (0.298), length of the floret (0.282), number of florets per spike (0.175) and rachis length (0.050), while the spike length (-0.141) had highly significant but negative correlation with this trait (Table 50).

4.14.1.6. Spike length

The spike length showed highly significant and positive correlation with rachis length (0.565), number of spikes per m^2 (0.435), yield of florets per plot (4 * 1 m) (0.423), weight of and florets per spike (0.373). However, the length of the floret (-0.332) and number of florets per spike (-0.150) had significant but negative correlation with this trait (Table 50).

4.14.1.7. Rachis length

The rachis length had positive and significant correlation with yield of florets per plot (4 * 1 m) (0.474), weight of florets per spike (0.440), number of spikes per m^2 (0.385) and number of florets per spike (-0.150). This trait however showed negative correlation with length of the floret (-0.263) (Table 50).

4.14.1.8. Number of florets per spike

The number of florets per spike showed highly significant and positive correlation with weight of florets per spike (0.256), yield of florets per plot (4 * 1 m) (0.178), number of spikes per m^2 (0.100) and length of the floret (0.094) (Table 50).

4.14.1.9. Floret length

The floret length showed positive and highly significant correlation with weight of florets per spike (0.430), number of spikes per m^2 (0.236) and yield of florets per plot (4 * 1 m) (0.225) (Table 50).

4.14.1.10. Weight of the florets per spike

The weight of florets per spike showed significant and positive correlation with yield of florets per plot (4 * 1 m) (0.864) and number of spikes per m^2 (0.856) (Table 50).

4.14.1.11. Number of spikes per m^2

Number of spikes per m^2 showed significant and positive correlation with yield of florets per plot (4 * 1 m) (0.752) (Table 50).

4.14.2. Genotypic correlation in double types

4.14.2.1. Days taken for sprouting of bulb

Days taken for sprouting of bulb showed highly significant positive correlation with days to spike emergence (0.671). This trait however showed negative correlation with flowering duration (-0.978), number of leaves per plant (-0.954), number of spikes per m^2 (-0.952), plant height (-0.939), spike length (-0.927), weight of florets per spike (-0.874), yield of florets per plot (4 * 1 m) (-0.803), rachis length (-0.802), length of the floret (-0.761) and number of florets per spike (-0.736) (Table 51).

4.14.2.2. Plant height

Highly significant and positive correlations for plant height was observed with spike length (1.001), yield of florets per plot (4 * 1 m) (0.975), number of florets per spike (0.971), number of leaves per plant (0.958), flowering duration (0.944), weight of florets per spike (0.904), number of spikes per m^2 (0.695), rachis length (0.652) and length of the floret (0.623). The trait however showed negative correlation with days to spike emergence (-1.574) (Table 51).

4.14.2.3. Number of leaves per plant

The number of leaves per plant had highly significant and positive correlation with weight of the florets per spike (0.994), yield of florets per plot (4 * 1 m) (0.987), number of florets per spike (0.981), spike length (0.965), flowering duration (0.941), length of the floret (0.730), number of spikes per m^2 (0.665) and rachis length (0.438). The trait however showed negative correlation with days to spike emergence (-1.759) (Table 51).

4.14.2.4. Days to spike emergence

Days to spike emergence showed highly negative correlation with flowering duration (-1.706), spike length (-1.518), yield of florets per plot (4 * 1 m) (-1.430), number of spikes per m^2 (-1.418), weight of florets per spike (-1.406), rachis length (-1.384), length of the floret (-2.136) and number of florets per spike (-1.276) (Table 51).

4.14.2.5. Duration of Flowering

Duration of flowering showed positive correlation with spike length (0.944), yield of florets per plot (4 * 1 m) (0.899), weight of florets per spike (0.875), number of spikes per m^2 (0.862), number of florets per spike (0.859), length of the floret (0.851) and rachis length (0.695) (Table 51).

4.14.2.6. Spike length

The spike length showed highly significant and positive correlation with yield of florets per plot (4 * 1 m) (0.974), number of florets per spike (0.967), weight of florets per spike (0.905), number of spikes per m^2 (0.696), rachis length (0.653) and length of the floret (0.604) (Table 51).

4.14.2.7. Rachis length

The rachis length had positive and significant correlation with number of spikes per m^2 (0.742), length of the floret (0.566), yield of florets per plot (4 * 1 m), number of florets per spike (0.481) and weight of florets per spike (0.347) (Table 51).

4.14.2.8. Number of florets per spike

The number of florets per spike showed highly significant and positive correlation with of florets per plot (4 * 1 m) (0.996), weight of florets per spike (0.950), number of spikes per m^2 (0.500) and length of the floret (0.489) (Table 51).

4.14.2.9. Floret length

The floret length showed positive and highly significant correlation with number of spikes per m^2 (0.918), weight of florets per spike (0.630) and yield of florets per plot (4 * 1 m) (0.561) (Table 51).

4.14.2.10. Weight of the florets per spike

The weight of florets per spike showed significant and positive correlation with yield of florets per plot (4 * 1 m) (0.965) and number of spikes per m^2 (0.534) (Table 51).

4.14.2.11. Number of spikes per m^2

Number of spikes per m^2 showed significant and positive correlation with yield of florets per plot (4 * 1 m) (0.565) (Table 51).

4.14.3. Phenotypic correlation in single types

4.14.3.1. Days taken for sprouting of bulb

Days taken for sprouting of bulb showed highly significant positive correlation with days to spike emergence (0.465). This trait however showed negative correlation with number of leaves per plant (-0.646), weight of florets per spike (-0.638), number of spikes per m^2 (-0.294), yield of florets per plot (4 * 1 m) (-0.287), flowering duration (-0.283), plant height (-0.197), spike length (-0.182), length of the floret (-0.043) and rachis length (-0.027) (Table 52).

4.14.3.2. Plant height

Highly significant and positive correlation for plant height was observed with spike length (0.996), rachis length (0.587), number of spikes per m^2 (0.435), weight of florets per spike (0.393), yield of florets per plot (4 * 1 m) (0.337), number of leaves per plant (0.271) and length of the floret (0.048). But it showed negative correlation with days to spike emergence (-0.527), flowering duration (-0.137) and number of florets per spike (-0.128) (Table 52).

4.14.3.3. Number of leaves per plant

The number of leaves per plant had highly significant and positive correlation with weight of the florets per spike (0.571), flowering duration (0.345), number of spikes per m^2 (0.263), yield of florets per plot (4 * 1 m) (0.173), spike length (0.172), rachis length (0.072). But this trait showed negative correlation with number of florets per spike (-0.189) and days to spike emergence (-0.169) (Table 52).

4.14.3.4. Days to spike emergence

Days to spike emergence showed highly positive correlation with length of the floret (0.375) and number of florets per spike (0.014). The trait however showed negative correlation with yield of florets per plot (4 * 1 m) (-0.401), number of florets per spike (-0.189) and days to spike emergence (-0.719), weight of florets per spike (-0.685), number of spikes per m^2 (-0.650), spike length (-0.511), rachis length (-0.407) and flowering duration (-0.211) (Table 52).

4.14.3.5. Duration of Flowering

Duration of flowering showed positive correlation with weight of florets per spike (0.424), number of spikes per m^2 (0.287), length of the floret (0.232), number of florets per spike (0.167) and rachis length (0.039). The trait however showed negative correlation with spike length (-0.125) (Table 52).

4.14.3.6. Spike length

The spike length showed highly significant and positive correlation with rachis length (0.558), number of spikes per m^2 (0.418), weight of florets per spike (0.364), yield of florets per plot (4 * 1 m) (0.297)), and length of the floret (0.016). The trait however showed negative correlation with number of florets per spike (-0.131) (Table 52).

4.14.3.7. Rachis length

The rachis length had positive and significant correlation with weight of florets per spike (0.434), yield of florets per plot (4 * 1 m) (0.424), number of spikes per m^2 (0.371), number of florets per spike (0.086) and length of the floret (0.010) (Table 52).

4.14.3.8. Number of florets per spike

The number of florets per spike showed highly significant and positive correlation with weight of florets per spike (0.248), yield of florets per plot (4 * 1 m) (0.176), length of the floret (0.161) and number of spikes per m^2 (0.080) (Table 52).

4.14.3.9. Floret length

The floret length showed positive and highly significant correlation with weight of florets per spike (0.306), yield of florets per plot (4 * 1 m) (0.138) and number of spikes per m^2 (0.032) (Table 52).

4.14.3.10. Weight of the florets per spike

The weight of florets per spike showed significant and positive correlation with number of spikes per m^2 (0.859) and yield of florets per plot (4 * 1 m) (0.846) (Table 52).

4.14.3.11. Number of spikes per m^2

Number of spikes per m^2 showed significant and positive correlation with yield of florets per plot (4 * 1 m) (0.722) (Table 52).

4.14.4. Phenotypic correlation in double types

4.14.4.1. Days taken for sprouting of bulb

Days taken for sprouting of bulb showed highly significant positive correlation with days to spike emergence (1.036). This trait however showed negative correlation with number of spikes per m^2 (-0.852), flowering duration (-0.830), spike length (-0.792), plant height (-0.772), yield of florets per plot (4 * 1 m) (-0.698), number of florets per spike (-0.690), rachis length (-0.646), number of leaves per plant (-0.637), weight of florets per spike (-0.623), length of the floret (-0.477) (Table 53).

4.14.4.2. Plant height

Highly significant and positive correlations for plant height was observed with spike length (0.999), yield of florets per plot (4 * 1 m) (0.971), number of florets per spike (0.955), days to spike emergence (0.940), number of leaves per plant (0.939), weight of florets per spike (0.900), number of spikes per m^2 (0.685), rachis length (0.639) and length of the floret (0.581). This trait also showed negative correlation with days to spike emergence (-0.347) (Table 53).

4.14.4.3. Number of leaves per plant

The number of leaves per plant had highly significant and positive correlation with weight of the florets per spike (0.946), yield of florets per plot (4 * 1 m) (0.931), spike length (0.928), flowering duration (0.914), number of florets per spike (0.897), number of spikes per m^2 (0.649), length of the floret (0.608) and rachis length (0.413). This trait also showed negative correlation with days to spike emergence (-0.157) (Table 53).

4.14.4.4. Days to spike emergence

Days to spike emergence showed highly significant negative correlation with flowering duration (-0.445), number of spikes per m^2 (-0.427), number of florets per spike (-0.416), rachis length (-0.411), yield of florets per plot (4 * 1 m) (-0.401), spike length (-0.389), weight of florets per spike (-0.364) and length of the floret (-0.125) (Table 53).

4.14.4.5. Duration of Flowering

Duration of flowering showed positive correlation with spike length (0.940), yield of florets per plot (4 * 1 m) (0.896), weight of florets per spike (0.868), number of spikes per m^2 (0.859), number of florets per spike (0.854), length of the floret (0.806) and rachis length (0.683) (Table 53).

4.14.4.6. Spike length

The spike length showed highly significant and positive correlation with yield of florets per plot (4 * 1 m) (0.974), number of florets per spike (0.961), weight of florets per spike (0.899), number of spikes per m^2 (0.685), rachis length (0.639) and length of the floret (0.595) (Table 53).

4.14.4.7. Rachis length

The rachis length had positive and significant correlation with number of spikes per m^2 (0.734), length of the floret (0.544), yield of florets per plot (4 * 1 m) (0.500), number of florets per spike (0.470) and weight of florets per spike (0.320) (Table 53).

4.14.4.8. Number of florets per spike

The number of florets per spike showed highly significant and positive correlation with yield of florets per plot (4 * 1 m) (0.993), weight of florets per spike (0.946), number of spikes per m^2 (0.490) and length of the floret (0.456) (Table 53).

4.14.4.9. Floret length

The floret length showed positive and highly significant correlation with number of spikes per m^2 (0.812), weight of florets per spike (0.621) and yield of florets per plot (4 * 1 m) (0.554) (Table 53).

4.14.4.10. Weight of the florets per spike

The weight of florets per spike showed significant and positive correlation with yield of florets per plot (4 * 1 m) (0.963) and number of spikes per m^2 (0.516) (Table 53).

4.14.4.11. Number of spikes per m^2

Number of spikes per m^2 showed significant and positive correlation with yield of florets per plot (4 * 1 m) (0.550) (Table 53).

4.15. Path analysis

4.15.1. Path analysis for single types

The genotypic correlation co-efficients of single types were apportioned into direct effects and indirect effects by path analysis and results are given in table 54.

4.15.2. Direct effects

Out of the twelve traits studied, the maximum positive direct effect on flower yield was through weight of florets per spike (0.6380) followed by number of leaves per plant (0.5784), flowering duration (0.3746), rachis length (0.2776), days taken for sprouting of bulb (0.2460), length of the floret (0.2286), number of florets per spike (0.2077) and days to spike emergence (0.0203).

Number of spikes per m^2 (-0.0511), spike length (-0.3784), plant height (-0.3190) and and and showed negative direct effect on yield.

4.15.3. Indirect effects

4.15.3.1. Days taken for sprouting of bulb

Days taken for sprouting of bulb recorded strong positive indirect effect through days to spike emergence (0.1827) and weight of florets per spike (0.0087). Whereas the other characters exerted a negative indirect effect.

4.15.3.2. Plant height

The indirect effect contributed by plant height through days to spike emergence (0.1775) and weight of the florets per spike (0.0302) was positive while its influence through other characters was negative.

4.15.3.3. Number of leaves per plant

The number of leaves per plant recorded a strong positive indirect effect through number of florets per spike (0.5626), plant height (0.5140), rachis length (0.4974), number of spikes per m^2 (0.3593), spike length (0.3262) and flowering duration (0.2995) whereas other characters exhibited negative indirect effect.

4.15.3.4. Days to spike emergence

Days to spike emergence recorded strong positive indirect effect through days to sprouting of bulb (0.0150), whereas the other characters showed a negative indirect effect.

4.15.3.5. Duration of Flowering

The indirect effect contributed by duration of flowering through number of florets per spike (0.2618), plant height (0.2506), spike length (0.2466), rachis length (0.2113), number of leaves per plant (0.2506), length of the floret (0.1016), weight of the florets per spike (0.0348) and number of spikes per m^2 (0.0118) was positive while its influence through other characters was negative.

4.15.3.6. Spike length

Spike length recorded strong positive indirect effect through days taken for sprouting of bulb (0.3492), days to spike emergence (0.3310) and number of leaves per plant (0.2134), whereas the other characters exerted a negative indirect effect.

4.15.3.7. Rachis length

The indirect effect contributed by rachis length through number of florets per spike (0.2822), number of leaves per plant (0.2387), plant height (0.1990), spike length (0.1927), number of spikes per m^2 (0.0720), length of the floret (0.0254) and weight of the florets per spike (0.0186) was positive, while its influence through other characters were negative.

4.15.3.8. Number of florets per spike

The number of florets per spike recorded strong positive indirect effect through days taken for sprouting of bulb (0.1685), days to spike emergence (0.1138), weight of the florets per spike (0.0015), whereas the other characters showed a negative indirect effect.

4.15.3.9. Floret length

The indirect effect contributed by floret length through spike length (0.1448), weight of florets per spike (0.1376), flowering duration (0.0630), plant height (0.0537), number of florets per spike (0.0343) and rachis length (0.0209) was positive, while its influence through other characters were negative.

4.15.3.10. Weight of the florets per spike

The weight of florets per spike recorded strong positive indirect effect through flowering duration (0.0592), rachis length (0.0428), length of the floret (0.3841), number of spikes per m^2 (0.3614), days taken for sprouting of bulb (0.0592) and spike length (0.1397), whereas the other characters exerted a negative indirect effect.

4.15.3.11. Number of spikes per m^2

The indirect effect contributed by number of spikes per m^2 through days taken for sprouting of bulb (0.1065) and spike length (0.0933) was positive, while its influence through other characters was negative.

4.15.4.1. Path analysis for double types

The genotypic correlation co-efficients of double types were apportioned into direct effects and indirect effects by path analysis and results are given in table 55.

4.15.4.1.1. Direct effects

Out of the twelve traits studied, the maximum positive direct effect on Number of spikes per m^2 was through weight of florets per spike (0.6378) followed by number of leaves per plant (0.5782), flowering duration (0.3756), rachis length (0.2779), days taken for sprouting of bulb (0.2580), length of the floret (0.2277) and day to spike emergence (0.0213).

Yield of florets/ plot (4 * 1 m) (-0.0518), spike length (-0.3784), plant height (-0.3297), number of florets per spike (-0.2078) and showed negative direct effect on yield.

4.15.4.1.2. Indirect effects

4.15.4.1.2.1. Days taken for sprouting of bulb

Days taken for sprouting of bulb recorded strong positive indirect effect through days to spike emergence (0.1838) and weight of florets per spike (0.0088). Whereas the other characters showed a negative indirect effect.

4.15.4.1.2.2. Plant height

The indirect effect contributed by plant height through days to spike emergence (0.1785) and weight of the florets per spike (0.0312) was positive while its influence through other characters was negative.

4.15.4.1.2.3. Number of leaves per plant

The number of leaves per plant recorded a strong positive indirect effect through number of florets per spike (0.5627), plant height (0.5242), rachis length (0.4984), number of spikes per m^2 (0.3592), spike length (0.3272) and flowering duration (0.2998) whereas other characters showed negative indirect effect.

4.15.4.1.2.4. Days to spike emergence

Days to spike emergence recorded strong positive indirect effect through days to sprouting of bulb (0.0152), whereas the other characters showed a negative indirect effect.

4.15.4.1.2.5. Duration of Flowering

The indirect effect contributed by duration of flowering through number of florets per spike (0.2629), plant height (0.2608), spike length (0.2467), rachis length (0.2123), number of leaves per plant (0.1941), length of the floret (0.1055), weight of the florets per spike (0.0349) and number of spikes per m^2 (0.0119) were positive while its influence through other characters were negative.

4.15.4.1.2.6. Spike length

Spike length recorded strong positive indirect effect through days taken for sprouting of bulb (0.3429), days to spike emergence (0.3320) and number of leaves per plant (0.2143). Whereas the other characters showed a negative indirect effect.

4.15.4.1.2.7. Rachis length

The indirect effect contributed by duration of rachis length through number of florets per spike (0.2823), number of leaves per plant (0.2388), plant height (0.1994), spike length (0.1932), number of spikes per m^2 (0.0725), length of the floret (0.0249) and weight of the florets per spike (0.0187) was positive, while its influence through other characters were negative.

4.15.4.1.2.8. Number of florets per spike

The number of florets per spike recorded strong positive indirect effect through days taken for sprouting of bulb (0.1676), days to spike emergence (0.1149), weight of the florets per spike (0.0014). Whereas the other characters showed a negative indirect effect.

4.15.4.1.2.9. Floret length

The indirect effect contributes of floret length through spike length (0.1457), weight of florets per spike (0.1377), flowering duration (0.0632), plant height (0.0538), number of florets per spike (0.0344) and rachis length (0.0212) was positive, while its influence through other characters were negative.

4.15.4.1.2.10. Weight of the florets per spike

The weight of florets per spike recorded strong positive indirect effect through flowering duration (0.0693), rachis length (0.0437), length of the floret (0.3851), number of spikes per m^2 (0.3615), days taken for sprouting of bulb (0.0243) and spike length (0.1399). Whereas the other characters showed a negative indirect effect.

4.15.4.1.2.11. Yield of florets/ plot (4 * 1 m)

The indirect effect contributes of yield of florets/ plot through days taken for sprouting of bulb (0.1056) and spike length (0.0943) was positive, while its influence through other characters were negative.

4.16. D^2 analysis for single types

D^2 analysis showed significant difference among the genotypes for all the characters studied (Table 56). The per plant mean of ten genotypes were transformed into standardized uncorrelated mean values and the D^2 values were computed for all the possible pairs of types.

Through clustering, the ten genotypes were grouped into 4 clusters or group constellations. The details regarding each clusters, source of origin of the types included in each cluster are furnished in Table. The clustering pattern was at random with regard to their geographical origin. Among the four clusters, cluster I was the largest consisting of four types followed by cluster IV with 3 types. Cluster II had two types and cluster III had one type.

4.16.1. Inter and Intra – cluster difference

Inter and Intra – cluster values among the twelve characters are presented in Table 57. The intracluster distance ranged from 0.00 (Clusters II, III) to 12.56 (Cluster IV).

The inter cluster D^2 values was maximum (38.24) between cluster II and IV, while it was minimum between cluster I and III (1.85).

4.16.2. D^2 mean values for character

The cluster means for the various characters are presented in Table 58. A wide range was observed among the different clusters for all the cluster means. Wide range of mean values among the clusters was noticed for the characters *viz*., days taken for sprouting of bulb and spike length. Cluster II had the highest values for plant height (45.69), number of leaves per plant (27.83), spike length (128.10), number of florets per spike (36.58), number of spikes per m^2 (1.82), floret length (6.62), weight of florets per spike (0.98) and yield of florets per plot (4 * 1 m) (36.18) while it had the lowest mean values for days taken for sprouing of bulb (12.24) and days to spike emergence (3.19). Cluster III had the highest mean values for days to sprouing of bulb (16.25) and days to spike emergence (43.28). Cluster IV had the highest mean values for rachis length (22.36) and flowering duration (22.06). While cluster I had the lowest values for plant height (30.62), number of leaves per plant (19.00), number of florets per spike (29.00), flowering duration (16.06), spike length (24.46) and floret length (4.06). Similarly cluster III had the lowest mean values for rachis length (15.46), weight of florets per spike (0.72) and yield of florets per plot (4 * 1 m) (21.63).

4.16.3. Relative contribution of each character

The rank given to each character in each combination was utilized for estimating the relative contribution of each character. The number of times each character appeared in the first rank is presented in Table 59.

It was observed that yield of florets per plot (4 * 1 m) contributed the maximum (36.25 %) towards genetic divergence followed by weight of florets per spike (28.84 %), whereas the contribution by plant height (2.38 %), number of leaves per plant (0.26 %), spike length (8.20 %), rachis length (0.26 %), number of florets per spike (0.53 %), days

to spike emergence (0.26 %), flowering duration (0.79 %), number of spikes per m^2 (1.85 %), floret length (10.58 %) was less towards divergence. There was no genetic diversity towards days taken for sprouting of bulb.

4.17. D^2 analysis for double types

D^2 analysis showed significant difference among the genotypes for all the characters studied (Table 60). The per plant mean of ten genotypes were transformed into standardized uncorrelated mean values and the D^2 values were computed for all the possible pairs of types.

Through clustering, the five genotypes were grouped into 3 clusters or group constellations. The details regarding each clusters, source of origin of the types included in each cluster are furnished in Table. The clustering pattern was at random with regard to their geographical origin. Among the four clusters, cluster I was the largest consisting of three types followed by cluster II and III had one type each.

4.17.1. Inter and Intra – cluster difference

Inter and Intra – cluster values among the twelve characters are presented in table 61. The intracluster distance ranged from 0.00 (Clusters I, II) to 11.23 (Cluster III).

The inter cluster D^2 values was maximum (35.23) between cluster II and III, while it was minimum between cluster I and III (1.76).

4.17.2. D^2 mean values for character

The cluster means for the various characters are presented in Table 62. A wide range was observed among the different clusters for all the cluster means. Wide range of mean values among the clusters was noticed for the characters *viz*., days taken for sprouting of bulb and spike length. Cluster II had the highest values for plant height (43.69), number of leaves per plant (24.73), spike length (126.15), number of florets per spike (35.48), number of spikes per m^2 (1.81), floret length (6.63), weight of florets per spike (0.86) and yield of florets per plot (4 * 1 m) (35.28), while it had the lowest mean values for days taken for sprouing of bulb (10.34) and days to spike emergence (2.91). Cluster III had the highest mean values for days to sprouing of bulb (15.32) and days to spike emergence (41.28) respectively. Cluster I had the highest mean values for rachis

length (20.36) and flowering duration (22.08). The lowest cluster mean values for plant height (32.63), number of leaves per plant (15.00), number of florets per spike (27.00), spike length (22.46) and floret length (4.01) were registered by cluster I. Similarly, Cluster III had the lowest mean values for flowering duration (12.98), rachis length (13.461), weight of florets per spike (0.72), number of spikes per m^2 (1.08) and yield of florets per plot (4 * 1 m) (19.62).

4.17.3. Relative contribution of each character

The rank given to each character in each combination was utilized for estimating the relative contribution of each character. The number of times each character appeared in the first rank is presented in Table 63.

It was observed that yield of florets per plot (4 * 1 m) contributed the maximum (35.67 %) towards genetic divergence followed by weight of florets per spike (24.54 %), whereas the contribution by plant height (2.27 %), number of leaves per plant (0.24 %), spike length (2.22 %), rachis length (0.25 %), number of florets per spike (0.51 %), days to spike emergence (0.23 %), flowering duration (0.72 %), number of spikes per m^2 (1.85 %), floret length (9.57 %) was less towards divergence. There was no genetic diversity towards days taken for sprouting of bulb.

Table 1. Performance of tuberose genotypes (single) for growth parameters (2011- 2013)

S.NO	Genotypes	Days taken for sprouting of bulb (days)	Plant height (cm)	No. of leaves per plant	Days to spike emergence	Flowering duration (days)
1.	Calcutta Single	14.38	78.89	243.00	94.00	8.26
2.	Hyderabad Single	16.48	80.96	220.00	90.00	6.37
3.	Kahikuchi Single	13.67	77.30	252.00	86.00	10.00
4.	Mexican Single	15.78	91.77	238.00	88.00	7.00
5.	Navsari Local	14.59	92.85	242.00	92.00	8.98
6.	Phule Rajani	13.79	72.50	251.00	85.00	8.90
7.	Prajwal	12.12	113.05	260.00	78.00	17.00
8.	Pune Single	15.97	110.07	232.00	82.00	9.28
9.	Shringar	14.26	91.75	245.00	83.00	15.00
10.	Variegated Single	13.23	117.50	253.00	81.00	7.12
	SE(D)	0.42	2.57	6.74	1.45	0.28
	CD (0.5)	0.88	5.39	14.15	2.93	0.58

Table 2. Performance of tuberose genotypes (single) for spike and yield parameters (2011- 2013)

S.No.	Genotypes	Spike length (cm)	Rachis length (cm)	Number of florets/ spike (Nos.)	Length of the floret (cm)	Weight of florets per spike (g)	Number of spikes/m^2 (Nos.)	Yield of florets/ plot (4* 1 m) (kg)
1.	Calcutta Single	63.89	16.75	25.00	6.30	29.25	31.00	2.53
2.	Hyderabad Single	65.96	15.30	43.00	6.20	32.76	34.50	2.72
3.	Kahikuchi Single	62.30	18.38	38.00	6.30	44.46	33.00	4.01
4.	Mexican Single	76.77	21.27	17.00	6.20	28.32	32.20	2.51
5.	Navsari Local	77.85	27.30	45.00	6.30	33.93	25.75	2.79
6.	Phule Rajani	52.50	23.48	40.00	6.30	47.20	35.00	4.06
7.	Prajwal	98.05	28.52	47.00	6.40	74.80	47.00	4.40
8.	Pune Single	95.07	35.75	37.00	6.10	43.66	39.00	3.90
9.	Shringar	76.77	22.32	42.00	6.30	51.48	40.00	4.26
10.	Variegated Single	102.50	20.86	28.00	6.20	39.78	33.65	3.78
	SE(D)	2.15	3.62	1.12	0.08	1.11	8.11	0.09
	CD (0.5)	4.51	7.24	2.36	0.17	2.34	16.24	0.19

Table 3. Performance of tuberose genotypes (double) for growth parameters (2011- 2013)

S.NO	Genotypes	Days taken for sprouting of bulb (days)	Plant height (cm)	No. of leaves per plant (Nos.)	Days to spike emergence	Flowering duration (days)
1.	Calcutta Double	13.25	73.92	246.00	88.00	10.48
2.	Hyderabad Double	16.15	68.87	235.00	89.00	9.12
3.	Pearl Double	14.62	69.70	238.00	86.00	10.39
4.	Suvasini	12.32	86.25	270.00	84.00	12.40
5.	Vaibhav	12.67	81.38	250.00	85.00	11.43
SE(D)		0.38	1.96	6.45	0.62	0.28
CD (0.5)		0.87	4.53	14.88	1.26	0.64

Table 4. Performance of tuberose genotypes (double) for spike and yield parameters (2011- 2013)

S.No.	Genotypes	Spike length (cm)	Rachis length (cm)	Number of florets/ spike (Nos.)	Length of the floret (cm)	Weight of florets per spike (g)	Number of spikes/m^2 (Nos.)	Yield of florets/ plot (4 * 1 m) (kg)
1.	Calcutta Double	58.92	38.75	35.00	7.10	112.36	32.00	2.57
2.	Hyderabad Double	53.87	33.95	34.00	6.70	108.56	21.01	2.48
3.	Pearl Double	54.70	42.67	30.00	7.40	109.32	31.50	2.42
4.	Suvasini	71.25	44.00	54.00	7.50	146.88	34.10	3.42
5.	Vaibhav	66.38	54.00	44.00	7.20	119.24	33.75	3.26
SE(D)		1.57	5.08	6.02	0.08	6.07	0.25	0.07
CD (0.5)		3.62	11.12	12.34	0.19	14.08	1.52	0.16

Table 5. Performance of tuberose genotypes (single) for quality parameters (2011- 2013)

S.No.	Genotypes	Concrete recovery (%)	Vase life (days)
1.	Calcutta Single	0.12	10.45
2.	Hyderabad Single	0.11	10.76
3.	Kahikuchi Single	0.12	10.87
4.	Mexican Single	0.13	8.91
5.	Navsari Local	0.12	10.97
6.	Phule Rajani	0.14	12.07
7.	Prajwal	0.16	12.97
8.	Pune Single	0.11	9.12
9.	Shringar	0.15	12.78
10.	Variegated Single	0.12	12.80
	SE(D)	0.02	2.76
	CD (0.5)	0.08	5.31

Table 6. Performance of tuberose genotypes (double) for quality parameters (2011-2013)

S.No.	Genotypes	Concrete recovery (%)	Vase life (days)
1.	Calcutta Double	0.06	9.10
2.	Hyderabad Double	0.06	9.00
3.	Pearl Double	0.06	8.62
4.	Suvasini	0.09	12.40
5.	Vaibhav	0.07	12.25
	SE(D)	0.03	2.35
	CD (0.5)	0.07	5.12

Table 7. Chemical composition of tuberose (Prajwal) concrete obtained through solvent extraction (GC-MS)

S.No	% Relative area	Compounds available
1.	0.08	Octacosone
2.	0.12	5- Isopropyl- 4- (trifluoromethyl)- 1H- Pyrimidin-2-one
3.	0.11	1-cyclopropyl-3,4-dimethyloxy-eugenol
4.	0.10	Hentriacontane
5.	0.10	2,3-Dihydroxyhex-2-enylbenzoate
6.	0.10	2-octenol
7.	0.19	Eicosanoic acid
8.	0.84	Tetradecanoic acid
9.	0.11	Octadecyl fluoride
10.	0.27	1-(Benzyloxy)-2-fluoro-2-phenyl-3(p- toluenesulsufonyloxy propane)
11.	0.20	10-octadecenoic acid, methyl ester
12.	0.53	Tetradecanoic acid
13.	0.31	Docosane
14.	0.21	2-octen-1-01
15.	1.10	7-Thiocyanato-6-demethoxythebaine
16.	3.71	Octacosane
17.	0.25	Hexanedioic acid
18.	0.11	Ether, hexyl pentyl
19.	4.36	Docosane
20.	0.18	1,11-Bis(methoxycarbonylethenyl)-10,2-dihydroxycycloeicosane

S.No	% Relative area	Compounds available
21.	6.98	3,-(4-pyridyl carbonylamino) quinazolin-2,4-dithione
22.	57.22	9-(4'-Aminobenzo-18-crown-6) methyl anthracene
23.	0.08	Hexacosane
24.	0.10	Octahydronaphthalene- (1 R)- carbonitrile
25.	9.79	Epoxy-9a-H-labda-8a-hydroxy-13(16), 14-die n-3-one
26.	0.21	Tritetracontane
27.	0.10	1,9b-Di(phenyl)-4,5,6,7,8,9,9b,10,11,12,13- undecahydro-2,2a-diazacycoocta(a)cyclohexa (e) indecene-3,3-dicarbonitrile
28.	10.56	3a,4,5,6,7,7a-Hexahydro-2-ethoxybenzimidazole
29.	1.65	2-Tridecen-1-ol
30.	0.34	4-methoxycarbonyl-1-methyl-2,3,5-endo-triphenylbicyclo (2.2.1) hept-2-en-7-one

Table 8. Chemical composition of tuberose (Suvasini) concrete obtained through solvent extraction (GC-MS)

S.No	% Relative area	Compounds available
1.	0.08	Dotriacontane
2.	0.12	5- Isopropyl-4- (trifluoromethyl)-1 H- pyrimidine-2-one
3.	0.11	Benzene-1,2-dimethoxy-4-(1-propenyl)
4.	0.10	Hexadecane-2,6,10,14-tetramethyl
5.	0.10	5-Benzoxy-2-methylhexanenitrile
6.	0.10	Dihexylsulfide
7.	0.19	Methyl iso-palmitate
8.	0.84	Tetradecanoic acid
9.	0.11	Dotriacontane
10.	0.27	1,2-Benzenedicarboxylic acid
11.	0.20	Methyl 11- octadecenoate
12.	0.53	Decanoic acid
13.	0.31	Pentatriacontane
14.	0.21	2-methyldodecan-1-ol
15.	1.10	2-d-cyclohexanone
16.	3.71	Dotriacontane
17.	0.25	Hexanedioic acid
18.	0.11	Ether, hexyl pentyl
19.	4.36	Pentatriacontane
20.	0.18	1,2- Benzenedicarboxylic acid
21.	6.98	Pyrrolidine,1-(1-cyclopenten-1-yl)

S.No	% Relative area	Compounds available
22.	57.22	2-propoxyl-4-phenylthiophene
23.	0.08	Octadecane
24.	0.10	1-methyleneprop-2-enyl thiocyanate
25.	9.79	Ethyl 3-(2'- furanyl) propionate
26.	0.21	Dotriacontane
27.	0.10	Pyrazinecarboxamide
28.	10.56	Thiophene,2-butyl
29.	1.65	Pentadecanal
30.	0.34	N-butyl-2-deuterio-2-cyclopentenylamine

Table 9. Floral biology of tuberose genotypes (single)

S.No.	Genotypes (Single)											
	Calcutta Single	Hyderabad Single	Kahikuchi Single	Mexican Single	Navsari Local	Phule Rajani	Prajwal	Pune Single	Shringar	Variegated Single	SE(D)	CD (0.5)
Days to first floret opening (days)	101.23	101.05	99.01	98.02	101.03	98.21	98.01	99.04	99.01	101.05	2.31	5.64
Floret diameter (cm)	4.65	4.72	4.81	4.76	4.63	4.75	4.89	4.73	4.71	4.64	0.26	0.58
Bud length (cm)	6.32	6.25	6.46	6.31	6.31	6.37	6.45	6.12	6.36	6.35	0.23	0.48
Hundred flower weight (g)	115.18	117.78	117.42	118.31	117.32	118.21	170.31	118.21	117.42	117.32	2.62	5.31
Bud colour	green	green	green colour with dark pink tinch at the tip of the floret	green	green	green colour with slight pink tinch at the tip of the floret	green colour with dark pink tinch at the tip of the floret	green	green	green		
Floret colour	White	White	White	White	White	White	White	slight pink tinch at the tip of the floret	White	White	White	
Row of petals	1	1	1	1	1	1	1	1	1	1	1	
Number of petals per floret (nos.)	6	6	6	6	6	6	6	6	6	6	6	

S.No.	Genotypes (Single)											
	Calcutta Single	**Hyderabad Single**	**Kahikuchi Single**	**Mexican Single**	**Navsari Local**	**Phule Rajani**	**Prajwal**	**Pune Single**	**Shringar**	**Variegated Single**	**SE(D)**	**CD (0.5)**
Number of pistils (nos.)	1	1	1	1	1	1	1	1	1	1	1	
Length of the stamen (cm)	1.12	1.15	1.21	1.22	1.21	1.11	1.12	1.16	1.21	1.12	0.09	0.19
Length of the pistil (cm)	3.83	3.43	3.45	3.62	3.43	2.62	3.59	3.53	3.67	3.13	0.18	0.37
Filament length (cm)	3.12	3.11	3.09	3.12	3.15	2.11	3.12	3.13	3.13	2.98	1.11	2.34
Anthesis time	3.45 to 4.50 p.m	3.45 to 4.50 p.m	3.45 to 4.50 p.m	3.45 to 4.50 p.m	3.45 to 4.50 p.m	3.45 to 4.50 p.m	3.45 to 4.50 p.m	3.45 to 4.50 p.m	3.45 to 4.50 p.m	3.45 to 4.50 p.m	3.45 to 4.50 p.m	
Stigma receptivity	three days after anthesis	three days after anthesis	three days after anthesis	three days after anthesis	three days after anthesis	three days after anthesis	three days after anthesis	three days after anthesis	three days after anthesis	three days after anthesis	three days after anthesis	
Anther dehiscence	5.45 pm to 7.30 pm	5.45 pm to 7.30 pm	5.45 pm to 7.30 pm	5.45 pm to 7.30 pm	5.45 pm to 7.30 pm	5.45 pm to 7.30 pm	5.45 pm to 7.30 pm	5.45 pm to 7.30 pm	5.45 pm to 7.30 pm	5.45 pm to 7.30 pm	5.45 pm to 7.30 pm	

Table 10. Floral biology of tuberose genotypes (double)

S.No.	Genotypes (Double)						
	Calcutta Double	**Hyderabad Double**	**Pearl Double**	**Suvasini**	**Vaibhav**	**SE(D)**	**CD (0.5)**
Days to first floret opening (days)	107.76	108.21	106.32	104.56	105.63	1.34	3.21
Floret diameter (cm)	5.34	5.08	5.23	5.78	5.67	0.24	0.49
Bud length (cm)	7.06	7.32	7.29	7.56	7.41	0.16	0.37
Hundred flower weight (g)	267.73	265.42	269.87	272.20	271.98	1.75	4.32
Bud colour	green	green	Green	green colour with dark pink tinch at the tip of the floret	green colour with dark green tinch at the tip of the floret		
Floret colour	White	White	White	white with slight pink tich at the tip of the floret	White	White	
Row of petals	3-4	3-4	3-4	3-4	3-4	3-4	
Number of petals per floret (nos.)	13 - 14	13 - 14	13 - 14	13 - 14	13 - 14	13 - 14	
Number of pistils (nos.)	1	1	1	1	1	1	

S.No.	Genotypes (Double)						
	Calcutta Double	**Hyderabad Double**	**Pearl Double**	**Suvasini**	**Vaibhav**	**SE(D)**	**CD (0.5)**
Number of anthers (nos.)	-	-	-	-	-	-	
Length of the stamen (cm)	-	-	-	-	-	-	
Length of the pistil (cm)	-	-	-	-	-	-	
Filament length (cm)	-	-	-	-	-	-	
Anthesis time	3.45 to 5.30 p.m	33.45 to 5.30 p.m	3.45 to 5.30 p.m	3.45 to 5.30 p.m	3.45 to 5.30 p.m	3.45 to 5.30 p.m	
Stigma receptivity	-	-	-	-	-	-	-
Anther dehiscence	-	-	-	-	-	-	-

Table 11. Size, equivalent diameter, radius and perimeter of the pollen grain in tuberose genotypes (single)

S.No	Genotypes	Size (μm^2)	Equivalent diameter (microns)	Radius (microns)	Perimeter (microns)
1.	Calcutta Single	15261.53	140.12	69.65	524.17
2.	Hyderabad Single	15961.53	141.29	69.76	546.07
3.	Kahikuchi Single	15142.46	138.71	68.40	528.18
4.	Mexican Single	13193.75	136.13	68.71	490.30
5.	Navsari Local	17987.98	161.94	80.06	559.50
6.	Phule Rajani	11501.90	125.16	59.84	459.04
7.	Prajwal	17557.53	152.55	71.64	565.10
8.	Pune Single	13319.98	134.20	66.21	502.16
9.	Shringar	23464.24	185.81	90.10	645.10
10.	Variegated Single	17567.62	153.55	72.66	566.11
SE(D)		1.41	2.97	1.48	69.65
CD (0.5)		2.95	6.20	3.08	69.76

Table 12. Pollen output, pollen viability and pollen germination in different genotypes of tuberose

S.No	Genotypes	Pollen output	Pollen viability (%)	Pollen germination (%)
1.	Calcutta Single	6875	88.08	72.31
2.	Hyderabad Single	5000	87.23	76.99
3.	Kahikuchi Single	5625	79.93	63.08
4.	Mexican Single	8125	89.11	71.27
5.	Navsari Local	5375	88.38	78.52
6.	Phule Rajani	9375	90.52	90.96
7.	Prajwal	6250	39.83	22.12
8.	Pune Single	7500	75.23	74.42
9.	Shringar	8750	89.21	86.74
10.	Variegated Single	10000	96.73	99.21
SE(D)		1.41	0.16	1.65
CD (0.5)		2.95	0.33	3.45

Table 13. Standardization of *in vitro* pollen germination media

Sucrose (%)	Boric acid (ppm)	Calcium Nitrate (ppm)	Magnesium Sulphate (ppm)	Potassium Nitrate (ppm)	Pollen germination (%)
10	10	200	200	100	75.02
10	20	200	200	100	74.12
10	30	200	200	100	74.20
10	50	200	200	100	71.09
10	100	200	200	100	70.23
15	10	200	200	100	60.12
15	20	200	200	100	61.75
15	30	200	200	100	62.21
15	50	200	200	100	63.54
15*	100	200	200	100	65.98
20	10	200	200	100	92.12
20	20	200	200	100	95.34
20	30	200	200	100	96.42
20	50	200	200	100	97.12
20	100	200	200	100	97.30
30	10	200	200	100	77.40
30	20	200	200	100	75.87
30	30	200	200	100	74.86
30	50	200	200	100	60.32
30	100	200	200	100	58.14

* Original medium

In all the treatments, pollen grain of Variegated Single was used

Table 14. Pollen tube length (microns) in certain tuberose genotypes

S.NO.	Treatments	Hours after dehiscence of anther					
		1	**5**	**10**	**15**	**20**	**25**
1.	Calcutta Single	6.62	10.35	18.94	23.52	29.78	36.94
2.	Hyderabad Single	35.15	70.31	151.84	216.85	389.14	446.03
3.	Kahikuchi Single	12.66	36.88	53.27	97.34	113.88	126.76
4.	Mexican Single	4.98	18.64	25.00	46.27	78.36	72.55
5.	Navsari Local	43.84	160.93	242.61	383.00	473.52	500.78
6.	Phule Rajani	76.82	98.18	210.44	452.23	625.756	726.07
7.	Prajwal	4.73	16.95	26.68	49.81	76.72	78.25
8.	Pune Single	24.34	174.67	238.07	500.18	580.41	611.55
9.	Shringar	65.00	181.50	240.53	410.63	581.55	636.32
10.	Variegated Single	118.85	520.43	839.33	1020.63	1234.95	1292.64
SE(D)		0.08	43.84	160.93	242.61	383.00	473.52
CD (0.5)		0.17	12.66	36.88	53.27	97.34	113.88

Table 15. Standardization of strength of NaOH and softening period for microscopic observation of pistils

Duration (hours)	NaOH (Normality)				
	6	**8**	**10**	**12**	**14**
1	IS	IS	IS	IS	ES
2	IS	IS	ES	ES	ES
3	IS	OS	ES	ES	ES
4	IS	ES	ES	ES	ES

IS – Insufficient softening

OS – Optimum softening

ES – Excess softening

Table 16. Standardization of strength of K_3PO_4 and concentration of aniline blue for *in vivo* pollen germination studies

Aniline blue (%)	K_3PO_4 (Normality)		
	0.1	**0.2**	**0.3**
0.1	**PSPF**	**PSPF**	**OSBF**
0.2	PSPF	OSPF	OSPF
0.3	OSPF	OSPF	OSPF

PSPF – Poor staining, Poor Fluorescence

OSPF – Optimum staining, Poor Fluorescence

OSBF – Optimum staining, Better Fluorescence

Table 17. Abundance of pollen grain germination on stigma upon selfing and crossing

	Genotypes	C.S	H.S	K.S	M.S	N.L	P.R	Pr	P.S	Sr	V.S
1.	C.S	3	3	3	3	3	3	3	3	3	3
2.	H.S	3	3	3	3	3	3	3	3	3	1
3.	K.S	3	3	3	3	3	3	3	3	3	3
4.	M.S	NA	NA	NA	3	NA	NA	NA	NA	NA	NA
5.	N.L	3	3	3	3	3	3	3	3	3	1
6.	P.R	3	1	1	1	3	3	3	3	3	1
7.	Prajwal	3	3	3	3	3	3	3	3	3	3
8.	P.S	3	3	3	3	3	3	3	3	3	3
9.	Sr	3	3	1	3	3	3	3	3	3	1
10.	V.S	1	3	1	1	1	1	3	1	3	3

1=high(above 150)

2= medium (76-150)

3=low (1-75)

NA=Not Attempted

C.S Calcutta Single
H.S Hyderabad Single
K.S Kahikuchi Single
M.S Mexican Single
N.L Navsari Local
P.R Phule Rajani
Prajwal Prajwal
P.S Pune Single
Sr Shringar
V.S Variegated Single

Table 18. Pollen tube length (microns) under *in vivo* conditions in cross combinations at different time intervals

Crosses/ Time	1 (hrs)	5 (hrs)	10 (hrs)	15 (hrs)	20 (hrs)	25 (hrs)
V.S X C.S	76.82	98.19	210.44	452.23	627.76	726.07
V.S X K.S	78.23	99.21	215.76	457.34	628.92	728.96
V.S X M.S	75.24	97.23	208.24	450.12	625.65	724.64
V.S X N.L	75.67	97.43	208.36	450.38	625.78	724.79
V.S X P.S	74.65	97.23	208.67	451.72	624.25	724.81
V.S X P.R	118.85	520.43	839.33	1020.63	1292.64	1234.95
P.R X K.S	54.23	85.14	198.85	402.52	598.76	706.23
P.R X M.S	67.87	89.74	214.90	459.75	643.63	727.64
P.R X H.S	47.26	80.52	204.85	431.82	621.65	706.73
P.R X V.S	78.92	97.85	231.76	465.97	634.52	738.42
Sr X V.S	43.21	75.94	186.98	401.46	597.65	698.83
Sr X K.S	36.98	71.58	180.72	399.35	586.64	695.76
N.L X V.S	43.84	160.93	242.61	383.00	473.52	500.78
SE(D)	34.30	48.91	76.33	211.13	211.13	117.91
CD (0.5)	98.02	136.80	220.44	622.87	622.87	350.35

V.S X C.S	Variegated Single x Calcutta Single
V.S X K.S	Variegated Single x Kahikuchi Single
V.S X M.S	Variegated Single x Mexican Single
V.S X N.L	Variegated Single x Navsari Local
V.S X P.S	Variegated Single x Pune Single
V.S X P.R	Variegated Single x Phule Rajani
P.R X K.S	Phule Rajani x Kahikuchi Single
P.R X M.S	Phule Rajani x Mexican Single
P.R X H.S	Phule Rajani x Hyderabad Single
P.R X V.S	Phule Rajani x Variegated Single
Sr X V.S	Shringar x Kahikuchi Single
Sr X K.S	Shringar x Variegated Single
N.L X V.S	Hyderabad Single x Variegated Single
V.S X C.S	Navsari Local x Variegated Single

Table 19. Number of pollen tubes at stigmatic and stylar regions and entry of ovary in single genotypes upon crossing

S.No	Cross combinations	Stigmatic region		Middle of the style		Entry of ovary	
		Range	Mean	Range	Mean	Range	Mean
1	Variegated Single x Calcutta Single	180- 250	231	116-192	129	46-65	52
2	Variegated Single x Kahikuchi Single	206- 216	213	106-159	110	40-52	45
3	Variegated Single x Mexican Single	198- 258	240	127-175	158	50-55	53
4	Variegated Single x Navsari Local	219- 278	237	127-173	140	41-61	46
5	Variegated Single x Pune Single	229- 265	254	138-170	151	37-64	47
6	Variegated Single x Phule Rajani	180- 250	231	116-192	129	46-65	52
7	Phule Rajani x Kahikuchi Single	212- 247	235	102-157	139	42-55	46
8	Phule Rajani x Mexican Single	229- 265	254	138-170	151	32-55	47
9	Phule Rajani x Hyderabad Single	202- 271	265	123-177	160	56-79	69
10	Phule Rajani x Variegated Single	296-318	310	175-212	198	73-95	80
11	Shringar x Kahikuchi Single	180- 250	231	116-192	129	46-65	52
12	Shringar x Variegated Single	198- 258	240	127-173	140	41-61	46
13	Hyderabad Single x Variegated Single	190- 290	278	135-182	179	55-82	68
14	Navsari Local x Variegated Single	197-247	231	133-172	160	37-64	16
	SE(D)	8.94		8.44		2.07	
	CD (0.5)	28.16		27.52		6.96	

Table 20. Number and percentage of ovules with pollen tube at micropylar end after 14/24 HAP in crosses

S.No	Cross combinations	Number of ovules in the ovary	Number of ovules with pollen tube at micropylar end	Percentage
1	Variegated Single x Calcutta Single	16	12	75
2	Variegated Single x Kahikuchi Single	14	8	57
3	Variegated Single x Mexican Single	16	13	81
4	Variegated Single x Navsari Local	16	8	50
5	Variegated Single x Pune Single	16	12	75
6	Variegated Single x Phule Rajani	17	15	88
7	Phule Rajani x Kahikuchi Single	15	12	80
8	Phule Rajani x Mexican Single	16	15	94
9	Phule Rajani x Hyderabad Single	16	12	75
10	Phule Rajani x Variegated Single	17	16	94
11	Shringar x Kahikuchi Single	15	8	53
12	Shringar x Variegated Single	16	12	75
13	Hyderabad Single x Variegated Single	14	8	57
14	Navsari Local x Variegated Single	14	9	64

Note: In other cross combinations among single genotypes, pollen tube did not reach ovary.

Table 21. Growth parameters, soil population and root knot index for tuberose genotypes (Single) to *Meloidogyne incognita*

Genotypes	Root length (cm)	Root weight (g)	Soil nematode population (per 200 cc of soil)	Root knot index	Level of resistance
Calcutta Single	53.21	62.98	160.32	4	S
Hyderabad Single	52.98	61.53	162.93	4	S
Kahikuchi Single	54.89	63.75	122.61	3	MR
Mexican Single	51.76	60.99	165.84	4	S
Navsari Local	46.72	61.78	288.35	5	HS
Phule Rajani	46.99	61.89	286.25	5	HS
Prajwal	48.23	63.97	291.98	5	HS
Pune Single	47.26	63.11	279.63	5	HS
Shringar	45.32	61.23	283.12	5	HS
Variegated Single	47.63	63.32	280.44	5	HS
SE(D)	1.58	0.75	1.9		
CD (0.5)	3.17	1.61	3.7		

Table 22. Reaction of tuberose genotypes (Single) to *Meloidogyne incognita*

Genotypes	No. of galls/ 10 g of roots	No. of egg masses/ g of root	No. of eggs/ egg mass	No. of RKN females/ g of root
Calcutta Single	56.89	8.72	127.73	41.82
Hyderabad Single	56.89	8.72	127.73	41.82
Kahikuchi Single	30.61	5.21	94.55	25.80
Mexican Single	56.89	8.72	127.73	41.82
Navsari Local	125.56	32.86	218.81	88.21
Phule Rajani	124.56	31.82	201.84	82.20
Prajwal	122.78	30.27	204.61	83.96
Pune Single	124.65	33.61	213.81	86.22
Shringar	127.96	33.86	220.78	88.22
Variegated Single	124.42	31.81	218.81	87.43
SE(D)	2.3	0.24	1.96	0.04
CD (0.5)	4.5	0.51	3.88	0.08

Table 23. Total Phenol content in tuberose genotypes (Single) inoculated with root knot nematode

Genotypes	Total phenol						
	Hours after inoculation						
	0	24	48	72	96	120	Mean
Calcutta Single	7.38	8.46	10.01	10.98	11.54	11.21	9.93
Hyderabad Single	7.36	8.39	10.05	10.99	11.56	11.24	9.95
Kahikuchi Single	12.54	14.68	17.41	18.59	19.84	19.21	17.05
Mexican Single	7.26	8.37	10.25	10.96	11.65	11.42	9.99
Navsari Local	5.41	7.89	9.92	10.59	10.98	10.54	9.22
Phule Rajani	5.42	7.84	9.96	10.53	10.92	10.55	9.20
Prajwal	5.31	7.28	9.65	10.29	10.87	10.48	8.98
Pune Single	5.27	7.98	9.29	10.95	10.89	10.45	9.14
Shringar	5.46	7.82	9.98	10.52	10.97	10.44	9.20
Variegated Single	5.51	7.94	9.99	10.59	10.45	10.41	9.15

Table 24. Ortho-dihydroxy phenol content in tuberose genotypes (Single) inoculated with root knot nematode

Genotypes	Ortho-dihydroxy phenol (mg g^{-1})						
	Hours after inoculation						
	0	24	48	72	96	120	Mean
Calcutta Single	6.48	6.94	7.45	7.95	8.05	7.94	7.47
Hyderabad Single	6.62	6.86	7.64	7.84	8.52	7.87	7.56
Kahikuchi Single	9.52	10.04	12.56	14.21	15.84	15.50	12.95
Mexican Single	6.84	6.94	7.54	7.69	8.50	7.49	7.50
Navsari Local	3.21	3.56	3.98	4.50	5.21	5.14	4.27
Phule Rajani	3.34	3.76	3.88	4.05	5.28	5.18	4.25
Prajwal	3.28	3.59	3.84	4.50	5.16	5.09	4.23
Pune Single	3.22	3.57	3.89	4.45	5.17	5.04	4.21
Shringar	3.29	3.56	3.98	4.50	5.26	5.14	4.29
Variegated Single	3.34	3.56	3.89	4.50	5.28	5.02	4.27

Table 25. Peroxidase activity in tuberose genotypes (Single) inoculated with root knot nematode

Genotypes	Peroxidase (changes in OD min^{-1} g^{-1} of sample)						
	Hours after inoculation						
	0	24	48	72	96	120	Mean
Calcutta Single	1.05	1.28	1.39	1.45	1.56	1.50	1.37
Hyderabad Single	1.06	1.30	1.41	1.44	1.53	1.51	1.38
Kahikuchi Single	2.49	3.01	3.14	3.30	3.65	3.54	3.19
Mexican Single	1.03	1.25	1.35	1.46	1.54	1.52	1.36
Navsari Local	0.65	0.74	0.81	0.86	0.89	0.84	0.79
Phule Rajani	0.61	0.72	0.84	0.87	0.89	0.85	0.80
Prajwal	0.63	0.74	0.82	0.86	0.88	0.85	0.80
Pune Single	0.64	0.73	0.84	0.87	0.89	0.81	0.81
Shringar	0.66	0.72	0.85	0.87	0.89	0.85	0.82
Variegated Single	0.65	0.76	0.83	0.85	0.89	0.83	0.80

Table 26. Polyphenol oxidase activity in tuberose genotypes (Single) inoculated with root knot nematode

Genotypes	Polyphenol oxidase (changes in OD min^{-1} g^{-1} of sample)						
	Hours after inoculation						
	0	24	48	72	96	120	Mean
Calcutta Single	1.08	1.37	1.48	1.64	1.85	1.71	1.52
Hyderabad Single	1.06	1.42	1.51	1.65	1.89	1.78	1.55
Kahikuchi Single	2.57	3.04	3.18	3.27	3.58	3.45	3.18
Mexican Single	1.07	1.38	1.49	1.62	1.87	1.74	1.53
Navsari Local	0.79	0.84	0.89	0.90	0.91	0.90	0.88
Phule Rajani	0.72	0.82	0.87	0.94	0.92	0.91	0.86
Prajwal	0.74	0.83	0.88	0.94	0.92	0.91	0.85
Pune Single	0.72	0.84	0.87	0.91	0.92	0.90	0.86
Shringar	0.79	0.83	0.88	0.93	0.92	0.90	0.88
Variegated Single	0.78	0.85	0.87	0.91	0.93	0.91	0.88

Table 27. Phenylalanine ammonia lyase activity in tuberose genotypes (Single) inoculated with root knot nematode

Genotypes	Phenylalanine ammonia lyase (nmol of transcinnamic acid min^{-1} g^{-1} of fresh tissue)						
	Hours after inoculation						
	0	24	48	72	96	120	Mean
Calcutta Single	9.04	9.38	9.65	9.84	9.98	9.89	9.63
Hyderabad Single	9.00	9.42	9.76	9.82	9.97	9.82	9.64
Kahikuchi Single	14.24	14.92	15.55	16.04	16.10	15.48	15.39
Mexican Single	9.03	9.31	9.64	9.89	9.92	9.84	9.61
Navsari Local	5.42	5.54	5.60	5.64	5.68	5.60	5.58
Phule Rajani	5.40	5.58	5.67	5.72	5.85	5.81	5.67
Prajwal	5.24	5.54	5.67	5.74	5.85	5.79	5.64
Pune Single	5.32	5.47	5.58	5.64	5.78	5.68	5.58
Shringar	5.41	5.52	5.61	5.66	5.72	5.69	5.60
Variegated Single	5.45	5.54	5.63	5.68	5.73	5.65	5.61

Table 28. Acid phosphatase activity in tuberose genotypes (Single) inoculated with root knot nematode

Genotypes	Acid phosphatase (*m*moles p-nitrophenol min^{-1} mg^{-1} protein)						
	Hours after inoculation						
	0	24	48	72	96	120	Mean
Calcutta Single	70.01	103.46	118.96	125.12	131.88	128.06	112.92
Hyderabad Single	69.02	102.54	117.92	126.21	132.86	125.62	112.36
Kahikuchi Single	95.42	103.46	118.96	125.12	131.88	128.06	117.15
Mexican Single	72.02	104.56	117.98	124.16	132.84	126.05	112.94
Navsari Local	43.54	51.94	55.18	63.11	67.13	60.37	56.88
Phule Rajani	42.53	54.42	55.21	64.15	68.17	61.38	57.64
Prajwal	44.56	52.98	56.35	62.17	69.18	62.37	57.94
Pune Single	45.54	53.94	57.18	64.11	69.13	60.37	58.38
Shringar	42.54	50.93	55.97	62.28	68.87	60.53	56.87
Variegated Single	43.45	51.49	55.81	63.11	67.31	60.73	56.98

Double types

Table 29. Growth parameters, soil population and root knot index for tuberose genotypes (double) to *Meloidogyne incognita*

Genotypes	Root length (cm)	Root weight (g)	Soil nematode population (per 200 cc of soil)	Root knot index	Level of resistance
Calcutta Double	53.52	62.86	280.44	5	HS
Hyderabad Double	54.21	63.23	284.12	5	HS
Pearl Double	53.87	62.34	283.52	5	HS
Suvasini	55.21	63.21	280.98	5	HS
Vaibhav	56.89	64. 85	286.42	5	HS
SE(D)	1.65	1.62	2.31		
CD (0.5)	3.35	3.24	4.60		

HS – Highly Susceptible

Table 30. Reaction of tuberose genotypes (double) to *Meloidogyne incognita*

Genotypes	No. of galls/ 10 g of roots	No. of egg masses/ g of root	No. of eggs/ egg mass	No. of RKN females/ g of root
Calcutta Double	124.62	32.81	218.91	87.33
Hyderabad Double	126.76	34.32	222. 78	86.52
Pearl Double	123.55	30.68	214.18	88.31
Suvasini	124.68	31.72	203.68	83.76
Vaibhav	123.56	32.92	202.49	82.10
SE(D)	2.08	1.4	3.87	0.98
CD (0.5)	4.15	2.9	7.74	1.96

Table 31. Total Phenol content in tuberose genotypes (double) inoculated with root knot nematode

Genotypes	Total phenol						
	Hours after inoculation						
	0	24	48	72	96	120	Mean
Calcutta Double	5.31	7.28	9.65	10.29	10.87	10.48	8.98
Hyderabad Double	5.42	7.84	9.96	10.53	10.92	10.55	9.20
Pearl Double	5.27	7.98	9.29	10.95	10.89	10.45	9.14
Suvasini	5.51	7.94	9.99	10.59	10.45	10.41	9.15
Vaibhav	5.46	7.82	9.98	10.52	10.97	10.44	9.22

Table 32. Ortho-dihydroxy phenol content in tuberose genotypes (double) inoculated with root knot nematode

Genotypes	Ortho-dihydroxy phenol						
	Hours after inoculation						
	0	24	48	72	96	120	Mean
Calcutta Double	3.29	3.56	3.98	4.50	5.26	5.14	4.29
Hyderabad Double	3.34	3.56	3.89	4.50	5.28	5.02	4.27
Pearl Double	3.22	3.57	3.89	4.45	5.17	5.04	4.21
Suvasini	3.34	3.76	3.88	4.05	5.28	5.18	4.25
Vaibhav	3.28	3.59	3.84	4.50	5.16	5.09	4.23

Table 33. Peroxidase activity in tuberose genotypes (double) inoculated with root knot nematode

Genotypes	Peroxidase (changes in OD min^{-1} g^{-1} of sample)						
	Hours after inoculation						
	0	24	48	72	96	120	Mean
Calcutta Double	0.64	0.73	0.84	0.87	0.89	0.83	0.81
Hyderabad Double	0.63	0.74	0.82	0.86	0.88	0.85	0.80
Pearl Double	0.61	0.72	0.84	0.87	0.89	0.85	0.80
Suvasini	0.65	0.76	0.83	0.85	0.89	0.83	0.80
Vaibhav	0.66	0.72	0.85	0.87	0.89	0.85	0.82

Table 34. Polyphenol oxidase activity in tuberose genotypes (double) inoculated with root knot nematode

Genotypes	Polyphenol oxidase (changes in OD min^{-1} g^{-1} of sample)						
	Hours after inoculation						
	0	24	48	72	96	120	Mean
Calcutta Double	0.72	0.84	0.87	0.91	0.92	0.90	0.86
Hyderabad Double	0.78	0.85	0.87	0.91	0.93	0.91	0.88
Pearl Double	0.72	0.82	0.87	0.94	0.92	0.91	0.86
Suvasini	0.74	0.83	0.88	0.94	0.92	0.91	0.87
Vaibhav	0.79	0.83	0.88	0.93	0.92	0.90	0.89

Table 35. Phenylalanine ammonia lyase activity in tuberose genotypes (double) inoculated with root knot nematode

Genotypes	Phenylalanine ammonia lyase (nmol of transcinnamic acid min^{-1} g^{-1} of fresh tissue)						
	Hours after inoculation						
	0	24	48	72	96	120	Mean
Calcutta Double	5.24	5.54	5.67	5.74	5.85	5.79	5.64
Hyderabad Double	5.45	5.54	5.63	5.68	5.73	5.65	5.61
Pearl Double	5.32	5.47	5.58	5.64	5.78	5.68	5.58
Suvasini	5.41	5.52	5.61	5.66	5.72	5.69	5.60
Vaibhav	5.40	5.58	5.67	5.72	5.85	5.81	5.67

Table 36. Acid phosphatase activity in tuberose genotypes (double) inoculated with root knot nematode

Genotypes	Acid phosphatase (*m*moles p-nitrophenol min^{-1} mg^{-1} protein)						
	Hours after inoculation						
	0	24	48	72	96	120	Mean
Calcutta Double	42.53	54.42	55.21	64.15	68.17	61.38	57.64
Hyderabad Double	44.56	52.98	56.35	62.17	69.18	62.37	57.94
Pearl Double	45.54	53.94	57.18	64.11	69.13	60.37	58.38
Suvasini	42.54	50.93	55.97	62.28	68.87	60.53	56.88
Vaibhav	43.45	51.49	55.81	63.11	67.31	60.73	56.98

Table 37. Self compatibility studies in hybrids and cultivars of tuberose pollinated second day after anthesis (Single x Single)

S.No	Genotypes	Number of flowers pollinated	Capsule retained at	
			7 days (%)	14 days (%)
1.	Calcutta Single	20	13.23	0
2.	Hyderabad Single	42	12.67	0
3.	Kahikuchi Single	40	14.98	0
4.	Mexican Single	18	5.98	0
5.	Navsari Local	30	19.98	0
6.	Phule Rajani	40	6.94	0
7.	Prajwal	56	7.24	0
8.	Pune Single	45	19.21	0
9.	Shringar	42	21.74	0
10.	Variegated Single	31	23.62	0

Table 38. Self compatibility studies in hybrids and cultivars of tuberose pollinated third day after anthesis (Single x Single)

S.No	Genotypes	No. of flowers pollinated	Capsule retained at maturity	
			7 days (%)	14 days (%)
1.	Calcutta Single	20	23.23	0
2.	Hyderabad Single	42	24.67	0
3.	Kahikuchi Single	40	25.98	0
4.	Mexican Single	18	10.98	0
5.	Navsari Local	30	23.98	0
6.	Phule Rajani	40	26.94	0
7.	Prajwal	56	26.74	0
8.	Pune Single	45	32.24	0
9.	Shringar	42	33.62	0
10.	Variegated Single	31	28.21	0

Table 39. Self compatibility studies in hybrids and cultivars of tuberose pollinated fourth day after anthesis (Single x Single)

S.No	Genotypes	No. of flowers pollinated	Capsule retained at maturity
			7 days (%)
1.	Calcutta Single	20	0
2.	Hyderabad Single	42	0
3.	Kahikuchi Single	40	0
4.	Mexican Single	18	0
5.	Navsari Local	30	0
6.	Phule Rajani	45	0
7.	Prajwal	40	0
8.	Pune Single	56	0
9.	Shringar	42	0
10.	Variegated Single	31	0

Table 40. Successful crosses

S.No	Crosses
1	Calcutta Single x Variegated Single
2	Kahikuchi Single x Variegated Single
3	Mexican Single x Variegated Single
4	Navsari Local x Variegated Single
5	Pune Single x Variegated Single
6	Phule Rajani x Variegated Single
7	Kahikuchi Single x Phule Rajani
8	Mexican Single x Phule Rajani
9	Hyderabad Single x Phule Rajani
10	Variegated Single x Phule Rajani
11	Kahikuchi Single x Shringar
12	Variegated Single x Shringar
13	Variegated Single x Hyderabad Single
14	Variegated Single x Navsari Local

Table 41. Cross compatibility studies in hybrids and cultivars of tuberose pollinated on fourth day after anthesis (Single x Single)

S.No	Crosses	Percent success
1	Calcutta Single x Variegated Single	24.00
2	Kahikuchi Single x Variegated Single	25.00
3	Mexican Single x Variegated Single	21.42
4	Navsari Local x Variegated Single	25.02
5	Pune Single x Variegated Single	28.07
6	Phule Rajani x Variegated Single	50.40
7	Kahikuchi Single x Phule Rajani	10.00
8	Mexican Single x Phule Rajani	70.00
9	Hyderabad Single x Phule Rajani	6.66
10	Variegated Single x Phule Rajani	78.40
11	Kahikuchi Single x Shringar	29.10
12	Variegated Single x Shringar	19.71
13	Variegated Single x Hyderabad Single	13.84
14	Variegated Single x Navsari Local	50.98

Table 42. Standardisation of soaking duration and concentration of tetrazolium solution for viability test

Incubation	Soaking duration (h)	Concentrations					
		0.25%			0.5%		
		Completely stained	Partially stained	Over stained	Completely stained	Partially stained	Over stained
40°C	2	62	38	-	85	14	1
	2½	86	14	-	94	1	5
Overnight	12	90	1	9	85	-	15

(Statistically not analysed)

Table 43. Influence of pre sowing treatment with growth regulators on seed and seedling characteristics in Tuberose

Growth regulators (G)	Germination (%)			Root length (cm)			Shoot length (cm)			Vigour index			Dry matter production ($g/seedling^5$)		
	Duration in hours (D)														
	8	16	Mean	8	16	Mean	8	16	Mean	8	16	Mean	8	16	Mean
GA_3 250 ppm	12.50	11.32	11.91	12.36	13.24	12.80	13.75	14.32	14.04	892.70	978.38	935.54	0.23	0.24	0.24
500 ppm	11.12	10.09	10.61	10.54	10.72	10.63	9.77	10.12	9.95	591.43	606.24	598.88	0.18	0.19	0.19
KNO_3 0.25 %	12.45	11.23	11.84	13.00	11.60	12.30	14.23	12.89	13.56	965.30	740.33	852.82	0.22	0.20	0.21
0.5 %	12.12	10.13	11.13	12.63	10.23	11.43	13.65	11.29	12.47	843.59	725.34	784.47	0.21	0.18	0.20
Thiourea 0.5%	09.21	09.05	9.13	10.53	9.82	10.18	9.98	9.29	9.64	496.55	440.49	468.52	0.17	0.15	0.16
1%	10.45	09.87	10.16	10.80	9.12	9.96	9.14	8.98	9.06	467.59	413.95	440.77	0.15	0.13	0.14
IBA 100 ppm	09.15	8.23	8.69	9.66	8.98	9.32	9.12	8.56	8.84	434.76	412.24	423.50	0.12	0.11	0.12
200 ppm	09.98	9.21	9.60	9.45	9.12	9.29	8.78	8.50	8.64	418.93	415.32	417.12	0.14	0.13	0.14
Water soaking	7.96	7.96	7.96	7.46	7.46	7.46	7.21	7.21	7.21	263.47	263.47	263.473	0.04	0.04	0.04
Mean	11.23	10.76	10.96	9.12	8.97	9.05	8.43	8.21	8.32	795.87	765.54	780.71	0.03	0.03	0.03
	G	D	GD	G	D	GD	G	D	GD	G	D	GD	G	D	GD
CD (P=0.05)	2.17	2.00	3.08	0.134	NS	NS	NS	NS	NS	0.261	NS	NS	0.217	NS	NS

Table 44. Hybridization data of different crosses in tuberose genotypes (Single x Single)

S.No	Crosses	Mean no. of days taken for fruit set	Mean number of days taken to fruit maturity	Mean number of seeds/capsule
1	C.S X V.S	7.50	80.21	17.75
2	K.S X V.S	7.75	78.97	20.00
3	M.S X V.S	7.45	80.75	21.25
4	N.L X V.S	8.25	76.75	22.75
5	P.S X V.S	7.96	81.86	32.96
6	P.R X V.S	7.85	81.00	38.00
7	K.S X P.R	7.50	80.00	19.00
8	M.S X P.R	8.45	85.08	16.23
9	H.S X P.R	8.07	82.13	28.97
10	V.S X P.R	7.75	81.00	41.00
11	V.S X Sr	8.50	81.50	24.00
12	K.S X Sr	8.76	83.74	25.00
13	V.S X H.S	8.95	85.98	26.00
14	V.S X N.L	8.76	85.43	32.00

C.S X V.S	Calcutta Single x Variegated Single	M.S X P.R	Mexican Single x Phule Rajani
K.S X V.S	Kahikuchi Single x Variegated Single	H.S X P.R	Hyderabad Single x Phule Rajani
M.S X V.S	Mexican Single x Variegated Single	K.S X Sr	Kahikuchi Single x Shringar
N.L X V.S	Navsari Local x Variegated Single	V.S X P.R	Variegated Single x Phule Rajani
P.S X V.S	Pune Single x Variegated Single	V.S X Sr	variegated Single x Shringar
P.R X V.S	Phule Rajani x Variegated Single	V.S X H.S	Variegated Single x Hyderabad Single
K.S X P.R	Kahikuchi Single x Phule Rajani	V.S X N.L	Variegated Single x Navsari Local

Table 45. Seed germination data of different crosses in tuberose genotypes (Single x Single)

S.No	Crosses	Mean no. of seeds sown	No. of seeds germinated	Germination percentage	Mean no. of days taken for germination
1	C.S X V.S	40	05	12.50	35.68
2	K.S X V.S	48	01	12.43	38.25
3	M.S X V.S	47	06	12.45	34.00
4	N.L X V.S	45	06	12.32	32.21
5	P.S X V.S	56	07	12.23	30.21
6	P.R X V.S	40	05	12.43	28.96
7	K.S X P.R	20	2	12.50	31.67
8	M.S X P.R	10	1	12.52	36.82
9	H.S X P.R	25	3	12.45	35.42
10	V.S X P.R	836	105	12.56	28.96
11	V.S X Sr	46	6	12.50	32.98
12	K.S X Sr	45	6	12.51	34.72
13	V.S X H.S	40	5	12.54	35.98
14	V.S X N.L	40	5	12.43	32.87

C.S X V.S	Calcutta Single x Variegated Single	M.S X P.R	Mexican Single x Phule Rajani
K.S X V.S	Kahikuchi Single x Variegated Single	H.S X P.R	Hyderabad Single x Phule Rajani
M.S X V.S	Mexican Single x Variegated Single	K.S X Sr	Kahikuchi Single x Shringar
N.L X V.S	Navsari Local x Variegated Single	V.S X P.R	Variegated Single x Phule Rajani
P.S X V.S	Pune Single x Variegated Single	V.S X Sr	variegated Single x Shringar
P.R X V.S	Phule Rajani x Variegated Single	V.S X H.S	Variegated Single x Hyderabad Single
K.S X P.R	Kahikuchi Single x Phule Rajani	V.S X N.L	Variegated Single x Navsari Local

Table 46. Micrometric observations during fruit development in selfed material

Stages (Days after anthesis)	Seed		Embryosac		Nucellus		Hypostase		Funiculus	Integument	Outer epidermis	Inner epidermis	Mesocarp
	L (µm)	W (µm)	L (µm)	W (µm)	L (µm)	W (µm)	L (µm)	W (µm)	T (µm)	T (µm)	T (µm)	T (µm)	T (µm)
D0	243	100	65	40	150	120	40	30	60	30	4	3	160
D4	281	161	70	40	200	160	50	45	80	50	5	4	260
D10	182	100	40	35	-	-	-	-	-	-	4.5	3.5	170

L= Length W=Width T=Thickness d=days

Table 47. Micrometric observations during fruit development in crossed material

Stages (Days after anthesis)	Seed		Embryosac		Embryo		Nucellus		Hypostase		Funiculus	Integument	Outer epidermis	Inner epidermis	Mesocarp	Tegma	Testa
	L (µm)	W (µm)	L (µm)	W (µm)			L (µm)	W (µm)	L (µm)	W (µm)	T (µm)	T (µm)	T (µm)	T (µm)	T (µm)	T (µm)	T (µm)
D0	290	135	60	45	ND	ND	140	100	45	35	60	20	4	2	150	-	-
D4	300	220	65	60	ND	ND	190	165	65	50	75	30	6	5	250	-	-
D10	460	280	70	50	ND	ND	340	280	125	80	120	40	12	8	280	-	-
D20	580	440	175	100	ND	ND	400	350	150	110	150	40	14	10	300	-	-
D30	700	600	280	150	200	130	-	-	-	-	-	-	-	-	-	30	70
D40	750	550	500	190	480	170	-	-	-	-	-	-	-	-	-	60	20

L= Length W=Width T= Thickness d=days ND-= Not Differentiated

Table 48. Estimates of variability and genetic parameters for tuberose flower yield and its components (cv. Single)

S.NO.	Characters	GCV	PCV	HERT	GA (%) OF MEAN
1	Days taken for sprouting of bulb	9.08	9.74	88.83	17.43
2	Plant height	17.16	17.50	**96.24**	34.69
3	Number of leaves per plant	4.34	5.50	62.16	17.05
4	Days to spike emergence	5.60	6.60	71.99	19.78
5	Flowering duration	**35.62**	**35.79**	**99.07**	**73.04**
6	Spike length	21.64	21.91	**97.58**	44.04
7	Rachis length	26.64	26.85	**98.47**	**54.46**
8	Number of florets/ spike	**31.80**	**32.01**	**98.72**	**65.08**
9	Length of the floret	1.45	3.11	78.23	15.39
10	Weight of florets per spike	**32.27**	**32.43**	**99.02**	**66.15**
11	Number of spikes/ m2	16.34	16.68	**95.00**	42.96
12	Yield of florets/ plot	21.71	21.96	**98.00**	44.22

Table 49. Estimates of variability and genetic parameters for tuberose flower yield and its components (cv. Double)

S.NO.	Characters	GCV	PCV	HERT	GA (%) OF MEAN
1	Days taken for sprouting of bulb	11.27	11.76	91.91	22.26
2	Plant height	9.78	10.28	90.52	19.17
3	Number of leaves per plant	5.25	6.14	73.06	9.25
4	Days to spike emergence	1.49	3.58	77.38	1.28
5	Flowering duration	11.28	11.71	92.83	32.39
6	Spike length	12.26	12.66	93.79	44.46
7	Rachis length	17.35	17.62	96.91	45.18
8	Number of florets/ spike	24.38	24.59	98.35	69.81
9	Length of the floret	3.93	5.04	60.68	6.30
10	Weight of florets per spike	13.29	13.66	94.67	46.64
11	Number of spikes/ m^2	17.64	17.92	96.93	65.78
12	Yield of florets/ plot	14.98	15.31	95.78	40.20

Table 50. Genotypic correlation coefficient among different characters in tuberose (single)

S.NO	1	2	3	4	5	6	7	8	9	10	11	12
1.	1.000	-0.292	-1.180	0.559**	-0.341	-0.258	-0.076	0.108**	-1.014	-0.725	-0.401	-0.549
2.		1.000	0.197	-0.755	-0.159	0.997**	0.595**	-0.152	-0.359	0.402**	0.457**	0.978
3.			1.000	-0.738	0.368**	0.229**	0.149**	-0.324**	0.705**	0.652**	0.326**	0.577**
4.				1.000	-0.307	-0.707	-0.558	0.068**	-0.526	-0.871	-0.910	-0.737
5.					1.000	-0.141**	0.050*	0.175*	0.282*	0.430*	0.298*	0.602*
6.						1.000	0.565**	-0.150	-0.332	0.373**	0.435**	0.423**
7.							1.000	0.097*	-0.263	0.440*	0.385*	0.474*
8.								1.000	0.094**	0.256**	0.100**	0.178**
9.									1.000	0.430**	0.236**	0.225**
10.										1.000	0.856*	0.864*
11.											1.000	0.752*
12.												1.000

1. Days taken for sprouting of bulb
2. Plant height
3. No. of leaves per plant
4. Days to spike emergence
5. Flowering duration
6. Spike length
7. Rachis length
8. Number of florets/ spike
9. Length of the floret
10. Weight of florets per spike
11. Number of spikes/m^2
12. Yield of florets/ plot (4 * 1 m)

Note:

* Significant at 5% level

** Significant at 1% level

Table 51. Genotypic correlation coefficient among different characters in tuberose (double)

S.NO	1	2	3	4	5	6	7	8	9	10	11	12
1.	1.000	-0.939	-0.954	0.671**	-0.978	-0.927	-0.803	-0.736	-0.761	-0.874	-0.952	-0.803
2.		1.000	0.958**	-1.574	0.944**	1.001**	0.652**	0.971**	0.623**	0.904**	0.695**	0.975**
3.			1.000	-1.759	0.941**	0.965**	0.438**	0.981**	0.730**	0.994**	0.665**	0.987**
4.				1.000	-1.706	-1.518	-1.384	-1.276	-2.136	-1.406	-1.418	-1.430
5.					1.000	0.944*	0.695*	0.859*	0.851*	0.875*	0.862*	0.899*
6.						1.000	0.653**	0.967**	0.604**	0.905**	0.696**	0.974**
7.							1.000	0.481*	0.566*	0.347*	0.742*	0.517*
8.								1.000	0.489**	0.950**	0.500**	0.996**
9.									1.000	0.630**	0.918**	0.561**
10.										1.000	0.534*	0.965*
11.											1.000	0.565*
12.												1.000

1. Days taken for sprouting of bulb
2. Plant height
3. No. of leaves per plant
4. Days to spike emergence
5. Flowering duration
6. Spike length
7. Rachis length
8. Number of florets/ spike
9. Length of the floret
10. Weight of florets per spike
11. Number of spikes/m^2
12. Yield of florets/ plot (4 * 1 m)

Note:

* Significant at 5% level

** Significant at 1% level

Table 52. Phenotypic correlation coefficient among different characters in tuberose (single)

S.NO	1	2	3	4	5	6	7	8	9	10	11	12
1.	1.000	-0.197	-0.646	0.465**	-0.283	-0.182	-0.027	0.075	-0.043	-0.638	-0.294	-0.287
2.		1.000	0.271**	-0.527	-0.137	0.996**	0.587**	-0.128	0.048**	0.393**	0.435**	0.337**
3.			1.000	-0.169	0.345**	0.173**	0.097**	-0.189	0.072**	0.571**	0.263**	0.173**
4.				1.000	-0.211	-0.511	-0.407	0.014**	0.375**	-0.685	-0.650	-0.719
5.					1.000	-0.125	0.039*	0.167*	0.232*	0.424*	0.287*	-0.141
6.						1.000	0.558**	-0.131	0.016**	0.364**	0.418**	0.297**
7.							1.000	0.086*	0.010*	0.434*	0.371*	0.424*
8.								1.000	0.161**	0.248**	0.080**	0.176**
9.									1.000	0.306**	0.032**	0.138**
10.										1.000	0.859*	0.846*
11.											1.000	0.722*
12.												1.000

1. Days taken for sprouting of bulb
2. Plant height
3. No. of leaves per plant
4. Days to spike emergence
5. Flowering duration
6. Spike length
7. Rachis length
8. Number of florets/ spike
9. Length of the floret
10. Weight of florets per spike
11. Number of spikes/m^2
12. Yield of florets/ plot (4 * 1 m)

Note:

* Significant at 5% level

** Significant at 1% level

Table 53. Phenotypic correlation coefficient among different characters in tuberose (double)

S.NO	1	2	3	4	5	6	7	8	9	10	11	12
1.	1.000	-0.772	-0.637	1.036*	-0.830	-0.792	-0.646	-0.690	-0.477	-0.623	-0.852	-0.698
2.		1.000	0.939**	-0.347	0.940**	0.999**	0.639**	0.955**	0.581**	0.900**	0.685**	0.971**
3.			1.000	-0.157	0.914**	0.928**	0.413**	0.897**	0.608**	0.946**	0.649**	0.931**
4.				1.000	-0.445**	-0.389**	-0.411**	-0.416**	-0.125**	-0.364**	-0.427**	-0.401**
5.					1.000	0.940*	0.683*	0.854*	0.806*	0.868*	0.859*	0.896*
6.						1.000	0.639**	0.961**	0.595**	0.899**	0.685**	0.974**
7.							1.000	0.470*	0.544*	0.320*	0.734*	0.500*
8.								1.000	0.456**	0.946**	0.490**	0.993**
9.									1.000	0.621**	0.812**	0.554**
10.										1.000	0.516*	0.963*
11.											1.000	0.550*
12.												1.000

1. Days taken for sprouting of bulb
2. Plant height
3. No. of leaves per plant
4. Days to spike emergence
5. Flowering duration
6. Spike length
7. Rachis length
8. Number of florets/ spike
9. Length of the floret
10. Weight of florets per spike
11. Number of spikes/m^2
12. Yield of florets/ plot (4 * 1 m)

Note:

* Significant at 5% level

** Significant at 1% level

Table 54. Path analysis (single)

S.NO	1	2	3	4	5	6	7	8	9	10	11	12
1.	**0.2460**	-0.2306	-0.1950	0.1827	-0.1432	-0.2271	-0.1614	-0.1996	-0.1170	0.0087	-0.1142	-0.4384
2.	0.3989	**-0.3190**	-0.2835	0.1775	-0.2134	-0.2278	-0.2287	-0.2775	-0.0750	0.0302	-0.1807	0.4212
3.	-0.4585	0.5140	**0.5784**	-0.2042	0.2995	0.3262	0.4974	0.5626	-0.0295	-0.0750	0.3593	0.4740
4.	0.0150	-0.0.113	-0.0071	**0.0203**	-0.0117	-0.0177	-0.0098	-0.0111	-0.0176	-0.0081	-0.0064	-0.6493
5.	-0.2180	0.2506	0.1940	-0.2153	**0.3746**	0.2466	0.2113	0.2618	0.1016	0.0348	0.0118	0.4484
6.	0.3492	-0.2702	0.2134	0.3310	-0.2471	**-0.3784**	-0.2627	-0.2743	-0.2397	-0.0829	-0.2053	0.5964
7.	-0.1821	0.1990	0.2387	-0.1340	-0.1566	0.1927	**0.2776**	0.2822	0.0254	0.0186	0.0720	0.6201
8.	0.1685	-0.1807	-0.2020	0.1138	-0.1452	-0.1533	-0.2112	**0.2077**	-0.0312	0.0015	-0.1299	0.6716
9.	-0.1087	0.0537	-0.0117	-0.1991	0.0630	0.1448	0.0209	0.0343	**0.2286**	0.1376	-0.1387	0.5904
10.	0.0224	-0.0603	-0.0827	-0.2564	0.0592	0.1397	0.0428	-0.0045	0.3841	**0.6380**	0.3614	0.9223
11.	0.1065	-0.0771	-0.0714	-0.0866	-0.0491	0.0933	-0.0782	-0.0766	-0.0621	-0.0337	**-0.0511**	0.5755
12.	-0.0265	0.1347	0.0354	-0.0179	0.0018	0.0309	0.0148	0.0356	0.0346	0.0323	0.0570	**0.9927**

Residual effect = 0.174

1. Days taken for sprouting of bulb
2. Plant height
3. No. of leaves per plant
4. Days to spike emergence
5. Flowering duration
6. Spike length
7. Rachis length
8. Number of florets/ spike
9. Length of the floret
10. Weight of florets per spike
11. Number of spikes/m^2
12. Yield of florets/ plot (4 * 1 m)

Table 55. Path analysis (cv. Double)

S.NO	1	2	3	4	5	6	7	8	9	10	11	12
1.	**0.2580**	-0.2408	-0.1951	0.1838	-0.1423	-0.2272	-0.1626	-0.1999	-0.1175	0.0088	-0.1143	-0.4385
2.	0.3998	**-0.3291**	-0.2834	0.1785	-0.2143	-0.2289	-0.2289	-0.2786	-0.0754	0.0312	-0.1806	0.4213
3.	-0.4865	0.5242	**0.5782**	-0.2052	0.2998	0.3272	0.4984	0.5627	-0.0259	-0.0752	0.3592	0.4742
4.	0.0152	-0.0117	-0.0072	**0.0213**	-0.0127	-0.0179	-0.0089	-0.0113	-0.0167	-0.0086	-0.0062	-0.6491
5.	-0.2183	0.2608	0.1941	-0.2143	**0.3756**	0.2467	0.2123	0.2629	0.1055	0.0349	0.0119	0.4485
6.	0.3429	-0.2805	0.2143	0.3320	-0.2481	**-0.3784**	-0.2637	-0.2747	-0.2388	-0.0830	-0.2051	0.5967
7.	-0.1812	0.1994	0.2388	-0.1351	-0.1576	0.1932	**0.2779**	0.2823	0.0249	0.0187	0.0725	0.6203
8.	0.1676	-0.1906	-0.2021	0.1149	-0.1443	-0.1534	-0.2109	**-0.2078**	-0.0334	0.0014	-0.1300	0.6723
9.	-0.1078	0.0538	-0.0127	-0.1993	0.0632	0.1457	0.0212	0.0344	**0.2297**	0.1377	-0.1389	0.5915
10.	0.0243	-0.0602	-0.0836	-0.2575	0.0693	0.1399	0.0437	-0.0046	0.3851	**0.6378**	0.3615	0.9232
11.	0.1056	-0.0774	-0.0713	-0.0875	-0.0592	0.0943	-0.0793	-0.0767	-0.0625	-0.0335	**-0.0518**	0.5756
12.	-0.0253	0.1348	0.0353	-0.0182	0.0019	0.0317	0.0158	0.0355	0.0345	0.0332	0.0573	**0.9929**

Residual effect = 0.182

1. Days taken for sprouting of bulb
2. Plant height
3. No. of leaves per plant
4. Days to spike emergence
5. Flowering duration
6. Spike length
7. Rachis length
8. Number of florets/ spike
9. Length of the floret
10. Weight of florets per spike
11. Number of spikes/m^2
12. Yield of florets/ plot (4 * 1 m)

Table 56. Composition of D^2 cluster and their geographical origin in tuberose (Single types)

Cluster	Total number of types	Name of types	Origin
I	4	Shringar	IIHR, Bangalore
		Calcutta Single	Calcutta
		Hyderabad Single	Hyderabad
		Kahikuchi Single	Assam
II	2	Phule Rajani	MPKV, Rahuri, Maharastra
		Prajwal	IIHR, Bangalore
III	1	Mexican Single	Mexico
IV	3	Pune Single	Pune
		Variegared Single	Local
		Navsari Local	Gujarat

Table 57. Intra and Inter cluster distance variation for different characters in tuberose (Single types)

Clusters	I	II	III	IV
I	10.71	17.17	1.85	34.28
II		0.000	24.90	38.24
III			0.000	22.36
IV				12.56

Table 58. Cluster means for different characters in tuberose (Single types)

Clusters	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12
I	14.76	**30.62**	**19.00**	**20.97**	**16.06**	124.46	18.76	**29.00**	**4.06**	0.91	1.53	35.78
II	**12.24**	**45.69**	**27.83**	30.19	18.76	**128.10**	20.13	**36.58**	**6.62**	**0.98**	**1.82**	**36.18**
III	**16.25**	37.64	24.53	**43.28**	20.98	123.45	**15.46**	32.23	4.07	**0.72**	**1.08**	**21.63**
IV	13.25	42.57	21.19	34.85	**22.06**	**92.53**	**22.36**	31.00	5.53	0.85	1.62	24.89

X1. Days taken for sprouting of bulb

X2. Plant height

X3. No. of leaves per plant

X4. Days to spike emergence

X5. Flowering duration

X6. Spike length

X7. Rachis length

X8. Number of florets/ spike

X9. Length of the floret

X10. Weight of florets per spike

X11. Number of spikes/m^2

X12. Yield of florets/ plot (4 * 1 m)

Table 59. Contribution of each character to divergence (Single types)

Characters	No. of first rank	Percentage of contribution
Days taken for sprouting of bulb	0	0.00
Plant height	9	2.38
Number of leaves per plant	1	0.26
Days to spike emergence	1	0.26
Flowering duration	3	0.79
Spike length	8	8.20
Rachis length	1	0.26
Number of florets per spike	5	0.53
Floret length	4	10.58
Weight of florets per spike	6	28.84
Number of spikes per m^2	7	1.85
Yield of florets per plot (4 * 1 m)	2	36.25

Table 60. Composition od D^2 cluster and their geographical origin in tuberose (Double types)

Cluster	Total number of types	Name of types	Origin
I	3	Suvasini	IIHR, Bangalore
		Calcutta Double	Calcutta
		Hyderabad Double	Hyderabad
II	1	Vaibhav	IIHR, Bangalore
III	1	Pearl Double	Mexico

Table 61. Intra and Inter cluster distance variation for different characters in tuberose (Double types)

Clusters	I	II	III
I	12.62	15.36	1.76
II		0.00	35.23
III			11.23

Table 62. Cluster means for different characters in tuberose (Double types)

Clusters	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12
I	14.76	**32.63**	**15.00**	20.97	**22.08**	**22.46**	**20.36**	**27.00**	**4.01**	0.81	1.53	32.78
II	**10.34**	**43.69**	**24.73**	**2.91**	18.76	**126.15**	18.12	**35.48**	**6.32**	**0.86**	**1.81**	**35.28**
III	**15.32**	36. 43	20.53	**41.28**	**12.98**	123.45	**13.46**	32.23	4.07	**0.72**	**1.08**	**19.62**

X1. Days taken for sprouting of bulb

X2. Plant height

X3. No. of leaves per plant

X4. Days to spike emergence

X5. Flowering duration

X6. Spike length

X7. Rachis length

X8. Number of florets/ spike

X9. Length of the floret

X10. Weight of florets per spike

X11. Number of spikes/m^2

X12. Yield of florets/ plot (4 * 1 m)

Table 63. Contribution of each character to divergence in tuberose (Double types)

Characters	No. of first rank	Percentage of contribution
Days taken for sprouting of bulb	0	0.00
Plant height	9	2.27
Number of leaves per plant	1	0.24
Days to spike emergence	1	0.23
Flowering duration	3	0.72
Spike length	8	7.22
Rachis length	1	0.25
Number of florets per spike	5	0.51
Floret length	4	9.57
Weight of florets per spike	6	24.54
Number of spikes per m^2	7	1.85
Yield of florets per plot (4 * 1 m)	2	35.67

CHAPTER V

DISCUSSION

Tuberose (*Polianthes tuberosa* L.*)* is one of the most important loose flowers and its double types are widely used as cut flowers. It is also used as an ornamental bulbous plant. It is cultivated on a large scale in Tamil Nadu, Karnataka, West Bengal and Maharastra. Waxy white flowering spikes of single as well as double types of tuberose impregnate the atmosphere with their sweet fragrance and because of longer keeping quality of flower spikes (Sandhu and Bose, 1973 ; Benschop, 1993) they are in great demand for making floral arrangement and bouquets in major cities of India. Looking at the importance of this crop, there is a prime need for its improvement through breeding. For a successful breeding programme, information on nature and magnitude of variability in germplasm collection is very essential.

The primary focus in the evaluation programme is to identify superior types that are adaptable to tropical conditions with high yield. This can be assessed by studying the *per se* performance of the genotypes. The genetic facts are inferred from phenotypic observations with the aid of suitable genetic parameters like estimates of heritability and genetic advance (Mishra and Choudhary, 1976). The correlation analysis help the breeders to define the selection indices for the breeding programme. Similarly, the quality parameters are used to assess the consumer preference of the particular genotype.

Therefore, the current study is centered on the evaluation of 10 single and 5 double genotypes for the comparative analysis of their mean performance in the morphological and yield traits under tropical condition. Genetic variations in yield are the major component in the diversity of the base population and the yield being a complex trait is influenced by genetic and environmental factors. Therefore magnitudes of variability, degree of association between the yield and the yield components and their relative contribution to flower yield $plot^{-1}$ of the genotypes are discussed in this chapter.

5.1. Evaluation of tuberose cultivars on growth and flowering parameters

5.1.1. Growth parameters

A significant variation in growth was observed among the genotypes with regard to days taken for sprouting of bulb, number of leaves per plant and plant height under tropical condition (Table 1). The variations among the growth parameters may be due to their diversified origin and also evolution of the particular genotype as a morphotype in their specific geographical location. This offers scope for selecting genotypes with better performance under tropical condition.

Mean performance of the cultivars for growth parameters reflected the variation among the cultivars. Significantly less number of days taken for sprouting of bulbs (12.12 days) was recorded in 'Prajwal', followed by 'Variegated Single' (13.23 days) while more number of days was taken by 'Hyderabad Single' (16.15 days). Maximum plant height (117.50, 113.05 cm) was noticed in 'Variegated Single and Prajwal'. This is in accordance with the results of Gudi (2006) and Vijayalaxmi *et al*., 2010. Prajwal produced maximum number of leaves/ plant (260) on first year after planting followed by Variegated Single (253), while minimum number of leaves was found in Hyderabad Single (220). The days taken for spike emergence was less (78 days) in 'Prajwal', followed by 'Variegated Single' (81 days) while it was more (94 days) in 'Calcutta Single'(94 days). The duration of flowering was significantly higher in Prajwal (17 days) followed by Shringar (15 days). This is in line with the findings of Patil *et al*. (2009). The differences among the varieties for vegetative characters are attributed to their variation in their genetic makeup (Swaroop, 2010).

Among the double genotypes, significantly less number of days taken for sprouting of bulbs (12.32) was recorded in 'Suvasini', followed by 'Vaibhav' (12.67) and more number of days was taken by 'Hyderabad Double' (16.15 days). Maximum plant height (86.25 cm) was noticed in 'Suvasini'. This is in accordance with the results of Gudi (2006). 'Suvasini' also produced maximum number of leaves/ plant (270) followed by 'Vaibhav' (250), while minimum number of leaves was recorded in 'Hyderabad Double' (235). The differences among the varieties for vegetative characters are attributed to their variation in their genetic makeup (Swaroop, 2010). The number of days

taken for spike emergence was less (84 days) in 'Suvasini', followed by 'Vaibhav' (85 days) while it was more in 'Hyderabad Double' (89 days). Similarly the duration of flowering was also significantly more in Suvasini (12.40 days) followed by Vaibhav (11.43 days). This is in line with the findings of Patil *et al*., (2009).

The present study revealed that, Prajwal (Single) and Suvasini (double) have shown their superiority with regard to growth traits like plant height (113.05, 86.25 cm), number of leaves per plant (260.00, 270.00) at three months after planting .

5.1.2. Flowering parameters

'Variegated Single' produced spikes with a maximum length of 102.50 cm followed by 'Prajwal' 98.05 cm while it was minimum in 'Phule Rajani' 52.50 cm (Table 2). The variation in spike length in different genotypes might be due to variation in their growth intrinsic factor. The rachis length was significantly more in 'Pune Single'(35.75 cm) followed by 'Prajwal' (28.52 cm) and it was minimum in 'Hyderabad Single' (15.30 cm). 'Prajwal' showed its superiority for number of florets/ spike (47.00) which were followed by 'Navsari Local' (45), while it was minimum in 'Mexican Single' (17). The increase in floret length also observed in 'Prajwal' (6.40) while it was the lowest in 'Pune Single' (6.10). This finding is in consonance with the findings of Vijayalaxmi *et al.,* 2010 in tuberose. Weight of florets/ spike was also higher in 'Prajwal' (74.80) followed by 'Shringar' (51.48). This might be due to the increased number of florets/ spike and bolder nature of florets. Among single genotypes of tuberose 'Prajwal' showed increase in number of spikes/ m^2 and yield of florets/ plot (4 * 1 m) (47 nos. and 4.40 kg) respectively. The maximum yield registered by 'Prajwal' might be due to its capacity to produce more number of florets per spike, increased floret length and weight of florets / spike.

Among double types, 'Suvasini' produced spike with maximum length of 71.25 cm followed by 'Vaibhav' 66.38 cm and it was minimum in 'Hyderabad Double' (53.87 cm). The rachis length was significantly higher in 'Vaibhav' (54.00 cm) followed by 'Suvasini' (44.00 cm) and it was minimum in 'Hyderabad Double' (33.95 cm). The variation in spike length and rachis length in different genotypes might be due to variation in their intrinsic factor. 'Suvasini' showed its superiority for number of florets/

spike (54.00) followed by 'Vaibhav' (44) and it was minimum in 'Pearl Double' (30). The increased floret length was noticed in 'Suvasini' (7.50) and it was lowest in 'Hyderabad Double' (6.70). This finding is in consonance with the findings of Patil *et al*., (2009). Weight of florets/ spike was maximum in 'Suvasini' (146.88 g) followed by 'Vaibhav' (119.24 g). This might be due to the increased number of florets/ spike. The increased number of spikes/ m^2 and yield of florets/ plot (4 * 1 m) were noticed in Suvasini (34.10 and 3.42 kg). The maximum yield may be accorded due to its capacity to produce more number of florets per spike, increased floret length and weight of florets / spike.

The present investigation resulted that Prajwal (Single) and Suvasini (Double) were found to be the best in respect of flowering traits *viz.,* number of florets/ spike (47.00, 54.00 nos.), length of the floret (6.40, 7.50 cm), weight of florets per spike (74.80, 146.88 g), number of spikes/m^2 (47.00, 34.10 nos.) and yield of florets/ plot (4 * 1 m) (4.40, 3.42 kg).

5.2. Quality parameters

Vase life is one of the important quality parameters, which decides the consumer's preference. Vase life among single types was maximum in genotype Prajwal (12.97 days) followed by Variegated Single (12.80 days) and it was minimum in Hyderabad Single (8.91 days). Whereas in double types, maximum vase life was noticed in the genotype Suvasini (12.40 days) followed by Vaibhav (12.25 days). Variations in vase life may be attributed to the difference in accumulation of carbohydrates due to variation in leaf production and sensitivity of cultivars to ethylene. In turn, variations in these aspects might be due to genetical makeup of genotypes and the existing environment. This finding is in accordance with the results given by Kamble *et al*. (2004) in gladiolus.

The concrete recovery among single types was maximum in the genotype Prajwal (0.16 %) followed by Shringar (0.15 %), whereas it was minimum in Hyderabad Single and Pune Single (0.11%). Among double types, maximum concrete recovery was noticed in the genotype Suvasini (0.09 %) followed by Vaibhav (0.07 %). Variations in concrete recovery may be attributed to the genetical makeup of genotypes and the existing environmental factors.

The promising genotypes suitable for tropical condition is Prajwal (single) and Suvasini (double) followed by Variegated Single (single) and Vaibhav (double) for loose flower and cut flower production respectively. Consumer preference for Prajwal and Suvasini were very high followed by Variegated Single. This may be due to the individual floret weight, floret colour and also due to strong, sturdy long stalk and concrete recovery. Increased longevity of flowers under ambient condition was also one of the reason for higher preference for these genotypes in the market.

5.3. Floral biology of tuberose genotypes

The studies on floral biology *viz*., flower morphology, pollination behavior and barriers in pollination of any crop is very important for crop improvement. Tuberose is a cross pollinated crop and the fundamental information *viz.,* anthesis, stigma receptivity, pollen viability and fertility etc., are much needed for programming crop improvement through hybridization (Plate 10 - 36).

In the present investigation on flower morphology of tuberose genotypes, there were six, long radiating anthers (1.11 to 1.16 cm) and pistils (2.62 to 3.83 cm). The stamens contain profuse yellow pollen (Plate 11). The pistil possessed three celled ovary which formed an ellipsoidal capsule. The individual flower weighed 2.52 g. Earlier floral description of tuberose was made by Shen *et al*., 1987 and Uma, 1990 and the reports are similar to the observations of the present study.

In the present study, the number of days taken for completion of flowering phases in all the tuberose genotypes was recorded right from the date of bud initiation to the date of fruit set. In general, number of days taken for appearance of first flower from planting varies from 98 to 101 days in single types and it varies from 103 to 105 days in double types.

In the process of normal flowering, the first splitting of the bud is referred as bud opening followed by anthesis. The bud opening or anthesis vary from variety to variety. They are also greatly influenced by environmental factors such as humidity, light, temperature and rain. Knowledge on bud opening/anthesis, mode of pollination is desirable in any crop improvement programme especially for making successful crosses.

In tuberose, the flower blooming occurs during evening hours. This speaks on to the magnitude role of sunlight in triggering the flower opening process and appears to be the nature's provision for ensuring pollination. In the present investigation, the maximum mean percentage of bud opening in tuberose genotypes (both single and double types) was between 3.45 to 5.30 pm and it increased gradually, reaching the peak at 6.30 pm beyond which no flowers opened. This is in agreement with the findings of Uma, 1990 who stated that the period of bud opening in tuberose was between 5.30 p.m. to 6.30 p.m.

In the present study, maximum mean percentage of anther dehiscence in tuberose genotypes (single) was observed between 3.45 to 5.30 pm and the stigma receptivity was assessed by carrying out artificial pollination of flowers under controlled conditions. In tuberose, 33 per cent fruit set was observed in flowers which were pollinated on third day of anthesis, indicating the maximum receptiveness of stigma during third day of anthesis. The flowers pollinated during first and second day after anthesis exhibited no fruit set indicating that the stigma was premature and are not ready for receptivity during that period. In general, the percentage of fruit set was higher in between 6.30 and 8.30 am irrespective of the pollination done on different days.

The loss of stigma receptivity can be identified from its dryness. Varying reports were made by Rajagopalan (1994) and Gupta and Raina (2001) who found that the stigma was receptive for about three days after anthesis. This may be due to the variation in environmental conditions such as temperature, humidity or dew, rainfall and season.

5.4. Pollen studies

Pollen size was studied under compound microscope by collecting samples of fresh pollen from tuberose (Plate 27 - 36). In the present investigation, the average pollen size of single tuberose genotypes was 11501.90 µm to 23464.24 µm.

Pollen viability is the ability of a pollen grain to germinate and develop as a pollen tube (Gerard, 1932). The growth of the pollen tube can be taken as the measure of pollen viability since the non-viable pollen could not make the growth of a pollen tube. Good pod set cannot be achieved unless pollen is viable with high germination percentage. The frequency of getting fertile pollen is another factor for ensuring the fruit set. Fertile pollen along with viability favour a better fruit set and consequently an acceptable yield.

Among the ten single genotypes, the highest percentage of pollen viability was observed in Variegated Single (96.73 %) followed by Phule Rajani (90.52%) and it was minimum in Prajwal (39.83 %). Likewise, pollen output studies showed that Variegated Single had higher pollen output of 10,000 and it was followed by Phule Rajani (9375). Similar studies on pollen viability were reported in roses by Lata (1971), Gowda *et al.,* (1977), Sandhya (1987), Gurumurthy (1990) and Sitharamu (1993). Pollen germination was estimated by acetocarmine test. This is the most commonly used procedure for germinating microelements of pollen to determine viability. The percentage of germinated and non germinated pollen grains were calculated (Table 12). Pollen germination status of ten genotypes varied from each other. The highest percentage of pollen germination was observed in Variegated Single (99.21 %) followed by Phule Rajani (86.74 %). The lowest percentage was recorded in Prajwal (22.12 %). Even though very good percentage of pollen viability was observed in all the genotypes, pollen germination was found to be comparatively lower. This may be probably due to the time of incubation period (4- 5 hours) allowed only to vigorous pollens to germinate during this period. In Iris, under *in vitro* condition pollen germination ranged from 27.7 to 88.5 per cent (Yabuya *et al.,* 1982). Similarly *in vitro* pollen germination by acetocarmine test was also reported in Gloriosa (Anandhi, 2012).

The crossability potential in distant crosses depends upon three kinds of interactions i) between the pollen grain wall and the male gemetophyte on the one hand and the tissues of female sporophyte on the other hand ii) between the parental genomes in the hybrid zygote, embyro and endosperm iii) between the hybrid tissues and surrounding maternal tissues.

In the present study, an attempt was made to study the pollen- pistil characters of 10 tuberose (single) genotypes and the barriers to the fertilization in the crosses. Each of the matching parental gene or gene complexes controlled a link in the chain of processes and interactions which are needed for successful hybridization. Incomplete matching may cause one or more missing link in the chain and may inhibit interbreeding through incompatibility. In this case, there is a lack of genetic information in one genotype about the mechanism prevailing in the other.

Hence successful fertilization involves a matching genetic system, gene regulated stepwise phyiological and biochemical processes and intimate pollen- pistil relationship. Therefore the biology of the pollen has been a matter of great concern because of its direct implication in plant breeding (Mulcahy and Ottaviano, 1983). Studies on morphology of pollen grains of different genotypes were carried out in the present investigation.

The study showed that, the size of the pollen grain was expressed by measuring the area as well as equivalent diameter of the pollen grain. The study also revealed that the wide range of pollen size observed in the parents. The diameter of the pollen grain was influenced primarily by the genotype of the mother plant with some effect of environment (Kumar and Sarkar, 1983). A significant variation in pollen grain size and perimeter was noticed between the genotypes. This is primarily due to differences in the genome constitution within the genotypes, may however be attributed to the environment. It has been found that the pollen grain size was controlled by polygenes which are sensitive to environment and pollen grain diameter was mainly controlled by additive components followed by dominance effect with greater influence of environmenal variations in maize (Kumar and Sarhar, 1984) and Sesame (Pfahler *et al.,* 1996). Such studies are not available in tuberose.

The relationship between pollen grain size and competitive ability to grow in pistil tissues upon self and cross pollination under *in vivo* conditions was studied. The germinated pollen grain grew faster in the stigmatic and stylar tissues and produced longer pollen tubes at a particular period which reveals the existence of positive relationship between size of the pollen and ability to grow. Such a correlation would appear logical since larger pollen grains contain more energy reserves for improved germination rate and better pollen tube growth on the stigma. This is in accordance with the studies made by Neuffer *et al.* (1968) in wheat.

The composition of *in vitro* pollen germination medium will vary from crop to crop and even between the genotypes within the species. The pollen germination medium mainly contains sucrose, boric acid and other salts. The stigma used in the medium acts as an osmotic regulant and also as nutrition for pollen germination and pollen tube growth (Johri and Vasil, 1961). Schmucker (1935) beleived that boron regulates the

hydration of colloids associated with polyhydroxyl compounds of the pollen membrane and is involved in the synthesis of pectin substances for the pollen tube wall. Voyiatizi (1995) studied the effect of sucrose, boric acid and calcium on *in vitro* pollen germination on rose cultivars. The optimum level of sucrose and boric acid enhances the germination whereas calcium has an inhibitory effect. He noticed negative correlation between calcium concentration and germination percentage.

The medium for *in vitro* pollen germination for tuberose has been standardized for the present study. Earlier the artificial medium was developed for tuberose pollen germination by Brewbaker and Kwack, 1963. But complete pollen germination was observed in the medium only after 24 hours of incubation. In the present study, it was found that higher concentration of boric acid had inhibitory effect on pollen germination as reported earlier in cotton (Gunasekaran, 1997) and hence the concentration of boric acid was optimised at 100 ppm level which showed immediate germination.

According to Bar- Shalom and Mattsson (1977) the pollen from plants of wet stigma type is often found to germinate readily in liquid media with the appropriate osmotic balance. The technique of the pollen culture *in vitro* therefore allows the investigator to germinate the pollen grains and study the growth without interference from the tissues of the pistil. It was reported that pollen tubes *in vitro* do not grow as fast or as long as they do *in vivo* and often exhibit erratic changes in the direction (Heslop- Harrison, 1987). In the present study, the genotype differences in respect of length of the pollen tube under *in vitro* conditions were observed for 10 single type genotypes. The pollen tube length was higher in the parent Variegated Single (Plate 37). It contains higher amount of reserve food than other genotypes which may be utilized for initial autotrophic pollen tube growth.

The rate of elongation and the total length of the pollen tubes obtained *in vitro* often fell far short of the actual length requirement to reach the ovule in pistil. *In vitro* pollen tubes emerged within few minutes after 24 hours of incubation. Thus the *in vitro* studies on pollen germination and pollen tube growth although, could explain the mechanism involved in the pollen- pistil interaction at stigmatic level and in incompatible crosses, the information on the growth of pollen tube could be obtained from *in vivo*

studies. Genetic data generated earlier revealed that gynoecial factors may determine the pattern of pollen tube behaviour such as order of fertilization, rate and direction of pollen tube growth (Ottaviano *et al.,* 1980). Hence the *in vivo* studies on pollen- pistil intercation would facilitate better understanding of the mechanism of crossability barriers in the crosses at the cellular level.

Aniline blue fluorescence technique is a recently developed procedure to study the *in vivo* pollen tube growth. In this technique, pistils are stained with water soluble aniline blue and ultra violet illumination callose plug present in the pollen tube fluoresce brightly. Pollen tubes are therefore clearly distinguishable from the stylar tissues and may be readily counted and measured. Sandra (2004) described the detailed procedure for *in vivo* studies of Hydrangea pistils using aniline blue fluorescence technique. Since the number and growth of the pollen tube could be traced from such fluorescence microscopic studies and such studies were not undertaken earlier in tuberose. Hence the investigations were taken up. As a prelude to actual study, the standardization of aniline blue fluorescence technique was attempted.

Insufficient softening of pistils leave the specimen hardy, which makes difficult to observe the pollen tube growth due to differential refraction. Excessive softening cause the breakage of pistils even at a gentle pressing rendering the observation of pollen tube difficult. Therefore the optimum strength of NaOH, concentration of aniline blue, K_3PO_4 and softening period of pistil were studied and determined to be 8 N, 0.1 N and 0.3 N, respectively.

The process of pollen germination on the stigma, growth of pollen tube in the style and the penetration of the tube into one of the synergid cells with the final discharge followed by double fertilization obviously require rather detailed precise adjustment between pollen and pistil (de Nattancourt, 1977). Kallo and Chowdhary (1992) reported that such adjustment was probably an integral component of the evolution of each species. So the pollen germinaion on the stigma is the first step to indicate favourable pollen pistil interaction for successful crossing.

In the present study, the lowest level of pollen germination upon selfing in parents indicated that all the genotypes showed self incompatibility. While in tuberose crosses, pollen germination on alien stigma ranged from scale 1 to 4 which indicated the presence

of wide variations in the pollen- pistil interactions within the cross compatible pollinations as well as between compatible and cross incompatible genotypes. High level of pollen germination was observed when Phule Rajani was crossed with Hyderabad single, Kahikuchi Single, Mexican Single and Variegated Single; Shringar x Kahikuchi Single and Variegated Single; Hyderabad Single x Variegated Single; Variegated Single x Calcutta Single, Kahikuchi Single, Mexican Single, Navsari Local, Phule Rajani, Pune Single and thus points to the existence of a positive and intimate pollen- pistil interaction between these genotypes (Tble 19, Fig 8 and Plate 38 a, b, c and d).

The pollen grain of Calcutta Single, Kahikuchi Single, Navsari Local Pune Single and Shringar germinated on the stigma of Variegated Single. Whereas the pollen grain of Variegated Single did not germinate on the Calcutta Single and Kahikuchi Single stigma. Likewise, Kahikuchi Single, Mexican Single and Hyderabad Single pollen grains germinated on the stigma of Phule Rajani but the Phule Rajani pollen grain did not germinate on the stigma of Kahikuchi Single, Mexican Single and Hyderabad Single. It points to the presence of unidirectional interspecific incompatibility or incongruity between the two genotypes. Significant differences were also observed in pollen germination between the reciprocal crosses. Successful fertilization involves a stepwise regulation of a series of physiological and biochemical processes and intimate pollen pistil relationship. The time required for the entry of pollen tubes into the ovule was variable depending on the distance between pollen (on the stigma) and ovule.

It was observed that for the pollen germination on the stigma, proper recognition substances are necessaary. In the present study, the pollen tube length at 2 HAP in majority of the crosses and selfing was lesser than the pollen tube length measured after successful crossing at the same time interval and showed that proper recognition substances might be lacking or not strong enough to promote pollen germination immediately in selfing and unsucessful crosses. Hence the pollen grains took longer time to germinate on the alien stigma.

In general, as the pollen start to grow, it utilizes endogenous reserves, then for further elongation the tube is dependent on stylar secretions which break various

substances down to release sugars which are ultimately used for tube wall synthesis (Gunasekaran, 1997). Arabinogalactans to be the major part of the pistil secretions that form the medium for pollen tube growth in lily.

The high rate of growth of pollen tube during the initial period was evident in all successful crosses. The faster germination brought about by the strong coordination between the pollen and pistil proteins. The less number of pollen tubes was resulted in all stylar and ovary regions (Table 20).

Apart from the arrest of pollen tubes at different levels in crosses, several abnormalities were noticed in the present investigation. In incompatible matings, there will be dense accumulation of callose at the tips which prevents further growth of pollen tube (Plate 39 b) (Shivanna and Heslop- Harrison, 1978). The strength of callose thickening in crosses depends on the taxonomic distance between the pollen and pistil genotypes as was reported by de Nattancourt, 1977.

The most common abnormalities was the formation of knot like structures at the tip of the pollen tube (Plate 39 c). The other abnormalities include twisting and bulging at the tip, bursting of pollen tube tip and breakage of pollen tube (Plate 39 d). A similar type of malformation in pollen tube was observed in Rhododendron (Williams *et al.,* 1982) and Maize (Manickam, 1996). However, such results were not reported earlier in tuberose.

Several attempts were made to relate the incompatibility with specific enzymes which act to prevent the growth of incompatible tubes (Oppositional mechanism) or the absence of which would not allow pollen tube growth (complementary mechanism). Peroxidase is one of the important isozymes reported to have a close link with 'S' allele in Nicotiana (Pandey,1967) which regulates protein activity, destroys IAA and catalyse a number of essential reactions. In plants, post pollination success is based primarily on two mechanisms. One is an intrasexual mechanism, the competition among the male gametophyte and the other is intersexual mechanism deciding female and male choice. In most plant species, the number of pollen grains deposited on the stigma greatly exceeds the number of ovules available for fertilization and consequently numerous pollen gametes fail inside the pistil during the process that extends from pollination to fertilization in many different plant species. Although this pattern of reduction appears to

be fixed with respect to a particular species, very little is known about how it is regulated. This interaction and tube attribution has been extensively studied in the self incompatibility reaction between closely related individuals (Lewis, 1994). In the present investigation, there was a severe reduction in the number of pollen tubes reaching the ovary being more or less similar. Although many authors have reported in other species on the reduction in the number of pollen tubes growing down the style (Kahn and De Mason, 1986; Cruzan, 1989; Herrero, 1992 a; Plitmann, 1993), only in few cases the pattern of the reduction has been studied.

In the present study, the main bottleneck seems to be in the upper portion of the style and few others stop growing between mid style and ovary as in Brassica (Ockendron and Gates, 1975) and *Curcurbita pepo* (Winsor and Stephenson, 1995). Similarly Sayers and Murphy (1966) reported that in *Medicago sativa* tha main determinent deciding the reduction of pollen tube number was the failure of pollen tubes to penetrate the stigma. In other species however, a high rate of pollen tube attition was observed in the lower regions of the pistil as in *Erythronium grandiflorum* (Cruzan, 1989). The present study suggests that the selection pressure occuring in parents upon selfing and also in few crosses appears along the entire style length and particularly, a higher level of pollen tube inhibition occured at stigmatic regions in the crosses using Prajwal as one of the parent (Plate 39 a).

It is beleived that two main forces could determine such a reduction of male gametophyte in the pistil. One force could be the differences in competitive ability of the pollen and the other could be a modulation of these differences by the pistil. This modulation would comprise both physical and physiological constraints and genetics of pollen pistil interaction as was reported by Hormaza and Herrero (1996). According to Herrero (1992 a) the width of the transmitting tissue of the style would be a physical limitation while physiological limitation include restriction in the nutrient supply. In the present study, greater percentage of reduction in pollen tube number in all the crosses and selfed plants may be due to the presence of inhibitory substances.

5.5. Screening of root knot nematode

In the present study, the tuberose genotypes were screened against root knot nematode using artificial inoculation method. The main objective of this study is to find out the resistant genotypes against root knot nematode. In the present investigation, *Meloidogyne incognita,* which was multiplied in the tomato roots, was used for screening the genotypes grown in the pots. Similar methods of artificial inoculation of plants with second stage juvenile larvae at the root part were followed in earlier screening studies (Ahmadi and Bac, 2005; Adegbite *et al*., 2006). Thus it is clear that screening technique employed in the present study was appropriate. Similar screening technique in wild *Solanum* species as well as grafts was reported against *Meloidogyne incognita* (Sherly., 2011).

In the present study, the effect of tuberose genotypes against root knot nematode on plant growth characters was assessed under pot culture. Significant reduction in growth parameters such as shoot length, root length, shoot weight and root weight were noticed in the susceptible varieties (Fig.1a). Though, there was a general reduction in plant growth, tuberose genotypes showed differences among them in the reduction of plant growth parameters.

Root length was found to be the highest in Kahikuchi Single, wherein the resistance level was moderate compared to others. Hence, the moderately resistant genotype had registered the highest length compared to other genotypes. Even under high nematode inoculums, the mean root length of Kahikuchi Single was not reduced. This might be due to the low nematode reproduction on Kahikuchi Single roots. Binks and Gowen (1997) also observed higher root weight with more primaries in resistant cultivars compared to susceptible cultivars. Thus root number and size are likely to be significant factors in plant tolerance to nematodes. Good root developmet potentially favours resistance to plant. In the present study also the moderately resistant genotype Kahikuchi Single showed higher number of roots with better root length.

The rate of growth in terms of root indicated that the resistance of Kahikuchi Single was good as compared to other genotypes. Invariably, this genotype showed superiority of growth rate under inoculated condition indicating the moderately resistant nature of this genotype to nematode infection. As compared to susceptible genotypes

(Calcutta Single, Hyderabad Single and Mexican Single) Kahikuchi Single showed its superiority in terms of root length (54.89 cm) and root weight (63.75 cm) under inoculated condition. This finding is supported from the results of Oda *et al.*, 1996 and Sherly, 2011.

Among the genotypes, the lowest values for root length was noticed in all the genotypes except Kahikuchi Single. It has also been reasoned that increased demand of nematode infested roots for nutrients, which redirect nutrients away from the developing tissues, resulting in poor plant development (McClure, 1977; Ploeg and Phillips, 2001). Root knot nematode infested plant had a very shallow and knotted root system which would have resulted in impaired plant growth due to reduced nutrient uptake and declined distribution of hormones, minerals and photosynthaates as indicated by Darekar and Mhase (1988).

Primary damage to the infected roots might be attributed to mechanical damage associated with feeding or invasion of *Meloidogyne incognita*, which caused withdrawl of nutrients and impaired physiological process. Generally, damage cause reduction in the rate of root extension and in turn the uptake of nutrients and water (Anwar and Din, 1986). In the present study, the shoot growth of plant was affected due to the impaired water relations. This might probably due to the development of giant cells which cause choking of xylem vessels interfering with the nutrient uptake. Secondary effects include reduced photosynthetic efficiency with a reduction in light interception and carbohydrate synthesis.

The resistance or susceptibility of the genotypes to root knot nematode is assessed by various methods and one such method is root knot indexing. In the present study, the lowest root knot index was found in the Kahikuchi Single compared to other genotypes. Index based on number of egg masses per gram of root was found to be the lowest in Kahikuchi Single. The statistical analysis indicated that there was a direct relationship between root gall and production of egg masses (Fig. 1b).

Egg production indicates the ability of the nematode to complete its life cycle or in other words the host status to the invading parasite. The mean performance for this trait showed that the genotype Kahikuchi Single produced less number of eggs per egg mass (94.55) and number of female per gram of root (25.80) as compared to other species. Roberts and May (1986) also found greater number of females, galls and eggs per plant in susceptible cultivars as compared to moderately resistant cultivars.

In pot culture, the buid up of *M.incognita* population was assessed taking into account of the soil nematode population 45 days after inoculation and root knot index. The lowest nematode population was noticed in the genotype Kahikuchi Single (122.61/ 200 cc of soil).

In the present investigation, Kahikuchi Single registered the least root knot index (3) which was grouped under the 'moderately resistant' category, whereas, the genotypes Calcutta Single, Hyderabad Single and Mexican Single were grouped under 'susceptible' category and the genotypes (Navsari Local, Phule Rajani, Prajwal, Pune Single, Shringar and Variegated Single in single types); (Calcutta Double, Hyderabad Double, Peral Double, Suvasini and Vaibhav in double types) were grouped under 'highly susceptible' category (Plate 40 and 41). The data on nematode population in the soil and indexing synthesized more comprehensive picture of the reaction of various genotypes. Resistance and susceptibility to plant parasitic nematodes reflected the effect of the plant on the nematode's reproducing ability (Cook and Evans, 1987). Resistant and moderately resistant plant species might be most valuable if they reduced nematode reproduction sufficient enough to affect the residual nematode population density in a field (Anita *et al*., 2006).

On the contrary, less reduction in plant growth parameters was also observed in Kahikuchi Single for the root parameters under scrutiny and could be considered as moderately resistant criterion. Inability of the other genotypes to produce more root resistant characters in the phase of nematode infection in Calcutta Single, Hyderabad Single and Mexican Single could be considered as a susceptibility criterion. The susceptibility was also expressed as the extent of damage to the root system. The pathogenicity of nematode to plants might have involved two main factors *viz.,* the reproductive rate and ability of a nematode to induce physiological interaction such as necrosis (Sarah *et al*., 1993).

Even though Kahikuchi Single was infested with nematode, it was ranked as moderately resistant, because of trachied discontinuity due to the formation of small thin walled giant cells. Therefore this genotype is considered to have a certain type of resistance mechanism against root knot nematode.

Although *Meloidogyne incognita* multiplication was noticed in all the genotypes, there was variation in pathogenicity which might be due to the presence of nematode resistant gene (Hadisoeganda and Sasser, 1982; Roberts and May, 1986). The genes made the plant less attractive for attack by nematodes. The resistant genotypes didn't show any wounding as against the susceptible genotypes. It was understood that the resistant plants fail to produce functional feeding sites in the host after invasion due to hypersensitive responses which led to failure to develop subsequently as reproducing females (Williamson and Kumar, 2006). Two types of mechanisms for root knot nematode resistance in plants have been reported including pre-infection resistance due to presence of toxic or antagonistic chemicals in root tissue, which would have prevented roots but failed to develop. It is often associated with an early hypersensitive reaction (HR) due to the death of the cells in root tissue around the nematode. This mechanism might have prevented the formation of a developed feeding site leading to resistance.

Boiteux and Charechar (1996) reported that resistant cultivars had gene of resistance in their gene pool that conferred resistance to *Meloidogyne incognita*. Development and reproduction of *Meloidogyne incognita* was reflected by resistance and susceptibility of the plant (Cook and Evans, 1987; Khan *et al.,* 2004). The results indicated that in Kahikuchi Single, reproduction of nematodes were found to be lower as compared to other genotypes. This study contributed information on the reaction of tuberose genotypes to *Meloidogyne incognita*.

By observing the overall performance of genotypes against root knot nematode, the plant growth parameters recorded in Kahikuchi Single showed improvement due to less infestation of nematodes.The probable reason suggested for stimulation in plant growth in the host is defense mechanism. The host root apparently putforth certain lateral rootlets at the site of infection, which not only increased the root length but also resulted in increased absorption of water and nutrients from soil. Due to the low level of nematode population in this genotype, any pathogenic effect on host might not be appearing. These results are in consonance with Raut (1981), Khan *et al.* (2004), Yadav *et al.* (2009) and Sherly (2011) in cluster bean and brinjal. Such reduction in plant growth characters and increased galls, egg masses, eggs and females in other genotypes might be due to the fact that more nematodes reached and penetrated the feeding site.

Plants have a repertoire of resistance genes that protect them from many pathogens, including viruses, bacteria, fungi and nematodes (Dewit, 1997; Kosack and Jones, 1997). In many cases, pathogen recognition by the host is mediated by single resistance genes (R genes) in the host and single gene in the pathogen called avirulence (Avr) genes. Recognition initiates a cascade of defense responses, often including a hypersensitive response (HR) consisting of localized cell necrosis at the infection site. Plant generally respond to nematode invasion by activation of a series of local and systemic defense mechanism (Lindgren *et al.,* 1992), inducible defenses against nematodes in turn accumulation of peroxidase (Ibrahim, 1991), polyphenol oxidase and superoxide dismutase (Zacheo and Zacheo, 1995), proteinase inhibitors (Bowles *et al*., 1991) and chitinase (Punja and Zhang, 1993).

Nematode resistance is usually associated with fewer egg masses or less gall formation in the host plant root system (Bouton *et al.* 1989) and is controlled by many genes (horizontal resistance) in many crop species. The mechanisms of horizontal resistance to nematode are poorly understood and the essential physiological and molecular events are not well characterized (Castagnone- Sereno, 2002). The present investigation has provided information that Kahikuchi Single is moderately resistant to root knot nematode and can be recommended for cultivation wherever the root knot nematode infestation is a serious problem.

5.5.1. Biochemical basis of resistance

The resistant/ tolerant/ susceptible genotypes possess various physical and chemical barriers, which restrict the entry and growth of a pathogen in the host cells. In many plant pathogen interactions, the phenomenon involved in the defense actions of plant to pathogen have been found and reported. Principal factors involved in the expression of resistance is based on genetic composition of the host and expression of the resistance genes which may be influenced by many factors like aggressiveness of pathogen, availability of specific nutrients, accumulation of inhibitory substances at the infection site, metabolism of the host constituents including the activity of numerous enzyme systems, host nutrition and environment (Barnett, 1959).

The induced multiple defense responses are elicited when plants are exposed to biotic stresses such as attack by herbivores or pathogens (Sarosh and Maiser, 2007). Higher plants have intricate mechanisms enabling them to respond to environmental changes, most likely established over a long period of evolution as sessile organisms (Wu *et al.,* 2007). These plant responses are controlled at the molecular level by changes in gene expression and many genes are involved in such stress responses (Tardif *et al.,* 2007). Phytohormones may participate in stress perception signaling and possibly initiate a cascade of stress-induced responses (Chandler and Robertson, 1994).

Many biochemical factors are known to be associated with biotic resistance in crop plants. In many cases, it is obvious that the biochemical factors are more important than morphological and physiological factors in conferring non- preference and antibiosis (Prabhu *et al.,* 2008). Occurence at lower concentration or total absence of such biochemicals leads to non-preference, a form of insect resistance (Singh, 1983). The biochemical constituents like glycoalkaloid (solasodine), phenols, ortho-dihydroxy phenols, phenolic oxidase enzymes *viz.,* polyphenol oxidase, peroxidase, phenylalanine ammonia lyase, IAA oxidase and acid phosphatase are available in brinjal and these biochemical constituents possess insect resistant properties (Kalloo, 1988).

5.5.2. Root knot nematode resistance:

Root knot nematodes spend most of their active life stages within plant roots. The infective stage is the second-stage juvenile (J_2), which penetrates the root and migrates to the vascular tissue to establish a permanent feeding site (Williamson and Hussey, 1996). Plants generally respond to nematode invasion by activation of a series of local and systemic defense mechanisms (Bouton *et al.,* 1989; Lindgren *et al.,* 1992; Trudgill, 1995). Inducible defense against nematodes included accumulation of peroxidase (Ibrahim, 1991), polyphenol oxidase and superoxide dismutase (Zacheo and Zacheo, 1995), proteinase inhibitors (Bowles *et al.,* 1991; Gheysen *et al.,* 1996) and perhaps chitinase (Punja and Zhang, 1993). Additionally, activation of pathways involved in phytoalexin biosynthesis resulted in a hypersensitive response that was characterized by rapid, localized necrosis of root tissues (Sijmons *et al.,* 1994).

In the present investigation, the biochemical mechanism in roots was established by analysing total phenols, ortho-dihydroxy phenol, ascorbic acid, protein and enzymes, *viz.,* peroxidase, polyphenol oxidase, phenyl alanine ammonia lyase, IAA oxidase and acid phosphatase at different intervals after artificial inoculation in tuberose genotypes.

Proteins play a role in eliciting the response in resistant plants. The increased protein content observed in the present study might be either due to stimulation in nitrogen metabolism of the host or by oxidation of certain phenols by the host from quinones that combine and thereby, this integrated some proteins as reported by Fieldman and Hanks (1964).

Phenolic compounds played a major role in the defense mechanism of the plants against various infectious agents. Total phenol content in roots is yet another indication of plant's resistance to root knot nematode. The accumulation of phenolic compounds in the injured area and the activation of associated oxidative enzymes have been reported by Balasubramanian and Purushothaman (1972). Acedo and Rohde (1971) also reported role of phenol contents towards the resistant mechanism against various nematode infections. The oxidized phenols are principal factors responsible for browning and production of necrotic tissues. In the resistant species, the non-toxic phenolic glycosides have been shown to be hydrolysed by β-glycosidase enzyme from the nematode and the resultant product might prevent localized parasitization or even cause the death of the nematode (Star, 1981; Hussey and Williamson, 1998). It is also possible that active phenols might have been released from glycosides by increased activities of β-glycosidases and later get oxidized in resistant genotypes. Narayana and Reddy (1980) also inferred that an increased phenolic content was considered to be contributory factor in the resistance to various nematode infections.

The higher amount of total phenols (12.54, 14.68, 17.41, 18.59, 19.84 and 19.21) observed in the present study with the Kahikuchi Single might be responsible for checking the infection of root knot nematode at different hours after artificial inoculation (Fig. 2). The accumulation of phenolic compounds in the nematode injured area and the activity of associated oxidative enzymes have been reported by Mountain (1965). Similar observation was made by Bajaj *et al.* (1983) in tomato and Sherly (2011) in brinjal.

Similarly, total phenol content in roots showed a negative association with root knot index, number of females per gram of roots and number of egg masses per gram of roots. Nematicidal activity of phenolic compounds was also reported by Mahajan *et al.* (1985). Post infectional increase of phenols in the roots might be due to the tendency of phenols to accumulate at the site of infection, which is involved in the defense mechanisms of plants through the interference in the metabolic activities of pathogens that might have resulted in higher root length (Gopinatha *et al.,* 2002).

The enzymes polyphenol oxidase and peroxidase oxidizes the colourless dihydroxy phenols into coloured ortho quinines. While, certain dihydroxy phenols get conjugated with each other or with glucose hydroxyl groups to form tannins, both are constituents of plant melanins (Bell, 1981). These tannins and ortho quinines have toxicity to a wide range of microorganisms. In the present investigation, estimation of ortho-dihydroxy phenols at different hours after inoculation showed that Kahikuchi Single showed higher ortho-dihydroxy phenol (9.52, 10.04, 12.56, 14.21, 15.84 and 15.55) than the other genotypes when challenged with *M. incognita*. An increasing trend in the ortho-dihydroxy content was noticed and attained the peak at 96 hours. The results revealed that higher ortho-dihydroxy phenol in Kahikuchi Single was responsible to confer resistance to root knot nematode (Fig. 3). This finding is in line with the reports of Indu Rani *et al.* (2008), Janani (2009) and Sherly (2011) who observed a higher ortho-dihydroxy phenol content in the galled roots than in healthy tissues of tomato, pepper and brinjal respectively.

Nematode infestation had a negative but significant association with peroxidase activity and had clearly brought out the fact that the better activity of peroxidase might have been involved in the defense mechanism of resistant/tolerant species. Peroxidase played a vital role in alleviating free radical toxicity in plant tissues. Following the entry of nematode, an increased peroxidase level in the infested plant suggested a great *de novo* synthesis of peroxidases since nematode do not exudate peroxidate (Glasiov *et al.,* 1967). In the present study, the higher peroxidase activity was observed in the Kahikuchi Single (2.49, 3.01, 3.14, 3.30, 3.65, 3.54 and 3.19) at different hours of interval after artificial inoculation. These enzymes gradually increased when the nematode infestation started and it attained the peak at 96 hours after inoculation and then decreased gradually (Fig. 4).

This falls in line with the findings of Mohanty *et al.* (1986) in cowpea and Shukla and Chakraborty. 1988, Indu Rani *et al.* (2008) and Sundharaiya (2008) in tomato; Janani (2009) in pepper and Sherly (2011) in brinjal.

In the present study, higher polyphenol oxidase activity was noticed in Kahikuchi Single (2.57, 3.04, 3.18, 3.27, 3.58, 3.45 and 3.18) at different hours of interval after artificial inoculation. These enzymes gradually increased when the nematode infestation started and it attained the peak at 96 hours after inoculation (Fig. 5). About 95 per cent of the O_2 taken up by the higher plants is consumed through cytochrome oxidases which are sensitive to cyanide. The other 5% was utilized by polyphenol oxidase and ascorbic acid oxidase, which were insensitive to cyanide poisoning (Huang, 1985). The role of PPO and ascorbic acid in impairing root knot nematode resistance had been well documented by several workers (Hassan *et al.,* 1994; Indu Rani *et al.,* 2008; Janani *et al.,* 2009 and Sherly*,* 2011).

In the present study, higher phenylalanine ammonia lyase activity was noticed in Kahikuchi Single (14.24, 14.92, 15.55, 16.04, 16.10, 15.48 and 15.39) at 0, 24, 48, 72, 96 and 120 hours of interval after artificial inoculation. These enzymes gradually increased after inoculation and it attained the peak at 96 hours (Fig. 6). The activity levels of phenylalanine ammonia-lyase and anionic peroxidase induced early resistance response to many other pathogens and also increased in tomato (Brueske, 1980; Zacheo *et al.,* 1993), brinjal (Sherly*,* 2011) after nematode inoculation.

Estimation of residual IAA in the roots is an indirect way of measuring the IAA oxidase activity. The lower IAA oxidase activity per se in the resistant genotype Kahikuchi Single indicated higher activity of this enzyme, which prevented the accumulation of IAA synthesized by the plant during nematode attack and resulted in less number of root galls compared to other genotypes.

This could have helped in less accumulation of IAA thereby preventing the formation of giant cells. The highest IAA oxidase was noticed in all genotypes except Kahikuchi Single where the nematode population was found high.This is in line with the findings of Towers (1964) in brinjal. The susceptibility might have been enhanced by secretion of proteolytic enzymes by the nematodes (Hussey, 1989), which might have free protein- bond IAA or break down protein to supply IAA precursor, tryptophan and

IAA synthesis activator such as phenylalanine (Huang, 1985). This would stimulate increase in IAA or produce an active growing or modified tissue that would provide the nematodes with adequate nutrition (Crammer *et al.,* 1993; Grundler and Wyss, 1994). This might have promoted more rapid maturation of females and greater fecundity and resulted in high reproduction and damage by the nematodes.

Acid phosphatase is yet another important enzyme closely related to nematode resistance. Increase in acid phosphatase activity in the roots was found to be a resistant mechanism to root knot nematode. In the present study, increase in acid phosphatase activity after artificial inoculation was noticed in the moderately resistant genotype Kahikuchi Single. Increasing trend was observed in acid phosphatase activity from the day of inoculation and it attained the highest at 96 hours and after which started to decline.

Root knot nematode incidence showed significant negative association with root ascorbic acid and acid phosphatase activity. This suggested the involvement of acid phosphatase in defense mechanism of plants to nematode attack.

A great deal of evidence has been advanced in recent years indicating a dramatic increase in the activity of several key enzymes like IAA oxidase and acid phosphatase and their secondary metabolites of phenylpropanoid pathway soon after the infection of host plants with nematodes (Swain *et al.,* 2004).

5.6. Seed set

Detailed seed setting behaviour was studied in 'Single' tuberose genotypes as 'double types' found to lack properly developed ovules and stigma. Seed setting behaviour of the ten single genotypes were studied under following two types of pollination *viz.,* artificial selfing and artificial crossing.

5.6.1. Self- compatibility

5.6.1.1. Artificial selfing

Fruit set as influenced by pollination on different days after anthesis

In the present study, artificial selfing has shown no improvement in fruit set of single tuberose genotypes. No fruit set was obtained when flowers were pollinated on second, third and fourth day after anthesis. This showed the existance of complete

self- incompatibility in these genotypes. This also rules out the possibility of protandry postulated by Shen *et al.* (1987) in tuberose cv. 'Single'. The results of self - incompatibility studies are also in confirmity with Shen *et al.* 1987 and Uma (1990) in tuberose cv. 'Single'. Similar results indicating self- incompatibility were reported in inter - varietal hybrids of *Agave lechuguilla* (Maksimov and Novikov, 1986) and *Yucca aloifolia* (Maksimov *et al*., 1987). According to Uma (1990) the self-incompatibility in tuberose cv. 'Single' may probably due to the low rate of pollen tube growth inside the style of self-pollinated pistils and its inhibition for further elongation in the style. This study confirms the self- incompatibility mechanism prevalent in tuberose cv. 'Single'. In the genus Gasteria, Brandham and Owens (1978) showed that self- incompatibility reaction was controlled atleast by two genes and confirmed that site of pollen tube inhibition was the ovule. Occurance of gemetophytic self-incompatibility was also reported in Narcissus, Hemerocallis, Lilium, Gasteria (Bateman, 1954; Arasu, 1968) and Freesia (Pandey, 1970). According to Fett *et al.* (1976) and Ascher (1978), the localization of incompatibility barrier is neither in the stigma nor in the style but ovary itself in *Lilium longiflorum.*

5.6.2. Cross – compatibility

5.6.2.1. Artificial crossing (Single type x Single type)

Fruit set as influenced by pollination on fourth day after anthesis

The studies were conducted on fruit set by pollinating on fourth day after anthesis. Among the single type tuberose crosses, the highest percentage of fruit set was recorded in crosses using Variegated Single as pollen parent. The cross between Phule Rajani X Variegated Single showed increased value (50.40 %) followed by crosses between Pune Single X Variegated Single (28.07 %). The crosses using Calcutta Single, Hyderabad Single, Kahikuchi Single, Mexican Single and Navsari Local with Variegated Single as pollen parent resulted successful fruitset. But other single types did not set fruits. This is in accordance with the studies of Uma (1990) and Sitharamu (1993) wherein she observed good fruit set when cv. 'Single' X cv. 'Variegated Single' and cv. 'Variegated Single' X cv. 'Single'. Similarly, Sazak and Tomosa (1979) studied 13 Hippeastrum cultivars as breeding parents in various cross combinations. They observed highest fertility under cross-pollination between Japanese cultivars. Similarly, higher

percentage of fruit set was obtained by artificial crossing in other crops like roses (Sonderhonson, 1974; Hansen, 1985; Sandhya, 1987; Gurumurthy, 1990) and Hibiscus (Markose and Aravindakshan, 1987).

The maximum percentage of fruit set was also noticed in crosses using Phule Rajani as pollen parent. The cross between Variegated Single X Phule Rajani showed the highest set (78.40 %) followed by crosses between Mexican Single X Phule Rajani (70 %) and it was minimum in crosses between Hyderabad Single X Phule Rajani (6.66 %) followed by Kahikuchi Single X Phule Rajani (10 %) at seven days after pollination. But the crosses in other single types using the same Phule Rajani as pollen parent did not set fruits.

A fruit set of 29.10 per cent was recorded in crosses between Variegated Single X Shringar followed by 19.71 per cent in the cross between Kahikuchi Single X Shringar. Other crosses using Shringar as pollen parent did not set fruits.

A higher fruit set was noticed in the cross between Variegated Single X Navsari Local (50.98 %) while it was low (13.84 %) in Variegated Single X Hyderabad Single. The crosses in other single types using Hyderabad Single and Navasari Local as pollen parents did not set fruits.

In tuberose, there are hardly any reports on possible causes for low fruit retention on artificial crossing. The studies conducted in other crops have revealed that, the low fruit set and low retention could be due to limitation of pollen grains (Garwood and Horovitz, 1985; Wheelan and Goldingay, 1989). Limited resource availability within the proper translocation of resources (Bawa and Webb, 1984; Lee and Bazaz, 1982) and lack of fertilization despite abundant pollen grains (Guth and Wellar, 1986; Cruzan, 1989) are also shown to cause poor fruit set. Aswath et al. (1989) attributed that failure of seed setting in ornamental climber Pyrostegia venusta as due to the development of non-viable pollen grains and also due to the occurance of degenerating ovules at megaspore tetrad stage.

5.6.3. Standardization of tetrazolium test

At 40^0C, the embryos of tuberose seed immersed in 0.25 per cent tetrazolium solution and incubated for 2 h recorded 62 per cent complete staining and 38 per cent partial staining of seeds. However, this was 86 and 4 per cent, respectively after 2½ h

incubation period. Whereas the embryos immersed in 0.5 per cent tetrazolium solution and incubated for 2 h registered 85, 14 and 1 per cent completely, partially and over stained seed, respectively. While at 2½ h incubation period 94 per cent completely staining, 1 per cent partial staining and 5 per cent over staining of seeds.

5.6.4. Influence of pre-sowing seed treatment with growth regulators

The seed germination study of tuberose did not show much response under favourable climatic condition. The major hurdle in crossing of tuberose genotypes is that fruit setting and seed setting is arrested in many crosses. Among the crosses made, only few crosses could produce few seeds, but later these seeds failed to germinate. These findings are in line with the results of study conducted earlier (Krishnamurthy, 2000). Hence assessing seed germination in the genotypes is very much essential.

In order to enhance the germination, pre- sowing seed treatments were given for open pollinated seeds of tuberose genotypes with varying chemicals. Sharga *et al*. (1970), Austin (1972), Miller and Holcomb (1982) with their studies with different crops emphasised that pre-sowing treatment with growth regulators such as GA_3, IBA is warranted as they enhanced the germination both at field and laboratory level and the seedling emergence and establishment at field.

Jouret (1977) also reported that growth and development of a plant is under the control of various growth regulating substances and external application of a particular growth regulator could cause promotion or inhibition of growth depending upon the concentration of the applied solution (hormone or growth regulator) and many growth regulating substances had been tried to boost up the growth of various crops with varying degree of success (Hashim *et al.*, 1991).

The present study conducted to evolve a suitable growth regulator for pre-sowing seed treatment with a different growth regulators *viz.,* GA_3, IBA, thiourea and KNO_3 at different concentrations (250 and 500 ppm; 100 and 200 ppm; 0.5% and 1%; 0.25% and 0.5%) along with water soaking for two different soaking durations (8 and 16h) revealed that the seed treatment with GA3 @ 250 ppm for 8 h improved the germination by 12.50 per cent which is 63.68 % compared to control.

The increase in germination due to GA_3 treatment was claimed as due to breakdown of starch and other substrates that induced the enzyme action, the first step of the germination process which created an ability to overcome a metabolic block in the embryonic axis of endosperm (Cneudt and Boseman, 1983) and also they demonstrated that low concentration of GA_3 could stimulate amylase production in the absence of germination. But Jouret (1977) reported that GA_3 did not influence the synthesis of total RNA in aleurone tissue. However, Heit (1971) concluded that GA_3 promoted the synthesis of specific mRNA responsible for *in vitro* synthesis of α amylase between 5-8 h of GA_3 treatment. Hence this could be the reason for the enhanced germination obtained in the present study with GA_3 compared to other growth regulators. Thompson (1970) also reported that GA_3 enhanced the activity of endo-β-1, 4-xylanase in the aleurone layer. The multiplicity of the effects of GA_3 in the regulation of enzyme synthesis and secretion in aleurone cells indicates that this hormone has the potential to regulate germination in numerous ways.

Wilfret and Green (1976) also demonstrated that GA_3 could enter into chemical combination with phospholipids (Wlamer *et al*., 1974) and thus increased the germination. In the present study, when GA_3 was used at higher concentration it decreased the germination significantly due to the lethal activity stimulated on enzyme reaction at supra optimal condition as reported by Natarajan (2003) in petunia. Thus the study indicated that, optimum concentration of GA_3 for enhancing the seed germination of tuberose is 250 ppm. The other characters evaluated on seedling quality *viz.,* root length, shoot length, drymatter production and vigour index values were also higher in seeds treated with GA_3 which was attributed to the increase in cell division and proliferation to root and apical meristem tissues by Lal *et al.* (1971) and Das *et al*. (1999).

Next to the treatment involving GA_3, seeds treated with KNO_3 0.5 per cent for 8 hours resulted higher germination percentage. The improvement in the seed germination may be attributed to the presence of nitarte formed during imbibitions. It would have provided additional substrate for aminoacid and protein synthesis for the enhancement of germination. Such promotary effects of potassium nitrate have also been reported in African marigold (Selvaraju and Selvaraj, 1994).

5.6.5. Number of days taken for fruit set

The number of days taken for fruit set was less in crosses between Mexican Single X Variegated Single (7.45) followed by Calcutta Single X Variegated Single (7.50), Kahikuchi Single X Phule Rajani (7.50) and Variegated Single X Phule Rajani (7.75), while it was more in Variegated Single X Hyderabad Single (8.95), Variegated Single X Navsari Local (8.76), Kahikuchi Single X Shringar (8.76), Variegated Single X Shringar (8.50), Mexican Single X Phule Rajani (8.45) and Navsari Local X Variegated Single (8.25). Similar results were obtained by Sitharamu, 1993 in tuberose.

5.6.6. Number of days taken for fruit maturity

The minimum number of days taken for fruit maturity was recorded in crosses between Navsari Local X Variegated Single (76.75) and Kahikuchi Single X Variegated Single (78.97), while the maximum number of days taken for fruit maturity was noticed in Variegated Single X Hyderabad Single (85.98), Variegated Single X Navsari Local (85.43), Mexican Single X Phule Rajani (85.08), Kahikuchi Single X Shringar (83.74), Pune Single X Variegated Single (81.86), Variegated Single X Phule Rajani (81.00) and Kahikuchi Single x Phule Rajani (80.00). Similar results were obtained by Sitharamu, 1993 in tuberose.

5.6.7. Number of seeds per capsule in different crosses

The number of seeds per capsule was higher in crosses between Variegated Single x Phule Rajani (41.00), Phule Rajani X Variegated Single (38.00) followed by crosses between Pune Single X Variegated Single (32.96), Variegated Single X Navsari Local (32.00), while it was lesser per capsule in the crosses between Mexican Single X Phule Rajani (16.23), Kahikuchi Single X Variegated Single (20.00), Variegated Single X Shringar (24.00), Kahikuchi Single X Shringar (25.00), Variegated Single X Hyderabad Single (26.00) and Hyderabad Single X Phule Rajani (28.97). Similar results were obtained by Sitharamu, 1993 in tuberose cv, 'Variegated Single' under crossing.

5.6.8. Seed germination in different crosses

The highest percentage of germination was recorded in crosses between Variegated Single x Phule Rajani (12.56 %), followed by crosses between Variegated

Single X Hyderabad Single (12.54 %), Mexican Single X Phule Rajani (12.52 %), Calcutta Single X Variegated Single (12.50 %), Variegated Single X Shringar (12.50 %), Kahikuchi Single X Shringar (12.51 %), Mexican Single X Variegated Single (12.45 %), Hyderabad Single X Phule Rajani (12.45 %) and Variegated Single X Navsari Local (12.43 %) while the lowest germination was recorded in Pune Single X Variegated Single (12.23 %). Similar results were obtained by Sitharamu,1993 in tuberose.

5.6.9. Number of days taken for germination

The number of days taken for germination was less in crosses using Variegated Single as pollen parent is between Phule Rajani X Variegated Single and Variegated Single x Phule Rajani (28.96) followed by crosses between Pune Single X Variegated Single (30.21), Kahikuchi Single X Phule Rajani (31.67) , Variegated Single X Navsari Local (32.87), Kahikuchi Single X Shringar (32.98), Variegated Single X Shringar (34.72), Variegated Single X Hyderabad Single (35.98), while it was more (38.25) in Kahikuchi Single X Variegated Single. Similar results were obtained by Sitharamu, 1993 in tuberose.

5.7. Post fertilization events

In the present investigation the early stages of development of seed (Zero and fourth DAA), there was normal development of the fruit, in both selfed and crossed materials. Normal development of fruit was evident from the healthy development of ovule, emrbyosac and fruitwall. However fusion nucleus did not undergo any divisions. However by the fourth day the degeneration symptoms noticed in egg apparatus and antipodals in the selfed ovules. On seventh DAA, all the tissues showed symptoms of degeneration and resulted in drooping of the fruits because of complete degeneration. This may be because of lack of pollen germination, pollen tube growth and lack of fertilization. Similar display in seed set between selfing and cross pollinations has been reported in Narcissus by Tammy *et al.,* 1999 and in tuberose by Krishnamurthy, 2000 wherein they have attributed the lack of seed set to absence of required stimulus for normal seed development. On the contrary, normal development of the fruit continued in cross material. Fusion nucleus failed to undergo division even on 20^{th} DAA is the key finding of the present investigation.

In the succesful crosses, by 30th DAA, there was complete differentiation of embryo and endosperm. Embryo was torpedo shaped. During this stage, there was intense accumulation of polysaccharides, RNA and proteins in embryo and endosperm indicating that they are metabolically highly acute. Major developmental stages occured between 20th and 30th DAA. Further, development of embryos and endosperm continued till 40th DAA. Thus the size of embryo and endosperm also got increased with the intense accumulation of polysaccharides, RNA and proteins. This represents a completely developed seed (Plate 42).

5.8. Genetic variability, heritability and genetic advance

In the crop improvement, germplasm serves as a valuable source of base population and provides scope for building up of genetic variability. Variability, heritability and genetic advance will help ascertain the real value of the genotypes and select superior ones. The exploration of genetic variability in the available germplasm is a pre-requisite in a breeding programme for effective selection of superior genotypes. The partitioning of total variability into heritable and non-heritable components by using suitable design will enable the breeder to know whether the superiority of selection is inherited by the progenies. Since natural genetic variation for most of the yield attributes is considerably high in tuberose, there is an urgent need for information on nature and magnitude of variation available in the material and part played by the environment in the expression of different characters. Moreover, heritability estimate along with genetic advance was more helpful than heritability estimate alone in predicting result and effect for the previous selection of best individual from any kind of plant population.

In single genotypes, the phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) (Table 48, Fig. 7) was the highest for flowering duration (35.79, 35.62) followed by weight of florets per spike (32.43, 32.27) and number of florets per spike (32.01, 31.80) suggesting that these characters are under genetic control. Hence, these characters can be relied upon selection for further improvement. The phenotypic coefficient of variation (PCV) was higher than genotypic coefficient of variation (GCV) for all the characters under study, indicating the role of environment in expression of genotype. Similar results were also reported by Mishra *et al.* (1987) in dahlia and Sheela

et al. (2005) in *heliconia*. Minimum values of phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) were recorded for characters like length of the floret (3.11, 1.45) number of leaves per plant (5.50, 4.34) and days to spike emergence (6.60, 5.60) and days taken for sprouting of bulb (9.74, 9.08). This type of findings indicated that very minimum variation existed among the genotypes with respect to these characters.

High heritability coupled with high genetic advance was observed for flowering duration (99.07, 73.04), weight of florets per spike (99.02, 66.15), number of florets per spike (98.72, 65.08) and rachis length (98.47, 54.46) (Table 48, Fig. 8). This indicates the lesser influence of environment in expression of these characters and prevalence of additive gene action in their inheritance. Hence, these traits are found suitable for selection. High heritability with moderate genetic advance was recorded for yield of florets/ plot (2* 2 m) (98.00, 44.22), spike length (97.58, 44.04), plant height (96.24, 34.69) and number of spikes/m^2 (95.00, 42.96) suggesting the presence of both additive and non-additive gene actions, and simple selection offers best possibility of improvement of this trait. The estimate of heritability was high with low genetic advance as percentage of mean for days to sprouting (88.83, 17.43), length of the floret (78.23, 15.39), days to spike emergence (71.99, 19.78) and number of leaves per plant (62.16, 17.05) which indicated that high heritability were due to non-additive gene effects and influence of environment. Hence, there is a limited scope for selection. Sheikh. *et al* (2005) reported similar kind of results in Iris.

In double genotypes, The phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) (Table 49, Fig. 9) was the highest for number of florets/ spike (24.59, 24.38), suggesting that this character is under genetic control. Hence, these characters can be relied upon selection for further improvement. The phenotypic coefficient of variation (PCV) was higher than genotypic coefficient of variation (GCV) for all the characters under study, indicating the role of environment in expression of genotype. Similar results were also reported by Misra *et al.* (1987) in dahlia and Sheela *et al*. (2005) in *heliconia*. Minimum values of phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) were recorded for days to

spike emergence (3.58, 1.49), length of the floret (5.04, 3.93) and number of leaves per plant (6.14, 5.25). This type of findings indicated that very minimum variation existed among the genotypes with respect to these characters.

High heritability coupled with high genetic advance was observed for number of florets per spike (98.35, 69.81), number of spikes/m^2 (96.93, 65.78), rachis length (96.91, 45.18) and yield of florets per plot (95.78, 40.20). (Table 49, Fig. 10). This indicates the lesser influence of environment in expression of these characters and prevalence of additive gene action in their inheritance. Hence, these traits are found suitable for selection. High heritability with moderate genetic advance was recorded for weight of florets per spike (94.67, 46.64), spike length (93.79, 44.46), flowering duration (92.83, 32.39), days taken for sprouting of bulb (91.91, 22.26) and plant height (90.52, 19.17) suggesting the presence of both additive and non-additive gene actions, and simple selection offers best possibility of improvement of this trait. The estimate of heritability was high with low genetic advance as percentage of mean for length of the floret (60.68, 6.30), days taken for spike emergence (77.38, 1.28) and number of leaves per plant (73.06, 9.25) which indicated that high heritability were due to non-additive gene effects and influence of environment. Hence, there is a limited scope for selection. These results are in accordance with the findings of Sheikh *et al.* (2005) in Iris.

5.9. Correlation coefficient studies

The goal of any crop improvement programme is to achieve high level of yield. Yield in plants is the end product of interaction of many correlated characters. Selection for these characters will be more effective when it is based on component characters that are highly heritable and positively correlated. When more number of variables is considered in correlation, the association becomes more complex and less obvious. Yield is a complex entity associated with a number of component characters. It is the prime concern of the plant breeder and it is the final factor on which selection programmes are to be envisaged. All changes in yield are accompanied by changes in one or more component characters. A study of association of these characters helps selection of genotypes and also suggests the advantage of a selection scheme for more than one character at a time, which could be explained that improvement of one character results in

the simultaneous improvement of all positively related characters. Similarly, non-existence of negative correlation between yield and quality traits (which normally do not happen in most of the crops) if identified, will be useful for the plant breeder to go in for selection of genotype derivatives in the segregating population for high yield coupled with quality factors. The relationship between genotypic, phenotypic and environmental correlations was discussed by Falconer (1981), which emphasized the characters having high heritability.

The genotypic and phenotypic correlation coefficients were computed in all possible combinations for twelve characters and are presented in Tables 50 and 52. Correlation coefficient analysis measures the mutual relationship between various plant characters and determines the component characters on which selection is based for genetic improvement for a particular character (Robinson *et al.,* 1949). A positive correlation between desirable characters is favorable to the plant breeder because it helps in simultaneous improvement of both the characters. In the present study, genotypic correlation coefficients were found to be higher than phenotypic correlation coefficients for most of the characters, indicating a strong inherent association between various characters and were masked by environmental component with regard to phenotypic expression.

The study showed a highly significant and positive correlation between days taken for sprouting of bulb and days to spike emergence (0.559) and number of florets/ spike (0.108). This trait however, showed negative correlation with weight of florets per spike (-0.725), yield of florets per plot (4 * 1 m) (-0.549), number of spikes per m^2 (-0.401), flowering duration (-0.341), plant height (-0.292), spike length (-0.258), number of leaves per plant (-1.180), length of the floret (-1.014) and rachis length (-0.076). Similar results were obtained by Vetrivel (2010) in gladiolus.

Highly significant and positive correlations for plant height was observed with spike length (0.997), yield of florets per plot (4 * 1 m) (0.978), rachis length (0.593), number of spikes per m^2 (0.457), weight of florets per spike (0.402) and number of leaves per plant (0.197). Prabhat Kumar *et al.,* (2011) also reported significant and positive association of plant height with spike length in gladiolus. There exists a positive and highly significant relationship of number of leaves per plant with length of the floret (0.705), weight of the florets per spike (0.652), yield of florets per plot (4 * 1 m) (0.577),

flowering duration (0.368), number of spikes per m^2 (0.326) and spike length (0.229). It also had positive significant correlation with rachis length (0.149), while the days to spike emergence (-0.738) had highly significant but negatively correlated.Similar findings were also reported by Vetrivel (2010) and Prabhat Kumar *et al.* (2011) in gladiolus.

Days to spike emergence in single genotypes showed highly significant positive correlation with number of florets per spike (0.068). This trait however, showed negative correlation with number of spikes per m^2 (-0.910), weight of florets per spike (-0.871), yield of florets per plot (4 * 1 m) (-0.737), spike length (-0.707), rachis length (-0.558), length of the floret (-0.526) and flowering duration (-0.307). Positive and significant association was observed for duration of flowering with yield of florets per plot (4 * 1 m) (0.602), weight of florets per spike (0.430), number of spikes per m^2 (0.298), length of the floret (0.282), number of florets per spike (0.175) and rachis length (0.050), while the spike length (-0.141) showed highly significant but negative correlation. This is in line with the findings of Rakesh Kumar *et al.* (2012) in snapdragon. The spike length showed highly significant and positive correlation with rachis length (0.565), number of spikes per m^2 (0.435), yield of florets per plot (4 * 1 m) (0.423), weight of florets per spike (0.373), while the length of the floret (-0.332) and number of florets per spike (-0.150) had significant but negative correlation with spike length. This is in consonance with the findings of Rakesh Kumar *et al.* (2012) in snapdragon. There exists a positive and highly significant relationship of rachis length with yield of florets per plot (4 * 1 m) (0.474), weight of florets per spike (0.440), number of spikes per m^2 (0.385) and number of florets per spike (-0.150). The trait however, showed negative correlation with length of the floret (-0.263). Similar such findings were reported by Rakesh Kumar *et al.* (2012) in snapdragon. The number of florets per spike showed highly significant and positive correlation with weight of florets per spike (0.256), yield of florets per plot (4 * 1 m) (0.178), number of spikes per m^2 (0.100) and length of the floret (0.094). These results are in conformity with the findings of Prabhat Kumar *et al.* (2011) in gladiolus. Length of the floret exhibited positive and significant association with weight of florets per spike (0.430), number of spikes per m^2 (0.236) and yield of florets per plot (4 * 1 m) (0.225). The weight of florets per spike showed significant and positive correlation with yield of

florets per plot (4 * 1 m) (0.864) and number of spikes per m^2 (0.856). Positive and significant association was also observed for number of spikes per m^2 with yield of florets per plot (4 * 1 m) (0.752). This is in accordance with the findings of Gurav *et al.* (2005).

In the double genotypes, the genotypic and phenotypic correlation coefficients were computed in all possible combinations for twelve characters and are presented in Tables 51 and 53. Correlation coefficient analysis measures the mutual relationship between various plant characters and determines the component characters on which selection is based for genetic improvement for a particular character (Robinson *et al.,* 1949). A positive correlation between desirable characters is favorable to the plant breeder because it helps in simultaneous improvement of both the characters. In the present study, genotypic correlation coefficients were found to be higher than phenotypic correlation coefficients for most of the characters, indicating a strong inherent association between various characters and were masked by environmental component with regard to phenotypic expression. Similar results were obtained by Singh (2011) in antirrhinum. The study showed a highly significant and positive correlation between days taken for sprouting of bulb with days to spike emergence (0.671). This trait however, showed negative correlation with flowering duration (-0.978), number of leaves per plant (-0.954), number of spikes per m^2 (-0.952), plant height (-0.939), spike length (-0.927), weight of florets per spike (-0.874), yield of florets per plot (4 * 1 m) (-0.803), rachis length (-0.802), length of the floret (-0.761) and number of florets per spike (-0.736).

Highly significant and positive correlations for plant height was observed with spike length (1.001), yield of florets per plot (4 * 1 m) (0.975), number of florets per spike (0.971), number of leaves per plant (0.958), flowering duration (0.944), weight of florets per spike (0.904), number of spikes per m^2 (0.695), rachis length (0.652) and length of the floret (0.623). The trait however, showed negative correlation with days to spike emergence (-1.574). Prabhat Kumar *et al.,* (2011) did similar studies and reported significant and positive association of plant height with spike length in gladiolus. Further the number of leaves per plant had highly significant relationship with weight of the florets per spike (0.994), yield of florets per plot (4 * 1 m) (0.987), number of florets per spike (0.981), spike length (0.965), flowering duration (0.941), length of the floret (0.730),

number of spikes per m^2 (0.665) and rachis length (0.438). The trait however, showed negative correlation with days to spike emergence (-1.759). Similar findings were also reported by Vetrivel. (2010) and Prabhat Kumar *et al*. (2011) in gladiolus.

Days to spike emergence in double types showed highly significant but negative correlation with flowering duration (-1.706), spike length (-1.518), yield of florets per plot (4 * 1 m) (-1.430), number of spikes per m^2 (-1.418), weight of florets per spike (-1.406), rachis length (-1.384), length of the floret (-2.136) and number of florets per spike (-1.276). This is line with the findings of Prabhat Kumar *et al*. (2011) in gladiolus for spike length and weight of florets per spike.

Positive and significant association for flowering duration was observed for spike length (0.944), yield of florets per plot (4 * 1 m) (0.899), weight of florets per spike (0.875), number of spikes per m^2 (0.862), number of florets per spike (0.859), length of the floret (0.851) and rachis length (0.695). This is in line with the findings of Rakesh Kumar *et al.* (2012) in snapdragon. The spike length showed highly significant and positive correlation with yield of florets per plot (4 * 1 m) (0.974), number of florets per spike (0.967), weight of florets per spike (0.905), number of spikes per m^2 (0.696), rachis length (0.653) and length of the floret (0.604). This is in consonance with the findings of Rakesh Kumar *et al.* (2012) in snapdragon. There exists a positive and highly significant relationship of rachis length with number of spikes per m^2 (0.742), length of the floret (0.566), yield of florets per plot (4 * 1 m), number of florets per spike (0.481) and weight of florets per spike (0.347). Similar such findings were reported by Rakesh Kumar *et al.* (2012) in snapdragon. Positive and significant association was observed for number of florets/ spike with yield of florets per plot (4 * 1 m) (0.996), weight of florets per spike (0.950), number of spikes per m^2 (0.500) and length of the floret (0.489). These results are in conformity with the findings of Prabhat Kumar *et al*., (2011) in gladiolus. Length of the floret exhibited positive and significant association with number of spikes per m^2 (0.918), weight of florets per spike (0.630) and yield of florets per plot (4 * 1 m) (0.561). In genotypic and phenotypic levels, weight of florets per spike exhibited positive relationship with yield of florets per plot (4 * 1 m) (0.965) and number of spikes per m^2 (0.534).

Positive and significant association was also observed for number of spikes/ m^2 with yield of florets per plot (4 * 1 m) (0.565). This is in consonance with the findings of Prabhat Kumar *et al*., (2011) in gladiolus for number of spikes/ m^2 .

5.10. Path coefficient analysis

An association between two traits is not a simple relationship but it is rather product of direct and indirect causes. Correlation co-efficient measures only the extent of association between any two characteristics involved in the complicated pathway leading to the end point. Therefore, significant correlation is no proof of direct or casual relationship. Indirect association becomes more complex and perplexing as more and more variables are considered. This correlation co-efficient together with path co-efficient values will be more useful in finding out character association.

A dependent character like yield of florets per plot and number of spikes/ m^2 is controlled by several component characters, which are interrelated in a complex manner and these mutual association will influence the net association observed between a component and yield and a change in any one of the component is likely to disturb the whole network of cause and effect relationship. So each component has two paths of action like direct effect on length of rachis and indirect effects through other components, which are not revealed from the mere correlation studies.

Path analysis is useful in unraveling these two effects and was first suggested by Wright (1921) and subsequently elaborated by Li (1956). This is simply a standardized partial regression analysis, which is based on cause and relationship, which serves to analyse by sub dividing correlation in a causal scheme. Dewey and Lu (1959) were perhaps the first to adopt this technique in the determination of yield components in crested wheat grass and they demonstrated the utility of this method in plant selection. Since then, it is being extensively utilized by plant scientists so as to get a clean picture of association of various plant characters.

In the present investigation on single types, it was observed that the maximum positive direct effect on flower yield was contributed by weight of florets per spike (0.6380) followed by number of leaves per plant (0.5784), flowering duration (0.3746), rachis length (0.2776), days taken for sprouting of bulb (0.2460), length of the floret

(0.2286) and days to spike emergence (0.0203). Number of spikes per m^2 (-0.0511), spike length (-0.3784), plant height (-0.3190) and number of florets per spike (-0.2077) showed negative direct effect on flower yield among the single types. Similar such findings were reported by Saravanakumar (2000) in tuberose for days to spike emergence and length of the floret .

Days taken for sprouting of bulb recorded strong positive indirect effect through days to spike emergence (0.1827) and weight of florets per spike (0.0087). Whereas the other characters exerted a negative indirect effect. The contribution of indirect effect of plant height through days to spike emergence (0.1775) and weight of the florets per spike (0.0302) was positive while its influence through other characters was negative. The number of leaves per plant recorded a strong positive indirect effect through number of florets per spike (0.5626), plant height (0.5140), rachis length (0.4974), number of spikes per m^2 (0.3593), spike length (0.3262) and flowering duration (0.2995) whereas other characters exerted negative indirect effect. This is in consonance with the findings of Saravanakumar (2000) in tuberose. Similarly, days to spike emergence recorded strong positive indirect effect through days to sprouting of bulb (0.0150), whereas the other characters exerted a negative indirect effect. The contribution of indirect effect of duration of flowering through number of florets per spike (0.2618), plant height (0.2506), spike length (0.2466), rachis length (0.2113), number of leaves per plant (0.2506), length of the floret (0.1016), weight of the florets per spike (0.0348) and number of spikes per m^2 (0.0118) was positive while its influence through other characters was negative. This is line with the findings of Saravanakumar (2000) in tuberose.

Spike length recorded strong positive correlation with days taken for sprouting of bulb (0.3492), days to spike emergence (0.3310) and number of leaves per plant (0.2134). Whereas the other characters exerted a negative indirect effect. This is in accordance with the results of Vetrivel (2010) who reported that marketable spikes had positive direct effect on days to spike emergence. The contribution of indirect effect of rachis length through number of florets per spike (0.2822), number of leaves per plant (0.2387), plant height (0.1990), spike length (0.1927), number of spikes per m^2 (0.0720), length of the floret (0.0254) and weight of the florets per spike (0.0186) was positive while its influence through other characters were negative. Similar results were obtained by Misra

and Saini (1990) and Hegde (1994) who have indicated a weak negative correlation between length of rachis and number of florets per spike. However, the findings of Sandhu *et al.* (1990) who observed high positive correlation between length of rachis and number of florets per spike and similar observations were also made by Basavaraddy (2004).

The number of florets per spike recorded strong positive indirect effect through days taken for sprouting of bulb (0.1685), days to spike emergence (0.1138), weight of the florets per spike (0.0015). Whereas the other characters exerted a negative indirect effect. The contribution of indirect effect of floret length through spike length (0.1448), weight of florets per spike (0.1376), flowering duration (0.0630), plant height (0.0537), number of florets per spike (0.0343) and rachis length (0.0209) was positive while its influence through other characters was negative. The weight of florets per spike recorded strong positive indirect effect through flowering duration (0.0592), rachis length (0.0428), length of the floret (0.3841), number of spikes per m^2 (0.3614), days taken for sprouting of bulb (0.0592) and spike length (0.1397). Whereas the other characters exerted a negative indirect effect. The contribution of indirect effect of number of spikes per m^2 through days taken for sprouting of bulb (0.1065) and spike length (0.0933) was positive while its influence through other characters was negative. Similar such findings were reported by Saravanakumar (2000) in tuberose for days taken for sprouting of bulb.

In double types, the maximum positive direct effect on number of spikes per m^2 was contributed by weight of florets per spike (0.6378) followed by number of leaves per plant (0.5782), flowering duration (0.3756), rachis length (0.2779), days taken for sprouting of bulb (0.2580), length of the floret (0.2277) and days to spike emergence (0.0213). Yield of florets/ plot (4 * 1 m) (-0.0518), spike length (-0.3784), plant height (-0.3297), number of florets per spike (-0.2078) showed negative direct effect on yield and similar observations were also made by Saravanakumar (2000) in tuberose for spike length and plant height.

Days taken for sprouting of bulb recorded strong positive indirect effect through days to spike emergence (0.1838) and weight of florets per spike (0.0088). Whereas the other characters exerted a negative indirect effect. The contribution of indirect effect of plant height through days to spike emergence (0.1785) and weight of the florets per spike

(0.0312) was positive while its influence through other characters was negative. The number of leaves per plant recorded a strong positive indirect effect through number of florets per spike (0.5627), plant height (0.5242), rachis length (0.4984), number of spikes per m^2 (0.3592), spike length (0.3272) and flowering duration (0.2998) whereas other characters exerted negative indirect effect. Days to spike emergence recorded strong positive indirect effect through days to sprouting of bulb (0.0152), whereas the other characters exerted a negative indirect effect. The contribution of indirect effect of duration of flowering through number of florets per spike (0.2629), plant height (0.2608), spike length (0.2467), rachis length (0.2123), number of leaves per plant (0.1941), length of the floret (0.1055), weight of the florets per spike (0.0349) and number of spikes per m^2 (0.0119) was positive while its influence through other characters was negative. This is in consonance with the findings of Saravanakumar (2000) in tuberose.

Spike length recorded strong positive indirect effect through days taken for sprouting of bulb (0.3429), days to spike emergence (0.3320) and number of leaves per plant (0.2143). Whereas the other characters exerted a negative indirect effect. The contribution of indirect effect of duration of rachis length through number of florets per spike (0.2823), number of leaves per plant (0.2388), plant height (0.1994), spike length (0.1932), number of spikes per m^2 (0.0725), length of the floret (0.0249) and weight of the florets per spike (0.0187) was positive while its influence through other characters was negative. The number of florets per spike recorded strong positive indirect effect through days taken for sprouting of bulb (0.1676), days to spike emergence (0.1149) and weight of the florets per spike (0.0014). Whereas the other characters exerted a negative indirect effect. The contribution of indirect effect of floret length through spike length (0.1457), weight of florets per spike (0.1377), flowering duration (0.0632), plant height (0.0538), number of florets per spike (0.0344) and rachis length (0.0212) was positive, while its influence through other characters was negative. The weight of florets per spike recorded strong positive indirect effect through flowering duration (0.0693), rachis length (0.0437), length of the floret (0.3851), number of spikes per m^2 (0.3615), days taken for sprouting of bulb (0.0243) and spike length (0.1399). Whereas the other characters exerted a negative indirect effect and similar observations were also made by Saravanakumar (2000) in tuberose.

It could be inferred from the results of genotypic correlation coefficient and path analysis in both single and double types that the characters *viz.,* weight of florets per spike, number of leaves per plant, flowering duration, rachis length, days taken for sprouting of bulb, length of the floret and days to spike emergence had significant positive correlation co-efficients and positive direct effects on yield which formed reliable indices for selection of genotypes for yield.

In the present study, residual effect made a marked contribution in both single and double types (0.174, 0.182) in determining the flower yield indicating some characters other than those included in present path co-efficient study might also play an important role in determing the total yield. Similar results of residual path value was reported by Saravanakumar (2000) in tuberose.

5.11. Mahanalobis D^2 analysis

The qualification of genetic diversity has made it possible to choose genetically diverse parents for a successful hybridization program. The D^2 technique based on multivariate analysis developed by Mahalanobis (1936) is the most effective method for quantifying the degree of genetic diversity among genotypes, which helps in selecting the parents for hybridization. In plant breeding, genetic diversity plays an important role because hybrids between genetically diverse parents manifest greater heterosis than those between closely related parents (Ramanujam, 1974 and Singh and Sharma, 1989). The scope for improvement in a crop is dependent on the genetic diversity present in available germplasm.

In the present study twelve characters were analysed. Analysis of variance showed significant differences among the types for all the characters indicating that the genotype varied significantly in respect of all the characters. The variability among the types are also confirmed by D^2 analysis, wherein ten single genotypes were grouped into four clusters and five double genotypes in three clusters indicating wide genetic diversity in the present material.

Among the four clusters in single types, cluster I was the largest with four genotypes followed by cluster IV had three types, cluster II had two genotypes and cluster III had only one genotype. In double types, cluster I had three genotypes and cluster II and III had only one genotype from the same geographical area and hence they

were not grouped into different clusters. In the present study, all clusters had genotypes from different geographical origin indicating factors other than geographical diversity might be responsible for such grouping. Moll *et al.*, 1962 regarded eco-geographical diversity as a reasonable index of genetic divergence. But eco-geographical diversity might be an inferential criterion which obviously could not always be used for the discrimination among the populations inhibiting the similar agroclimatic regions. Moreover, geographic distribution and genetic diversity as estimated by D^2 statistic need not be directly related (Murthy and Arunachalam, 1966).

Though the types chosen for the present study were from different geographical sources, they got themselves grouped in to four clusters only. This might be due to the exchange of breeding materials from one place to another (Verma and Mehta, 1976).

5.11.1. Intra and Inter cluster distance

The intra and inter cluster distance indicates that the crossing of genotypes between these four clusters in single types and three clusters in double types with high genetic divergence might result in a high degree of heterosis (Moll *et al.,* 1962).

A considerable divergence among Calcutta, Andhra Pradesh, Karnataka, Maharashtra and Gujarat types distributed into different clusters in both single and double types showed varying degree of inter cluster genetic divergence which might be desirable for heterosis breeding.

5.11.2. Cluster means for different characters in tuberose

In single types, cluster I had the lowest cluster mean values for plant height (30.62), number of leaves per plant (19.00), days to spike emergence (20.97), flowering duration (16.06), number of florets per spike (29.00) and floret length (4.06). The cluster II possessed high mean values for plant height (45.69), number of leaves per plant (27.83), spike length (128.10), number of florets per spike (36.58), floret length (6.62), weight of florets per spike (0.98), number of spikes per m^2 (1.82), yield of florets per plot (4 * 1 m) (36.18) while it had the lowest mean values for days taken for sprouting of bulb (12.24), days to spike emergence (3.19), flowering duration (18.76) and rachis length (20.13). Similarly, cluster III showed the high mean values for days taken for sprouting

of bulb (16.25), days to spike emergence (43.28) and low mean values for weight of florets per spike (0.72), number of spikes per m^2 (1.08) and yield of florets per plot (4 * 1 m) (21.63). Likewise, cluster IV possessed high mean values for flowering duration (22.06), rachis length (22.36) and low value for spike length (92.53) (Fig. 11).

Among the four clusters, cluster II has got high genetic divergence and could be useful in breeding programme. The genotypes from this cluster showed minimum flowering duration (18.76), days taken for sprouting of bulb (12.24) and days to spike emergence (3.19). This could be utilized in breeding for a short duration crop and for high yield. Similar such findings were reported by Saravanakumar (2000) in tuberose.

In double types, Cluster I had high mean values for flowering duration (22.08) and rachis length (20.36) and low mean values for plant height (32.63), number of leaves per plant (15.00), spike length (22.46), number of florets per spike (27.00) and floret length (4.01). The cluster II possessed high mean values for plant height (43.69), number of leaves per plant (24.73), spike length (126.15), number of florets per spike (35.48), floret length (6.32), weight of florets per spike (0.86), number of spikes per m^2 (1.81), yield of florets per plot (4 * 1 m) (35.28) while it was low for days taken for sprouting of bulb (10.34) and days to spike emergence (2.91). Cluster III showed the high mean values for days taken for sprouting of bulb (15.32), days to spike emergence (41.28) and low mean values for flowering duration (12.98), rachis length (13.46), weight of florets per spike (0.72), number of spikes per m^2 (1.08) and yield of florets per plot (4 * 1 m) (19.62) (Fig.12).

As in single types, the cluster II has got high genetic divergence and could be useful in breeding programme. The genotypes from cluster showed minimum days taken for sprouting of bulb (10.34) and days to spike emergence (2.91). This could be utilized in breeding for a short duration crop and for high yield. This is in consonance with the findings of Saravanakumar (2000) in tuberose.

5.11.3. Relative Contribution of each character

The results indicated that in single types of tuberose, yield of florets per plot (4 * 1 m) had contributed the maximum (36.25 %) towards genetic divergence, followed by weight of florets per spike (28.84 %). In double types too, the same trait contributed

maximum (35.67 %) towards genetic divergence, followed by weight of florets per spike (24.54 %). This is in consonanace with the findings of Vairavan *et al.* (1973). Further, yield of flowers per plot showed high heritability.

Based on the mean performance and genetic divergence the genotypes Prajwal (Single), Suvasini (Double) have been identified for commercial cultivation. In hybridization, among the ninety crosses made between ten single types, only fourteen crosses found to be successful as evidenced through seed set. These fourteen F1 hybrid progenies are slow growing and takes longer duration for attaining maturity (Plate 43 a, b and c). These progenies are now in initial vegetative stage. They have to be evaluated in future to assess the growth, yield, quality parameters and also its resistance to nematodes. For remaining 76 unsuccessful crosses, the techniques viz., shortening of the style, stylar pollination and the In-ovulo embryo culture/ embryo rescue have to be attempted in future to overcome the incompatibility barriers.

CHAPTER VI

SUMMARY

The present investigation on "Evaluation of tuberose genotypes for development of F_1 hybrids for quantitative, qualitative characters and nematode resistance" was carried out at the Department of Floriculture and Landscaping, Tamil Nadu Agricultural University, Coimbatore between 2011 – 2013. The experiment was conducted in Randomized Block Design with three replications using ten single types and five double types of tuberose. Studies on pollen viability, *in vitro* pollen germination, *in vivo* pollen germination, seed setting behaviour of tuberose genotypes after artificial selfing and crossing, post fertilization, seed germination and screening studies on tuberose genotypes against root knot nematode were carried out. The variables were assessed based on the performance, while the traits were examined for genotypic variability, genotypic / phenotypic correlation and path analysis of yield attributing traits. The salient findings of the research work are summarized below:

⇒ Fourteen crosses (Variegated Single x Calcutta Single, Variegated Single x Kahikuchi Single, Variegated Single x Mexican Single, Variegated Single x Navsari Local Single, Variegated Single x Pune Single, Variegated Single x Phule Rajani, Phule Rajani x Kahikuchi Single, Phule Rajani x Mexican Single, Phule Rajani x Hyderabad Single, Phule Rajani x Variegated Single, Shringar x Kahikuchi Single, Shringar x Variegated Single, Hyderabad Single x Variegated Single, Navsari Local x Variegated Single) were found to be compatible, in which the pollen tube successfully reached the ovule and seed set observed in seven days after crossing.

⇒ The pollen grain of Shringar (185.81microns) recorded the maximum equivalent diameter followed by Navsari Local (161.94 microns).

⇒ The modified Brewbaker and Kwack's medium resulted in good pollen germination. It was modified by substituting 20 per cent of sucrose and 200 ppm of boric acid instead of 15 per cent of sucrose and 100 ppm boric acid.

⇒ The pollen tube length varied from 4.73 and 118.85 microns within one hour after incubation in single types. The same trend was observed with respect to 5, 10, 15, 20 and 25 hours after incubations with the range of 16.95 to 520.43; 26.68 to 839.33; 49.81 to 1020.63; 76.72 to 1234.95 and 78.25 to 1292.64 microns, respectively. In general, the pollen tube was significantly longer in Variegated Single when compared to other genotypes.

⇒ For microscopic observations of pistils 3 hours of softening period in 8 N NaOH was required for tuberose genotypes. The optimum staining and better fluorescence could be obtained when 0.1 per cent aniline blue was dissolved in 0.3 N K_3PO_4

⇒ All the genotypes in the present study were self- incompatible and recorded low level of pollen germination on the stigma.

⇒ Various abnormalities such as knot like structure at the tip of the pollen tube, twisted and bulged tips, bursting at the tip of pollen tube were noticed in all the incompatible crosses

⇒ Though the present seed germination in tuberose is low, seeds treated with GA_3 250 ppm for 8 hours (T_3) recorded improved highest germination (12.50 %) and it was followed by the treatment T_7 (KNO_3 0.5 per cent for 8 hours) which registered a germination of 12.45 per cent. The minimum germination (7.96 %) was recorded in the treatments T_{17} and T_{18} (water soaking for 8 and 16 hours) respectively.

⇒ The results of the quick viability (tetrazolium) test expressed that the concentration of 0.25 % 2,3,5-triphenyl tetrazolium chloride was found optimum to test the viability of seeds

⇒ The screening of both single and double tuberose genotypes exhibited significant variation for all the traits studied under tropical condition. Among the genotypes, the bulbs of Prajwal (Single) and Suvasini (double) sprouted early (12.12, 12.32 days).

⇒ Prajwal (Single) and Suvasini (Double) have shown their superiority with regard to growth traits like plant height (113.05, 86.25 cm, respectively), number of leaves per plant (260.00, 270.00, respectively) at three months after planting .

⇒ The genotypes Prajwal (Single) and Suvasini (Double) took minimum days for spike emergence (78, 84 days) while Calcutta Single and Hyderabad Double took maximum days for spike emergence (94, 89 days), respectively.

⇒ Variegated Single and Suvasini (Double) showed increased spike length (102.50, 71.25 cm) over the other genotypes.

⇒ Pune Single and Vaibhav (Double) exhibited increased rachis length (35.75, 54.00 cm) over the other genotypes.

⇒ Prajwal (Single) and Suvasini (Double) were found to be the best in respect of flowering traits *viz*., number of florets/ spike (47.00, 54.00 nos.), length of the floret (6.40, 7.50 cm), weight of florets per spike (74.80, 146.88 g), number of spikes/m^2 (47.00, 34.10 nos.) and yield of florets/ plot (4 * 1 m) (4.40, 3.42 kg).

⇒ Increased concrete recovery (0.16 and 0.09 %) and vase life (12.97,12.40 days) were recorded in the genotypes Prajwal (Single) and Suvasini (double).

⇒ High heritability coupled with high genetic advance as per cent of mean were observed for flowering duration (99.07, 73.04), weight of florets per spike (99.02, 66.15), number of florets per spike (98.72, 65.08) and rachis length (98.47, 54.46) in single types while in double types it was observed for number of florets per spike (98.35, 69.81), number of spikes/m^2 (96.93, 65.78), rachis length (96.91, 45.18) and yield of florets per plot (95.78, 40.20).

⇒ High estimates of phenotypic and genotypic co-efficient of variation were observed for flowering duration (35.79, 35.62) followed by weight of florets per spike (32.43, 32.27) and number of florets per spike (32.01, 31.80) in single types while in double types it was observed for number of florets/ spike (24.59, 24.38) indicating that these characters are under genetic control. Hence, these characters can be relied upon selection for further improvement.

⇒ In single types, positive and significant association was observed for number of florets/ spike with weight of florets per spike (0.256), yield of florets per plot (4 * 1 m) (0.178), number of spikes per m^2 (0.100) and length of the floret (0.094) at genotypic level. Similarly positive and significant association was observed for

number of florets/ spike with yield of florets per plot (4 * 1 m) (0.996), weight of florets per spike (0.950), number of spikes per m^2 (0.500) and length of the floret (0.489) in double types.

⇒ Path analysis showed that the weight of florets per spike (0.6380), number of leaves per plant (0.5784), flowering duration (0.3746), rachis length (0.2776), days taken for sprouting of bulb (0.2460), length of the floret (0.2286) and days to spike emergence (0.0203) found to be the dominating contributors towards the flower yield in single types while in double types, the traits *viz.,* weight of florets per spike (0.6378), number of leaves per plant (0.5782), flowering duration (0.3756), rachis length (0.2779), days taken for sprouting of bulb (0.2580), length of the floret (0.2277) and days to spike emergence (0.0213) found to be the dominating contributors towards the number of spikes per m^2.

⇒ A wide genetic diversity have been observed through the D^2 analysis among the genotypes. The ten single genotypes were grouped into four clusters and five double genotypes into three clusters. The varieties from IIHR, Bangalore (Shringar and Prajwal) in single types and (Suvasini and Vaibhav) in double types have been grouped under different clusters.

⇒ Cultivars chosen from the same ecographic region (IIHR, Bangalore) were found scattered in different clusters in both single and double types indicating the presence of wide genetic variability among these types.

⇒ The D^2 analysis revealed that the yield of florets per plot (4 * 1 m) contributed the maximum in both single and double types (36.25, 35.67 %) towards genetic divergence.

⇒ In screening studies against nematode, the genotype Kahikuchi Single was categorised under ‘moderately resistant’ (MR), while Calcutta Single, Hyderabad Single and Mexican Single were categorised under ‘susceptible’ (S). Whereas Navsari Local, Phule Rajani, Prajwal, Pune Single, Shringar and Variegated Single were brought under ‘highly susceptible’ group.

⇒ Based on the mean performance and genetic divergence the genotypes Prajwal (Single), Suvasini (Double) have been identified for commercial cultivation. In hybridization, among the ninety crosses made between ten single types, only fourteen crosses found to be successful as evidenced through seed set. These fourteen F_1 hybrid progenies are slow growing and takes longer duration for attaining maturity. These progenies are now in initial vegetative stage. They have to be evaluated in future to assess the growth, yield, quality parameters and also its resistance to nematodes. For remaining 76 unsuccessful crosses, the techniques *viz.,* shortening of the style, stylar pollination and the In-ovulo embryo culture/ embryo rescue have to be attempted in future to overcome the incompatibility barriers.

REFERENCES

Abad, P., B. Favery, M. Rosso and P. Castagnone- Sereno. 2003. Root- knot nematode parasitism and host response: molecular basis of a sophisticated interaction. **Mol. Plant. Path.,** 4: 217-224.

Abdul- Baki, A.A. and J.D. Anderson. 1973. Vigour determination in soybean seed by multiple criteria. **Crop Sci**., 13: 360- 363.

Abraham, V. and B.M. Desai, 1976. Radiation induced mutants in tuberose. **Indian J. Genetics and Plant Breeding**. 36: 375-377.

Acedo, J.R. and R.A. Rohde. 1971. Histochemical root pathology of *Brassica oleracea* var. Capitata L. Infected by Pratylenchus penetrans (Cobb) Filipjev and Schuurmans Stekhoven. **J. Nematol.,** 3:62-68.

Adegbite, A.A ., N.A. Amusa, G.O. Agbaje and L.B. Taiwo. 2006. Screening cowpea varieties for reaction to root-knot nematode under field conditions. **Veg. Sci**., 12: 21-27.

Ahmadi, A.R. and A.M. Bac. 2005. Reaction of some tomato cultivars to root-knot nematode (*Meloidogyne javanica*). **Iranian J. Plant Path**., 41 (3): 403- 414.

Anandhi. 2012. Enhancement of variability in *Gloriosa spp*. through mutation and hybridization techniques. Ph. D (Hort.) Thesis. Tamil Nadu Agricultural University, Coimbatore.

Anita, S., H. Akhtar and A. Syed. 2006. Screening of field pea (*Pisum sativum*) selections for their reactions to root- knot nematode (*Meloidogyne incognita*). **J. Zhejiang Univ Sci.,** 7(3): 209- 214.

Anonymous, 2000b. Annual Report. The AICRP on Floriculture. ICAR, New Delhi. pp. 232- 235.

Anonymous, 2000c. Annual Report. The AICRP on Floriculture. ICAR, New Delhi. pp. 235- 237.

Anonymous. 2008. Gladiolus **All India co-ordinated Research Project**.. pp. 71-76.

Anuradha, S. and J.V.N. Gowda. 2000. Path coefficient analysis in gladiolus. **Proc. national conference on Gladiolus, January**, p. 92.

Anwar, S.A. and G.M. Din. 1986. Nematodes: Biotic constraints to plant health. **Proc. Parasitol**., 3: 48- 53.

Arasu, N.N. 1968. Self-incompatibility in angiosperms: a review. **Genetica,** 39: 1-24.

Arisumi, T. 1975. Effect of pollination, temperature on seed yield of crosses of day lily. **Hort. Sci.,** 10: (4) 375- 376.

Ascher, P.D. 1978. Localization of the self and the interspecific incompatibility reaction in style sections of *Lilium longiflorum*. **Pl. Sci. Letters**, 10: (2): 199- 203.

Ascher, P.D. and S.J. Peloquin. 1968. Pollen tube growth and in-compatibility following intra and interspecific pollinations in *Lilium longiflorum*. **Am. J. Bot**., 55: 1230- 1234.

ASTA 1960. Official analytical methods of the American Spice Trade Association, New York, pp. 41-42.

Aswath, C. 1985. Reproductive biology, floral biology and propagation studies in some climbers of bignoniaceae. M.Sc., Thesis submitted to U.A.S., Bangalore.

Aswath, C., J.V. Narayana Gowda, and Shyamsunder Joshi. 1990. Studies on the floral biology and pollen fertility in ornamental climbers. **Mysore J. Agric. Sci**., 24: 482- 483.

Aswath, C., Narayana Gowda, J.V. and Shyamsunder Joshi. 1989. Histochemical studies on anther and ovule development in *Pyrostegia venusta* Lindee. **Mysore J. Agric. Sci**., 23: 497- 500.

Austin, R.B. 1972. **Effect of environment before harvesting on viability**. In: 'Viability of Seeds' Chapman and Hall Ltd., London. pp.115-149.

Babeley, G.S. and A.K. Kandya. 1986. Use of TTC for rapid testing of the viability of *Lagerstroemia parviflora* seeds. **J. Trop. For**., 2(3): 226-231.

Bailey, L. H. 1919. The Standard Cyclopedia of Horticulture. 3rd ed. MacMillan, London.

Bailey, L. H. 1930. *Polianthes*. The Standard Cyclopedia of Horticulture. MacMillan Publishing Co. Inc., New York. 3 pp. 2731-33.

Bajaj, K.L., P.Singh and R. Mahajan. 1985. Changes induced by *Meloidogyne incognita* in Super oxidase dismutase, peroxidase and polyphenol oxidase activity in tomato roots. **Biochem. Physiol. Pflanz.,** 180: 543- 546.

Bajaj, K.L., Y.K. Arora and R. Mahasan. 1983. Biochemical differences in tomato cultivars resistant and susceptible to *Meloidogyne incognita.* **Rev. Nematol.,** 6(1): 143- 154.

Balamurugan. K., Rengaswamy, P. and Arumugam, T. 2002. Variability studies in gladiolus**. J. Ornamental Hort., 5(1):** 38-39.

Balasubramanian, M. and D. Purusothaman. 1972. Phenolic contents of root-knot affected tissues.**Indian J. Nematol.,** 2: 77-79.

Banker, G.J. and A. Mukhopadhyay. 1980. Varietal trail on tuberose (*Polianthes tuberosa* L.). **South Indian Hort.,** 28(4): 150- 151.

Bar- Shalom, D. And O. Mattsson. 1977. Mode of hybridization as an important factor in the germination of trinuclear pollen grains. **Bot. Tiddskr**., 71: 251- 254.

Barkar, G.J. 1980. Effect of seed treatments with gibberellic acid on germination of winter annuals. **South Indian Hort., 28**(2) : 60-61.

Barnett, H.L. 1959. Plant disease resistance. **Rev. Microbiol.,** 13:191-210.

Basavaraddy, M., 2004. Evaluation of elite hybrids of gladiolus for cut flower production under transition tract of Karnataka. M.Sc.(Agri.) Thesis, University of Agricultural Sciences, Dharwad.

Bateman, A. J. 1954. Self- incompatibility systems in angiosperms. *Iberis amara*. **Heredity,** 8: 305- 332.

Bawa, K.S. and C.J. Webb. 1984. Flower fruit and seed abortion in tropical forest trees: Implication for evolution of paternal and maternal reproductive patterns. **Am. J. Bot**., 71: 736 – 751.

Bell, A.A. 1981. Biochemical mechanisms of disease resistance. **Ann. Rev. Plant Physiol**., 32: 21-88.

Belorkar, P. V., B. N. Patil, B. S. Dhumal, V. J. Golliwar and S. D. Dalal. 1993. Effect of N-levels and GA_3 on growth, flowering and yield of tuberose. **J. Soils and Crops, 3(2):** 102-108.

Benschop, M. 1993. Polianthes. In: De Hertogh, A., Le Nard, M. (Eds.), The physiology of flower bulbs. **Elsevier,** Amsterdam, The Netherlands, pp. 589-601.

Bhat, B.K and A.K.Dhar.1971. Seed dormancy in Atropa belladonna. **Indian J. Agric. Sci**., **49(9):** 761-764.

Bhattacharjee, S.K., L.P. Yadav and T.P. Mukhopadhay. 1979. Effect of bulb size, planting depth and spacing on tuberose(*Polianthes tuberosa* L.). **The Lal Bagh**, 24(4): 24- 29.

Bichoo, G. A., John, A. O. and Wani, S. A. 2002. Genetic variability in some quantities characters of gladiolus. **J. Ornamental Hort., 5(1)**: 22-24.

Binks, R.H. and G.R. Gowen. 1997. Eraly screening of banana plantain varieties for resistance to *Radopholus similes.* **Inter. J. Nematol**., 7(1): 57- 61.

Blamowski, Z.K. and E. Borowski. 1995. Effects of long chairm aliphatic alcohols on seed germination and seedlings growth of *Allium crpa* L. and *Zinnia elegans* Jacq. **Seed Abstr., 20** (8) : 351.

Boiteux, L.S. and J.M. Charechar. 1996. Genetic resistance to root knot nematode (*Meloidogyne javanica*) in egg plant (*Solanum melongena*). **Plant Breed**., 115 (3): 198-200.

Borah, A. and P. N. Phukan. 2000.Effect of VAM fungus, *Glomus fasciculatum* and root-knot nematode, *Meloidogyne incognita* on brinjal. **J. Agrl. Sci. Soc.North-East India, 13: 212- 214.**

Bouton, J. H., R.S. Hussey and S.R. Smith. 1989. Greenhouse screening of alfalfa cultivars for resistance to southern root- knot nematode. **Crop Sci**., 29: 823- 825.

Bowles, D. J., S.Gurr, S. Scollan, H.J. Atkinson and K. Hammond- Kosack. 1991. **Local and systemic changes in plant gene expression**. pp. 225-236 in C. J. Smith, ed. Biochemistry and molecular biology of plant- pathogen interactions. Oxford, UK: Oxford University Press.

Bradley, D.J., P. Kjellborn and C. Lamb. 1992. Elicitor and wound induced oxidative cross linking of plant cell wall proline-rich protein- a novel rapid defense response. **Cell**, **70**: 21-30.

Brandham, P.E. and S.I. Owens. 1978. The genetic control of self- incompatibility in the genus *Gasteria* (Liliaceae). **Heredity**, 40: 165- 169.

Bray, H.G. and W.V. Thrope. 1954. Analysis of phenolic compounds of interest in metabolism. Meth. Biochem. **Ana**l., 1: 27- 52.

Brewbaker, J.L. 1957. Biology of the angiosperm pollen grain. **Indian J. Genet. Pl. Breed**, 19: 121- 138.

Brewbaker, J.L. 1959. Biology of the angiosperm pollen grain. **Indian J. Genet. Pl. Breed**, 19: 121- 133.

Brewbaker, J.L. and B.K. Kwack. 1963. Essential role of calcium in pollen germination and pollen tube growth. **Am. J. Bot**., 50: 747- 858.

Brock, R.D. 1954. Fertility in Lilium hybrids. **Heredity**. 8: 409- 420.

Brudrant, L.A. 1985. Herbertia. 41: 55-60.

Brueske, C.H. 1980. Phenylalanine ammonia-lyase activity in tomato roots infected and resistant to the root- knot nematode, *Meloidogyne incognita.* **Physiol. Plant Path.,** 16: 409-414.

Burton, G.W. 1952. Quantitative inheritance in grasses. **Proc. 6th Intl. Grassld. Congr., 1:** 227-283.

Burton, G.W. and E.R. Devane. 1953. Estimating heritability in Tall fescue (*Festuca arundinaceae*) from replicated clonal material. **Agron. J**., 45: 478- 481.

Castagnone-Sereno, P. 2002.Genetic brinjal onto the rootstock of *Solanum torvum* to save it from root knot nematodes. **Indian Hort., 32: 31-32.**

Chakrabarti, U. And S.D. Mishra. 2002. Evaluation of biochemical parameter for screening resistance of chickpea cultivars against *Meloidogyne incognita.* **Indian J. Nematol**., 32 (1): 26-29.

Chakrabarty, S. 2001. Intergrated management of root- knot nematode in brinjal. **Indian J Nematol.**, 31: 79- 98.

Chandler, P.M. and M. Robertson. 1994. Gene expression regulated by abscisic acid and its relation to stress tolerance. **Ann. Rev. Plant Physiol. Plant. Mol. Biol.,** 45: 113-141.

Cho, M.R., J.Y. Kim, C. Song, J.Y. Ko, S.Y. Na and M.S. Yiem. 1996. Screening of carnation cultivars for resistance to *Meloidogyne incognita.* **Journal of Nematology** 28(4S): 639- 642.

Cneudt, G. and G. Boseman. 1983. Effect of gibberellic acid and chlormequat on the growth and flowering of *Celosis argentea* var. *Cristata* (L.) **Rijks universiteit Gent., 48**(1) : 79-83.

Cobb, N.A. 1918. Estimating the nematode population of soil. U.S. Department of Agricultural Technology Circular, 1: 1-48.

Constabel, C.P., D.R. Bergery and C.A. Ryan. 1995. System in activates synthesis of wound- inducible tomato leaf polyphenoloxidase via the octadecanoid defense signalling pathways. **Proc. Natl. Acad. Sci.** USA, 92: 407- 412.

Cook, R. and K. Evans. 1987. **Resistance and Tolerance**. In: Brown, R.H., Kerry, B.R. (Eds.), Principles and practices of nematode control in crops. Academic Press, marrickville, NSW, Australia, p.179-231.

Crammer, C.L., D. Weisseenborn, C.K. Cottingham, C.J. Denbow, J.D. Eisenback, D.N. Radin and X. Ku. 1993. Regulation of defence- related gene expression during plant pathogen interaction. **J. Nematol., 25:** 507- 518.

Criley, R.A. 1988. Propagation methods for gingers and heliconias. Bulletin, Heliconia Society International 3(2):6-7.

Crop Production Manual, TNAU, 2012.

Cruzan, W.B. 1989. Pollen tube attribution in *Erythronium grandiflorum.* **Am. J. Bot**., 76: 562 – 570.

Dadlani, N.K. and More, T.A. 1988. Studies on pollen fertility and viability in *Jasminum.* **Ann. agric. Res**., 9(2): 247- 251.

Darekar, K.S. and N.L. Mhase. 1988. Assessment of yield loss due to root knot nematode *Meloidogyne incognita* race 3 in tomato, brinjal and bitter gourd. **Inter. Nematol. Network Newsl.,** 5: 7-9.

Darwin, C. 1876. Effect of cross and self fertilization in the vegetable kingdom. 2nd Ed., New York.

Darwin, C. 1877. The different forms of flowers on plants of the same species. London.

Das, B.C., P. Behera, P.K. Pando and B. Nayak. 1999. Studies on the effect of growth regulators on growth of salvia. **Orissa J. Horticulture, 27(2)** : 5-9.

De Nattancourt, D. 1977. Incompatibility in angiosperms. **Springer**, Berlin Heidelberg, New York.

Denial Sundaraj, D., G.Balasubramanyam and G. Soundrapandian. (1971). Effect of pre-treatment on germination of kolinji seeds. **Madras Agric. J**., **58(1):**1-4.

Desai, B.M. 1975. Some observations of gamma irradiation in growth and flowering of tuberose. **Curr. Sci**., 44(11): 406- 407.

Deshraj, R.L., H.C. Misra, Saini and S.R. Dohare. 1997. Correlation and path-coefficient studies in gladiolus over different plant densities and seasons on flowering of China aster. **J. Soils and crops, 7:** 136-138.

Devarajan, K. and N. Seenivasan. 2002. Biochemical changes in banana roots due to *Meloidogyne incognita* infected with *Paecilomyces lilacinus.* **Curr. Nematol.,** 13: 1-5.

Devi, G. and N.S.A. Thakur. 2007. Screening of rice germplasm/ varieties for resistance against root knot nematode (*Meloidogyne graminicola*). **Indian J. Nematol.,** 37(1): 86.

Dewey, D.R. and Lu .1959. A correlation and path coefficient analysis of components of crested wheat grass seed production. **Agron. J**., 1: 515- 518.

Dewit, P. 1997. Pathogen avirulence and plant resistance: a key role for recognition. **Trends Plant Sci., 2:** 452- 458.

Dhaduk, B.K., Brijendra Singh and N.K. Dadlani. 1989. Factors affecting seed germination in gladiolus. **Prog. Hort.,** 21(1&2): 38- 43.

Dharmalingam, C., S. Madhava Rao and D. Denial Sundaraj.1973. Pre-germination treatment of kolingi seeds to improve germination. **Seed Res., 1:** 58-62.

Dhawan, S.C. and C. L. Sethi. 1976. A comparative study on the life cycle of Meloidogyne in an apparently healthy and little leaf- attached egg plant roots. **Indian J. Nematol.,** 6: 236-240.

Dikerson, D.P., S.F. Pascholati, A.E. Hagerman, L.G. Butler and R.L. Nicholson. 1984. Phenyl alanine ammonia-lyase and hydroxy cinnamate- CoA ligase in maize mesocotyls inoculated with *Helminthosporium maidis* (or) *H. carbonum.* **Physiol.Plant Path**., 25: 111-123.

Drewes, F.E. and J. Van Staden. 1990. Germination of *Tagetus minuta* L. II. Role of gibberellins. **J. Plant Growth Regul.,** 9: 285 – 291.

Edens, R.M., S.C. Anand and R.J. Bolla, 1995. Enzymes of phenylpropanoid pathway in soybean infected with *Meloidogyne incognita* or *Heterodera glycins.* **J. Nematol.,** 27: 292- 303.

Falconer, D. S. 1981. **Introduction to Quantitative genetics**. Ronalds press Company, New York.

Farkas, G.L. and Z. Kiraly. 1962. Role of phenolic compound in physiology of plant disease and disease resistance. **Phytopath.,** 44: 104- 105.

Fett, W.F., J.D. Poxton and D.B. Dickinson. 1976. Studies on the SI response of *Lilium longiflorum*. **Am. J. Bot**., 63 (8): 1104- 1108.

Fieldman, A.N. and R.W. Hanks. 1964. Quantitative changes in the free ascorbic acid and protein in the roots of healthy *Radopholus similis* infected and recovered grape fruit seedlings. **Phytopath., 54(10): 1210- 1215.**

Finch-Savage, W.E.,D. Gary and G.M. Dickson. 1991. Germination responses to seven bedding plant species to environmental condition and gibberallic acid. **Seed Sci. &Technol., 19** : 487-494.

Fisher, C.A. 1918. The correlation between relatives in supposition of mendelian inheritance. Trans. Roy. Soc. Edinburgh., 52: 399- 433.

* Galton, F. 1889. Natural inheritance, Mc. Millan and Co., London.

Ganesh, S. 2010. Influence of growth regulators and micronutrients on growth, yield and quality of tuberose (*Polianthes tuberosa* l.) cv. Prajwal. M.Sc. (Hort.) Thesis. Tamil Nadu Agricultural University, Coimbatore.

Ganguly, A. K. and A. R. Dasgupta. 1979. Sequential development as peroxidase (EC 1.11.17) and IAA oxidase activities in relation to resistant and susceptible responses in tomatoes to the root-knot nematode, *Meloidogyne incognita.* **Indian J. Nematol.,** 9: 143- 151.

Ganguly, A. K. and A. R. Dasgupta. 1984. Sequential development of polyphenol oxidase (EC 1.14.18.1) in resistant and susceptible tomatoes with the root-knot nematodes, *Meloidogyne incognita.* **Nematol. Medit.,** 12: 15-22.

Garwood, N.C. and C.C. Horovitz, 1985. Factors to limit fruit and seed production of a temperate shrub, *Staphyla trifoliata L. (*Staphylaaceae*)*. **Am. J. Bot**., 72: 453 – 466.

Gatehouse, J.A. 2002. Plant resistance towards insect herbivores: a dynamic interaction. **New Phytol., 156:**145- 169.

Geetha, C.K., P.K. Valsalakumari and P.K. Rajeevan. 1997. Effect of seed priming on germination and seedling vigour in *Cassia spp*. **J. Applied Hort. Navsari, 3(1/2):** 89-92.

Gerard, B. 1932. The effect of heat on the germination of date pollen. **Date Growers' Inst. Report, 9:** 15.

Gheysen, G., W. Van der Eycken, N. Barthels, M. Karimi and M. Van Montagu. 1996. The exploitation of nematode- responsive genes in novel nematode control methods. **Pesticide Sci.,** 47: 95-101.

Giebel, J. 1970. Phenolic content in roots of some solanaceae and its influence on IAA oxidase activity as an indicator of resistance to Heterodera rostochinensis. **Nematologica,** 16: 22-32.

Gilissen, L.J.W. and N.B.M. Brantjes. 1978. Function of the pollen coat in different stages of fertilization process. **Acta. Bot. Neerl**., 27 (3/4): 205 -212.

Gilissen, L.J.W. 1978. Post X- irradiation effects on petunia pollen germination *in vitro* and *in vivo.* **Environ. Expt. Bot.,** 18(2): 81 - 86.

Glasiov, K.T., K.R. Gayler and J.C. Waldron. 1967. Effects of auxin and GA on the regulation of enzyme synthesis in sugarcane tissue. **Proceedings of 6th international conference on plant growth substances.** pp: 442-443.

Gopinath, G. and B.B. Madalageri. 1986. Bacterial wilt resistance in egg plant. **Veg. Sci**., 13: 189- 195.

Gopinatha, K.V., M. Nagesh and D. Nanjegowda. 2002. Biochemical estimation of resistance in tomato cultivars to root knot nematode, *Meloidogyne incognita.* **Indian. J. Nematol**., 32(2): 183- 233.

Gordon, S.A., R.P. Webber. 1951. Colorimetric determination of IAA. **Plant. Physiol.,** 26: 192- 195.

Gowda, J.V.N., Nalawadi, U.G. and U.V. Sulladmath. 1977. Studies on pollen fertility and stainability in eight hybrid tea roses. **Curr. Res**., 6: 46- 47.

Gregory, R.N. and A.M. McClure. 1978. Peroxidase and 6- phosphogluconate dehydrogenase in resistant and susceptible cotton infected by *Meloidogyne incognita*. J. Nematol., 10: 34 – 39.

Grundler, F.M.N. and U. Wyss. 1994. Strategies of root parasitism by sedentary nematodes. In: Kohmot, K., Singh, R.P., Singh, U.S. and Zeigler, R. (Eds) Pathogenesis and host parasite specificity in plant diseases, histopathological, genetics and molecular basis Vol. 11: Eukaryotes. **Perjamon Press.:** 304- 319.

Guar, H.S. and S.K. Prasad. 1980. Population studies of *Meloidogyne incognita* on egg plant (*Solanum melongena*) and its effect on the host. **Indian J. Nematol**., 10: 40-52.

Gudi, G. 2006. Evaluation of tuberose varieties. Thesis submitted to University of Agricultural Sciences, Dharwad, Karnataka.

Gudian, S., L. Arene and C. Pellegrino. 1991. Influence of temperature on hygrometry of rose pollen germination. **Adv. Hort. Sci.,** 5(3): 96- 98.

Gunasekaran, M. 1997. Studies on palynological features as related to crossability barriers in some diploid and tetraploid species of genus *Gossypium*. Ph. D (Hort.) thesis submitted to Tamil Nadu Agricultural University, Coimbatore.

Gupta, L.M. and R. Raina. 2001. Significance of sequential opening of flowers in *Gloriosa superba* L. **Current Science, 80(10):** 1266-1267.

Gupta, M.N., V. Lakshmi and S.K. Dutta. 1984. Cytomorphological studies on control of gamma induced mutants in tuberose. **J. Agri. Nuclear Bio**., 13(4): 140- 141.

Gupta, P., A.C. Pathak, and R.N.S. Banafar, 2002, Studies on the performance of gladiolus cultivar in Malwa region of Madhya Pradesh. **South Indian Horticulture**, **50**(4-6): 641-644.

Gupta, S.R., A.K. Singh and , O.P. Singh. 2001, Variation for flowering characters and their vase life in gladiolus (*Gladiolus floribundus* L.). **Advances of Plant Science**, **14**(1): 133-136.

Gurav, S.B., S.M. Katwate, B.R. Singh, D.S. Kahade, A.V. Dhane and R.N. Sabale, 2005. Quantitative genetic studies in tuberose. **Journal of Ornamental Horticulture**, 8(2): 124-127.

Gurumurthy, K.R. 1990. Hybridization studies in some rose cultivars. M.Sc. Thesis submitted to U.A.S., Bangalore.

Guth, C.J. and S.G. Wellar, 1986. Pollination, fertilization and ovule abortion in *Oxalis magnifica.* **Am. J. Bot**., 73: 246 – 253.

Hadisoeganda, W.W. and J.N. Sasser. 1982. Resistance of tomato, bean, southern pea and garden pea cultivars to root knot nematodes based on host suitability. **Plant Dis**., 66: 145- 150.

Hansen, B. 1985. Fertilization experiments in species of *Rosa.* **Tidsskr. Plavl. AVT**., 89(4): 381- 384.

Harborne, J.B. 1988. Plant phenolics. In: Bell E A, Charlwood B V, eds, secondary plant products. Springer- Verlag. pp. 329 – 402.

Hashim, M.E., S.M. Hassan and W.M. Elginay. 1991. Effect of some growth regulators on the growth and flowering of *Senecio cruentus* – hybrid plants. **Annals. Agrl. Sci., 36**(1) : 203-208.

Hassan, H.M., A.M.K. Aliah., I.K.A. Ibrahim and M.M. Badr. 1994. Free amino acids and oxidative enzymes in infested roots of tomato genotypes resistant and susceptible to *Meloidogyne incognita.* **Nematol. Medit.,** 22: 179- 183.

Hassan, S.M. E., M.Sq. Rahaman, M.R. Amin, U.K. Majumdar and M.U. Ahmad. 2000. Effect of neem (*Azadirachta indica*) on the root -knot nematode (*Meloidogyne incognita*) of brinjal. **Pak. J. Bio. Sci.,** 3: 1853-1854.

Hazarika, K. 1990. Pathogenicity and management of *Meloidogyne incognita* on brinjal (*Solanum melongena* L.). **M.Sc. (Agri.) Thesis, Assam Agricultural University, Jorhat.**

Heald, C.M., B.D. Bruton and R.M. Davis. 1989. Influence of *Glomus intradices* and soil phosphorus on *M.incognita* infecting *Cucumis melo*. **J. Nematol**., 21: 69- 73.

Hegde, M. V., 1994. Studies of variability, correlation, path analysis and performance of gladiolus hybrids. **M.Sc., (Agri) Thesis**. University of Agricutural scinces, Dharwad Karnataka, India.

Heit, C.E. 1971. Germination studies with geranium seed. **Proceedings of the Association of official seed Analysts, 61** : 105-111.

Henny, R.J. 1977. Effect of sucrose level, medium composition and pH on the *in vitro*-germination of pollen from the *Spathiphyllum floribundam* (Linden and Andre). **Proc. Flo. St hort. Soc.,** 90: 304-306.

Herrero, M. 1992 a. Mechanisms in the pistil that regulate gametophytic population in peach (*Prunus persica*). In: Ottaviano, E., D.L. Mulcahy (eds.). Angiosperm pollen and ovules. **Springer**, New York Berlin Heidelberg, pp. 377 – 381.

Heslop – Harrison, J. 1987. Pollen germination and pollen tube growth. **Int. Rev. Cytol.,** 107: 1 – 78.

Heslop- Harrison, J. 1983. Self incompatibility. **Phenomenology and physiology**. Proc. R. Soc. London Ser. B., 218: 317- 395.

Hiratsuka, S., T. Tezuka and Y. Yamamoto. 1983. Use of longitudianally bisected pistils of *Lilium longiflorum* for studies on self- incompatibility. **Pl. Cell Physiol**. 24(4): 765- 768.

Hormaza, J.I . and M. Herrero. 1996. Dynamics of pollen tube growth under different competition regimes. **Sex. Plant Reprod**., 9: 153 – 160.

Howard, T.M. 1977. Inter specific hybrids in tuberose. **Plant life**. 33: 82-84.

Howard, T.M. 1978. Inter specific hybrids in tuberose. **Plant life**. 33: 126- 128.

Huang, C.W., L.H. Lin and S.P. Huang. 1971. Changes in peroxidase isoenzymes in tomato galls induced by *Meloidogyne incognita.* **Nematol,** 17: 460- 466.

Huang, J.S. 1985. **Mechanism of resistance to root knot nematodes**. In: Carter, C.C. and Sasser, J.N. (Eds) An Advanced Treatise on *Meloidogyne* Vol. I: Biology and control. A Co operative Publication of Department of Plant Pathology and USAID, North Carolina University, Graphics; 165- 174.

Hunter, R.E. 1978. Effects of catechin in culture and in cotton seedlings on growth and polygalacturonase activity of *Rhizoctonia solani*. **Phytopathology,** 68: 1032- 1036.

Hussey, R.S. 1989. Disease including secretion of plant parasitic nematode. Ann. Rev. **Phytopath.**, 27: 123- 141.

Hussey, R.S. and V.M. Williamson. 1998. **Physiology and molecular aspects of nematode parasitism.** In: Bigham, J.M. (Eds) Plant and nematode interactions. Madison, Winsconsin, U.S.A.

Ibrahim, K.S. 1991. Peroxidase isoenzymes from *Meloidogyne* cultured on different hosts. **Rev. Nematol**., 14: 335-344.

Indu Rani, C., D. Veeraragavathatham and S. Sanjutha. 2008. Analysis on biochemical basis of root knot nem atode (*Meloidogyna incognita*) resistance min tomato (*Lycopersicum esculentum* Mill.). Res. J. Agrl. Bio. Sci., 4(6): 866-870.

Irulappan, I., A. Doraipandian and S. Muthuswamy. 1980. Varietal evaluation in tuberose (*Polianthes tuberosa* L.). National seminar on production technology for commercial flower crops. India, pp: 66-70.

ISTA. 1993. International rules for seed testing. **Seed Sci. and Technol.** 21: 1- 288.

Jagadish, C., K. Kem sujit, Yadav and Satya Kumar. 2003. Performance of gladiolus cultivars under valley conditions of Uttaranchal. **Progressive Horticulture**, **35**(1): 108-110.

Jain, L.P. 1962. Statistical techniques in quantitative genetics.

Jana, B.K. and T.K. Bose. 1981. Effect of bulb size, depth of planting and spacing on growth and flowering in *Hippeastrum hybridum***. South Indian Hort**., 29: 215-217.

Janani, P. 2009. Studies on grafting in black pepper (*Piper nigrum* L.) with reference to resistance to quick wilt and slow wilt diseases. M.Sc. (Hort.), Thesis, tamil Nadu Agricultural University, Coimbatore.

Jenkins, W.R. and D.P. Taylor. 1967. **Plant Nematology**. Reinhold Pub. Co, New York.

Jensen, W.A. 1962. Botanical histochemistry: Principles and practice. Freeman and San Francisco.

Jhon, A.Q. and F.U. Khan. 2002. Evaluation of exotic tulip genotypes under Kashmir Valley conditions**. J. Ornam. Hort. (New series).** 5(2): 27- 30.

Johansen, D.A. 1940. **Plant microtechniques.** Mc Grew Hill Book. Co. Tuc. New York. pp: 121- 154.

John, A.Q., S.S. Saini and P.P. Sharma. 1988. Floral biology studies in Pansy (*Viola wittrockianagans*). **Harvana J. Hort. Sci**., 17 (1-2): 1-8.

John. A.Q., G.A. Bichoo, and M.A.A. Siddique. 1996. Performance of gladiolus cultivars in Kashmir. **Flora and Founa of Jhansi, 2(1):** 75-77.

Johnson, H.W., H.F. Robinson and R.E. Comstock. 1955. Estimation of genetic variability and environmental variability in soybean. **Agron. J., 47:** 314-318.

Johri, B.M. and I.K. Vasil. 1961. Physiology of pollen. **Bot. Rev**., 27: 325- 381.

Joshi, A.C. and J.V. Pantulu. 1941. **Indian Bot. Soc.** 20: 37-69.

Jothi, G. and Rajeswari Sundarababu.2002. Peroxidase and chitinase activities in brinjal inoculated with *Meloidogyne incognita* and endomycorrhiz**. J. Biol. Control**., 16: 161-164.

Jouret, M.f. 1977. Gibberellic acid and seed dormancy breakig in *Impatiens parviflora, I. glanduliflora* and *I. bulfourii*. **Seed Abstr., 1**(7) : 178.

Kahn, T.N. and D.A. Demason. 1986. A quantitative and structural comparison of citrus pollen tube development in cross- compatible and self- incompatible gynoecia**. Can. J. Bot.,** 64: 2548- 2555.

Kallo, G. and J.B. Chowdhury. 1992. Distant hybridization of crop plants. **Springer-**verlag Berlin Heidelbers, New York.

Kalloo, G. 1986. Tomato (*Lycopersicon esculentum* Mill.) Allied Publishers (P) Ltd., New Delhi. p. 121.

Kalloo, G. 1988. Biochemical basis of insect resistance in vegetables. In: **Vegetable Breeding**. Vol. II. C.R.C. Press. Inc. Boca Raton, Florida.pp 125.

Kamble, B.S., 2001, Evaluation of gladiolus (*Gladiolus hybridus* Hort.) varieties. M.Sc.(Hort.) Thesis, University of Agricultural Sciences, Dharwad.

Kamble, B.S., B.S. Reddy, P.M. Gangadharappa and B.S. Kulkarni. 2004. Evaluation of gladiolus varieties for quality parameters, flower and corm yields. **Haryana Journal of Horticulture Science**, **33**(1&2): 74-75.

Kancalova, M.N., 1977. Studies oin Rose pollen II. Branched pollen tubes. **Prestia.** 47(2): 111- 116.

*Kandpal, K. and Chandra, R. 2010. Performance of gladiolus cultivars under valley conditions of Dehradun. **Abstract book of National Symposium on Life style floriculture: Challenges and Opportunities**. Solon, March 19-21. p.22.

Kannan, P., G.V. Rajalingam and K. Haripriya. 1998. Correlation and path co-efficient analysis in tuberose (*Polianthes tuberosa* L.). J. Spices and Aromatic Crops, 7(2): 149- 153.

Katwate, S.M., C.A. Nimbalkar, U.T. Desai and S.D. Warade. 2002. Variability analysis in gladiolus hybrids. **South Indian Horticulture**, **50**(4-6): 629-634.

Kawase, K and Tsukamoto, Y. 1977. Studies on breeding in chrysanthemum, *Chrysanthemum morifolium,* Ramet. I. Self- fertility**. J. Jap. Soc. Hort. Sci**., 46 (1): 101- 112.

Khan, R. M. and P. Parvatha Reddy, 1992. **Nematode problems of ornamental crops and management**. In: "Nematode pests of crops", pp. 250-257. DBS publishers and Distributors, Delhi.

Khan, T.A., S. Nair and M.S. Ashraf. 2004. Effect of population levels of *Meloidogyne javanica* on plant growth and nematode multiplication on cucurbits. **Pak. J. Nematol**., 22(1): 83-87.

Kishan, S., Krishan, P. and Singh, K.P. 2005. Performance of gladiolus under Delhi conditions. **J. Ornamental Hort., 8(1):** 32-35.

Kohli, R.K and A. Kumari.1986. Cause and cure of dormancy in *Cassia occidentalis* L. seeds. **Proc.Internal.Workshop on Special problems in physiological investigations of tree crop**s. R.R.I.I, Kottayam pp.75-81.

Koike, S.T., K.V. Subbarao, R.M. Davis and T.A. Turini. 2003. Vegetable disease caused by soil borne pathogens. **Publications on California University. http:// anrcatalorg.ucdavis.edu.**

Kosack, H.K.E and J.D.G. Jones. 1997. Plant disease resistance genes. **Ann. Rev. Plant Physiol.,** 48: 575-607.

Krishnamurthy, K.B. 2000. Hybridization and micropropagation studies in tuberose (*Polianthes tuberosa* L.). M.Sc thesis. Univeristy of Agricultural Sciences, Bangalore.

Kumar, A. and R.K. Jain. 2008. Comparative reaction of tomato hybrids against root knot nematodes (*Meloidogyne spp.*). **Indian J. Nematol.,** 38 (2): 259- 261.

Kumar, D. And K.R. Sarkar. 1983. Factors affecting pollen grain diameter in maize. **Indian J. Genet**., 43: 156- 159.

Kumar, D. And K.R. Sarkar. 1984. Genotype, environment interaction in determining pollen grain diameter in maize. **Indian J. Genet**., 44: 476 – 479.

Lal, N., S.N. Yadav and L.S. Srivastava. 1971. Effect of some plant growth regulators on rooting behaviour of cutting of *Bougainvillea* 'Thimma'. **Punjab Hort. J., 11(3/4)** : 278-279.

Lal,S.D. and D.S.Singh. 1978. Performance of some gladiolus varieties under Chakrata conditions. **Plant Sci**., 10: 177-178.

Lata, P. 1971. Hybridization in modern roses. **Curr. Sci**., 40: 4-6.

Lata, P. 1980. Effect of ionizing radiation on roses: Induction of somatic mutations. **Environ. Exp. Bot**., 20(4): 325- 353.

Lata, P. and M.N. Gupta. 1975. Effect of ionizing radiations on roses: Meiotic studies on control and gamma ray induced mutants of rose cultivar (Mantezuma), **Cytologia**. 40: 213- 219.

Lee, T.D. and F.A. Bazaz. 1982. Regulation of fruit and seed production in an annual legume, *Cassia fasciculata.* **Ecology.**, 63: 1374- 1388.

Leela, N.K., P.M. Shafi, S.P. Johm and B. Chempakam. 2002. Chemical composition of essential oils of turmeric (*Curcuma longa* L.). **Acta Pharm., 52**: 137-141.

Lepcha, B., M.C. Nautiual and V.K. Rao. 2007. Variability studies in gladiolus under mid hill conditions of Uttarkhand. **J. Ornamental Hort., 10(3):** 169-172.

Lewis, D. 1949. Incompatibility in flowering plants. **Ecol. Rev**., 24: 472- 494.

Lewis, D. 1994. Gametophytic sporophytic incompatibility. In: Williams E.G., B.R. Knox and

A.E. Clarke (eds.). Genetic control of development in flowering plants. **Kluwer, Dordrecht,** pp 88 – 101.

Lewis, D. and Crowe, L.K. 1958. Unilateral interspecific incompatibility in flowering plants. **Heredity**, 12: 233- 256.

Li, C.C. 1956. The concept of path co-efficient and impact on population genetics. **Biometrics**. 12, 190-210.

Lindgren, P.B., J.L. Jakobek and J.A. Smith. 1992. Molecular analysis of plant defence responses to plant pathogens. **J. Nematol.,** 24: 330- 337.

Linskens, H.F. 1964. **The influence of castration on pollen tube growth after self-pollination**. In: Pollen physiology and Fertilization. Linskens, H.F. (ed.) pp. 230- 236.

Linskens, H.F. 1975. **Incompatibility in Petunia**. Proc. Roy. Soc. B. London, 188: 299- 311.

Liu, X.H., Q. Lin and Y. Cai. 1983. A study on the improvement of germination of litchi pollen. Fujian agric. **Sci. Tech**., No.3, pp: 26-28.

Loganathan, M. 2002. Development of bioformulation for the management of major fungal- nematode complex disease of cabbage and cauliflower in Tamil Nadu. **Ph.D (Thesis), TNAU, Coimbatore**.

Luckwill, L.C. 1949. Fruit development in relation to hormones. **Endeavour**, 8: 188- 193.

Lush, J.L. 1940. Intra-sire correlation and regression of offspring on dams as a method of estimating heritability of characters. **Proc. Amer. Soc. Animal Prodn., 33**: 293-301.

Luzmy and J. Burivalova. 1989. A contribution to the determination of pollen viability in selected garden rose and root stock collections. **Facultas Hort**., 4(2): 39-48.

Mahadevan, A. 1966. Biochemistry of infection and resistance. **Phytopathology**, 57; 96-97.

Mahadevan, A. 1970. Proinhibitors and disease resistance. **Phytopathology,** 68: 73-80.

Mahajan, R., P. Singh and L. Bajaj. 1985. Nematicidal activity of some phenolic compounds against *Meloidogyne incognita*. **Rev. Nematol.,** 8: 161- 164.

Mahalonobis, P.C. (1936). **Proc. Natn. Inst. Sci. India***,* 2: 49 - 55.

Maksimov, A.P. and V.M. Novikova. 1986. Bio- ecological characteristics and qualities of seeds and seedlings of *Yucca* I.

Maksimov, A.P., T.G. Mukhortova, V.M. Novikova and V.N. Kuznetsov. 1987. Bio- ecological characteristics and qualities of seeds and seedlings of *Yucca* II.

Malhotra, S.K. 1991. Role of phenolic contents in resistance to Fusarium wilt of tomato. **Indian. J. Plant Pathol.,** 9(1&2): 57-58.

Malik, C.P. and M.B. Singh. 1980. **In:** Plant enzymology, Histoenzymology. Kalyani Publication, Delhi, p.53.

Manickam, S. 1996. Fertilization barriers in intergeneric crosses involving maize as the female parent. Ph. D. Thesis. Indian Agricultural Research Institute. New Delhi.

Maraite, H. 1973. Changes in the polyphenol oxidase and peroxidase in muskmelon (*Cucumis melo* L.) infected by *Fusarium oxysporum* f.sp. *melonis.* **Physiol. Plant Path**., 3: 29-49.

Markose, B.L. 1996. Genetic and biochemical basis of resistance to bacterial wilt in chilli. **Ph.D. Thesis, Kerala Agricultural University, Vellanikkara, Kerala**, P.140.

Markose, B.L. and M. Aravindakshan. 1987. Pollen biology in shoe- flower. **Agric. Res. J**. Kerala, 25 (1): 5-10.

Masood, A. and S.I. Hussain. 1976. Phenolic and ortho dihydroxy phenolic chnges and their role in the resistance and susceptibility of three tomato varieties to *Meloidogyne incognita*. **Indian.J. Nematol**., 6: 86-93.

McClure, M.A. 1977. *Meloidogyne incognita.* A metabolic sink. **J. Nematol., 9**: 88-90.

Meenakshi Srinivas, S., Niranjan Murthy and J.L. Karihaloo. 1995. Tuberose hybrids Shringar and Suvasini. **Indian Hort**., 40 (3): 5-7.

Mehta, M and D.N. Sen.1991. Seed germination studies of *Cassia Italia* in Indian desert. **Annals of arid zone, 30(1):** 67-70.

Miller, E.A. and E.J. Holcomb. 1982. Effect of GA_3 on germination of *Primula vulgaris* Huds. and *Primula x pulyantha*. **Hort. Sci., 17**(5) : 814-815.

Misra, R.L. and B. Choudhary.1976. Selected gladiolus for the hills. **Indian Hort**., 20: 25-27.

Misra, R.L. and H.C. Saini. 1990. Correlation and path coefficient studies in gladiolus. **Indian Journal of Horticulture**, **47**(1): 127-132.

Misra, R.L., T.S. Verma, P.C. Thakur and B. Singh. 1987. Variability and correlation studies in dahlia. **Indian J. Hort., 44(3&4):** 269-273.

Mohanty, K.C., A.K. Ganguly and D.R. Dasgupta. 1986. Development of peroxidase C.E.C.1.11.1.7. activities in susceptible and resistant cultivars of cowpea inoculated with the root knot nematode. *Meloidogyne incognita.* **Indian J. Nematol., 16 (2): 252-256.**

Molinari, S. 1991. Induction of iso peroxidase in resistant and susceptible tomato cultivars by *Meloidogyne incognita.* **Indian J. Nematol.,** 23: 254-258.

Moll, R.H., W.S. Salhuana and H.F. Robinson. 1962. Heterosis and genetic diversity in varietal crosses of maize. **Crop. Sci., 2:** 197-198.

Mountain, W.B. 1965. **Pathogenesis by nematodes**. In: K.F. Baker and W.C. Snyder (eds.). Ecology of soil borne pathogens. Prelude to biological control. Univ. California Press, Berkeley.p. 285-301.

Mukhopadhyay, A. and G.J. Bankar. 1981. A priliminary note on the effect of different spacing, depth of planting and bulb sizes on growth and flowering of tuberose cv. Double**. The Lal Bagh.** 26: 63-65.

Mulcahy, D.L. and E. Ottaviano. 1983. Pollen: biology and implications for plant breeding. **Elsevier Bromedical**, New York.

Murthy, B.R. and V. Arunachalam. 1966. The nature of divergence in relation to breeding system in some crop plants. **Indian J. Genet., 26**: 188-198.

Nagaraja, G.S. and J.V. Narayana Gowda, 2002. **Correlation studies in tuberose**. National symposium on Indian Floriculture in the New Millennium, Bangalore, India. pp.54.

Nagaraja, G.S., J.V. Narayana Gowda and A.A. Farooqi. 1999. Correlation studies in the tuberose (*Polianthes tuberosa*) cv. Single. **Indian Perfumer**. 43 (3): 131- 133.

Nagesh, M., P. Parvatha Reddy and M.S. Rao. 1997. Integrated management of *Meloidogyne incognita* on tuberose using *Paecilomyces lilacinus* in combination with plant extracts. **Nematol. Medit.** (1997), 25: 3-7.

Nair, S.A. and K.N. Shiva. 2003. Performance of selected gladiolus (*Gladiolus floribundus*) varieties under Bay island conditions. **Indian Journal of Agricultural Science**, **73**(7): 397-398.

Narayana, Y.D. and D.D.R. Reddy. 1980. Chemical constituent root- knot nematode resistant varieties of tomato. **Nematol. Medit**., 8: 51.

Natarajan, K. 2000. Seed technological studies in marigold (*Tagetes erecta* L.) cv. African Giant. M.Sc., (Ag.) Thesis, Tamil Nadu Agricultural University, Coimbatore.

Natarajan, K. 2003. Seed production, processing and storage techniques in petunia (*Petunia grandiflora*) cv. Misc. M.Sc., (Ag.) Thesis, Tamil Nadu Agricultural University, Coimbatore.

Nazir, M., V.K. Dwivedi and K.L. Bhat. 2003. Correlation studies in gladiolus over different environments. **National symp. recent adv. Indian floriculture.** KAU, Vellanikara, Kerala. November. 12-14. P.280.

Neeraj, H.P., Misra and B.P. JHA. 2000, Evaluation of gladiolus germplasm under north Bihar conditions. **Indian Journal of Horticulture**, **57**(2): 178-181.

Neeraj, J., H.P. Mishra and P.B. Jha. 2005. Variability study in gladiolus. **Haryana J. Hort., 34(1-2):** 70-72.

Negi, S.S., S.P.S. Raghava and T.V.R.S. Sharma. 1982. New cultivars of gladiolus. **Indian Hort**., 26: 19-20.

Neuffer, M.G., L. Jones and M.S. Zuber. 1968. The mutants of maize. **Crop Science**. Society of America, Madison, Wis.

Nimi, Y., L. Tong Hua and K. Matsuo. 1997. Self- compatible and incompatible reactions in Asiatic hybrid lilium x Enchantment: influence of pistil age on seedset**. J. Jap. Soc. Hort. Sci**., 65(4): 835- 842.

Niranjanmurthy and S. Meenakshi Srinivas. 1997. Genotypic performance and character association studies in tuberose (*Polianthes tuberosa* L.). **J.of ornamental Hort**., 5(1-2): 31-34.

Nirmal Johnson, S.B. 2000. Studies on nematode pests of cut flowers with special reference to carnation, gerbera, gladiolus and asiatic lily. Ph. D (Hort.) Thesis. Tamil Nadu Agricultural University, Coimbatore.

Nithya Devi, A., V. Ponnuswami, P. Sundararaju, H. Soorianathasundaram, S. Sathiamoorthy, S. Uma and I. Van Den Bergh. 2007. Phenylalanine ammonia lyase and total phenol content in resistant banana *to Pratylenchus coffeae*. **Indian J. Nematol.,** 37 (2): 149- 155.

Ockendon, D. J. and P.J. Gates. 1975. Growth of cross and self pollen tubes in the styles of *Brassica oleraceae.* **New Phytol**., 75: 155 – 160.

Oda, M., M. Nagata, K. Tsuji and H. Sasaki. 1996. Effects of scarlet eggplant rootstock on growth, yield and sugar content of grafted tomato fruits**. J. Japan Soc. Hort. Sci.,** 65: 531- 536.

Olowe, T. 2009. Cow pea germpalsm resistant to *Meloidogyne arenaria* race 1, *Meloidogyne incognita* race 4 and *Meloidogyne javanica.* **Europ. J. Sci. Res**., 28 (3): 338- 350.

Osman Karaguzel, Sadik Cakmakci, Veli Ortacesme and Bilal Aydinoglu. 2004. Influence of seed coat treatments on germination and early seedling growth of *Lupinus varius.* **Pak. J. Bot.,** 36(1): 65 – 74.

Ostrolucka, M.G. and F. Bencat. 1987. Pollen viability of some introduced woody species *in-vitro* conditions. **Lesnictvi**., 33(9): 799- 810.

Ottaviano, E., M. Sari – gorla and D.L. Mulcahy. 1980. Pollen tube growth rates in *Zea mays* .Implications for genetic improvement of crops. **Crop Science**, 210: 437 – 438.

Owens, R.G. and H.N. Specht. 1966. Biochemical alterations induced in host tissue by root- knot nematode contribution. **Boyce Thompson Inst**., 23: 181- 198.

Pandey, K.K. 1959. Mutations of the self-incompatibility gene (S) and pseudo compatibility in angiosperms Lloydia, 22: 222-2334.

Pandey, K.K. 1967. Origin of genetic variability combinations peroxidase isozymes determine multiple allelism of the 'S' gene. **Nature** (Lond.), 213: 669 – 672.

Pandey, K.K. 1970. Time and site of the S gene action, breeding system and relationship in in-compatibility. **Euphytica.** 19: 364- 372.

Pankaj, A.K., A. Shrohi and V. Singh. 1998. Ascorbic acid oxidase (AAO) activity in roots of resistant and susceptible tomato cultivars infested with *Meloidogyne incognita*. **Indian J. Nematol., 28**: 91- 92.

Panse, V.G. and P.V. Sukhatma. 1967. **Statistical Methods for Agricultural Workers.** Indian Council of Agricultural Research, New Delhi. P. 381.

Panse, V.G. and Sukhatme, P.V., 1985, Statistical Methods for Agricultural Workers. Indian Council of Agricultural Research, New Delhi, p.158.

Paric, A., J. Hindija, E. Muratovic, N. Pojskic and K. Bajrovic. 2008. Breaking dormancy of two endemic *Lilium* species: *Lilium bosniacum* (G. Beck) Beck ex Fritsch and *Lilium martagon* L. var. Cattaniae Vis**. Seed Science and Technology**, 36 (3): 788 – 791.

Parvathareddy, P. 1986. Analysis of crop losses in vegetables due to *Meloidogyne incognita.* **Int. Nematol. Network Newsl**, 3(4): 3-5.

Pasha, M.K. and R.K. Das. 1982. Quick viability test of soybean seeds by using tetrazolium chloride. **Seed Sci. & Technol., 10:** 651-655.

Patil, J.D., B.A. Patil, B. Chougule and N.R.Bhat. 1990. Performance of different tuberose (*Polianthes tuberosa* L.) cultivars under pune condition. **Curr. Res. Rep**., 3(1): 118.

Patil, K.B., 2003, Study of some exotic varieties of gladiolus. **Orissa Journal of Horticulture, 31**(1): 42-48.

Patil, V.S., P.M. Munikrishnappa and S. Tirakannanavar, 2009. Performance of growth and yield of different genotypes of tuberose under transitional tract of north Karnataka. **Journal of Ecobiology***,* 24(4): 327-333.

Paul, S. 1998. Biological and biological cases of resistance in solanaceous vegetables against bacterial wilt infected by *Ralstonia solanacearum* (Smith). **Ph.D. Thesis, Kerala Agricultural University, Vellanikkara, Kerala**.

Persson, B. 1993. Enhancement of seed germination in ornamental plants by growth regulators infused *via* acetone. **Seed Sci and Tech.,** 21(2): 281 – 290.

Pfahler, P.L., M.J. Pereira and R.D. Barnett. 1996. Genetic and environmental variation in anther, pollen and pistil dimensions in sesame. **Sex Plant Reprod**., 9: 228 – 232.

Plitmann, U. 1993. Pollen tube attrition as related to breeding system in Brassicaceae. Plant syst. Evol., 188: 65- 72.

Ploeg, A.T. and M. S. Phillips. 2001. Damage to melon (*Cucumis melo* L.) cv. Durango by *Meloidogyne incognita* in Southern California. **Nematol.**, 3: 151- 158.

Podile, A.R. and V.D.V. Laxmi. 1998. Seed bacterization with *Bacillus subtilis* AF1 increases PAL and reduces the incidence of Fusarium wilt in pigeon pea**. J. Phytopath.,** 146(5/6): 224-259.

Powell, N.T. 1971. Interaction of plant parasitic nematodes with other disease causing agents. **In Plant parasitic nematodes vol. II** (Eds. Zuckerman, H.M.; W.F. Mai and R.A. Rhode). Academic press, New York. pp. 119-136.

Prabhat Kumar, Maurya Ravi Kumar, Chakraborty Binayak, Mer Rakesh and D. S. Mishra. 2011. Genetic variability and correlation studies in *Gladiolus hybrida* L. under tarai condition of Uttarakhand. **Progressive Horticulture**. 43 (2): 323- 327.

Prabhu, M., A. Ramesh Kumar and V. Ponnuswami. 2008. Breeding for shoot and fruit borer resistance in brinjal. **Asian J. Hort.,** 3(2): 456- 459.

Pratap, M. and A. Manohar Rao. 2006. Assessment and variability studies in gladiolus. **J. Ornamental Hort., 9(2):** 145-147.

Punja, Z.K. and Y.Y. Zhang. 1993. Plant chitinases and their role in resistance to fungal diseases. **J. Nematol., 25**: 526-540.

Radhakrishna, K.N. 2002. Genetic studies in tuberose *(Polianthes tuberosa. L.*). M.Sc. (Agri.) Thesis, University of Horticultural Sciences, Bangalore.

Rahman, M.A., M.A. Rashid, M.A. Salam, M.A.T. Masad, A.S.M.H. Masum and M.M. Hossain,. 2002a. Performance of some grafted eggplant genotypes on wild *Solanum* rootstocks against root knot nematode**. Pak**. **J. Biol. Sci.,** 2(7): 446- 448.

Rahman, M.A., M.A. Rashid, M.M. Hossain, M.A. Salam and A.S.M.H. Masum. 2002. Grafting compatibility of cultivated egg plant varieties with wild *Solanum* species. **Pak. J. Biol. Sci.,** 5(7): 755-757.

*Rahul Kumar, Sanjay Kumar, Yogesh Chandra Yadav and Adesh Kumar. 2010. Genetic variability in gladiolus (Gladiolus species L.). **Abstract National Symp. Life Style Floriculture: Challenges and Opportunities.** Solon, March 19-21. p. 26.

Rai, S.K., R.S. Katiyar and S.P. Singh, 2000, Prospects of gladiolus crops on sodic wasteland. **Proceedings of the National Conference on Gladiolus**, January, pp.104-106.

Rajendran, G., P. Veluswamy, T.S. Muthukrishnan and R. Sivagami. 1975. Susceptibility of crossandra and related genera to the root – knot nematode *Meloidogyne incognita.* **South Indian Horticulture**.23: 3/4.

Rajagopalan, A. 1994. Investigation on certain aspects of growth, development, crop production and quality in Glory lily (*Gloriosa superba* L.). **Ph.D., (Hort.) Thesis**, Tamil Nadu Agricultural University, Coimbatore, India.

Rajagopalraman, P., A.K. Ganguly and D.R. Dasgupta. 1992. Studies on two oxido reductase and polyphenol oxidase from cowpea infected by *Meloidogyne incognita.* **Indian J. Nematol**., 22: 139- 141.

Rajan, S. 1985. Selection, efficiency, genetic and biochemical basis of resistance to bacterial wilt in tomato. **Ph.D. Thesis, Kerala Agricultural University**, Vellanikkara, Kerala, P.185.

Rajasekar, S.P., A.K. Ganguly and S.C. Swain. 1997. Quantitative changes in superoxide dismutase, catalase and peroxidase with reference to resistance in tomato to *Meloidigyne incognita.* **Indian Nematol.,** 27: 79- 85.

Rajiv Kumar and D.S. Yadav. 2005. Evaluation of gladiolus cultivars under sub-tropical hills of Meghalaya. **J. Ornamental Hort., 8(2):** 86-90.

Rakesh Kumar, Santosh Kumar, Awani Kumar Singh. 2012. Genetic variability and diversity studies in snapdragon (*Antirrhinum majus*) under tarai conditions of Uttarakhand.Indian **Journal of Ornamental Horticulture**. 82(6).

Ramanujam (1974). **Theoret. App. J. Genetics***,* 25 : 211- 214.

Ramzan, A., I.A. Hafiz, T. Ahmad and N.A. Abbasi. 2010. Effect of priming with potassium nitrate and dehusking on seed germination of gladiolus (*Gladiolus alatus*). **Pak. J. Bot.,** 42 (1): 247- 258.

Rao, C.R. (1952). Advanced Statistical Methods in Biometrical Research. John Wiley and Sons. New York.

Rao, C.R. 1960. Multivariate analysis - An indispensable statistical aid in applied research Sankhya. 22: 317-338.

Rao, T.M., T. Janakiram, S.S. Negi and S.P.S. Raghava. 2000, Arka Suvarna – Indian bred yellow gladiolus. **Proceedings of the National Conference on Gladiolus**, January, pp.59-61.

Raut, S.P. 1981. Effect of initial inoculum levels of *Meloidogyne incognita* on plant growth and *Rhizobium nodulation* of mung bean. **Indian phytopath**., 33(2): 353- 353.

Reddy, P.P., M.S. Rao and M. Nagesh. 1997. Nematode management in horticultural crops. **In: Neem in sustainable Agriculture, S.S. Narwal *et al.* (eds.), Scientific Publications, Jodhpur, India**. pp. 229-246.

Renard, H.A. and P. Clere. 1978. Dormany breaking with gibberellins in four species, *Impatiens balsamina, Lavandula angustifolia, Brassica rapa* and *Viola odorata*. **Seed Sci. and Technol., 6**(3) : 661-677.

Roberts, P.A. and D. May. 1986. *Meloidogyne incognita* resistance characteristics in tomato genotypes developed for processing. **J. Nematol**., 18: 173- 178.

Robinson, H.F., R.E. Comstock and P.H. Harvey. 1949. Estimates of heritability and the degree of dominance in Corn. **Agron, J., 41:** 353-359.

Rosen, W.G. 1961. Studies on pollen tube chemotrophism. **Am. J. Bot**., 48: 889- 895.

Roy, R.K. and Sharga, A.N. 2000. Studies on the performance of some exotic gladiolus cultivars under sub-tropical conditions: **A comparative analysis. Proc. National Conf. Gladiolus,** Lucknow. January, pp. 81-84.

Roy, T.K. 1981. Biochemical aspects of host parasite relationships in plant parasitic nematodes. **Proc. Indian National Sci. Academy,** 47(6): 919- 936.

Rupa Rani, K.K. Prasad and Rakesh Ranjan. 2007. Study on varietal performance in gladiolus. **The Orissa J. Hort**., **35(2):** 35-38.

Sadhankumar, P.G. 1995. Incorporation of resistance to fruit cracking in a bacterial wilt resistance genetic background in tomato. **Ph.D. Thesis, Kerala Agricultural University, Vellanikkara,** Kerala, P.151.

Sadhu, M.K. and Bose, T.K. 1973. Tuberose for most artistic garlands. **Indian Hort**., 18(3): 17-20.

Sadhu, M.K. and P.C. Das. 1978. Effect of bulb size, planting density and depth of planting on growth, flowering and bulb production of tuberose (*Polianthes tuberosa* L.). **Indian J. Hort**., 35: 147- 150.

Sahare, M.L. and S.V.S. Shastry. 1963. Meiosis in garden roses. Chromosoma, 13: 702- 704.

Saini, R.S., Gupta, A.K. and Yamadagni, R.V. 1991. Performance of different cultivars of gladiolus (*Gladiolus grandiflorus* L.) under Hissar conditions. **South Indian Hort., 39(2):** 99 -101.

Sakkeer Hussain, C.T., R.L. Mishra, S.K. Bhattacharjee and H.C. Saini. 2001. Correlation and path analysis in gladiolus. **J. Ornamental Hort. New Series, 4(1):** 13-16.

Sambandamurthi, S. 1983. Studies on induced mutation in tuberose (*Polianthes tuberosa*). Ph. D (Hort.) thesis submitted to Tamil Nadu Agricultural University, Coimbatore.

Sambandhamurthi, S. and K. Appavu. 1980. Effect of chemicals on colouring of tuberose (*Polianthes tuberosa* L.). **In:** National seminar on production Technology for commercial Flower crops. TNAU, pp. 73-75.

Sandhu, M.R. and Bose, T.K. 1973. Tuberose for most artistic garlands. **Indian Hort.,** 18(3): 17 – 20.

Sandhu, G.P.S., S.C. Sharma, S.C. and J.S. Arora .1990. Association among morphological traits in gladiolus. **Punjab Horticulture Journal**, **30**(1-4): 191-194.

Sandhya, M.G. 1987. Studies on pollen fertility, seed set and seed germination in some rose cultivars. M.Sc., Thesis submitted to U.A.S., Bangalore.

Sandra M. Reed. 2004. Self- incompatibility and time of stigma receptivity in two species of *Hydrangea.* **Hort Science**. 39(2): 312- 315.

Sanjai, K.D. and Brahma Singh. 2000. Gladiolus: A potential crop in Ladakh. **Proc. National Conf. Gladiolus**. January, pp. 75-80.

Sankari, A. 2000. Studies on the development of F1 hybrids in tomato (*Lycopersicon esculentum* Mill.) with high yield and resistance to tomato leaf curl virus. **Ph.D. Thesis, TNAU,Coimbatore.**

*Sankari, A., Nagalakshmi, S., Renuga, M. and Lakshmanan, V. 2010. Performance of gladiolus varieties under Shevaroys hills. **Abstract book of National Symposium on Life Style Floriculture: Challenges and Opportunities.** Solan. March 19-21. p. 18.

Sarah, J.L., Sabatini and M. Boiseau. 1993. Differences in pathogenicity to banana (*Musa sp* cv. poyo) among isolates of *Rodopholus similis* from different population areas of the world. **Nematropica**, 23: 75-79.

Sarosh, B.R. and J. Meiser. 2007. Transcriptional profiling by cDNA- AFiP reveals novel insights during methyl jasmonate, wounding and insect attack in *Brassica napus*. **Plant Mol. Biol.**, 64: 425- 438.

Saravanakumar. 2000. Crop improvement in tuberose (*Polianthes tuberosa*). Ph. D (Hort.) Thesis. Tamil Nadu Agricultural University, Coimbatore.

Sasser, J.N., H.R. Powers and G.B. Lucas. 1957. Effect of root knot nematodes on the expression of black shank resistance in tobacco. **Physiopath., 43**: 483.

Sathiamoorthy,S. 1973. Preliminary investigations on breeding potential and certain aspects of breeding bananas. Ph.D. (Hort.) Thesis, Tamil Nadu Agricultural Univeristy, Coimbatore.

Sathyanarayana Reddy and Kartar Singh. 1997. Effect of planting on bulb size and bulb production in tuberose cv. Double. **Karnataka J. Agric. Sci**., 10(1): 90- 92.

Sathyanarayanan, G. 2000. Seed Technological studies in phlox (*Phlox drummondii*) cv. 'Globe Mix'. M.Sc., (Ag.) Thesis. Tamil Nadu Agricultural University, Coimbatore.

Sayers, E.R. and R.P. Murphy. 1966. Seed set in alfafa as related to pollen tube growth,

fertilization frequency and post fertilization ovule abortion. **Crop Sci.,** 6: 365 – 368.

Sazak, M. And T. Tomosa. 1979. Studies on the breeding of *Hippeastrum hybridum*. Cross- compatibility of cultivars and character of F1 seedlings. **Stud. Inst. Hort. Kyoto**. Unit, 9: 108- 118.

Schindler, A.F. 1961. A simple substitute for a Baermann funnel. **Plant. Dis. Reporter, 45**: 747 - 748.

Schmucker, T. 1935. Planta, 23: 264 – 283.

Seetharamu, G.K., D.P. Kumar, E. Mohan and P. Sridhara harle. 2003. Evaluation of gladiolus varieties under low-cost polyhouse for hill zone. **National Symposium on Recent Advances in Indian Floriculture**, November 12-14, p.89.

Selvakumari, A. 2005. Seed Technological studies in China Aster (*Callistephus chinensis*) cv. Poornima. M.Sc. (Ag.) Thesis, Tamil Nadu Agricultural University, Coimbatore.

Selvaraju, P. 1985. Studies on certain aspects of production, grading, treating and storage of seed in marigold. (*Tagetes erecto* L.) cv. Spungold. M.Sc. (Ag.) Thesis, Tamil Nadu Agricultural University, Coimbatore.

Selvaraju, P. And J.A. Selvaraj. 1994. Effect of pre-sowing treatments on germination and vigour of seed in marigold (*Tagetes patula* L.). **Madras Agric. J.,** 81 (9): 496 – 497.

Semeniuk, U.K. 1979. Spotless gold, Spotless Yellow and Spotless Pink rose: blackspot resistant breeding lines. **Hort. Adv**., 14(6): 764- 765.

Serfoji, P., S. Rajeshkumar and T. Selvaraj. 2010. Management of root- knot nematode, *Meloidogyne incognita* on tomato cv Pusa Ruby by using vermicompost, VAM fungus, *Glomus aggregatum* and mycorrhiza helper bacterium, *Bacillus coagulans***. J. Agric. Tech**., 6(1): 37- 45.

Sethi, C.L. and N.K. Sharma. 1976. Nature of resistance of cowpea to the root knot nematode *Meloidogyne incognita and the pigeonpea cyst nematode Heterodera cajani.* **Indian J. Nematol., 6: 81-85.**

*Shanmugasundaram, K.A., Shanthi, A. and Monoj Nazir. 2010. Performance of gladiolus under coastal conditions of Karaikal. **Abstract book of National Symposium on Life Style Floriculture: Challenges and Opportunities**. Solan. March 19-21. pp. 35.

Sharga, A.N., U.S. Motial and K.K. Basario. 1970. The effect of GA_3 (Seed treatment) on the germination, vegetative growth, quantity and quality of marigold flowers. **Sci. and cult., 36** : 279-280.

Sharma, J.L., P.C. Trivedi, M.K. Sharma and B. Tiagei. 1990. Alteration in proline and phenol content of *Meloidogyne incognita* infected brinjal cultivars. **Pak. J. Nematol**., 8: 33- 38.

Sharma, M.P., S. Bhargava, M.K. Varma and A. Adholeya. 1994. Interaction between the endomycorrhizal fungus *Glomus fasciculatum* and the root knot nematode *Meloidogyne incognita* on tomato. **Indian J. Nematol.,** 24: 133- 139.

Sheela, V.L., C.S. Jayachandran Nair, R. Rakhi and P.R. Geetha lakshmi. 2006. Correlation studies in heliconia. **J. Ornamental Hort., 9(1):** 29-32.

Sheela, V.L., R. Rakhi, C.S. Jayachandran Nair and T. Sabina George. 2005. Genetic variability in heliconia. **J. Ornamental Hort., 8(4):** 284-286.

Sheikh, M.Q., A.Q. John, M.A.A. Siddique and T.M. Paul. 2005. Genetic variability in Iris. **J. Ornamental Hort., 3(1-2):** 23-25.

Shen, J. M., Huang, K.K., Huang, T.S. 1987. Study of tuberose hybridization. **Acta Hort**. 205:71-74.

Sherly, J. 2011. Studies on grafting of brinjal accessions (*Solanum melongene* L.) with wild solanum rootstocks. Ph. D (Hort.) Thesis. Tamil Nadu Agricultural University, Coimbatore.

Shevchenko, S.V. and Feofilova, G.B. 1981. Pollen viability in *canna* hybrids and original species and cultivars. **Bull. Gosud. Nikits. Bot. Sad**., 13 (46): 94- 98.

Shivanna, K.R. and J. Heslop – Harrison. 1978. Inhibition of the pollen tube in the self- incompatibility response of grasses. **Incompatibility Newsl**., 10: 5 -7.

Shukla, Y.M. and M.K. Chakraborty. 1988. Biochemical studies on response of tobacco and tomato plants to root knot nematode infection. **Tobacco Res.,** 14: 43- 50.

Sidhu, G. S. and Arora, J. S. 2000. Evaluation of gladiolus varieties for summer flower production, exploring the gladiolus in India. **Proc. National Conf. Gladiolus**, Lucknow. January, pp. 115-117.

Sijmons, P.C., H.J. Atkinson and U. Wyss. 1994. Parasitic stategies of root knot nematodes and associated host cell responses. **Ann. Rev. Phytopath**., 32: 235-259.

Silva, H.S.A., R.D.S. Romeiro, D. Macagnam, B.D.A. Halfeld, M.C.B. Pereira and A. Mounteer. 2004. Rhizobacterial induction of systemic resistance in tomato plants: non- specific protection and increase in enzyme activities. **Biol. Con.** 29: 288- 295.

Sindhan, G.S. and R.D. Parashar. 1984. A comparative study of pea varieties resistant and susceptible to powdery mildew by leaf curl and mosaic disease. **Prog. Hort.,** 20 (3 & 4): 185 – 189.

Singh and Manoj Kumar. 1999. Effect of spacing, depth and time of planting on growth, flowering and bulb production of tuberose cv. Double. **J. Orn. Hort.,** 2(2): 127- 130.

Singh, B. and B. Choudhary. 1973. The chemical characteristics of tomato cultivars resistant to root-knot nematodes. **Nematologica**, 19: 443-448.

Singh, B.D. 1983. **Breeding for resistance to biotic stresses II**. Insect resistance In: Plant breeding principles and methods. pp. 494.

Singh, D.K. and A.B. Abidi. 1988. Biochemical transformation in leaves of tomato (*Lycopersicon esculentum* Mill.) varieties affected by leaf curl and mosaic disease. **Prog. Hort.**, 20 (3 and 4): 185-189.

Singh, G. and P. Srivastava. 1990. Seeds germination improvement in lavender with acid treatment. **Seed Res., 18(1):** 86-87.

Singh, K. P. 1995. Improved production technologies for tuberose (*Polianthes tuberosa* L.). A review of research done in India. **Agric. Rev., 16(3):** 141-166.

Singh, K. P. and S. Uma. 1995. Studies on ratoon crop in tuberose (*Polianthes tuberosa* L.) cv. 'Single' and 'Double'. **Indian Perfumers, 39(4):** 158-160.

Singh, K., P. Singh and J.S. Arora. 2000. Effect of cultivar response, season, stage of harvest and sucrose on keeping quality of gladiolus spikes. **Proceeding of the National Conference on Gladiolus**, January, pp.143-149.

Singh, R.K. 2003. Variability studies in dahlia for some quantitative traits. **J. Ornamental Hort., 6(1):** 58-60.

Singh, S.P. and Sharma, J.R. (1989). Theort. *Appl. Genetics,* 79 : 841- 846.

Singh. A.K. 2011. Assessment of snapdragon germplasm for various traits.Indian Journal of Ornamental Horticulture. Vol 76 (8)

Sirohi, A. and D.R. Dasgupta. 1993. Mechanism of resistance in cowpea to the root knot nematode, *Meloidogyne incognita* race 1. Early induction of Phnylalanine ammonia lyase (EC 4.3.1.5.) and chlorogenic acid. **Indian J. Nematol**., 23: 31-41.

Sitch, L.A. 1990. Incompatibility barriers operating in crosses of *Oryza sativa* with related species and genera. In: Gustafsson, J.P. (ed.). Gene manipulation in plant improvement II. Plenum Press, New York, pp: 77-93.

Sitharamu,G.K. 1993. Hybridization studies in tuberose. M.Sc (Hort.) Thesis, University of Agricultural Sciences, Bangalore.

*Snedecor, G.W. and C.W.G. Cockhran. 1967. Statistical Methods. The IOWA State University Press, IOWA, USA.

Som, M.G. and B. Choudhary. 1976. Changes in phenol content of resistant and susceptible lines of tomato in response to leaf curl virus infection. **Soc. Exptl. Agrits**., 1(2): 53-54.

Sonderhonson, E. 1974. Raising *Rosa multiflora* from seed. **Gertner Tidende**. 90 (29): 428- 429.

Srinivas, P.T. 1993. Genetic variability studies in dahlia (*Dahlia variabilis*) **Ph.D. (Hort.) Thesis**, University of Agricultural Sciences, Bangalore, India.

Sriram, I. 2004. Studies on standardization of seed testing methods and storability of some important medicinal crops. **Seed Tech News, 34(4):** 7.

Srivastava, S.K. 1987. Peroxidase and polyphenol oxidase in *Brassica juncea* plants infected with *Macrophomina phaseolina* (Tassi)Goid and their implication in disease resistance. **J.Phytopath**., 120: 249- 254.

Star, J.L. 1981. Beta-glycosidases from *Meloidogyne incognita and Meloidogyne javanica.* J. Nematol., 13: 413- 414.

Stebbins, G.L. 1976. Seed, seedlings and the origin of angiosperms**. In: Origin and early evolution of angiosperms,** New York: Columbia University Press.

Sudha, R. 1991. Evaluation in interspecific hybrid derivatives of tomato (*Lycopersicon esculentum* Mill.) for resistance to tomato spotted wilt virus (TSWV) and economic characters. **M.Sc. (Hort) Thesis, TNAU,Coimbatore.**

Sunderababu, R. And S. Vadivelu. 1988. Pathogenicity of *Meloidogyne* species to tuberose (*Polianthes tuberosa* L.). **Indian J. Nematol**., 18: 146 - 148.

Sundharaiya, P. 2008. Stability analysis for combined resistance to leaf curl virus and root knot nematode (*Meloidogyne incognita race 3*) in tomato (*Solanum lycopersicum* L.). **Ph.D. (Hort.). Thesis, TNAU, Coimbatore.**

Swain, P.K., J.C. Rath and S.K. Mishra. 1987. Interaction between *Meloidogyne incognita* and *Pseudomonas solanacearum* on brinjal. **Indian J. Nematol.,** 17: 61-71.

Swain, S.C., A.K. Ganguly and Umarao. 2004. Race specific biochemical responses in differential hosts against the root knot nematode, *Meloidogyne incognita.* **Indian J. Nematol**., 34(1): 26-32.

Swain, S.C., Rath, S. and Sethi, B.K. 2008. Evaluation of gladiolus cultivars for quality flowers and corm yield in Eastern Ghats high land zone of Orissa. **The Orissa J. Hort., 36 (1):** 120-123.

Swaroop, K. 2010. Morphological variation and evaluation of gladiolus germplasm. *Indian* **Journal of Agricultural Sciences**, 80 (8): 742-745.

Swarup, V. Kaicker, V.S., R.S. Malik and A.P. Singh. 1973. Rose breeding in India. Indian **Journal of Genetics and Plant Breeding**. 33(1): 43- 51.

Syamal, M.M. and A. Kumar. 2002. Genetic variability and correlation studies in dahlia. **J. Ornamental Hort., 5(1):** 40-42.

Syed atif shaukat , Syed zulfiqar ali shah , Syed kashif shaukat , Syed waasif shoukat. 2012. Evaluation of Different Gladiolus Cultivars under Union Council Bangoin Poonch J&K Conditions . **Journal of Agricultural Science and Applications**. 1(4): 139- 142.

Takami, T., K. Matsumoto, N. Nakata and F. Tamura. 2009. Effects of wing removal treatment on seed germination of *Lilium formolongi* hort. **Horticultural Research (Japan).,** 8(4): 433 – 237.

Tammy, L., Sage, Fanny, William, W., Cole and Barrett. 1999. Differential ovule development following self and cross pollination. The basis of self- sterility in *Narcissus triandrus.* **Amer. J. Bot., 86** (6): 855- 870.

Tardif, G., N.A. Kane, H. Adam, L. Labrie, G. Major, P. Gulick, F. Sarhan and J.F. Laliberte, 2007. Interaction network of proteins associated with abiotic stress response and development in wheat. **Plant Mol. Biol**., 63: 703-718.

Taylor, A.L. and J.N. Sasser. 1978. Biology, identification and control of root knot nematode *Meloidogyne spp.* North Carolina State Univeristy Graphics, Raleigh, NC.111 PP.

Thomas L. Sims and Timothy P. Robbins. 2009. Gametophytic Self-Incompatibility in Petunia.

T. Gerats, J. Strommer (eds.), *Petunia*, DOI 10.1007/978-0-387-84796-2-5, **Springer Science Business Media**, LLC .

Thompson, P.A. 1970. Effect of temperature, chilling and treatment with gibberellins on the germination of *Primula species*. **J. Hort. Sci., 45** : 175-85.

Towers, G.H.M. 1964. Metabolism of phenolics in higher plants and micro organism. In **J.B. Horbone ed. Biochemistry of phenolics**, Academic Press, London, pp: 249- 294.

Trudgill, D. L. and V. C. Block. 2001. Apomictic, polyphagous root- knot nematodes: exceptionally successful and damaging biotrophic root pathogens. **Ann. Rev. Phytopath**., 39: 53- 77.

Trudgill, D.L. 1995. Origins of root-knot nematodes (*Meloidogyne spp. Nematoda*) in relation to their cultural control. **Phytoparasitica**, 23: 191- 194.

Tul Bahadur Poon,T.M. Rao, D.P. Kumar, R. Venugopalan and M.V. Dhananjaya. 2009. Study on floral biology of gladiolus genotypes. **Nepal Journal of Science and Technology**. 10: 37 – 43.

Uma, S. 1990. Studies on causes of non- seed setting in some ornamental crops. Ph.D thesis, University of Agricultural Sciences, Bangalore.

Uma, S. and Gowda, J.V.N. 1990. Self fertile tuberose - A possibility. International seminar on new frontiers in Horticulture. Bangalore, pp:125.

Umamaheswari, R. and Rajeswari Sundarababu. 2001. Mangement of root-knot nematode *Meloidogyne incognita* infecting cow pea by calotropis leaf extract. **Indian J. Nematol**., 31: 133- 135.

Vairavan, S., V.A. Siddia, V. Arunachalam and M.S. Swaminathan. 1973. A study on the nature of genetic divergence. **Appl. Gent., 43:** 213-221.

Valeri, M.W. and C. Greggory. 1991. Acid phosphatise-1 from nematode resistant tomato, isolation and characterization of its gene. **Plant Physiol.,** 97: 139- 146.

Varma, T.S., B. Choudary and G. Swarup. 1979. Root knot nematode nature of damage and relationship of initial larval penetration to resistance in brinjal. **Indian J. Hort**., 36: 105- 109.

Verma, V.S. and R.K. Metha, 1976. Genetic divergence in Lucerne. J**. Maharastra Agri. Univ., 1:** 23-28.

Vetrivel, T. 2010. Evaluation of gladiolus (*Gladiolus spp* l.) varieties suitable for shevaroys conditions. M.Sc. (Hort.) Thesis. Tamil Nadu Agricultural University, Coimbatore.

Vidhyasekaran, P., K. Sethuraman, K. Rajappan and K. Vasumathi. 1997. Powder formulations of *Pseudomonas fluorescens* to control pigeonpea wilt**. Biol. Control, 8**: 166- 171.

Viglierchio, D.R. and P.K. Yu. 1965. Plant parasitic nematodes a new mechanism for injury of hosts. **Science**, 147: 1301-1303.

Vijayalaxmi, M., A. Manohar Rao, A.S. Padmavatamma and A. Siva Shanker, 2010. Evaluation and variability studies in tuberose (*Polianthes tuberosa* L.) single cultivars. **Journal of Ornamental Horticulture**, 13 (4): 251- 256.

Vijayan, R. 2002, Seed Handling and conditioning in Zinnia (*Zinnia elegans* Jacq.) and Gaillardia (*Gaillardia pulchella* Foug.) M.Sc., (Ag.) Thesis. Tamil Nadu Agricultural University, Coimbatore.

Visser, T., D.P. Vries, De, G.W.H. Wells and J.A.M. Scheurink. 1977. Hybrid Tea rose pollen germination and storage. **Euphytica**, 26(3): 721-728.

Voyiatzi, C.I. 1995. An assessment of the *in vitro* germination capacity of pollen grains of five tea hybrid rose cultivars. **Euphytica,** 83: 199- 204.

Weber, G.P. and L.E. Wiesner. 1980. Tetrazolium testing procedures for native shrubs and forbs. **J. Seed Technol., 5(2):** 23-34.

Wheelan, R.J. and R.L. Goldingay, 1989. Factors affecting seed set in *Telopea speciossima:* The importance of pollen limitation. **J. Ecol**., 77: 1123- 1134.

Wilfret, G.J. and J.L. Green. 1976. Optimum gibberellic acid concentration to accelerate flowering and increasing yield of statice. **Proceedings of the Florida state Horticultural Society, 88** : 527-530.

Williams, E.G., R.B. Knox and J.L. Rouse. 1982. Pollination sub systems distinguished by pollen tube arrest after incompatible interspecific crosses in Rhododendron (Ericaceae). **J. Cell. Sci**., 53: 255- 277.

Williamson, V.M. and A.Kumar. 2006. Nematode resistance in plants: the battle underground. **Trends Genetics**, 22: 396-403.

Williamson, V.M. and R.S. Hussey. 1996. Nematode pathogenesis and resistance in plants. **Plant cell,** 8: 1735- 1745.

Winsor, J.A. and A.G. Stephenson. 1995. Demographics of pollen tube growth in *Cucurbita pepo.* **Can. J. Bot.,** 73: 583 – 589.

Wlamer, R.E., L.C. Stephen. and M.U. Angell. 1974. Gibberillin accelerates flowering of *Cyclamen persicum* Mill. **Hort. Science, 9**(5) : 476-477.

*Wright, S., 1921, Correlation and causation. **Journal of Agricultural Research**, **20**: 557-585.

Wu, G., H.B.Shao, L.Y. Chu and J.W. Cai. 2007. Insights into molecular mechanisms of mutual effect between plants and environment. **Rev. Agron. Sustain. Dev**., 27: 69-78.

Yabuya, T., H. Takatsugi, T. Adachi and T. Nagatomo. 1982. Some characteristics of *in vitro* germination of *Iris ensata* Thumb. **Pollen Bull.** Fac. Agric. Miyazki.

Yadav, L.P. and R.G. Maity. 1989. **Commercial Flowers- Tuberose**: 519- 544. Ed. T,K, Bose, Naya Prakash, Calcutta.

Yadav, P.K., B.L. Baheti and B.S. Rathore. 2009. Pathogenicity of root-knot nematode, *Meloidogyne incognita* on cluster bean (*Cyamopsis tetragonoloba*). **J. Mycol. Plant Path**., 39(1):172-173.

Yono, F., S. Tokumasu and M. Kato. 1975. The behaviour of pollen tubes and the developmental process of ovules in Pelargonium. **Euphytica,** 24: 251- 259.

Zacheo, G. and T.B. Zacheo. 1988. Involvement of superoxide dismutase and superoxide radicals in the susceptible and resistant tomato plants to *Meloidogyne incognita* attack. **Physiol. Mol. Plant Path**., 321: 313- 322.

Zacheo, B.T., M.T. Matill and G. Zacheo. 1990. Ultra structural response of potato root resistant to *Globodera rostochinensis* pathotype Race 1. Rev. Nematol., 13: 29 - 36.

Zacheo, G. and T.B. Zacheo. 1995. **Plant-nematode interactions**. Histological, physiologica;l and biochemical interactions, pp: 321- 353. In K. Kohmoto, U.M. Singh and R.R. Singh (eds.). Pathogenesis and host specificity in plant diseases Elsevier Science, Oxford, UK.

Zacheo,B. T., M.T. Melillo, M.T. Matill and G. Zacheo. 1995. **The biology of giant cells**. In: Fenoll C, Grundler FMW, Ohl SA, editors. Cellular and Molecular aspects of Plant –Nematode Interactions. Dordrecht: Kluwer Academic Publishers, 1997.p. 65-79.

* Originals not seen

Fig.1.1. Root characters (root length and root weight) of RKN infected tuberose genotypes (single)

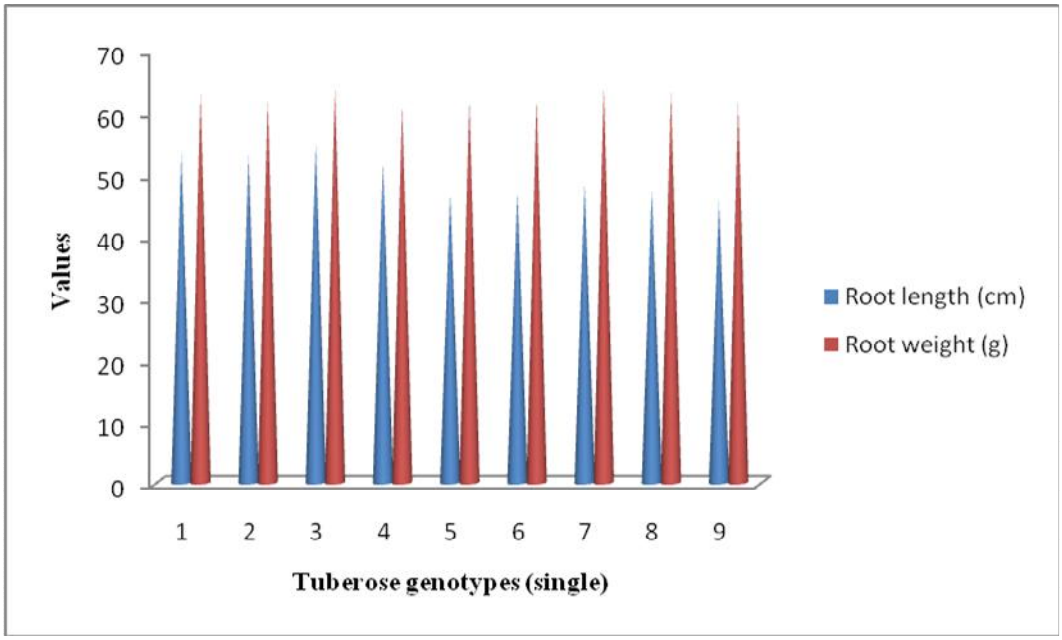


Fig.1.2. No. of RKN females/ g root, No. of eggs/ egg mass, No. of egg masses/ g of root and No. of galls/ 10 g infected roots after inoculation in tuberose genotypes (single)

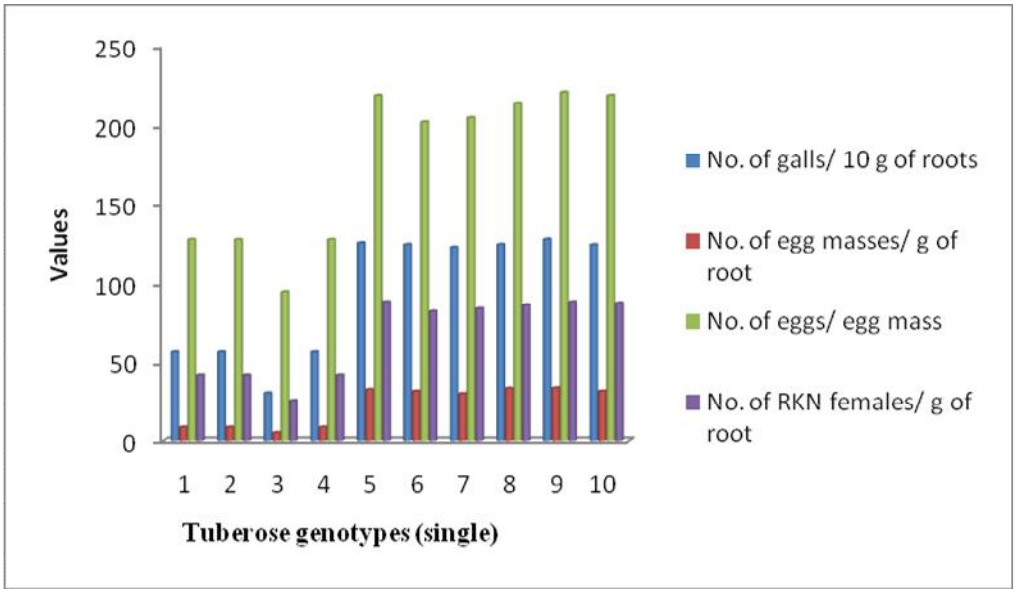


Fig. 2. Total phenol content in RKN infected tuberose genotypes (single) after inoculation

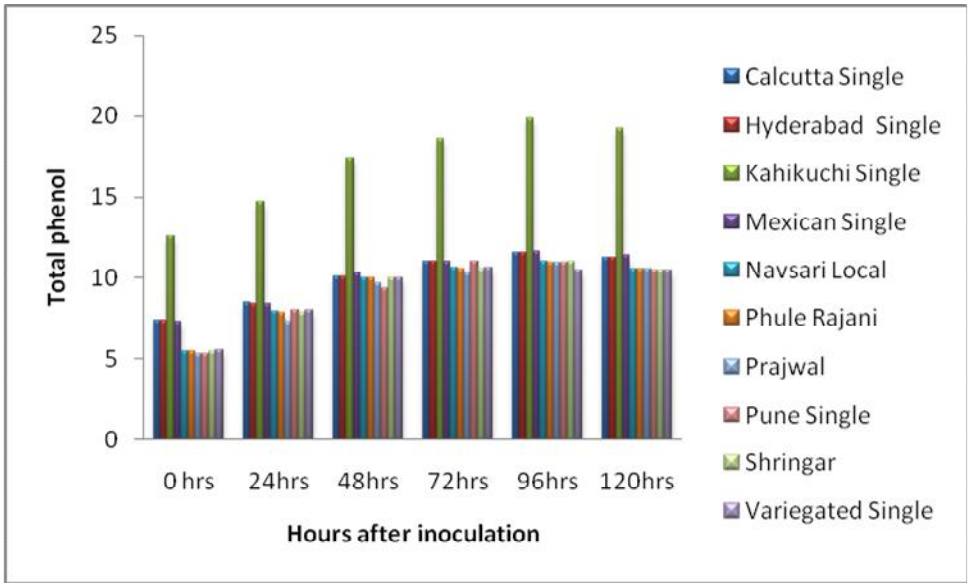


Fig. 3. Ortho di- hydroxy phenol content in RKN infected tuberose genotypes (single) after inoculation

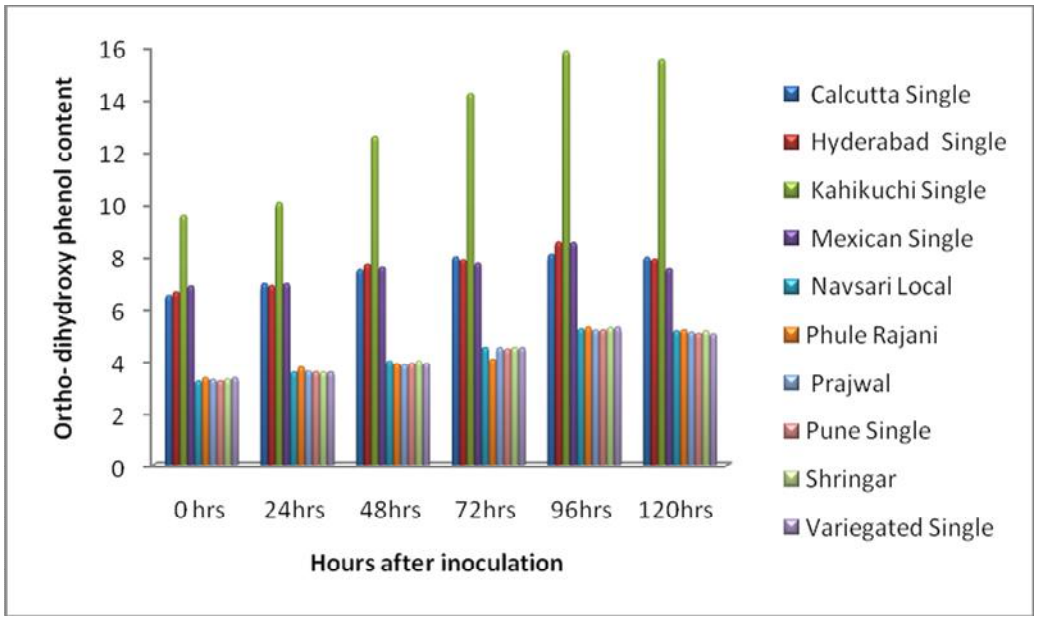


Fig. 4. Peroxidase activity in RKN infected tuberose genotypes (single) after inoculation

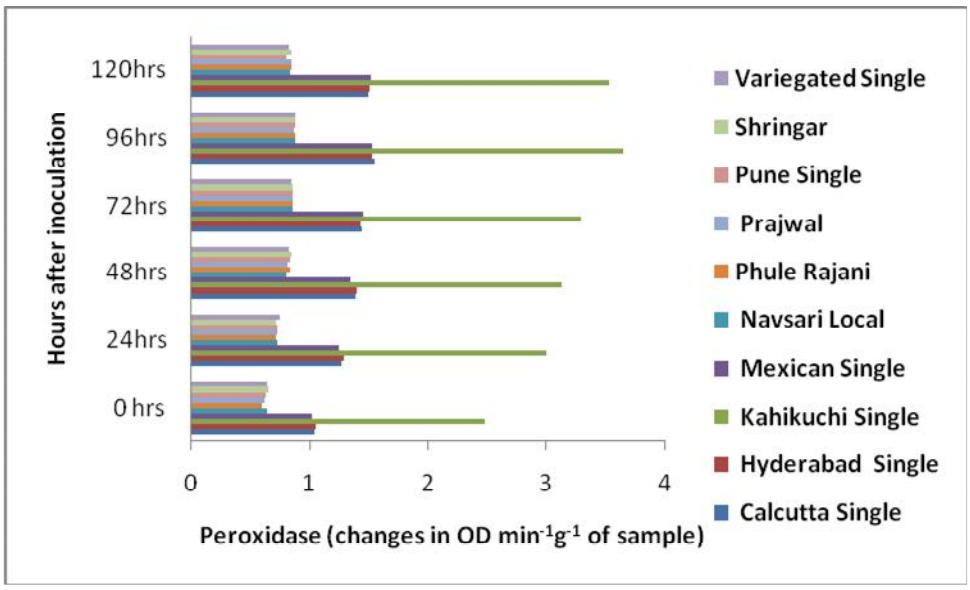


Fig. 5. Polyphenol oxidase activity in RKN infected tuberose genotypes (single) after inoculation

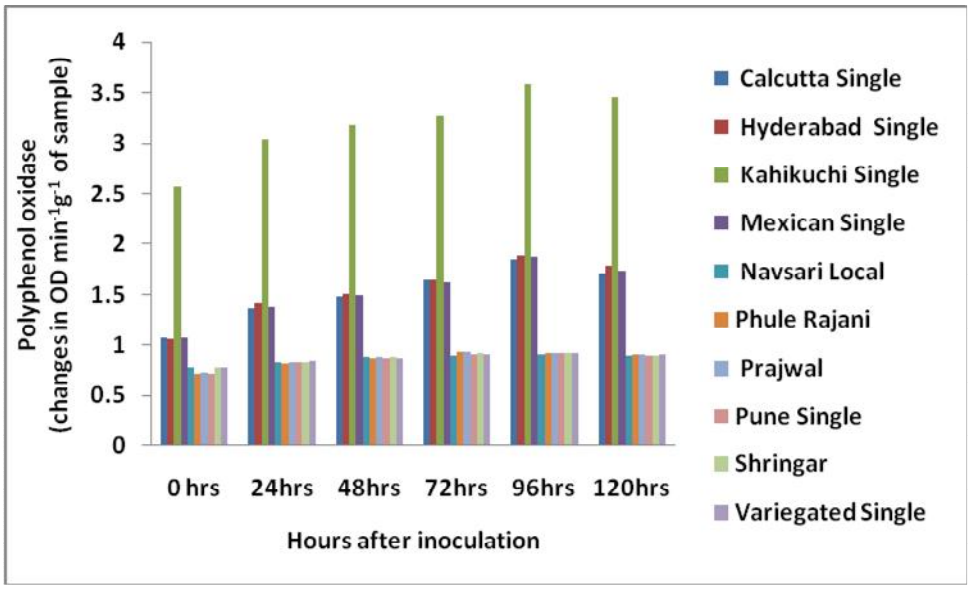


Fig. 6. Phenylalanine ammonia lyase activity in RKN infected tuberose genotypes (single) after inoculation

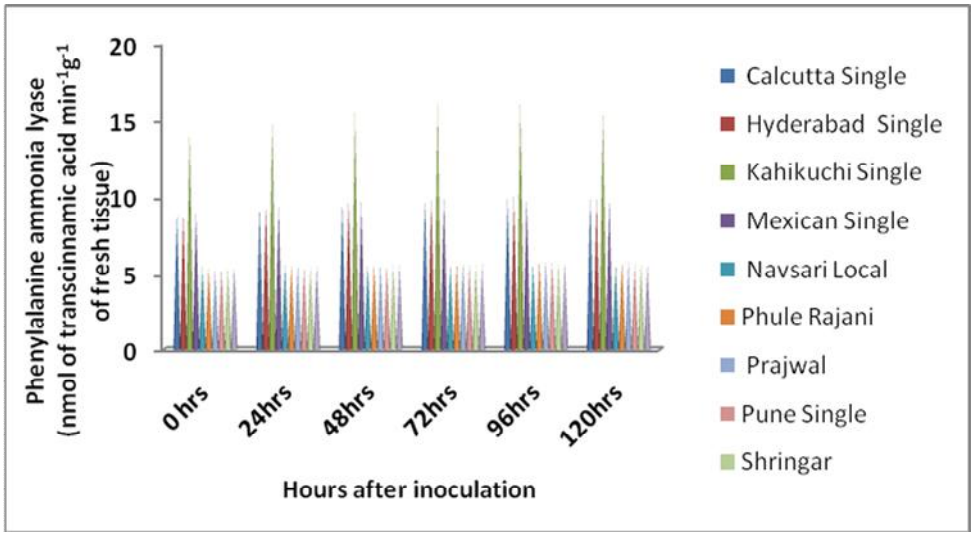
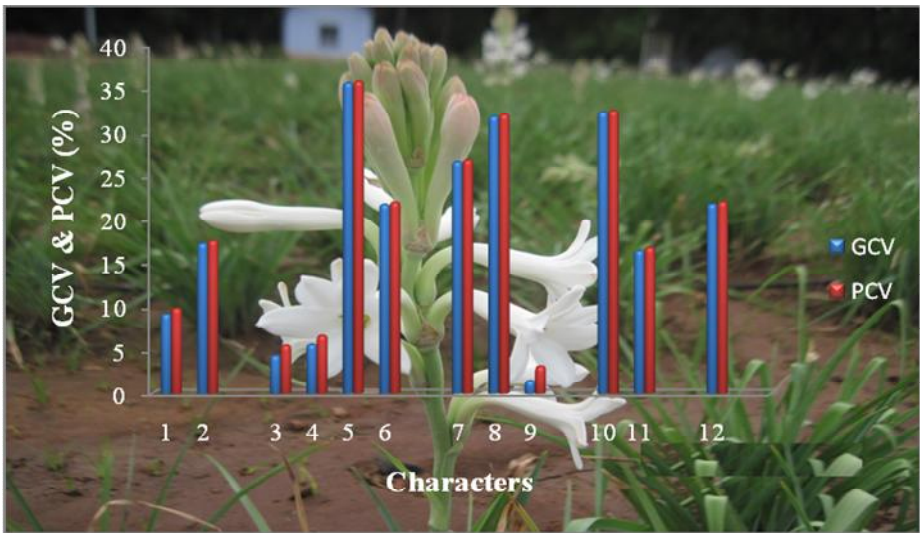


Fig. 7. Phenotypic co-efficient of variation and genotypic co-efficient of variation in vegetative and floral characters of tuberose (single types)



1. Days taken for sprouting of bulb
2. Plant height
3. No. of leaves per plant
4. Days to spike emergence
5. Flowering duration
6. Spike length
7. Rachis length
8. Number of florets/ spike
9. Length of the floret
10. Weight of florets per spike
11. Number of spikes/m^2
12. Yield of florets/ plot (2* 2 m)

Fig. 8. Heritability and Genetic Advane as percent mean in vegetative and floral characters of tuberose (single types)

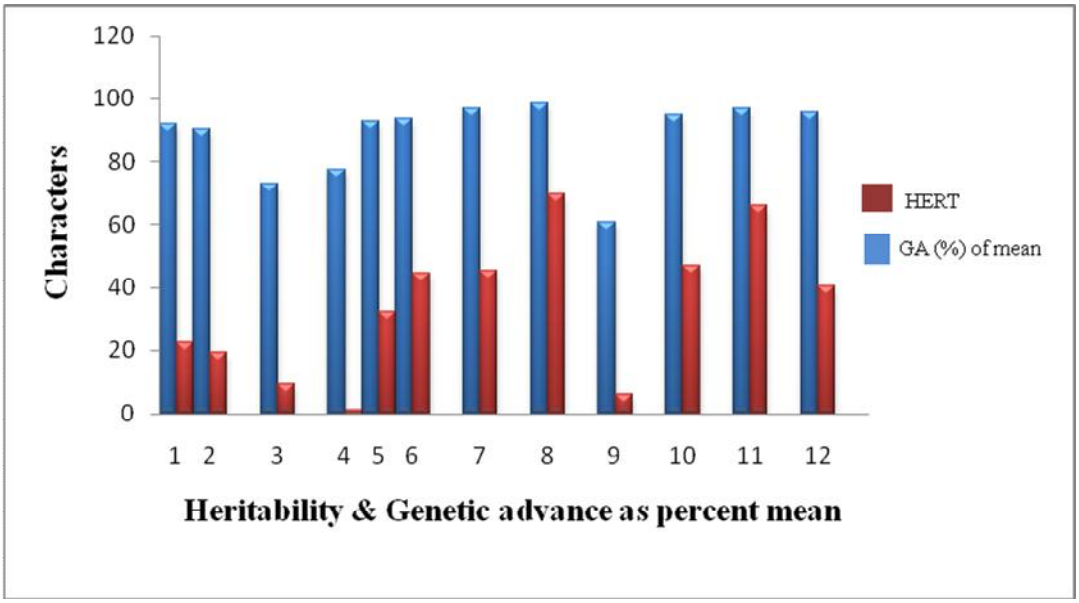
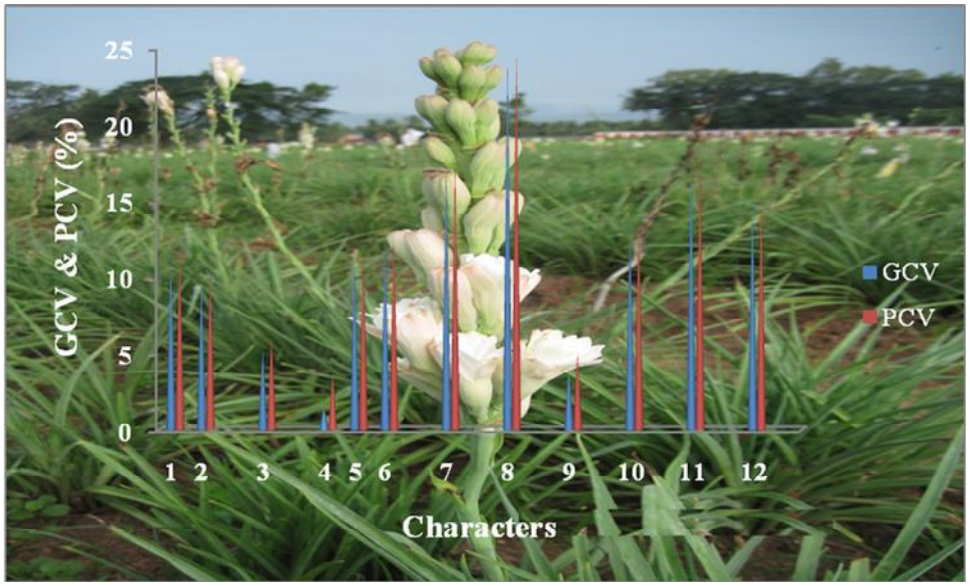
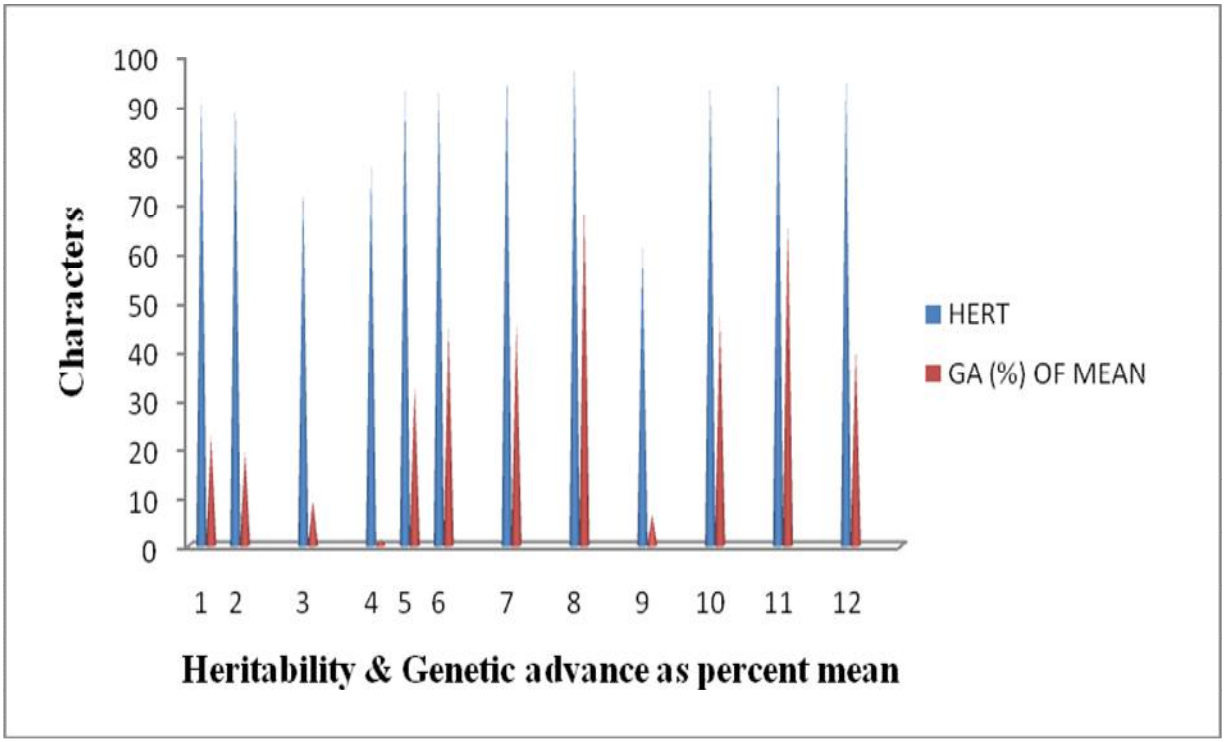


Fig. 9. Phenotypic co-efficient of variation and genotypic co-efficient of variation in vegetative and floral characters of tuberose (double types)



1. Days taken for sprouting of bulb
2. Plant height
3. No. of leaves per plant
4. Days to spike emergence
5. Flowering duration
6. Spike length
7. Rachis length
8. Number of florets/ spike
9. Length of the floret
10. Weight of florets per spike
11. Number of spikes/m^2
12. Yield of florets/ plot (2* 2 m)

Fig. 10. Heritability and Genetic Advance as percent mean in vegetative and floral characters of tuberose (double types)



1. Days taken for sprouting of bulb
2. Plant height
3. No. of leaves per plant
4. Days to spike emergence
5. Flowering duration
6. Spike length
7. Rachis length
8. Number of florets/ spike
9. Length of the floret
10. Weight of florets per spike
11. Number of spikes/m^2
12. Yield of florets/ plot (2* 2 m)

Fig. 11. Cluster mean for yield per plant in single types of tuberose

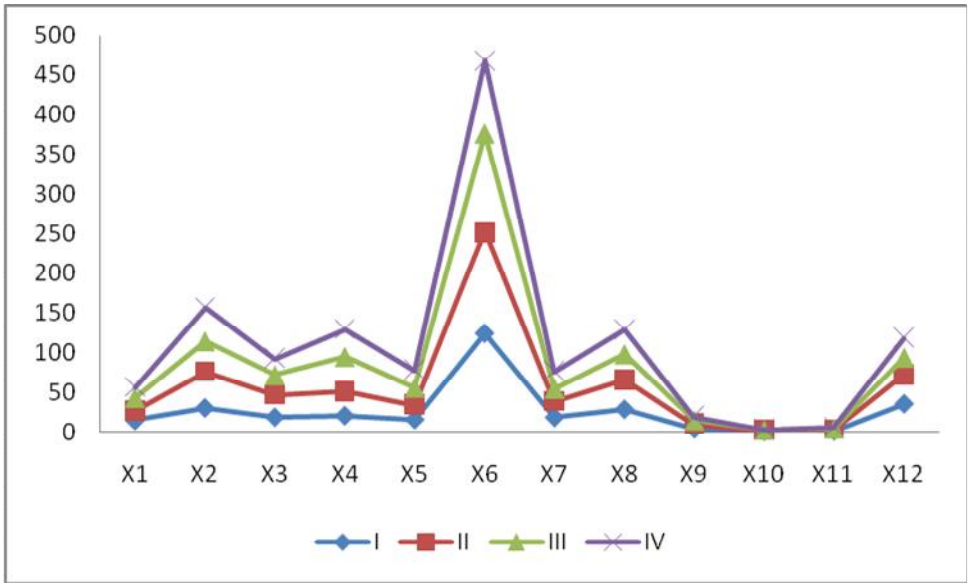
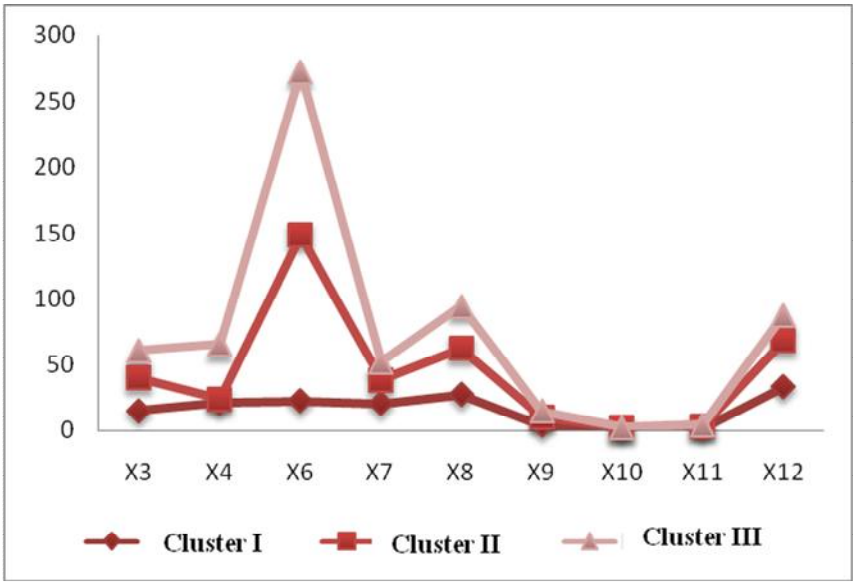


Fig. 12. Cluster mean for yield per plant in double types of tuberose



X1. Days taken for sprouting of bulb
X2. Plant height
X3. No. of leaves per plant
X4. Days to spike emergence
X5. Flowering duration
X6. Spike length
X7. Rachis length
X8. Number of florets/ spike
X9. Length of the floret
X10. Weight of florets per spike
X11. Number of spikes/m^2
X12. Yield of florets/ plot (2* 2 m)

TUBEROSE

Plate 1. Field view - Evaluation of parents

Plate 2. Field view - Crossing block

In vivo pollen germination and pollen tube growth of pollinated stigma stained with aniline blue and illuminated under fluorescent light (25 X)

Plate 3. Pollen germination on pollinated stigma (25x)

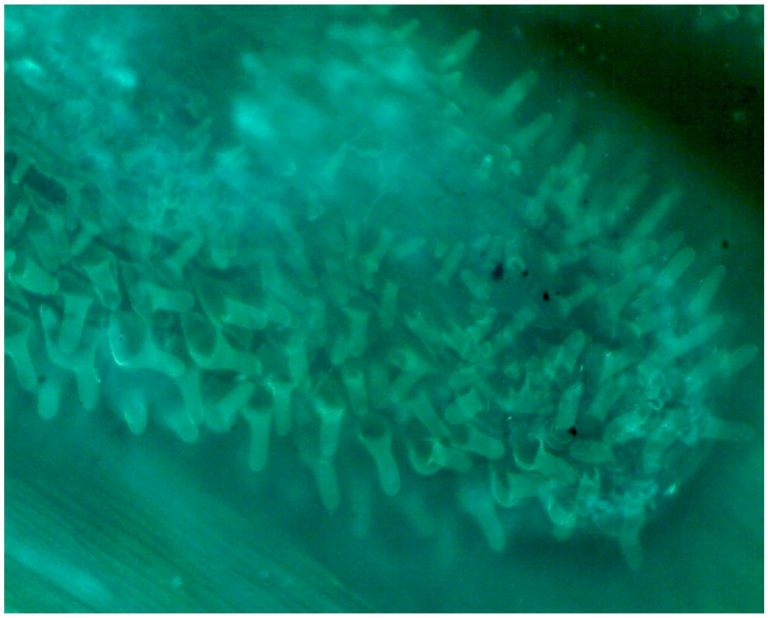


Plate 4. Pollen tube growth on pollinated stigma(25x)

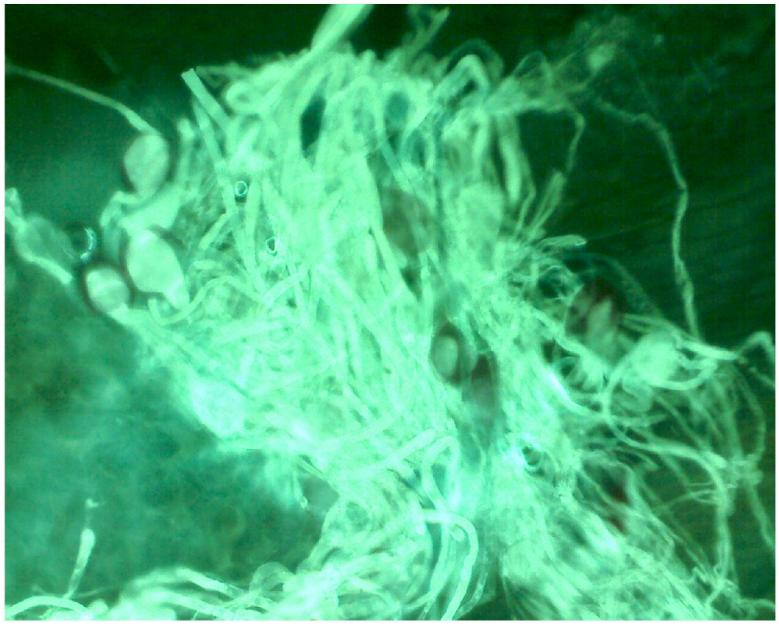


Plate 5. Maintaining pure culture of root knot nematode *(Meloidogyne incognita)* in tomato cultivar PKM 1

Plate 6. Screening of tuberose genotypes against root knot nematode

Plate 7. Collected egg mass of RKN from infected tomato roots

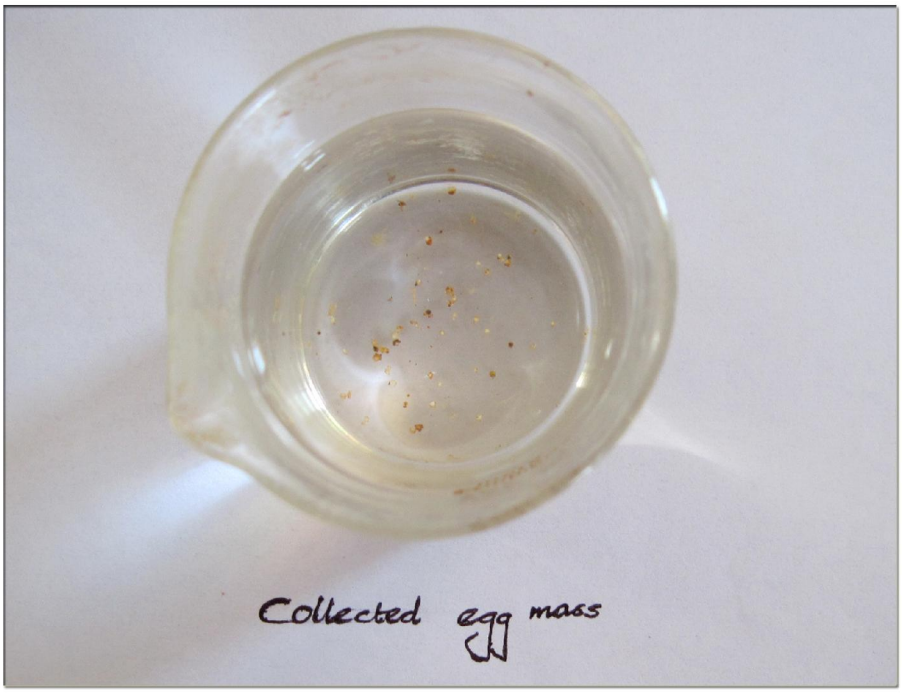


Plate 8. Artificial inoculation of root knot nematode

Plate 9. Crossing techniques

Florets at pre- anthesis stage

Emasculation

Dusting of pollen

Bagging

Fruit set –I stage

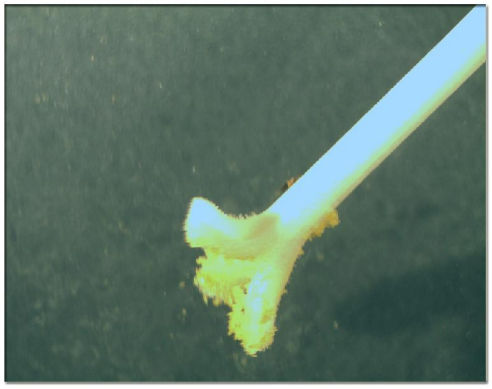
Fruit set – II stage

Fruit set – III stage

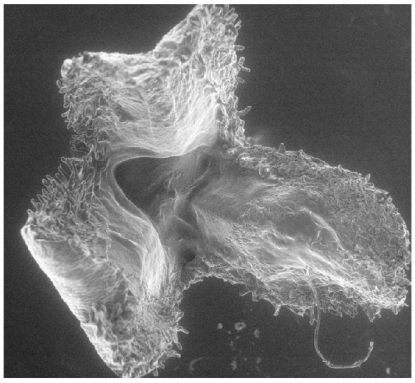
Hybrid seeds(F_1)

Plate 10. Microscopic view of pistil

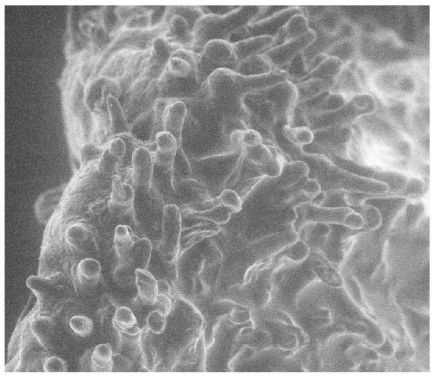
Pollinated stigma

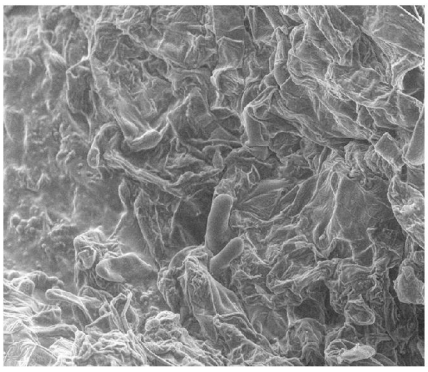


Trifid stigma



Wet stigma





Ovary

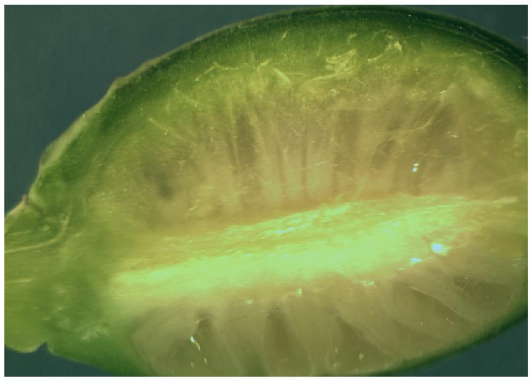
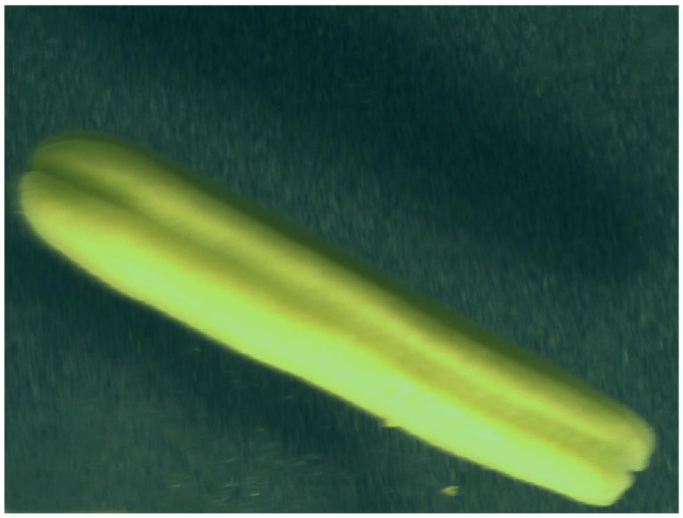


Plate 11. Microscopic view of stamen

Stamen before dehiscence



Stamen after dehiscence

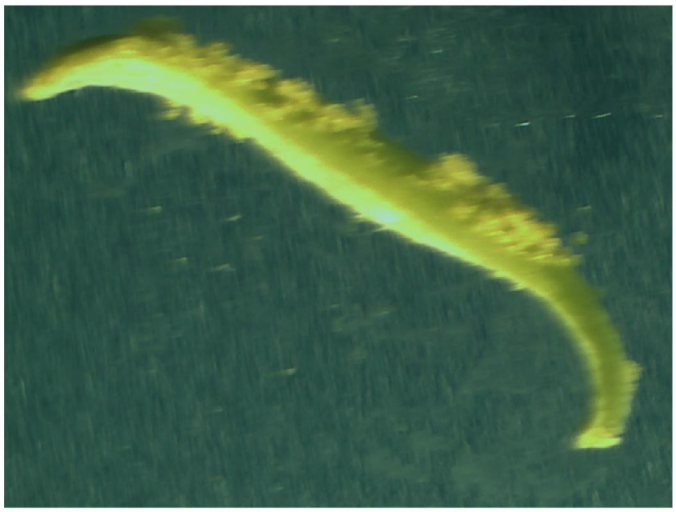
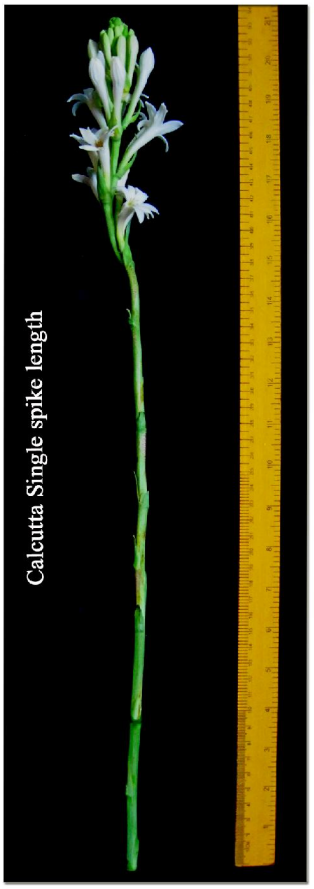


Plate 12. Floral characters and bulb size of Calcutta Single



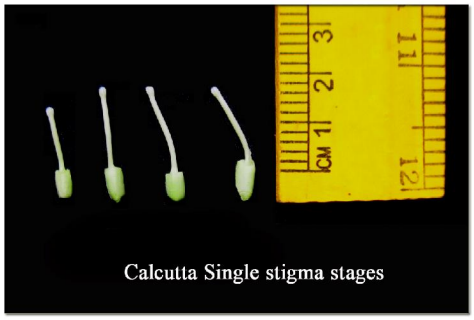


Plate 13. Floral characters and bulb size of Hyderabad Single

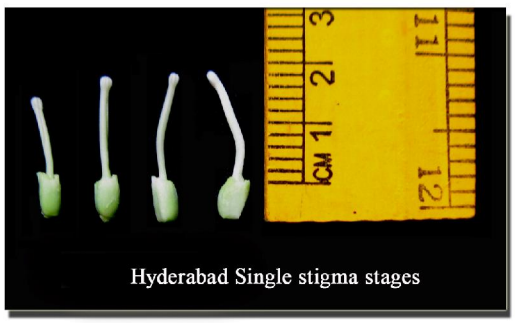
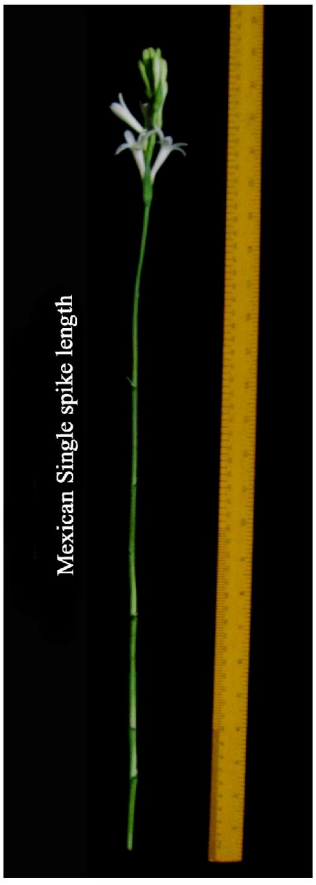


Plate 14. Floral characters and bulb size of Kahikuchi Single

Plate 15. Floral characters and bulb size of Mexican Single



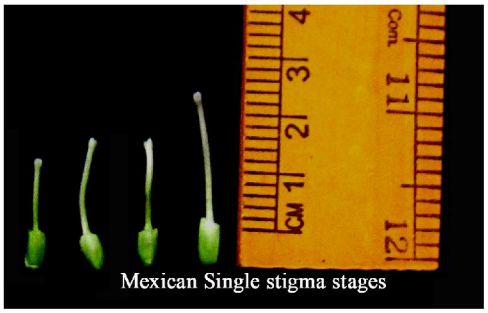
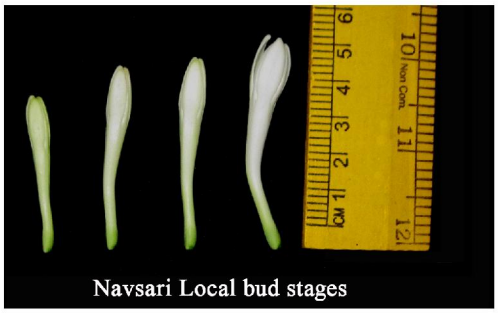
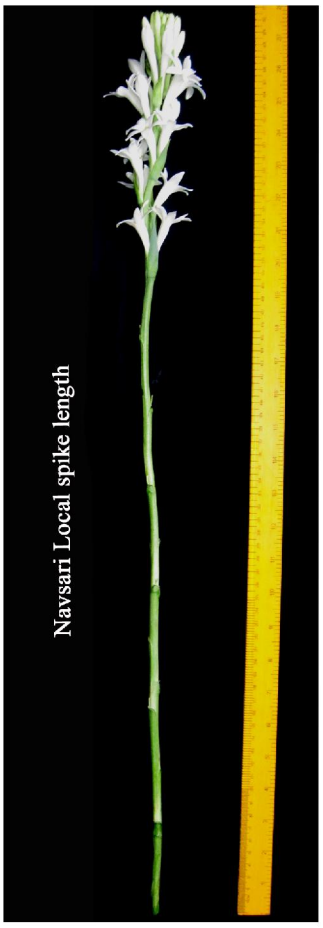


Plate 16. Floral characters and bulb size of Navsari Local





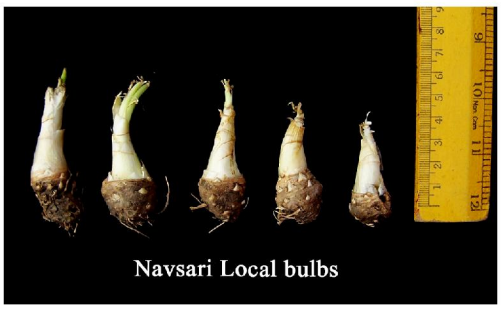


Plate 17. Floral characters and bulb size of Phule Rajani

Plate 18. Floral characters and bulb size of Pune Single

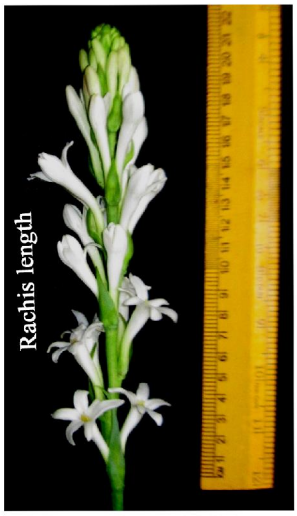
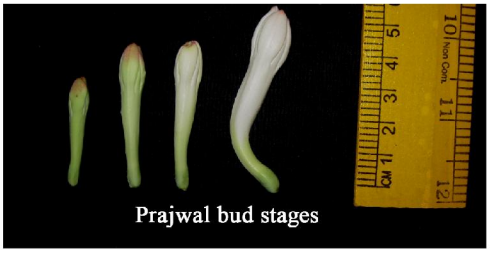


Plate 19. Floral characters and bulb size of Prajwal



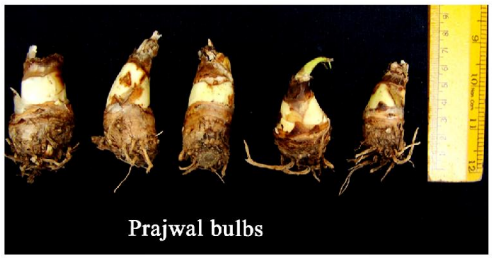


Plate 20. Floral characters and bulb size of Shringar

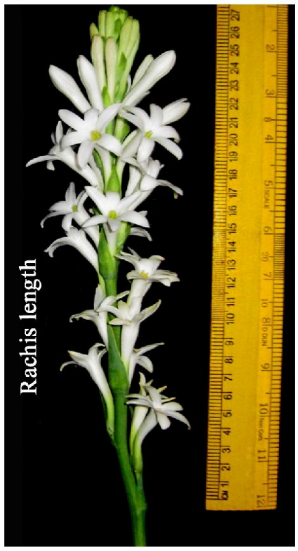


Plate 21. Floral characters and bulb size of Variegated Single

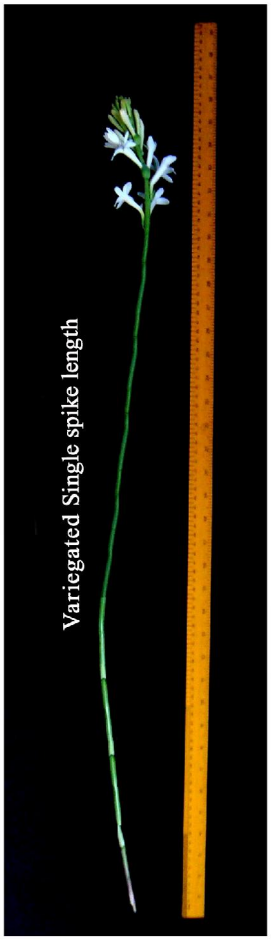
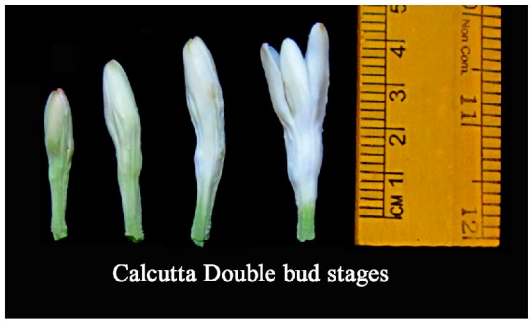


Plate 22. Floral characters and bulb size of Calcutta Double



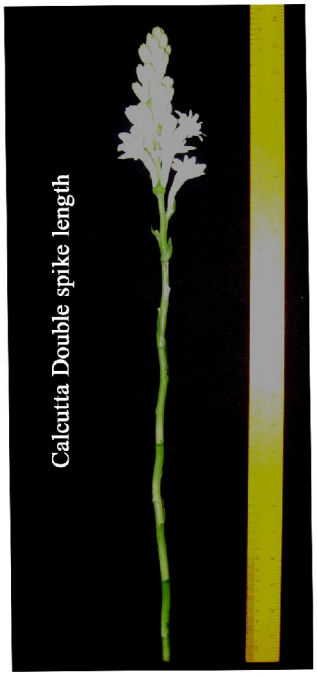
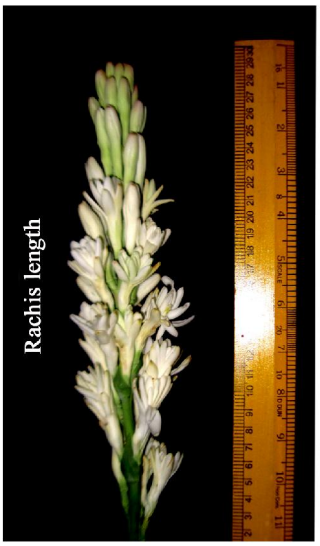


Plate 23. Floral characters and bulb size of Hyderabad Double



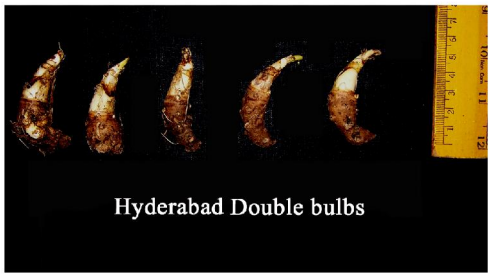


Plate 24. Floral characters and bulb size of Pearl Double

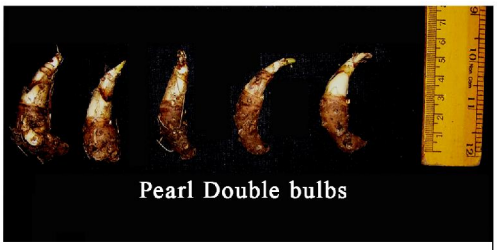


Plate 25. Floral characters and bulb size of Suvasini

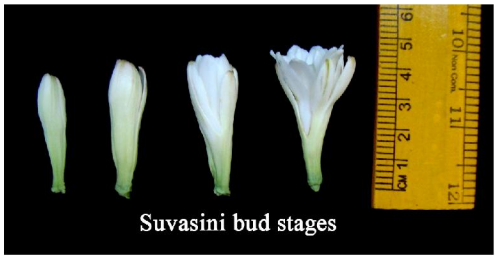


Plate 26. Floral characters and bulb size of Vaibhav

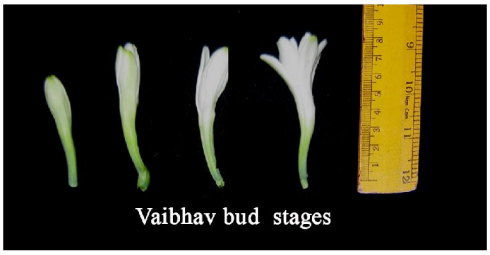
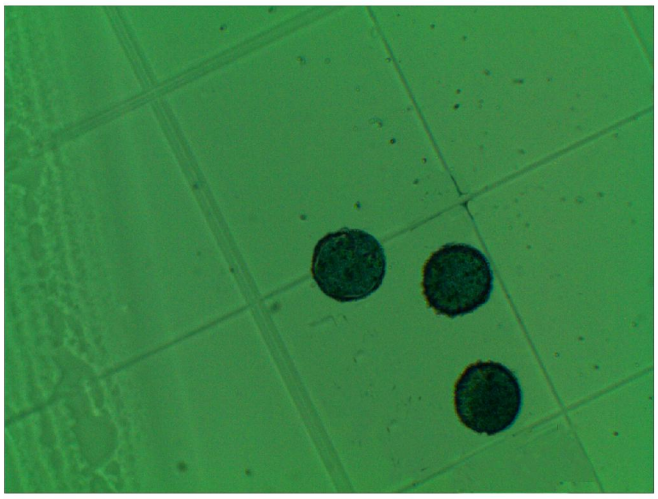
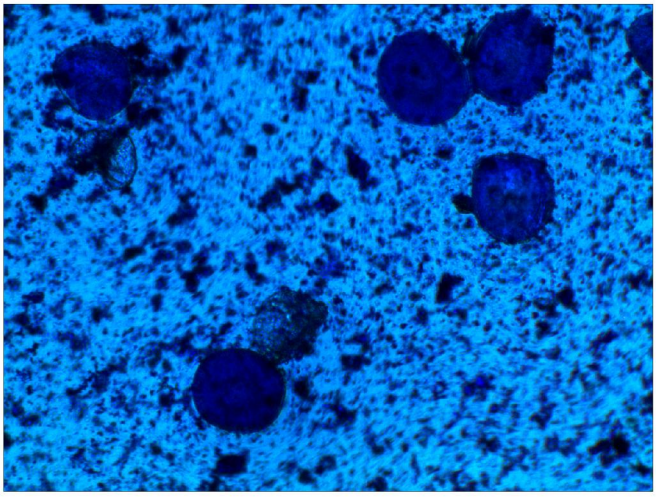


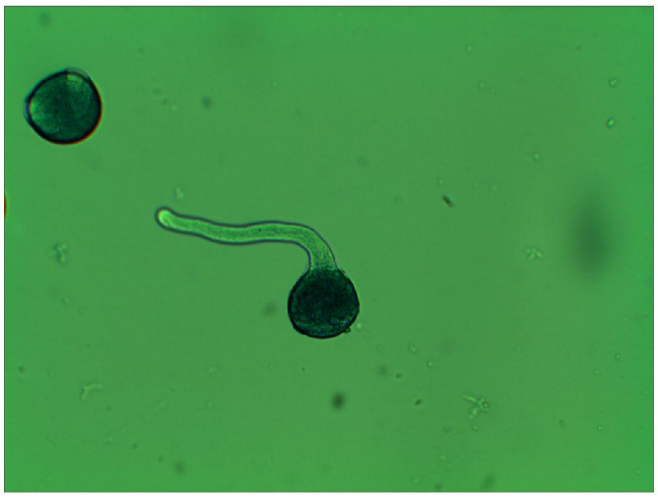
Plate 27. Pollen output, viability and germination in Calcutta Single



Pollen Output

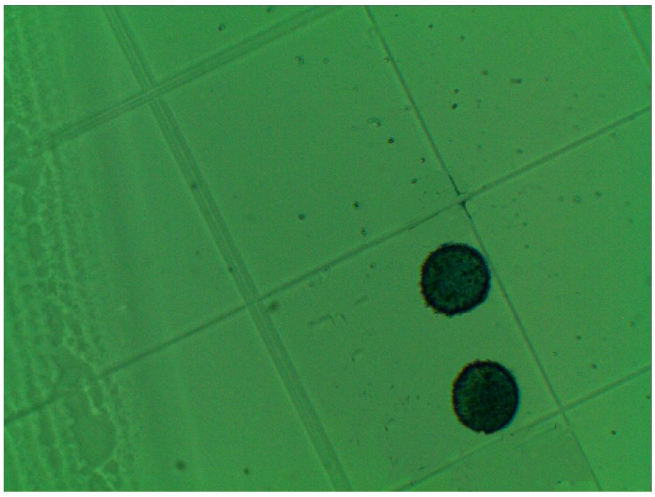
Pollen Viability





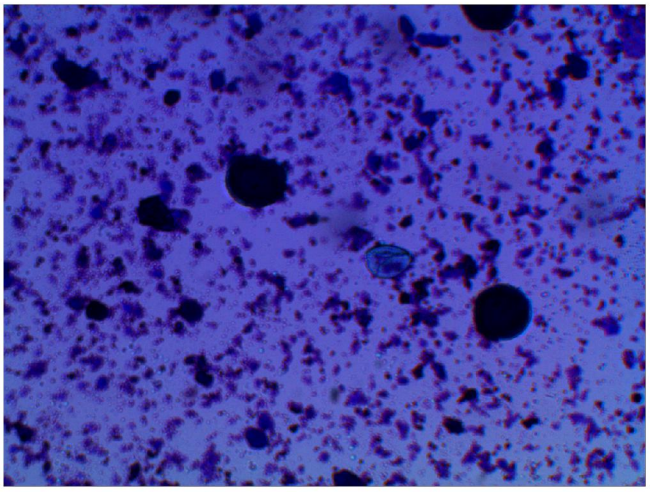
Pollen Germination

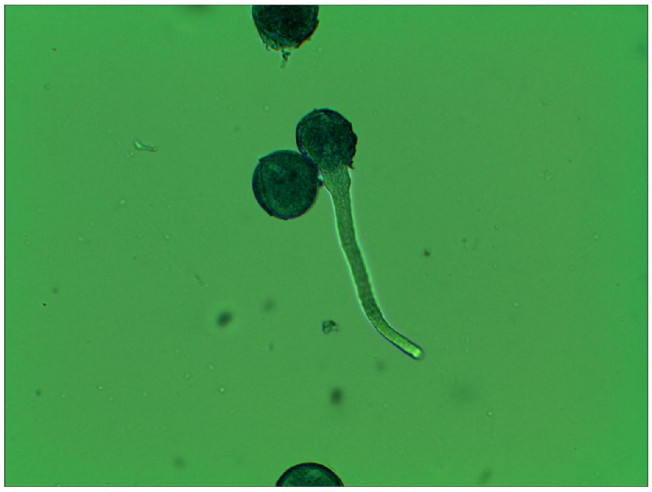
Plate 28. Pollen output, viability and germination in Hyderabad Single



Pollen Output

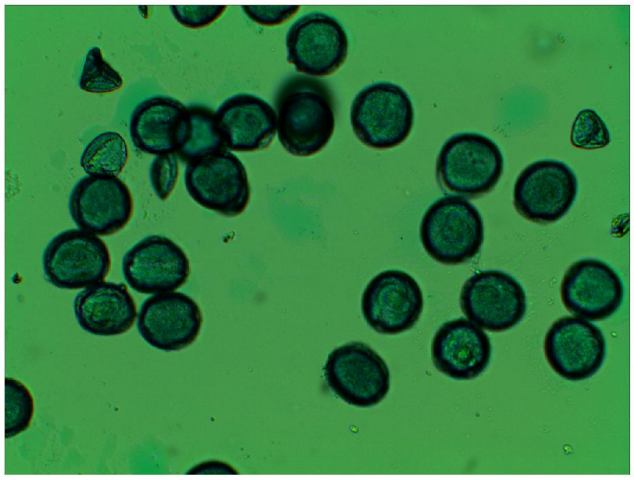
Pollen Viability





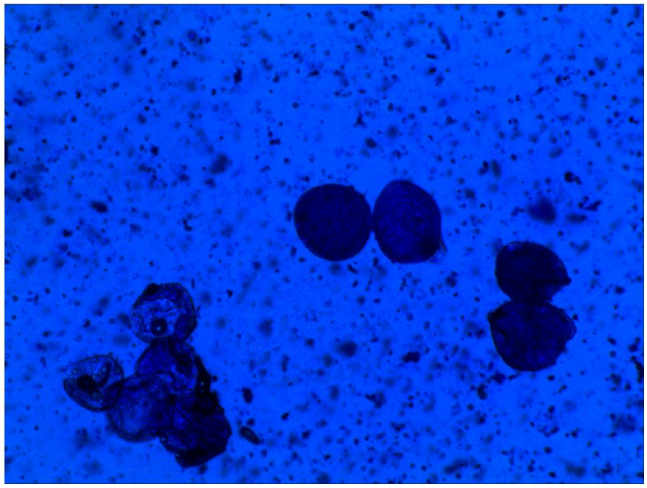
Pollen Germination

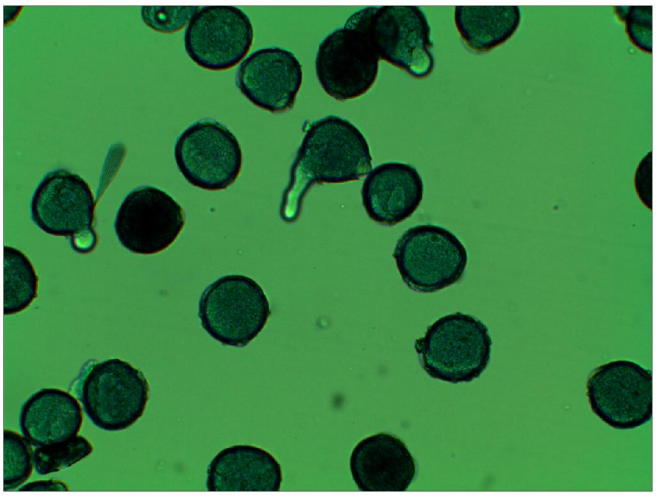
Plate 29. Pollen output, viability and germination in Kahikuchi Single



Pollen Output

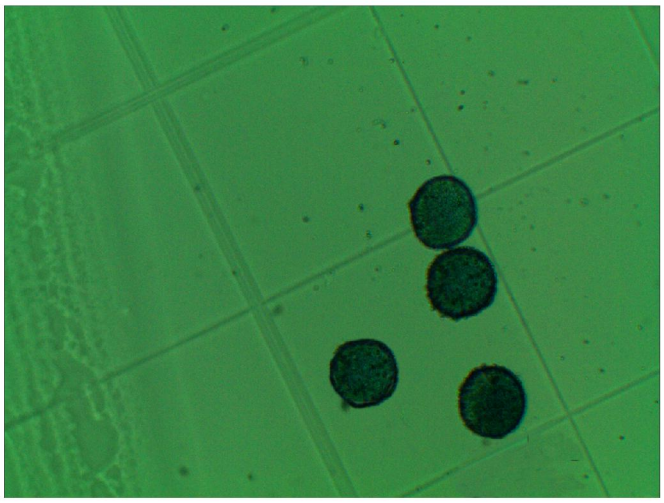
Pollen Viability





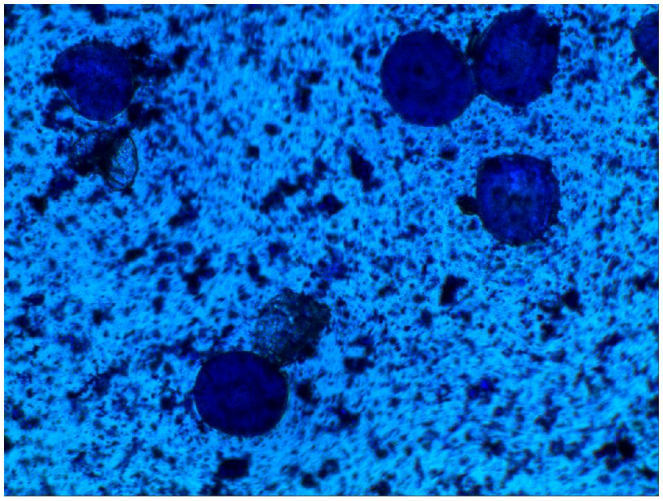
Pollen Germination

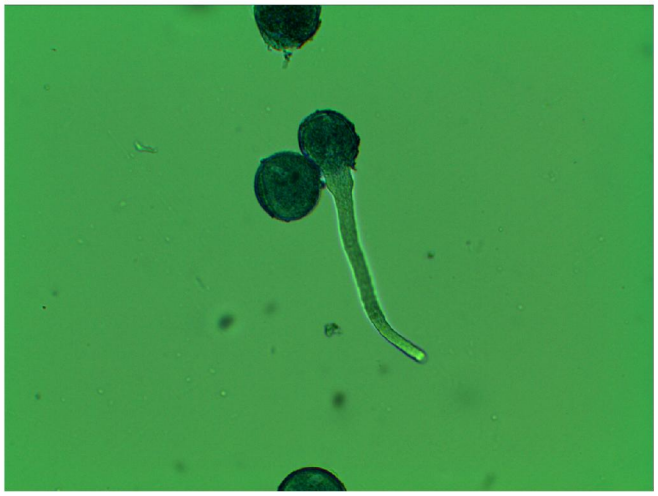
Plate 30. Pollen output, viability and germination in Mexican Single



Pollen Output

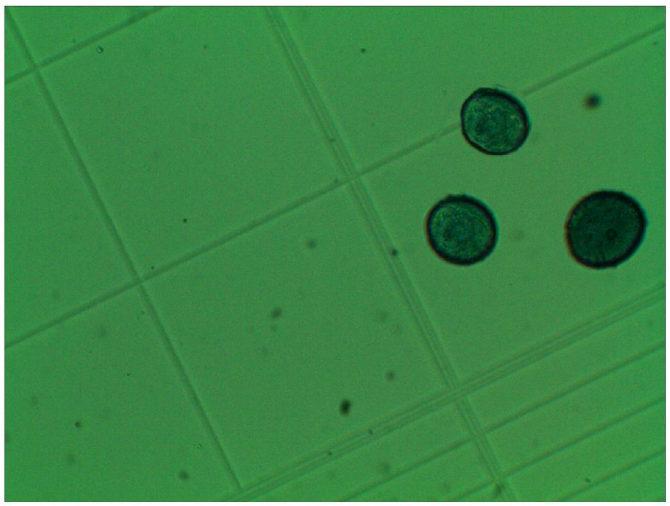
Pollen Viability





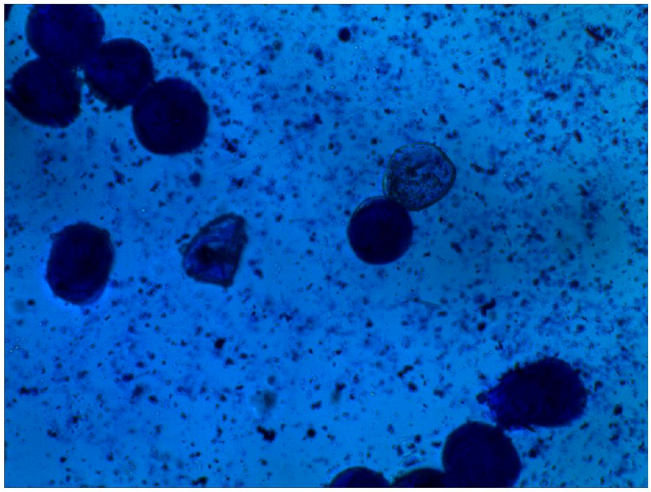
Pollen Germination

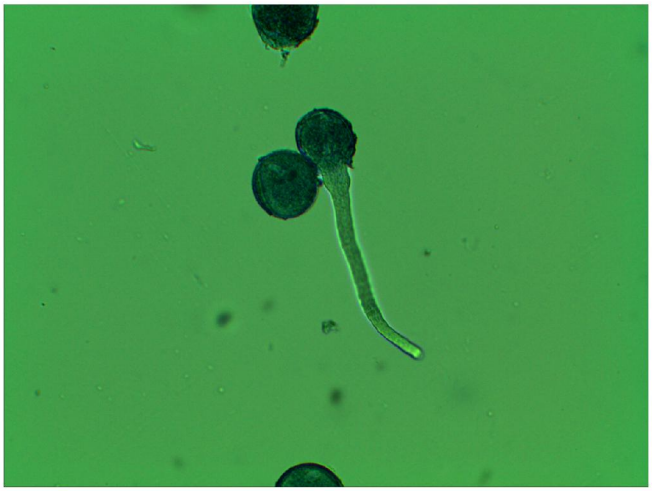
Plate 31. Pollen output, viability and germination in Navsari Local



Pollen Output

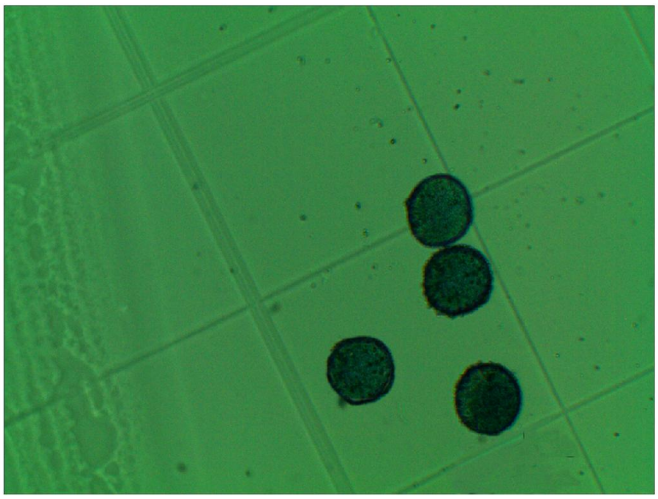
Pollen Viability





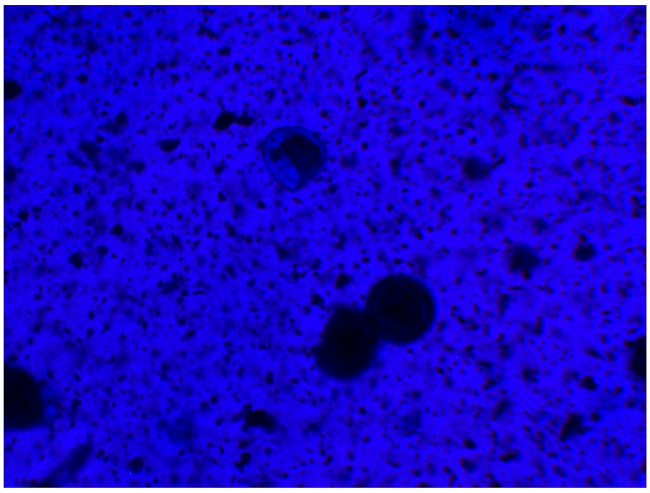
Pollen Germination

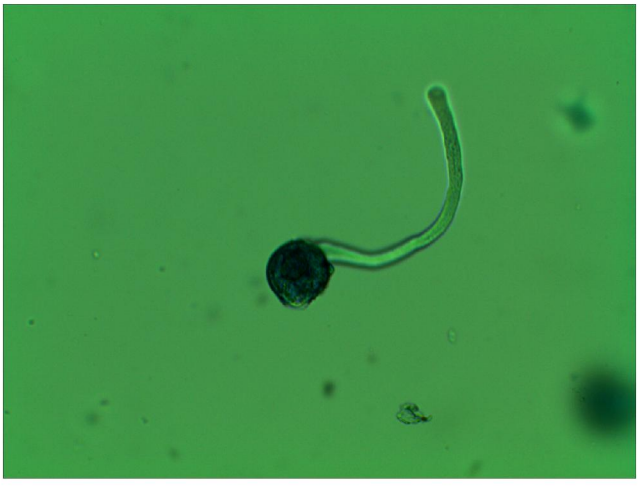
Plate 32. Pollen output, viability and germination in Phule Rajani



Pollen Output

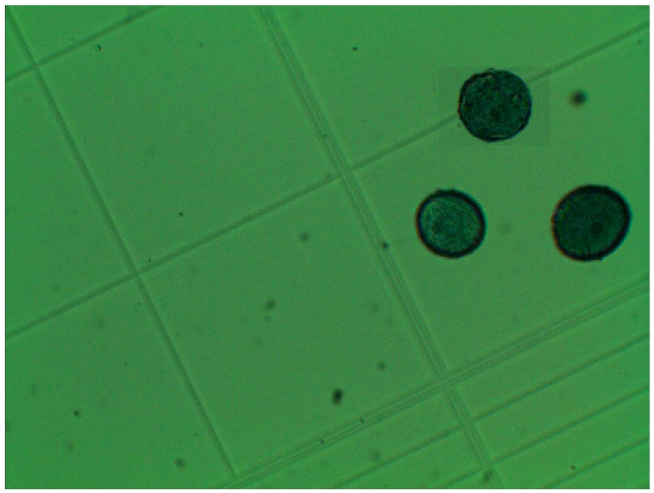
Pollen Viability





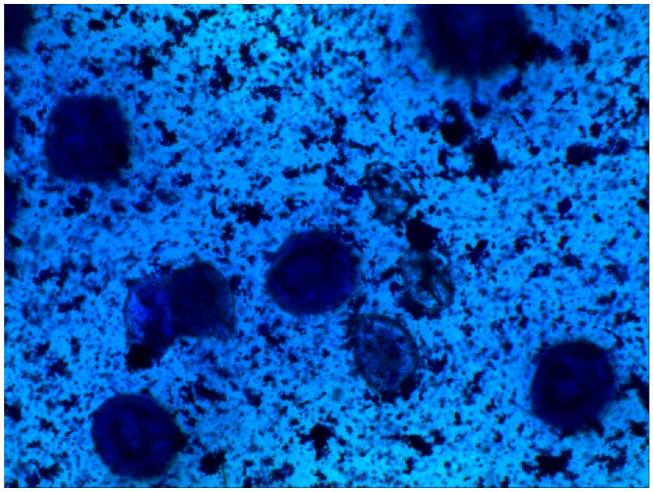
Pollen Germination

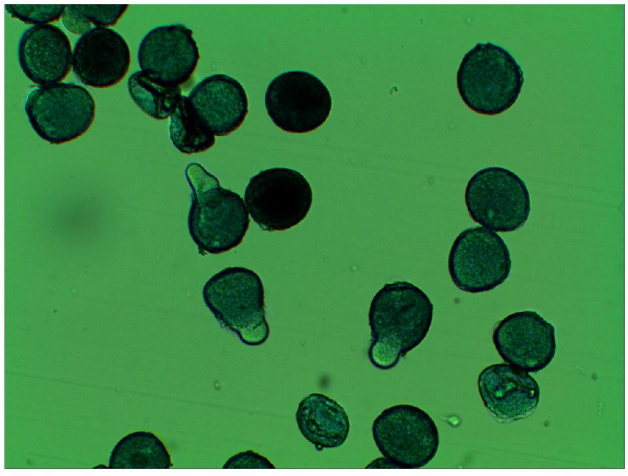
Plate 33. Pollen output, viability and germination in Prajwal



Pollen Output

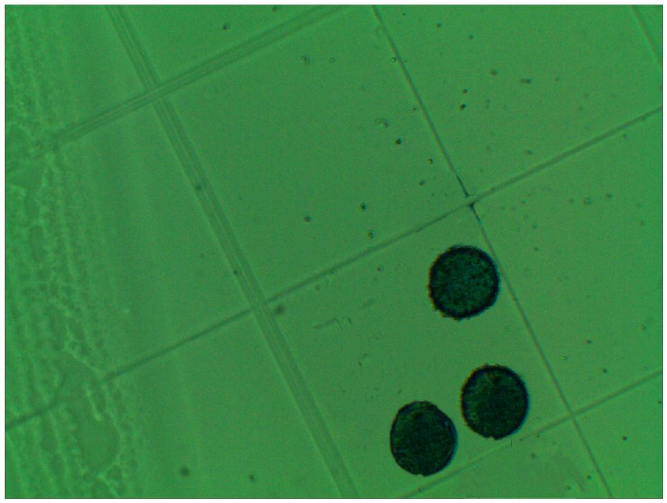
Pollen Viability





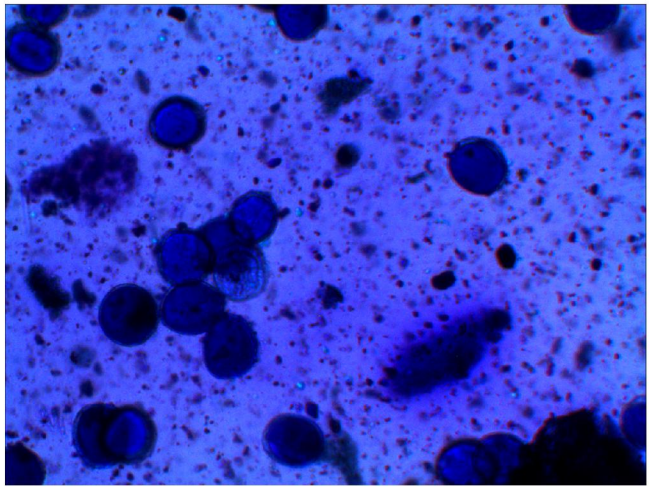
Pollen Germination

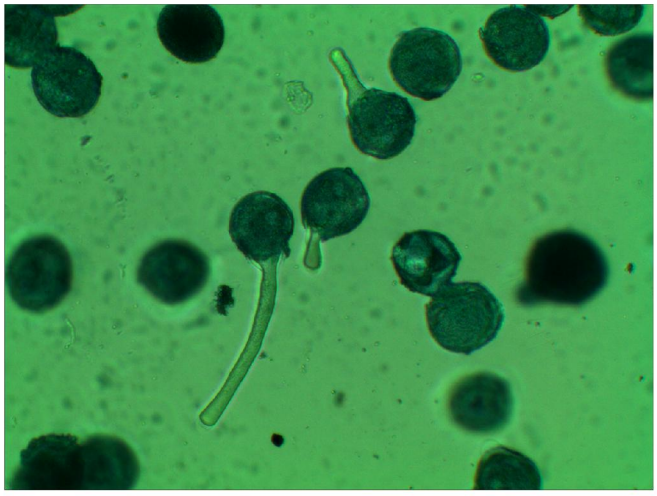
Plate 34. Pollen output, viability and germination in Pune Single



Pollen Output

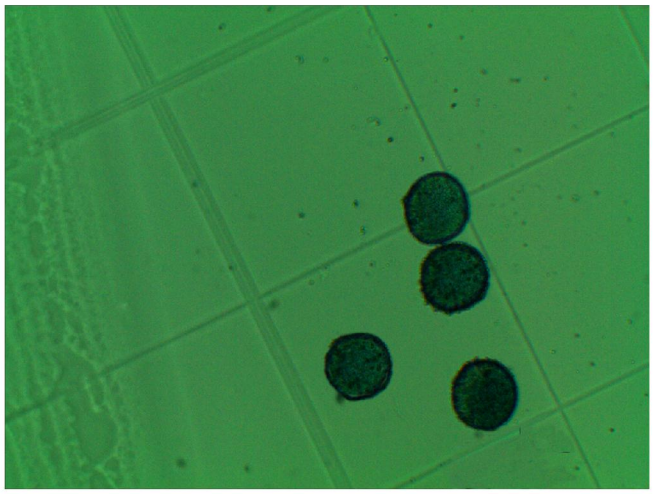
Pollen Viability





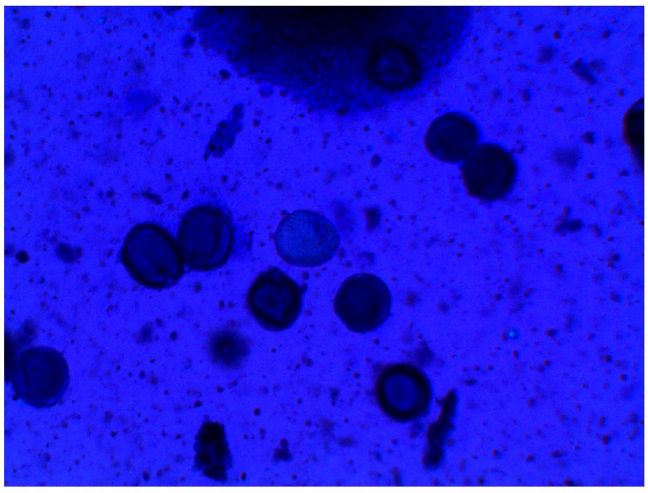
Pollen Germination

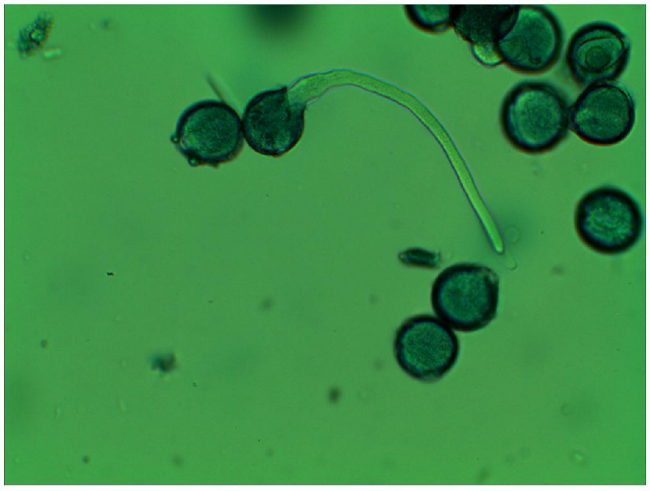
Plate 35. Pollen output, viability and germination in Shringar



Pollen Output

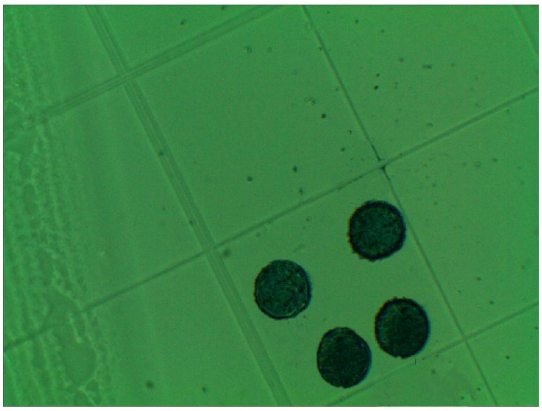
Pollen Viability





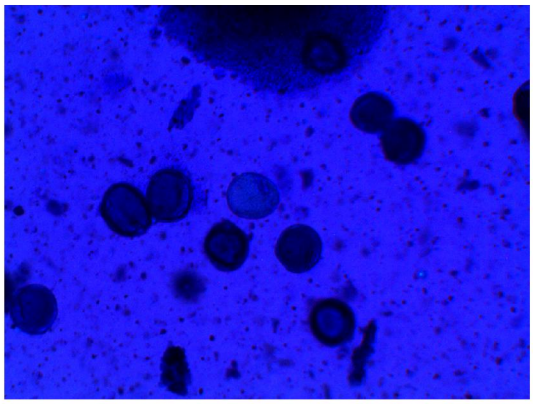
Pollen Germination

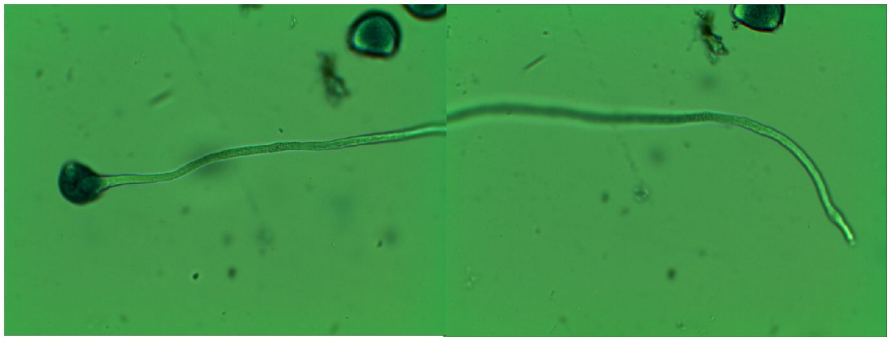
Plate 36. Pollen output, viability and germination in Vaibhav Single



Pollen Output

Pollen Viability





Pollen Germination

Plate 37. *In-vitro* pollen germination and pollen tube growth

Pollen germination & pollen tube growth of Variegated Single

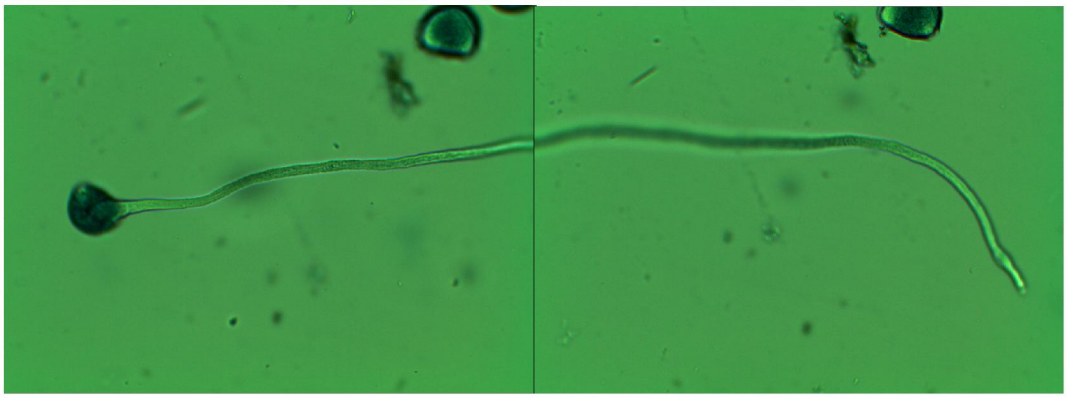


Plate 38. Pollen germination and pollen tube growth in successful crossses

Phule Rajani x Variegated Single

Plate 38a. Pollen germination on stigma

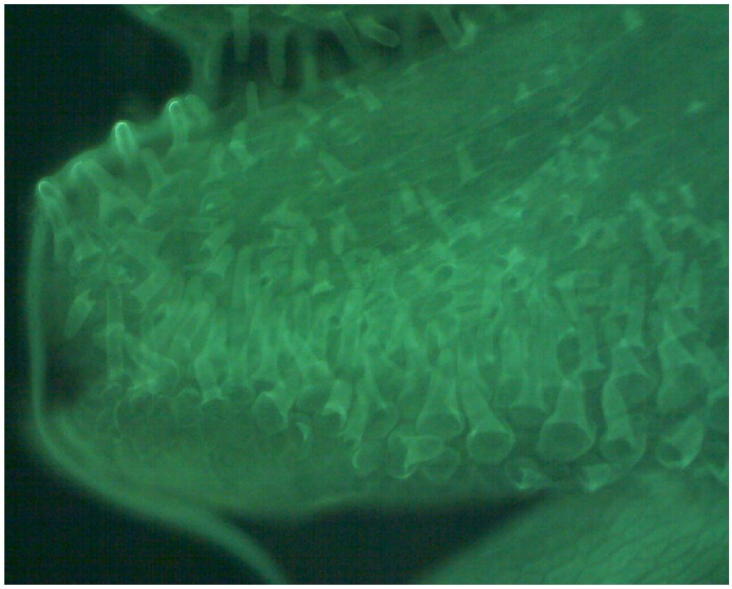


Plate 38b.Pollen tube growth on stigma (25 X)

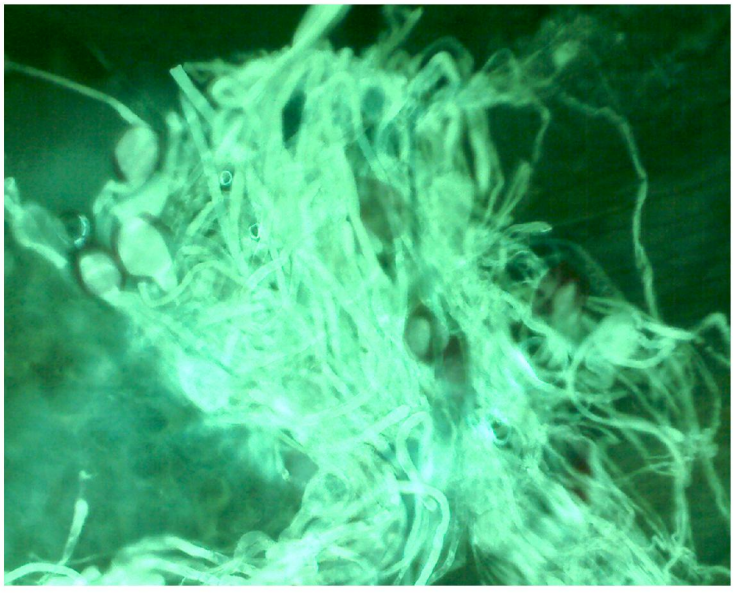


Plate 38c. Pollen tube growth on stylar region (25 X)

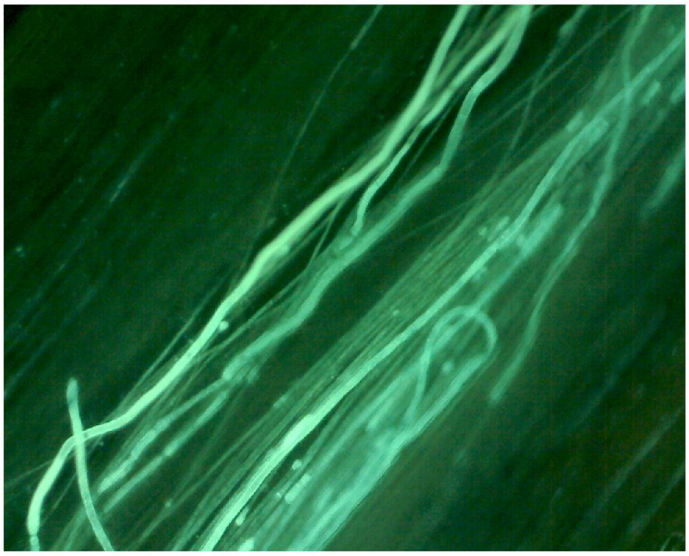


Plate 38d. Pollen tube growth in ovary and entry at the ovule

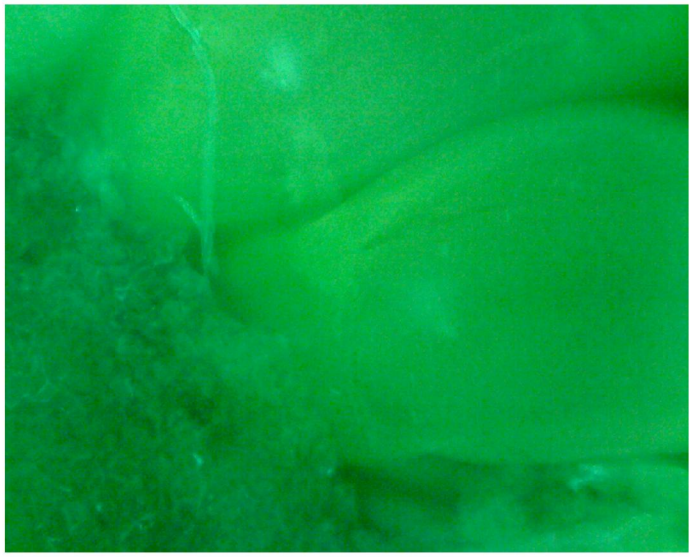


Plate 39. Pollen germination and pollen tube growth in incompatible crosses

Phule Rajani x Prajwal

Plate 39a. Pollen germination on stigma (25 X)

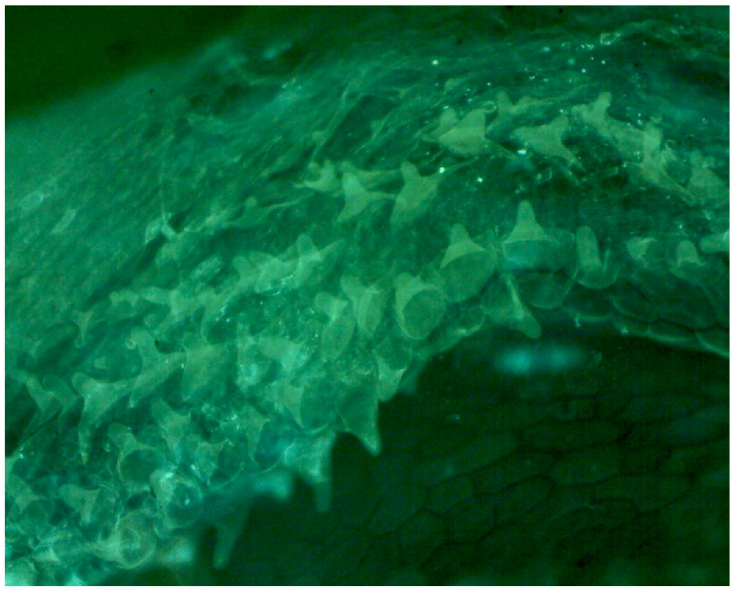


Plate 39b. Formation of callose plug during pollen tube growth (25 X)

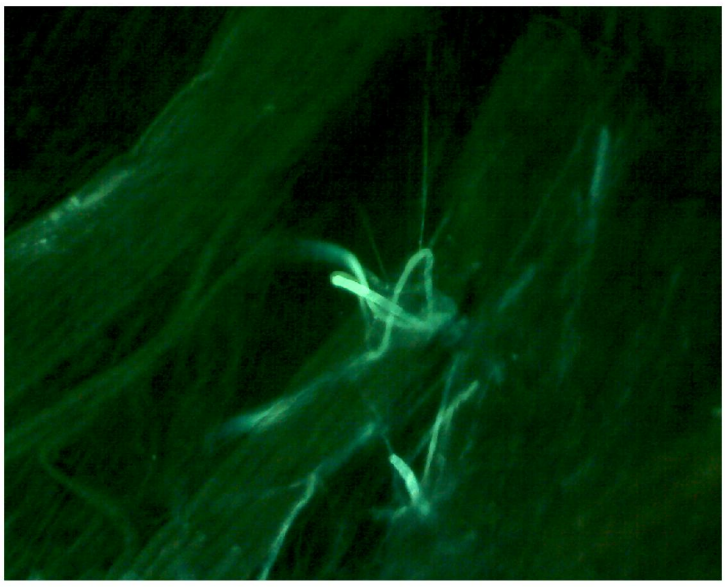


Plate 39c. Formation of knot like structure in the tip of pollen tube at the stylar region (25 X)

Plate 39d. Breakage of pollen tubes at the stylar region (25 X)

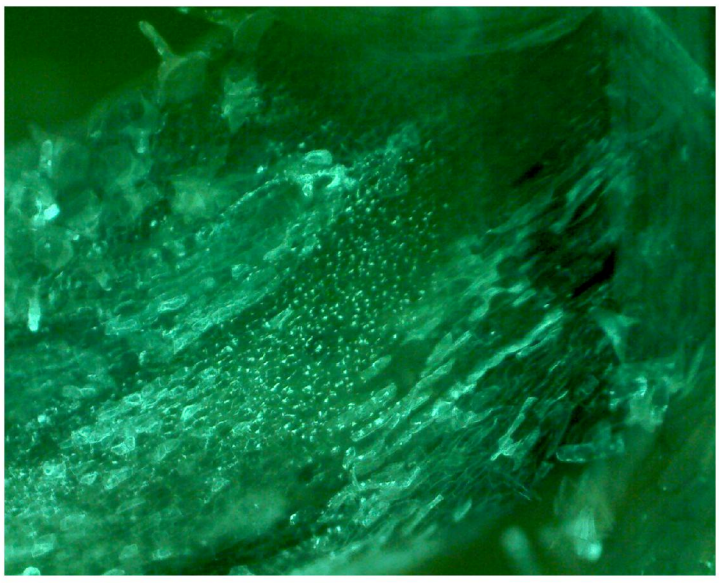
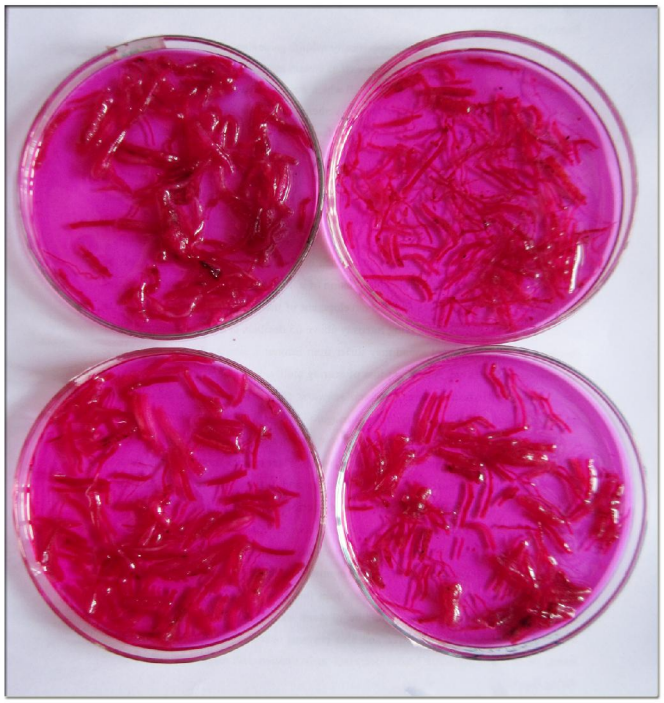
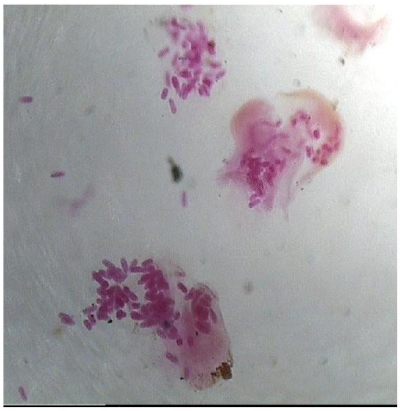


Plate 40. Infected roots stained with FAA



Microscopic view

Eggs of root knot nematode



Females of root knot nematode

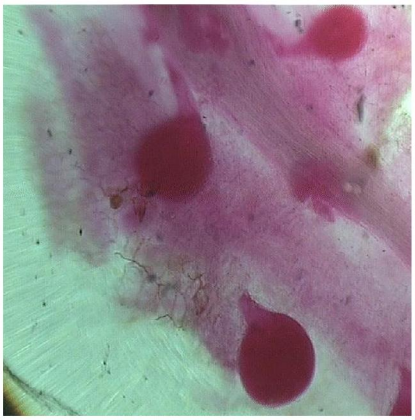
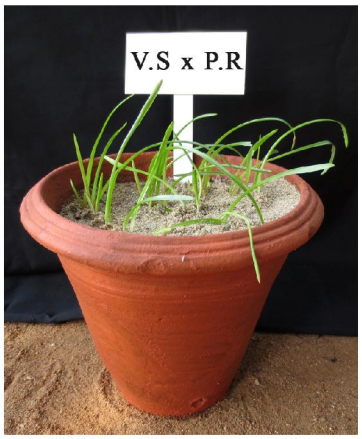


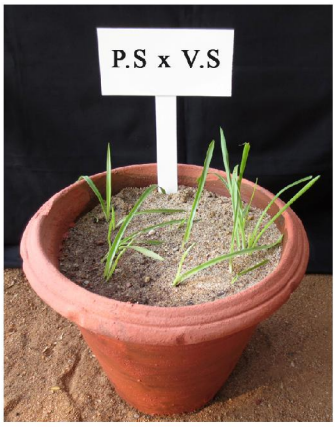
Plate 41. Root knot nematode gall index in tuberose genotypes

Characteristic symptoms of RKN infected tuberose roots

Plate 42a. Hybrid of various cross seedlings



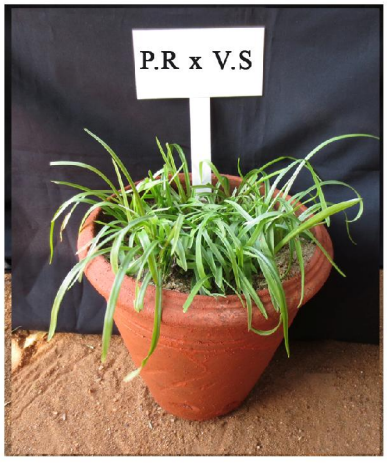
Variegated Single x Phule Rajani



Pune Single x Variegated Single

Calcutta Single x Variegated Single

Kahikuchi Single x Variegated Single

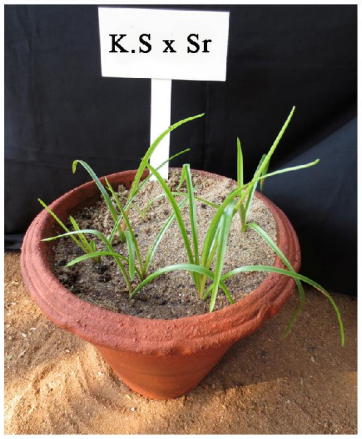


Phule Rajani x Variegated Single

Variegated Single x Hyderabad Single

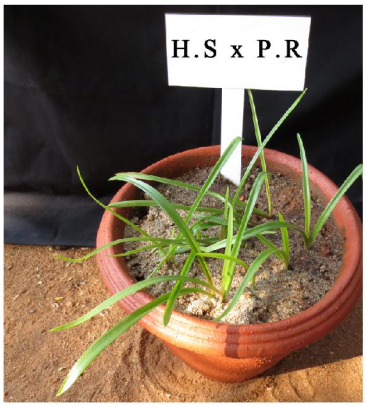
Plate 42b. Hybrid of various cross seedlings

Kahikuchi Single x Phule Rajani

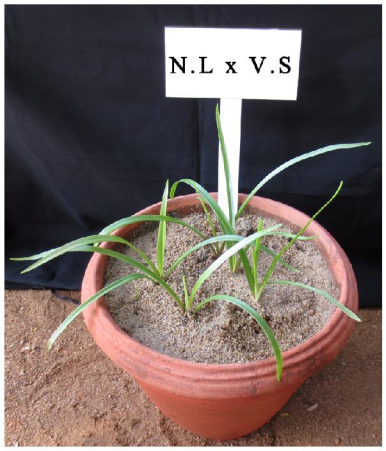


Kahikuchi Single x Shringar

Variegated Single x Shringar



Hyderabad Single x Phule Rajani



Navsari Local x Variegated Single

Mexican Single x Phule Rajani

Plate 42c. Hybrid of various cross seedlings

Mexican Single xVariegated Single

Variegated Single x Navsari Local

ANNEXURE I

WEATHER DATA DURING CROP GROWTH PERIOD (JUNE 2011 TO MARCH 2013)

S. No	Months	Temperature		Relative humidity (%)		Rainfall (mm)	No. of rainy days	Sunshine (hours)	Pan Evaporation (mm)	Solar radiation (Cal cm^{-2}/day	Wind speed (KMPH)
		Max. (0^0C)	Min. (0^0C)	Morning 07.22 hrs	Evening 14.22 hrs						
1	June'2011	30.6	23.0	83.50	57.0	92.1	7	5.1	4.7	345.5	9.3
2	July	31.0	23.2	82.8	56.3	31.5	4	5.0	5.2	338.3	10.0
3	August	31.3	22.8	87.2	56.4	7.1	1	4.3	4.9	315.7	8.5
4	September	30.3	22.2	88.1	58.5	48.2	4	6.9	5.4	356.2	8.2
5	October	31.6	22.6	90.6	59.3	305.3	14	6.5	4.3	356.9	4.4
6	November	28.7	20.8	89.7	61.0	243.1	10	5.4	1.8	329.3	4.7
7	December	29.3	19.1	89.2	52.0	11.6	1	6.4	3.3	372.2	4.7
8	Jan' 2012	29.7	18.4	88.5	45.9	1.0	-	8.2	3.7	406.4	4.9
9	February	32.3	19.3	83.5	35.3	-	-	8.4	4.8	416.9	6.0
10	March	34.9	22.5	83.1	35.7	1.2	-	8.3	5.4	420.9	4.8
11	April	35.2	24.3	85.7	42.5	78.4	2	7.3	5.4	369.1	4.6
12	May	34.5	24.1	87.0	49.1	25.6	2	7.8	5.4	382.8	5.6
13	June	32.4	23.8	77.5	49.2	11.1	1	5.6	6.4	368.2	11.3
14	July	31.5	23.5	78.7	51.7	27.5	3	5.3	6.1	353.2	11.6

15	August	31.2	23.0	83.0	54.7	28.3	4	4.8	5.5	323.2	9.8
16	September	32.4	22.6	83.7	49.6	6.1	1	7.1	6.3	360.5	8.5
17	October	30.6	22.3	87.2	58.7	165.2	5	5.7	4.3	313.4	5.7
18	November	30.7	20.5	89.0	48.0	22.4	2	7.2	4.0	372.3	4.2
19	December	30.5	20.3	85.0	43.0	6.9	2	6.6	4.7	383.52	6.2
20	Jan’ 2013	31.6	19.0	86.0	35.0	-	-	8.3	5.5	429.00	5.2
21	February	31.9	20.7	82.0	38.0	99.8	1	7.7	5.6	398.86	5.8
22	March	34.2	22.8	80.0	37.0	-	-	9.7	6.3	403.11	5.3

ANNEXURE - II

PROPERTIES OF THE SOIL AT EXPERIMENTAL SITE

S.No.	Particulars	Initial (Values)	Final (Values)
A.	**Chemical analysis**		
1.	Available nitrogen ($kg\ ha^{-1}$)	180.43	169
2.	Available phosphorus ($kg\ ha^{-1}$)	26.24	11.3
3.	Available potassium ($kg\ ha^{-1}$)	102.3	79
B.	**Micronutrient analysis**		
1.	Available boron (ppm)	0.500	0.862
2.	Available iron (ppm)	0.954	2.830
3.	Available zinc (ppm)	1.326	1.395
C.	**Other characteristics**		
1.	pH	8.38	
2.	EC (dSm^{-1})	1.04	