CHARACTERIZATION OF ONION GENOTYPES USING MOLECULAR MARKERS AND ITS FIELD PERFORMANCE IN THE PLAINS OF WEST BENGAL

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In

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By

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Dedicated
To
My beloved
Parents
&
Respected
Sir (Prof. T. K. Maity)

APPROVAL OF EXAMINERS FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHILOSOPHY (HORTICULTURE) IN VEGETABLE SCIENCE

We, the undersigned, having been satisfied with the performance of **Mr. TARIQUE ASLAM,** in the viva-voce examination conducted today, the January 3, 2019 recommend that the thesis be accepted for the award of the Degree of **Doctor of Philosophy (Horticulture)** in **Vegetable Science** of Bidhan Chandra Krishi Viswavidyalaya.

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Certificate

This is to certify that the work recorded in the thesis entitled **"CHARACTERIZATION OF ONION GENOTYPES USING MOLECULAR MARKERS AND ITS FIELD PERFORMANCE IN THE PLAINS OF WEST BENGAL"** submitted by **Tarique Aslam** for the award of the degree of **Doctor of Philosophy (Horticulture)** in **Vegetable Science** of Bidhan Chandra Krishi Viswavidyalaya, is the faithful and bonafide research work carried out under my personal supervision and guidance. The results of the investigation reported in the thesis have not so far been submitted for any other Degree or Diploma. The assistance and help received during the course of investigation have been duly acknowledged.

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Tarique Aslam

(Tarique Aslam)

List of Abbreviations and Symbols

@	at the rate of	Fig.	Figure
Cm	Centimeter	RH	Relative humidity
C.D.	Critical difference	pH	Concentration of active hydrogen ion
SEm(±)	Standard error ofmean	Max.	Maximum
^0C	Degree centigrade	Min.	Minimum
et al.	And others	No.	Number
etc.	Et cetera(= and the rest)	Viz	Videlicet(namely)
Fig.	Figure	h^2	Heritability(broad scence)
g	Gram	GCV	Genotypic co-efficient of variation
ha	Hectare	PCV	Phenotypic co-efficient of variation
i.e.	That is	TSS	Total soluble solids
kg.	Kilogram	°C	degree centigrade
/	Per	bp	Base pair
%	Percentage	cM	centi Morgan
µ	Miu	Ppm	Parts per million
µl	Microlitre	PCR	Polymerase Chain Reaction
mM	Millimolar	RP	Resolving power
m	Metre	RAPD	Randomly Amplified Polymorphic DNA
mm	Millimetre	SSR	Simple Sequence Repeat
M	Molar	PIC	Polymorphism Information Content
'	Prime	F value	Fixation indicate value
mg	Milligram	EDTA	Ethylene-diamine tetra acetic acid
ml	Millilitre	v/v	Volume per volume
%	per cent	dNTPs	deoxy Nucleotide Tri Phosphates
Mha	Million Hectre	PP	Percentage of Polymorphism
PEG	Polyethylene glycol	MI	Marker Index
Pg	Picogram	DI	Diversity Index
Dept.	Department	<	lesser than
DNA	Deoxyribo Nucleic Acid	>	greater than
Σ	Sum	×	Cross

Abstract

The present investigation entitled "**Characterization of onion genotypes using molecular markers and its field performance in the plains of West Bengal**" was carried out at the research field of All India Network Research Project on Onion and Garlic (ICAR), C- Block Farm, Bidhan Chandra Krishi Viswavidyalaya, Kalyani, West Bengal during *Rabi* seasons of 2015-16 and 2016-17. Twenty genotypes were laid out in Randomized Block Design with three replications. The genotypes were planted manually in flat beds of 2x1.5 m size at a spacing of 15x10 cm. Analysis of variance studies indicated significant difference among all the genotypes for all the characters under study. The varieties exhibit wide range of variation on different growth parameters and qualitative characters, yield components and yield. Among twenty varieties of onion, Bhima Kiran was the best in respect of highest yield (264.35 q/ha) in the year 2015-16 and (270.58 q/ha) yield in the year of 2016-17. The results indicated that PCV were greater than the corresponding GCV for all the traits in the both the years as well as over pooled analysis. Difference between GCV and PCV were found minimum for most of the characters except double bulb (%) which exhibited high difference between GCV and PCV. The characters like Double bulb (%), Neck thickness (mm), Days to maturity, Grade B bulb, Grade C bulb, Marketable yield (kg/plot), Total yield (kg/plot), Total soluble solids (^{0}Brix) and Physiological weight loss percentage after one month showed low to moderate heritability with low genetic advance, whereas Marketable yield (q/ha) and Total yield (q/ha) showed moderate heritability with high genetic advance in the year 2015-16. On the contrary, high heritability concomitant with low genetic advance had exhibited by most of the characters except Marketable (bulb/plot), Marketable yield (q/ha), Total yield (q/ha), Percent disease index, which showed high heritability with moderate to high genetic advance. Narrow difference between GCV and PCV and high heritability coupled with high genetic advance confirmed that least environmental effects on most of the characters. Individual bulb weight (g) consistently significantly positive associations with a number of characters like Marketable yield (kg/plot), Marketable yield (q/ha), Total yield (kg/plot) and Total yield (q/ha) at phenotypic level, therefore, this trait may be considered important and undeviating relationship with yield. When interrelationship among these three characters where considered, then selection of any other traits had capacity to generate a strong correlated response selecting individually as well as among them. Based on multivariate analysis, the varieties were grouped into 5 clusters. The genotypes of cluster I recorded the superior

performance for total yield, marketable yield, days to maturity, individual bulb weight, minimum double bulb percentage, Physiological weight loss percentage after one month, whereas the genotypes of cluster – II had higher days to maturity, total yield, marketable yield, desirable minimum values for PWL after one month and double bulb percentage. Similarly, the genotypes of clusters-III, clusters-IV cluster-V showed maximum values for days to maturity. In relation to Purple Blotch disease, lowest PDI observed in RO-636 followed by PKV White, Bhima Sweta and Arka Kirtiman, whereas, highest PDI observed in DOGR-546-DR followed by DOGR-571-LR, Line-355 and DOGR-HY-1. In the controlled condition after artificial inoculation of purple blotch, almost every plant got infected but disease severity was varies with the varieties; DOGR-546-DR, DOGR-571-LR and Line-355 got severe infection but genotype like Arka Kirtiman and Arka Sweta, PKV White and RO-636 showed less severity. Genetic divergence of 16 varieties was analyzed by using 10 RAPD and 9 SSRs primers. . Out of (10) Primers six primers showed 100% polymorphism (Oligo-02, Oligo-04, Oligo-05, OPC-04, OPQ-06 and OPG-13). Oligo-01 had (94.9%) moderate polymorphism and OPA-09 had the lowest polymorphism (78.4 %). The polymorphic bands proved to be useful in differentiating between the genotypes and group them into categories. Among SSR primers, ACM-004, ACM-068, ACM-187, ACM-326, ACM-046 ACM-240 and ACM-300 produced more bands and primer ACM-318 amplified the fewest. The SSR based grouping was more or less similar with phenotype and RAPD based grouping.

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CHAPTER I

Introduction

INTRODUCTION

Onion (*Allium cepa* L.) is an important bulbous vegetable crop grown around the world. It is consumed by common masses round the year in India and valued for its distinct pungent flavour and is an essential ingredient for the cuisine of many regions. Onion is liked for its flavour and pungency which is due to the presence of a volatile oil '*allyl propyl disulphide*'- organic compound rich in sulphur. Onion bulb is a rich source of minerals like phosphorus, calcium and carbohydrates. It also contains protein and vitamin C. It is being used in several ways as a fresh, frozen and dehydrated bulb. Dehydrated onion is in great demand which reduces transport cost and storage losses. Onion has got good medicinal value. It is stimulant, diuretic and having expectorant and anti bacterial properties. It prevents heart disease by lowering blood cholesterol and lipid level. It is one of the few versatile vegetable crops that can be kept for a fairly long period and can safely withstand the hazards of rough handling including long distance transport. The increasing demand of onion for domestic and export markets have encouraged farmers to grow the crop on larger scale.

India is the second largest producer of onion in the world after China. Of all 15 vegetable crops listed by FAO onion falls second to tomato in terms of production. Although onion is produced in all the States in India, the key onion producing states are Maharashtra, Gujarat, Madhya Pradesh, Karnataka, Orissa, Uttar Pradesh, Andhra Pradesh and Tamil Nadu in an area of 1.167 million hectare with the production of 20.21 million tonnes. The productivity of onion in India is 17.32 tonnes ha-1 (Anon., 2016). Onion is the leading agricultural commodity in export and earns valuable foreign exchange for the country. Of the total fresh vegetable exports, the share of onion is 67 per cent. During 2016-17, the export of onion from the country is around 2.4 million tonnes with the value to the tune of Rs. 3,386 crores (APEDA, 2017). The major importing countries of onion from India are UAE, Singapore, Gulf Countries, Sri Lanka and Bangladesh.

Onion is mainly grown as a Rabi crop in India, but the diverse climates and distinct seasons of country, making possible to grow an array of vegetable including onion in different seasons. In West Bengal, onion is generally grown during Rabi season and the bulb is made available from April onwards with production of 3.43 lakh metric tons during 2015-16 (Anon., 2016) from an area of about 22.0 thousand hectare.

The production and productivity of onion in India are very low as compared to other countries. The reasons for lower productivity of onion in India could be attributed to the limited availability of quality seed and lack of development of hybrids in onion is the major constraints (Kulkarni *et al.* 2012). Other reasons responsible for limiting the production and productivity of onion are unawareness of the farmers about suitable seasons, varieties for different seasons, climate, soil and improved cultivation techniques, as well as diseases and pests damaging the crop and their control measures and post harvest management, unawareness of characteristics of varieties, seasonality's and lack of proper post harvest management by marketing agencies.

The genus *Allium* has morphologically been relatively uniform and there is only little common morphological character for the delimitation of natural groups within the genus. Onion exhibits a good amount of morphological variation for plant height, bulb size and colour, bolting, purple blotch resistance, yield and quality traits. The phenotypic expression of the plant characters is mainly controlled by its genetic makeup and the environment, in which it is grown. Further, the genetic variance of any quantitative trait is composed of additive variance (heritable) and non-additive variance and include dominance and epistasis (non-allelic interaction). Therefore, it becomes necessary to partition the observed phenotypic variability into its heritable and non-heritable components with suitable parameters such as genotypic and phenotypic coefficient of variation, heritability and genetic advance. Further, genetic advance can be used to predict the efficiency of selection. Yield is a complex character controlled by a large number of contributing characters and their interactions. A study of correlation between different quantitative characters provides an idea of association that could be effectively exploited to formulate selection strategies for improving yield components. For any effective selection programme, it would be desirable to consider the relative magnitude of association of various characters with yield. The information on genetic divergence of various traits particularly of those that contribute to yield and quality would be of most useful in planning the breeding programme. D^2 statistics developed by Mahalanobis (1936) provides a measure of magnitude for divergence between two genotypes under comparison. It considers the variation produced by any character and their consequent effect that it bears on other characters. The technique was first used by Mahalanobis in an anthropometric survey of the united province in India. This technique has been applied in several crops to select genotypes for further breeding programmes. Grouping

of genotypes based on D^2 analysis will be useful in choosing suitable parental lines for heterosis breeding. Such studies are also useful in selection of parents for hybridization to recover superior transgressive segregants and it can further result into release of improved open pollinated varieties for commercial cultivation.

Purple blotch (*Alternaria porri* L.) is most destructive disease prevailing in almost all onion growing areas of the world, which causes heavy loss in onions under field conditions. The yield loss of onion in India due to this disease under favourable conditions varies from 5.0-96.5 percent (Gupta *et al.*, 1994).The disease occurs under favourable conditions of temperature 28-30°C and 80-90% relative humidity. It is more common in *Kharif* season. Small, sunken, whitish flecks with purple coloured centres are common symptoms occurring on leaves and flower stalks. Further, large purple area develops forming dead patches. The intensity of disease varies from season-to-season. It causes losses of 25% in *Rabi*, 50% in *Kharif* in Maharashtra, while in Northern and Eastern parts 25-90% damage in bulbs and seed crop occurs when the disease appears along with *Stemphylium* blight. This leads to reduction of onion production which adversely affects exports and also results price hike within the country. Keeping in view of the above reason management of purple blotch of onion has become an issue in present condition.

Characterization and grouping based on phenotype are influenced by environmental variations; molecular markers are preferred because of polymorphic nature, co-dominance, selective neutral behaviour, easy and fast assay, high reproducibility and easy exchange of data between laboratories (Joshi *et al.* 1999). A molecular marker is a DNA sequence that is readily detected and whose inheritance can easily be monitored. There are different marker systems available for crop plants such as restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), inter simple sequence repeat (ISSR), microsatellite or simple sequence repeat (SSR), amplified fragment length polymorphism (AFLP), sequence characterized amplified region (SCAR), cleaved amplified polymorphic sequences (CAPS) and single nucleotide polymorphism (SNP) *etc.* (Semagn *et al.* 2006). For diversity analysis, RAPD and ISSR markers are widely used by horticulturists due to their low cost, simplicity and no need of prior sequence information. The knowledge of genetic diversity helps in the efficient management of germplasm and selection of parents for crossing.

Therefore, the experiment entitled "Characterization of onion genotypes using molecular markers and its field performance in the plains of West Bengal" was planned with the following objectives:

- Evaluation of *Rabi* onion genotypes on the basis of morphological, biochemical characters and yield performance.
- Reaction of Purple blotch (*Alternaria porri)* disease to the onion genotypes under field and controlled conditions.
- Characterization of onion genotypes using molecular markers.

CHAPTER 2

Review of Literature

REVIEW OF LITERATURE

The most distinctive feature of living organism is the presence of variation, which is a natural phenomenon and is of much significance in future breeding programme for crop improvement. Vavilov (1951) was probably the first to realize that variability in the initial material ensures better chances of producing desirable types. Mather (1952) stated that with the help of statistical advances in past, it could be possible to elaborate methods of measuring variation, partition it into its various components, *i.e.*, heritable and non-heritable, which also help in measuring and predicting the effects of selection. Onion varieties show wide range of variations among themselves. This causes difficulty in identifying the superiority of particular variety. Thus, before embarking upon any crop improvement programme, comprehensive study of existing variability is very essential. The inter-relationship between different characters is also of considerable significance since this helps in making simultaneous selection for different economic traits.

The relevant research information has been reviewed here under the following sub-heads:

2.1 Varietal Evaluation

2.2 Genetic Parameter studies

2.3 Genetic Divergence studies

2.4 Purple Blotch studies

2.5 Molecular Markers

2.1. VARIETAL EVALUATION

Kalra *et al.* (1995) studied the growth, pungency and flavour characteristics of different varieties of onions during bulb development. They observed fresh weight and dry weight, bulb diameter, total pyruvic acid content increased throughout bulb development. Bulb size was greatest after 120 days in cultivars VL-3 and 102-1, they noted.

Pramanick *et al.* (1999) observed significant differences between the varieties/lines for all the characters. They reported that Pusa Red gave the highest yield followed by SI 126. However, SI 126 had the highest total soluble solids, dry matter (%) and ascorbic acid contents, making it suitable for the table, for dehydration as well as for

storage purposes, they observed. They reported that Early Grano was least pungent followed by N 53 and SI 106, and Early Grano was the poorest in keeping quality followed by SI 13. They reported that Early Grano was early among the varieties.

Mohanty *et al.* (2000) observed that Kharif onion variety N-53 produced the tallest plant (58.5 cm) with maximum number of leaves/plant (17.5) whereas var. Arka Pitambar recorded the most dwarf plants (37.4 cm) with minimum number of leaves/plant (7.8), least diameter of bulb (4.2 cm) and the lowest bulb yield (156.8 q/ha). The neck thickness of the bulb ranged from 0.6 to 1.1 cm. The varieties Pusa Madhavi (0.6 cm), Agrifound Dark Red (0.7 cm), Arka Kalyan (0.7 cm), Arka Niketan (0.7 cm), Agrifound Light Red (0.8 cm) and Punjab Red Round (0.8 cm) showed thinner neck than the other varieties while N-53 had maximum neck thickness (1.1 cm). The bulb diameter was highest for Arka Kalyan (6.0 cm) which was *at par* with N-53 (5.8 cm) while it was lowest for Arka Pitambar (4.2 cm) and N 2-4-1 (4.6 cm). The highest bulb weight (90.2 g) was exhibited by N-53 while Pusa Red produced the smallest bulb (48.4 g). The highest total bulb yield of (315.2 q/ha) was obtained from Agrifound Dark Red which was statistically *at par* with N-53 (302.5 q/ha). Moderate yield of 276.0 q/ha was obtained from Arka Kalyan followed by Nasik Red (274.7 q/ha). Out of the 4 moderate yielders, Agrifound Light Red and Arka Niketan had better keeping quality.

From a performance study on onion cultivars, Mohanty and Prusti (2001) recorded the highest yield (21.06 t/ha) from cv. Arka Kalyan which was *at par* with Arka Niketan (19.64 t/ha) and Pusa Madhavi (18.96 t/ha), while Agrifound Dark Red and N 53 displayed moderately high yield of (18.06 and 17.85 t/ha), respectively. They also opined that cultivars suitable for Rabi crop if grown in the Kharif season could confer equally good bulb yield as that of Kharif cultivars. Arka Niketan and Pusa Madhavi with medium bulb size, better storage quality and high yield are advocated for commercial cultivation in the Kharif season. Evaluation of 12 varieties of onion during Kharif season revealed that Pusa Madhavi, Arka Niketan, Punjab Red Round, Agrifound Dark Red, Arka Pitamber and Agrifound Light Red produced small to medium sized bulb with thinner neck depicting better storage quality. The varieties N 53 and Arka Kalyan produced significantly higher bulb yield (228.54 and 220.60 q/ha, respectively) than other varieties (Mohanty and Prusti, 2002a).

Jain and Sarkar (2002) reported that plant height, number of leaves per plant and bulb diameter was highest in Arka Niketan but the yield was highest in Agrifound Dark

Red followed by Arka Niketan. Mohanty *et al.* (2002) studied the performance of onion cultivars during Kharif season in Bhawanipatna, Orissa and reported that varieties like Agrifound Dark Red and N 53 recorded significantly higher yield (280.40 and 270.52 q/ha, respectively) than other varieties. Pusa Madhavi, Arka Niketan and Punjab Red Round had the greatest potential for better storage quality. Agrifound Dark Red was suggested for cultivation in Kharif season.

Cheema *et al.* (2003) studied the nine exotic onion genotypes and observed highest number of leaves (14.17) and the lowest bolting percentage in AC-383-I while Nashik Red showed the lowest neck diameter (1.27 cm).

Dubey *et al.* (2004) studied the yield and different yield and bulb quality characters, including bulb diameter and size index, weight of 20 bulbs, percentage of doubles and bolters, total soluble solids, dry matter, gross and marketable yield in onion. They reported that performance of Agrifound Light Red was stable for bulb development and bulb yield.

Onion cvs. Baswant-780, Arka Kalyan and Agrifound Light Red had higher weight & diameter of bulb and bulb yield as reported by Mahanthesh *et al.* (2005).

Gautam *et al.* (2006) studied the performance of different varieties of onion and their transplanting time for off-season production in mid hill conditions. They observed the highest fresh bulb yield (16.63 t /ha) was on the variety N-53.

According to Mahanthesh *et al.* (2007) plant height, number of leaves per plant, neck thickness, number and thickness of bulb, weight and volume of bulb had positive and significant correlation with the bulb yield. Thus, it would be rewarding to lay due emphasis on the selection of these characters for rapid improvement on bulb yield of onion.

Mahanthesh *et al.* (2008) carried out an experiment on the screening of different onion (*Allium cepa* L.) genotypes for bulb size and other characters of onion bulbs conducted during Kharif seasons under rainfed conditions at the Agricultural Research station, Hiriyur in Central Dry Zone of Karnataka. The genotypes Baswant -780 and Arka Kalyan which gave bigger sized bulbs with maximum weight, volume and diameter of bulb, number and thickness of rings per bulb.

In Regional Research and Technology Transfer Station, Keonjhar, Orissa, Das (2008) conducted an experiment during Kharif season to study the effect of seedling age

and variety on the yield of Kharif Onion. He observed that 45 days old seedlings of the variety Nasik Red out yielded all other varieties by producing the highest yield of 438.41 q/ha and gradually yield decreased when seedling age subsequently 75 days old.

Supe *et al.* (2008) were conducted experiment at Onion Research Station, Pimpalgaon Baswant (Nashik) to evaluate eight new onion genotypes during rangda (Late Kharif) season. The results revealed that among the different genotypes S-1 proved to be superior in terms of growth and yield parameters like days to 50% top fall after transplanting (86 DAT), neck thickness (1.19cm), maximum bulb to green top ratio (4.47), lowest premature bolting percentage (3.17%), less twin bulb percentage (5.03%) and highest bulb yield per ha. (525.31q/ha). The same selection also recorded minimum purple blotch index (12%) among all the selections and varieties tested.

Baswant-780 was the top yielded with a mean bulb production of 230.50 q/ha. Agrifound Dark Red (AFDR) and N-53 with a yield potential of 199.40 and 176.50 q/ha, respectively were the next best performers in that order. The study marked that highest yield manifested by B-780 was accompanied by better growth and larger size of bulbs. (Sharma, 2009). Onion varieties Baswant-780 and Agrifound Dark Red excelled the existing recommended Kharif onion variety N-53 by producing 200.7 q/ha with 74.7 g bulb size and 179.1 q/ha with 69.5 g bulb size, respectively; thus these varieties were also found suitable for cultivation during kharif season in the lower hills of Himachal Pradesh (Dev, 2009).

Bhat and Bhusan (2009) experimented on different onion genotypes (Agrifound Dark Red, L-28, Agrifound Light Red, N-53, Arka Niketan, Yellow Globe, Brown Spanish, Arka Kalyan, Patna Red and Local genotype). They reported that Agrifound Light Red recorded the maximum yield of 35.2 q/ha followed by Arka Niketan (33.9 q/ha) that was found significantly higher than all other cultivars.

Bhatia *et al.* (2009) evaluated the onion genotypes for growth and yield under Hisar conditions. They reported that variety Pusa White Round gave the highest yield (283.0 q/ha) and minimum yield was recorded in NRCWO-2 (153.5 q/ha). Sarada *et al.* (2009) evaluated the performance of 8 onion cultivars (Agrifound Dark Red, Agrifound Light Red, Arka Niketan, Arka Kalyan, Arka Pragati, Pusa Red, N 53 and Nasik Red) in black soils in the Krishna-Godavari zone of Andhra Pradesh, India. They noted that

cultivars varied significantly in growth and yield parameters. Arka Kalyan produced the tallest plants (61.14 cm) and Arka Niketan and Arka Kalyan were superior in terms of number of leaves per plant (16.7 and 15.4, respectively).

Giri *et al.* (2009) studied the performance of onion cultivars during Kharif season in the plains of West Bengal. The results revealed that cultivars like Agrifound Dark Red, Baswant-780, Agrifound Light Red and N-53 among other cultivars tested in this experiment have the better potentialities to grow as a Kharif crop. The highest bulb yield (170.30 q/ha) was obtained in Agrifound Dark Red followed by Baswant-780 (151.40 q/ha).

Hazra *et al.* (2009) studied on identification of suitable early *rabi* onion varieties under West Bengal condition. Early-*rabi* onion with planting of seedlings in the first week of October employing the varieties like, Baswant 780, Agrifound Dark Red, Arka Pragati and Phule Safed and harvesting the bulbs during last week of February appeared to be the best.

At Krishi Vigyan Kendra of Haveri, Karnataka, Hiremath and Nagaraj (2009) conducted front line demonstration trials on onion. They observed that Arka Kalyan recorded higher yield of 18.57 t/ha compared to Bellary red (10.33 t/ha). The percentage increase in the yield over respective local control was 28.96 and 21.53 for Arka Kalyan and Bellary Red respectively, they noted. They also reported that yield fluctuation was due to variation in soil moisture availability, rainfall, soil type and pest attack as well as change in the location of trials every year.

Birari *et al.* (2011) studied the performance of twelve genotypes of onion (*Allium cepa* var. *cepa* L.) under Parbhani agro-climatic conditions. They recorded maximum bulb yield in Agrifound Light Red (43.08 t/ha) followed by L-28 (39.19 t/ha), J.N. DOW-207 (37.03 t/ha). They reported that genotype Agrifound Dark Red was found superior for most of the traits viz., less bolting percentage, less twin bulb percentage, maximum shape index, and more number of bulb per kg.

Chandrika and Reddy (2011) studied the varietal performance of onion to varied planting patterns in the non-traditional area of Southern Agro-climatic zone of A.P. The genotype Arka Pragathi was superior to Agrifound Light Red, Arka Niketan and Arka Kalyan with taller plants, producing more leaves per plant, accumulated higher dry

matter, bulbs of superior quality with longer length, diameter and weight. It produced maximum bulb yield of 33.83 t/ha in 2004 and 24.64 t /ha in 2005 and fetched mean maximum profit of Rs. 85588 and benefit cost ratio of 3.74.

Under Akola condition Jogdande *et al.* (2011) evaluated the performance of ten onion genotypes (Local-1, Local-2, Local-3, Locals-4, Local-5, Local-6, Kalwan Local, Chandur Local, Akola Safed and Agrifound Light Red). They reported that Agrifound Light Red produced maximum plant height, maximum number of leaves per plant was produced by Local-1 and Local-6 took minimum period for harvesting of bulb. Maximum weight of fresh bulb, cured bulb, marketable yield per hectare was recorded in variety Akola Safed.

Lakshmi and Padma (2011) evaluated onion varieties in high altitude and tribal zone of Andhra Pradesh. The results revealed that B-780 followed by Phule Samarth recorded significantly higher yield (24.14 and 23.86 t/ha, respectively) compared to other cultivars due to the production of significantly larger size bulbs with more length, diameter and weight. It was found that kharif varieties if grown in rabi season could also confer good bulb yield. Though B-780, Agrifound Dark Red and Arka Kalyan are recommended for rainy season, the yields conferred that they could be grown in rabi season also under tribal zone of Andhra Pradesh.

Dwivedi *et al.* (2012) evaluated eight improved cultivars of onion (VL-1, VL-3, Arka Niketan, Arka Kalyan, Pusa Red, Pusa White flat, Pusa Hybrid 107 and Pusa Hybrid 102) for growth, yield and quality traits under agro-climatic conditions of Kymore Plateau region of Madhya Pradesh. They reported that variety Pusa Hybrid 102 recorded significantly highest yield along with width of bulb, weight of bulb and dry matter content followed by Pusa White Flat with highest TSS content.

Naik *et al.* (2012) evaluated onion varieties under Central Telengana conditions of Andhra Pradesh. They took observations on plant height, yield parameters (bulb diameter and bulb weight) and yield. They reported that Arka Pragati, followed by Light Red onion and Arka Kalyan, was well adapted to the agro-climatic conditions of the Central Telangana zone of Andhra Pradesh in terms of yield.

At Department of Horticulture, Marathwada Agricultural University, Parbhani (Maharashtra), Pardesi and Wasker (2012) investigated the performance of improved onion varieties. The results revealed that variety Arka Niketan (Check) was early in

maturity, while variety PKV Selection White was best for low twin bulb percent. The neck thickness was lowest in variety Sel-383. Yield of bulb was found to be highest in variety JNDWD-207.

Reddy *et al.* (2013) studied on evaluation and popularization of onion *(Allium cepa* var. *cepa*) varieties during *kharif* season under semi-arid conditions of Andhra Pradesh. They reported that Agrifound Dark Red, N-53 and Agrifound White produced bigger size bulbs leading to high bulb yield (37.57, 31.64 and 26.09 t/ha, respectively). Rabi varieties when grown during Kharif season could confer equally good bulb yield as that of kharif varieties

Tripathy *et al.* (2013) reported that the cultivars Bhima Super, NRCWO-3, NRCRO-4 and Arka Niketan (control) produced significantly high total bulb yield (325.41 to 376.00 q/ha).

Umamaheswarappa *et al.* (2014) studied the morphological and yield attributes of onion (*Allium cepa* L.) varieties under Central Dry Zone of Karnataka. They reported that the highest and significant polar diameter of bulb (5.40 cm), equatorial diameter of bulb (5.60 cm), bulb weight (74.20 g), total yield (370.50 q/ha) and marketable yield (328.9 q/ha) was noted in the genotype NRCWO-2, NRCWO-2 and HOS-4. They suggested these varieties are to be under cultivation during Kharif season in Central Dry Zone of Karnataka.

The highest bulb yield (313.5 q/ha) was recorded in genotypes AVT-I- BLRO-1229 and AVT-II-CLRO-1227, followed by IET hybrid-ALRO-1230 and IET-ALRO-1243(297.0 and 280.5 q/ha, respectively) whereas the lowest bulb yield (171.6 q/ha) was obtained in AVT-II-CLRO-1275 genotypes (Attri *et al.*, 2015).

Hirave *et al.* (2015) reported that the variety N-53 produced the maximum plant height (66.87 cm). While, Bhima Red and Phule Samarth were *at par* in terms of number of leaves per plant (14.33 and 14.20 cm). Regarding neck thickness, the variety Pune Red recorded minimum value (0.87 cm) which was *at par* with N-53 (0.93 cm), Bhima Super (0.97 cm) and Baswant-780 (1.03 cm). The variety N-53 produced the maximum bulb diameter (6.61 and 6.36 cm), fresh bulb weight (110.95 and 106.28 gm), cured bulb weight (99.53 and 95.30 gm). However, cultivar Bhima Red recorded

maximum marketable yield per hectare (328.57 quintal/ha) which was *at par* with Bhima Raj (298.41 q/ha) and Bhima Super (269.83 q/ha), respectively.

Tarai *et al.* (2015) reported that Bhima Kiran and Bhima Shakti recorded the highest bulb length (6.2 cm) and bulb diameter (5.4 cm) respectively. They further reported that better performance was observed in Bhima Shakti, Arka Kalyan and Agrifound Light Red with respect to bulb weight (77.5 g, 75.8 and 72.5 g), yield per plot (29 kg, 28.4 kg and 27.2 kg) and yield per ha (24.8 t/ha, 24.3 t/ha and 23.2 t/ha), respectively. The recorded lowest neck diameter was in N-2-4-1 followed by Arka Niketan and Agrifound Light Red indicating their potentiality for longer storage life.

Twenty high yielding varieties are evaluated and the biometric and yield parameters were analyzed. The study revealed that onion varieties Bhima Sakti, Agrifound Light Red, Agrifound White, Arka Kalyan, Bhima Super, and Agrifound Dark Red can be recommended for the plains of Thrissur distrct, Kerala. The results presented based on analysis of data reflects that all varieties tried were appreciable in the field conditions except Bhima Raj in terms of marketable bulb yield and percentage of productive bulbs (Menon *et al.*, 2016).

.Ganiger *et al.* (2018) conducted experiment with nine different varieties of onion. Among them, growth parameters like, more number of leaves was observed in Arka Bindu (12.62), highest plant height is seen in Arka Bheema (59.12 cm) and the highest leaf length reported in Arka Kalyan (50.37). Highest number of rings is seen in Arka Pragati (10.50) and lowest in Arka Kalyan (7.70). TSS was found highest in Arka Keetiman (14.70 ° B). Among yield parameters, bulb weight was the highest in Arka Pragati (212.62 g), total weight was reported highest in Arka Pragati (12.75 kg).

2.2. GENETIC PARAMETER STUDY

The effective selection depends upon the heritable components of genetic variation, which can be measured by heritability. Heritability is the portion of genetic variation which is transmitted from generation to generation. Lush (1949) classified heritability into broad sense and narrow sense. Heritability in broad sense is the portion of genetic variance to the total variance and therefore, gives the broad perspective whereas narrow sense heritability is the portion of additive genetic variance to the total variance and gives precise estimate of genetic variability. The knowledge of heritability is therefore essential for crop improvement. The estimates of heritability serve as a useful guide to the breeder. The breeder will be able to realize the proportion of

variation that is due to genotypic effect in case of broad sense heritability. If heritability of a character is very high, selections for this character would be fairly successful as there would be close correspondence between genotype and phenotype due to a relatively smaller contribution of environment to the phenotype. But for a character of low heritability, selection may be considerably difficult or virtually impractical due to the masking effect of environment on the genotypic effect (Singh,1990). Genetic advance is the measure of improvement that can be achieved by practicing selection in population. Since, the estimates of heritability give no indication of the amount of progress expected from the selection they are most meaningful when accompanied by estimates of genetic advance. High genetic advance coupled with high heritability is an indication of more additive gene action (Panse, 1957). Burton and Devane (1953) suggested that the GCV along with the heritability estimate could provide a better depiction of the amount of advance to be expected by phenotypic selection.

McCollum (1968) studied the heritability of bulb diameter and the shape index. He found that heritability was low for the bulb diameter, intermediate for bulb length and high for the shape index.

Randhwa *et al.* (1974) observed significant variation among genotypes for leaf number per plant. But, Sandhu and Korla (1975) observed low heritability and genetic advance for plant height in onion genotypes. Pandian *et al.* (1980) reported very high heritability for number of leaves per plant, coupled with low genetic advance. But Patil *et al.* (1986) noticed very high heritability with high value of genetic advance. Plant height had high heritability associated with low genetic advance (Vidyasagar *et al.*, 1993). Patil (1997) reported low variation among twenty five onion genotypes for neck thickness, the estimates of genetic and phenotypic variance were low, the GCV and PCV also reported to be low. He noticed lower heritability coupled with low genetic advance. Balareddy (1999) recorded low PCV and GCV and reported very high heritability associated with medium genetic advance.

Vidyasagar *et al.* (1993) recorded lower variation (10.4-15%) among genotypes studied for TSS. Sidhu *et al.* (1986) observed lowest genotypic coefficient of variation for TSS among all the characters studied among different genotypes of onion. The genotypic and phenotypic coefficients of variation were observed to be lowest (Trivedi *et al.*, 2006 (a) and Patil, 1997) for TSS. They also reported higher heritability associated with low genetic advance. Balareddy (1999) reported low to moderate GCV

and PCV, high heritability associated with moderate genetic advance for the character TSS.

Genotype environment interactions were significant for all characters, except bulb diameter. The highest genetic variation was observed in bulb yield (150.80-210.60 q/ha). Phenotypic variation was high for neck thickness (22.72%), but moderate for plant height (13.07%), bulb weight (10.65%) and number of leaves per plant (10.61%). High values of heritability coupled with moderate to high genotypic coefficient of variation and genetic gain were observed for the number of leaves per plant, neck thickness, and plant height and bulb weight. Bulb yield was significantly and positively correlated with the number of leaves per plant and bulb weight at phenotypic and genotypic levels. Neck thickness was positively correlated with plant height and bulb diameter, but was negatively correlated with bulb weight and yield at both levels. It was concluded that these two parameters, as well as the number of leaves per plant, were the most important components in the selection for high bulb yield. (Mohanty, 2001)

Fatema (2001) reported high heritability associated with high genetic advance as percentage of mean for plant height. Mohanty (2002) and Mohanty (2004) observed significant variation among twelve onion varieties reported high GCV and PCV for neck thickness, the heritability was estimated to be highest which was coupled with low genetic advance.

Pavlovic *et al.* (2003) recorded significant variability for bulb yield. Phenotype coefficient of variation (PCV) was greater than genotype coefficient of variation (GCV). Heritability confirmed that the genotype variability was stronger in the overall phenotype variability. The highest heritability coupled with genetic advance was recorded for sprouting losses, followed by rotting losses and total losses.

Prasad *et al.* (2005) observed significant variation in plant height among 209 onion lines. Monpara *et al.* (2005) observed non significant variation among the 106 genotypes for number of leaves per plant. However, bulb yield displayed significant and positive phenotypic and genotypic associations with number of leaves per plant, bulb length; bulb girth and 10-bulb weight (Golani *et al.*, 2006).

A study on genetic variability, correlation in storage life of onion *(Allium cepa* L.) was carried out by Trivedi *et al.* (2006). The results indicated that a positive direct effect was recorded for the characters like physical loss in weight, bulb weight, non-

reducing and total sugars, where as negative direct effect was registered for the attributes like rotting and sprouting losses, polar bulb diameter, equatorial bulb diameter, T.S.S., reducing sugars and marketable yield. High heritability was also recorded for total sugar, non-reducing sugar, marketable yield, polar bulb diameter, total soluble solids and physical loss in weight Trivedi *et al.* (2006 a).

Gurjar and Singhania (2006) evaluated thirty cultivars and local landraces of onion and revealed that the PCV were higher than the GCV for the different traits studied. High heritability with moderate to high GCV and genetic gain were recorded for bulb neck thickness, bulb weight and bulb yield, indicating that these traits could be improved by simple selection. Moderate to high heritability with low GCV and genetic gain were observed for plant height, days to maturity, number of leaves per plant, equatorial bulb diameter and dry matter content, indicating the prospective of heterosis breeding for their amelioration.

Bulb weight is an important character, directly related to bulb yield. Significant variation among onion genotypes for bulb weight was noticed by Aliyu *et al.* (2007). High heritability coupled with high genetic advance for the bulb weight was reported by Singh *et al.* (1995). McCollum (1971) noticed low heritability. While, Buso and Costa (1979) reported high heritability and Vidyasagar *et al.* (1993) observed moderate heritability associated with low genetic advance for bulb weight.

Haydar *et al.* (2007) reported non-significant variation for days to maturity among ten onion varieties. He recorded the highest genotypic and phenotypic coefficient of variation.

Pavlovic *et al.* (2007) reported that the genetic variance was higher than that of the environmental and the phenotypic coefficient of variation was higher than genetic coefficient of variation. The values of phenotype variance and heritability in broader sense showed that average dry matter was controlled more by genetic factors than the ecology conditions.

Sidhu *et al.* (1986) observed moderate heritability for days to maturity with low genetic advance. However, Vidyasagar *et al.* (1993) reported high heritability with low genetic advance for this character. Patil (1997) observed significant variation among genotypes for number of days to maturity and low PCV and GCV. High estimate of

genetic advance was recorded for TSS content with moderate to high values of heritability (Yaso, 2007). Moderate to high estimates of heritability coupled with high genetic advance were noticed for days to maturity (Yaso, 2007).

According to Hossain *et al.* (2008) higher genotypic coefficients of variations were recorded in number of seeds per scape (NSPS), final plant height (FPH), final scape height, fresh weight of bulb and bulb length. These characters also exhibited high heritability along with high genetic advance as percentage of mean. Phenotypic correlation coefficients showed that bulb length, bulb diameter and scape diameter were positively and significantly correlated with fresh weight of bulb. The number of seeds per scape, final scape height, final plant height and number of pseudostem branches at maximum flowering stage were also positively and significantly correlated with seed yield per scape.

Mahanthesh *et al.* (2008) observed that bulb weight was positively associated with polar diameter, length and girth of bulb. However, it was negatively correlated with days to maturity and total soluble solids.

Dhotre *et al.* (2010) reported high heritability coupled with high expected genetic advance for number of rings per bulb, TSS and dry matter content. Bulb yield exhibited positive and significant association with fresh bulb weight, equatorial diameter, TSS and number of rings per bulb and neck thickness was significantly correlated with rotting and total storage loss. Fresh bulb weight, equatorial diameter and bulb shape index exerted positive and direct effect and polar diameter and double/split bulb per cent showed negative direct effect on bulb yield. It was proposed to emphasize more on such characters to improve bulb yield.

Hosamani *et al.* (2010) reported variation was highest for bulb yield per hectare followed by average bulb weight, dry matter content and bulb neck thickness. The genotypic coefficient of variation and phenotypic coefficient of variation were high for yield and average bulb weight. Whereas, moderate variation for all the characters except total soluble solids and number of leaves per plant. The heritability in broad sense lowest in case of number of leaves per plant whereas, highest in dry matter content. Higher heritability estimates were obtained for the dry matter content yield per hectare, total soluble solids and average bulb weight and it was moderate for other traits.

Higher estimates of genotypic and phenotypic coefficients of variation were recorded for bulb weight, reducing sugars, non reducing sugars, total sugars, total loss and sulphur content, reducing sugars and sulphur content were registered the higher heritability coupled with high genetic advance was reported by Ananthan (2010).

The highest phenotypic and genotypic coefficient of variation was recorded on yield per plot, yield per hectare, bulb size, plant height and bulb weight. Similarly, the high heritability and genetic advance were also recorded on yield per hectare, plant height, bulb size, and bulb weight, being suggesting the major role of genetic constitution in the expression of the characters. Yield per hectare had positive and highly significant correlation with yield per plot, TSS and bulb size both at phenotypic and genotypic level, respectively (Navaldey *et al.*, 2011).

Dhaduk *et al.* (2011) reported that the genotypic correlation coefficient was higher than corresponding phenotypic ones for most of characters studied and there is predominant role of heritable factors. Bulb yield showed positive correlation with bulb polar diameter, equatorial diameter, average bulb weight, days to maturity at genotypic level while at phenotypic level yield showed significant and positive relation with average bulb weight. Path coefficient revealed that maximum positive direct effect was recorded by average bulb weight followed by plant height on bulb yield.

Morsy *et al.* (2011) reported Bulb weight had highly significant positive correlation with plant weight, dry matter and TSS in both seasons (Kharif and Rabi). The significant positive correlation was obtained between bulb weight and each of plant height and number of leaves per plant in both seasons. Marketable yield had highly significant positive correlation with plant height and total yield in both seasons. The significant positive correlation was obtained between marketable yield and each of number of leaves per plant, bulb length, dry matter and TSS content in both seasons. Total yield had highly significant positive correlation with plant height, plant weight, days to maturity and marketable yield in both seasons. Significant positive correlation was obtained between total yield and each of number of leaves per plant, bulb length, dry matter and TSS in both seasons.

Genetic variability and correlation in onion was studied by Ram *et al.* (2011) during Rabi seasons involving 16 genotypes. Results revealed that the genotype Pusa Madhvi, AOSDRB-0919 and AOSDRB-0913 performed better in terms of yield and

yield contributing traits and these lines may be use for breeding program. The highest phenotypic and genotypic coefficient of variations were noted for yield per plot, yield per ha, bulb size, plant height and bulb weight. The high heritability and genetic advance were recorded in yield per plot (86.9 and 0.60%), yield per ha, plant height, bulb size and bulb weight, suggesting the major role of genetic constitution in the expression of the character. Yield per ha had positive and highly significant correlation with yield per plot, TSS and bulb size both at phenotypic and genotypic level respectively.

GCV was higher than corresponding PCV for most of the traits. High heritability along with high genetic advance was observed for bulb yield and neck thickness. Correlation studies revealed significant positive correlation between yield per hectare with plant height, polar and equatorial bulb diameter, bulb weight and moisture content. Maximum direct positive effects were exhibited by equatorial diameter of bulb through average weight of bulb and TSS was reported by Chandanshive *et al.* (2011) in garlic.

From a study on genetic variability, Bharti *et al.* (2011) reported that the genotype cv. Pusa Madhavi, AOSDRB-0919 and AOSDRB-0913 performed better in terms of yield and yield attributing characters and therefore, these lines may be used for breeding programme. The highest phenotypic and genotypic coefficient of variations were recorded on yield per plot (16.96-15.81%), yield per ha (14.86-14.07%), bulb size (12.34-11.65%), plant height (12.00-11.57%) and bulb weight (14.27-11.57%). Similarly, the high heritability and genetic advance were also recorded on yield per plot (86.9 and 0.60%), yield per ha (89.6 and 7.30%), plant height (93.0 and 11.38%), bulb size (89.2 and 6.16%), and bulb weight (65.7 and 18.80%), suggesting the major role of genetic constitution in the expression of the characters. Yield per ha had positive and highly significant correlation with yield per plot, TSS and bulb size both at phenotypic and genotypic level, respectively.

Navaldey *et al.* (2011) conducted an experiment was on genetic variability studies in onion. They reported highest phenotypic and genotypic coefficient of variations on plant height (12.00-11.57%).

Singh *et al.* (2011) observed a wide range of variability in plant height (54.95 to 71.80 cm) of onion. They reported that marketable yield was significantly and positively correlated with plant height, neck thickness.

An investigation was carried out to study the genetic variability in late Kharif germplasm of onion at Nashik, Maharashtra by Singh *et al.* (2011). The results revealed a higher magnitude of coefficient of variation for bolters (112.78-112.65%), followed by doubles (86.35-86.16%), thrips/plant (37.55-37.36) and marketable yield (29.34 and 29.90%). Highest heritability was noted in doubles, gross yield, bulb diameter, plant height, bolters and thrips/plant. High genetic advance noted in bolters (231.73%), doubles (177.12%), thrips/plant (76.56%) and marketable yield (54.53%) and rest of others characters showed medium to low genetic advance. Marketable yield was significantly and positively correlated with plant height, neck thickness, bulb diameter, bulb size index, weight of 20 bulbs, and gross yield and negatively correlated with bolters, doubles and days for bulb initiation at genotypic and phenotypic levels.

Chattopadhyay *et al.* (2013) reported that all the traits except polar diameter had high heritability and high genetic advance as percent of mean. Traits with high values of heritability coupled with moderate genetic advance as percent of mean, *viz.* plant height suggest that selection for improvement of these characters may be rewarding.

Ibrahim *et al.* (2013) observed the existence of high genetic variability within the families studied with high negative heritability in some yield traits such as soluble solid content which shows the preponderance of this trait when selecting this type of local cultivar for breeding.

Jat *et al.* (2014) reported Pusa Madhavi was superior in terms of bulb yield per hectare. Large amount of variability was observed in the experimental for selection. Characters like plant height, leaf length, leaves per plant, bulb diameter, fresh weight of bulb, dry weight of bulb, bulb yield per plot, high and heritability coupled with high to moderate genetic advance.

Aditika *et al.* (2017) found the difference between phenotypic (PCV) and genotypic (GCV) coefficient of variation narrow for most of the traits except bulb weight and neck thickness. The GCV ranged from 4.82 (bulb diameter) to 14.60 per cent (bulb weight) while PCV ranged from 5.72 (number of marketable bulbs per plot) to 17.29 per cent (bulb weight) for the various characters studied. The high estimates of heritability were found for all the characters studied. Expected genetic advance over mean was observed high for the characters *viz* yield per plot, bulb weight and number of marketable bulb per plot.

Mohapatra *et al.* (2017) observed difference between GCV and PCV were minimum for plant height, polar diameter, equatorial diameter, total yield, whereas, difference between GCV and PCV was observed high for the other traits like, bolting percentage, double bulb percentage, split bulb percentage, neck thickness, number of leaves. The narrow difference between GCV and PCV indicated that these characters were least influenced by the environment. On the other hand, the wide difference between GCV and PCV designated that environment had major role for phenotypic expression of these traits. High heritability accompanied with high genetic advance was noticed for all characters except found for boliting per cent, number of leaves, split bulb percentage, neck thickness, showed low heritability with low genetic advance indicating that simple selection may be effective to fix and improve such traits. As a result, selection of these this character was not to be effective.

Dangi *et al.* (2018) assessed the degree of variability, heritability and genetic advance in onion (*Allium cepa* L.) using 13 quantitative and four biochemical traits. PCV was higher than GCV for all the traits but the difference was less in plant height, pyruvic acid, leaf length, number of leaves, pseudostem length, pseudostem width, leaf width and total phenols indicating higher contribution of genotypic effect towards phenotypic expression. Highest heritability was observed for plant height, number of leaves, leaf length, leaf width, pseudostem length, pseudostem diameter, total phenols and pyruvic acid. High heritability along with high genetic advance as percent of mean was recorded in number of leaves, leaf length, leaf width, pseudostem length, pseudostem diameter, total phenols and pyruvic acid indicating the presence of additive gene action for the expression of these traits.

2.3. GENETIC DIVERGENCE

The genetic variability which measures the forces of differentiations at two levels, namely intra-cluster and inter cluster level, is a prerequisite for any plant breeding program. Significant differences among genotypes in respect of agronomic traits are prerequisites of multivariate analysis. A considerable amount of genetic variability was observed and, therefore, diversity analysis was carried out through Principal component and D^2 statistics analysis.

Twelve cultivars of onion were evaluated during the Kharif season by Mohanty and Prusti (2002b) to study genetic diversity using Mahalanobis' D^2 statistic. The

populations were grouped into three clusters which included two monogenic groups. Maximum inter-cluster distance was recorded between cluster II and III (D^2=192.25). The cv. Arka Kalyan (Cluster II) was agronomically superior than the other cultivars. Genetic divergence was not parallel to geographic distribution. Weight of bulb and neck thickness contributed predominantly towards total divergence. Hybridization between cluster II (cv. Arka Kalyan) and III (cv. Pusa Madhavi) was suggested to recover transgressive segregates of kharif onion with high yield potential and other desirable characters.

Singh and Dubey (2011) found significant difference among twenty six onion lines for all the traits indicating sufficient genetic diversity among the cultivars. On the basis of D^2 values, the twenty six genotypes were grouped in six clusters. The cluster Ist was largest consisting twenty genotypes followed by cluster II with two genotypes (L-352, L-424), cluster III, IV, V and VI with one genotype were the smallest cluster. The highest intra-cluster value (D^2) and genetic distance was noted for cluster I (61.68), (7.83) followed by cluster II (39.63), while other clusters III, IV, V and VI had zero intra cluster value.

Adesoye *et al.* (2012) reported that the highest genetic distance (75%) was observed between light brown and white phenotypes. *A. ascalonicum* produced a distinct banding pattern from all other 14 genotypes belonging to *A. cepa.* Genetic diversity within and between the four phenotypic (colour) classes showed that highest variability exists within the purple cultivars. Nei's gene diversity ranged from 0.11 ± 0.19 in the light brown phenotypic groups to 0.27 ± 0.18 in the purple group. The observed number of alleles was 1.79 ± 0.41 and effective number of alleles was 1.46±0.35 for the purple phenotype, while 1.27±0.45 and 0.19±0.32 alleles represent the observed alleles and effective number of alleles in detecting polymorphism the light brown phenotype, respectively. Dendrogram constructed from similarity coefficient produced three clusters. The On8 and On10 cultivars from Borno and Kebbi states respectively were the closest probably as a result of exchange of genetic materials between farmers and for commercial purpose being an open pollinated crop, while On16 and On17 clustering together may be due to collection from same location and adaptation to the environment. Results are discussed in the light of current taxonomic delineations which consider *A. ascalonicum* as a subspecies of *A. cepa.* Interspecific Hybridization between *A. cepa* and *A. ascalonicum*is encouraged in order to introduce medicinally active compounds like

flavonoids into *A. cepa.*

An experiment was conducted by Singh *et al.* (2013) to study the variability and their interrelationship and divergence pattern based on quantitative and qualitative traits. All accessions were grouped into six different clusters. The highest inter cluster distance was observed between IV and V and lowest between II and VI. Based on cluster means the important cluster was I for plant height and percent marketable bulbs cluster IV for mean yield, total yield and equatorial diameter and cluster III for the number of leaves, collar thickness and average bulb weight. The greater part of the variance was accounted for other traits such as plant height, collar thickness, per cent marketable bulb yield, polar diameter of the bulb and neck thickness. The high diversity found in the accessions showed its great potential for improving qualitative as well as quantitative traits in long day onion.

From the study on genetic diversity Kale *et al.* (2014) found the maximum inter cluster D^2 value of 30.67 between cluster VI and IX, followed by the cluster IX and X. Cluster VII showed maximum intra cluster diversity (D^2=1 0.51) followed by cluster IX (D^2=10.17). Total soluble solids contributed maximum towards total divergence, followed by leaf area, dry matter, magnesium, calcium, vitamin C, bulb splitting per cent, pyruvic acid and bulb yield.

Khosa and Dhatt, (2015) analyzed 43 accessions of bulb onion for genetic variability studies based on morphological and biochemical traits. It was found that cluster-I was largest of all with 12 genotypes while cluster-IV and VI were the smallest with only two genotypes. Observations on cluster distances revealed that the intra-cluster distance were less than inter-clusters. In principal component analysis, first seven principal components explained 78.29% of the total variation and quantitative characters contributed significantly towards PC-1, whereas qualitative characters in PC-2.

Based on multivariate analysis, Mohapatra, (2017) grouped sixteen varieties into 5 clusters. The genetic divergence total soluble solids followed by pyruvic acid, vitamin C and individual bulb weight content the maximum contribution towards total divergence.

Dangi *et al.*, (2018) clustered all accessions into four groups based on Euclidean distance and Ward's minimum variance. Cluster I comprised of accessions with highest yield, yield related traits and high pungency. Cluster II was the largest cluster and

composed of accessions having highest TSS and dry matter content. Cluster III was the smallest cluster comprising of accessions having highest polar diameter and total phenols. Cluster IV comprised of accessions having low yielding potential. Five principal components (PC1 to PC5), having latent roots greater than one, accounted for 78.5% total variation. Cluster analysis and Principal component analysis (PCA) were in agreement for assigning genotypes into four clusters. In the first principal component, plant height, leaf length and pseudostem diameter were the most contributing traits, whereas dry matter, total soluble solids (TSS) and pyruvic acid were the principal traits of the second principal component. Based on squared cosine value (Cos2) for variables, average bulb weight, gross yield and marketable yield in positive direction and plant height, leaf length and pseudostem width in negative direction, were the major contributing traits. Squared cosine value (Cos2) for individual factor determined that Superex and Black Gold were the most prominent genotypes contributing towards PCA.

2.4. PURPLE BLOTCH STUDY

Onions like other vegetables are susceptible to numerous foliar, bulb and root pathogens that reduce yield and quality (Cramer, 2000). Purple blotch of onion caused by *Alternaria porri* is an important disease of onions worldwide (Chaput, 1995 and Schwartz, 2004) especially in warm and humid environments (Suheri and Price, 2000).

Among several factors, diseases are the most important factors associated with low productivity in onion. Purple blotch caused by *Alternaria porri* is one among the serious fungal diseases that affect onion, causing heavy yield loss ranging from 2.5 to 87.8 per cent during Kharif season (Srivastava *et al.*, 1994).

Dhiman *et al.* (1986) studied reaction of onion genotypes against purple blotch disease and was found that off the 18 genotypes raised for bulb crop and none was found to be resistant. Pathak *et al.* (1986) found only one line IR-56-1 as resistant and five lines viz., IHR-25, IHR-44, IHR-499, IHR-500 and Arka kalyan as moderately resistant. Sugha *et al.* (1992) evaluated 94 onion genotypes under natural conditions and designated just two varieties, IC39178 and IC49371 as resistant to purple blotch.

Bhonde *et al* (1992) screened 8 onion cultivars and recorded the lowest incidence and intensity of PLB on cv. Agrifound Light Red.

Sharma (1997) reported the lines IC48059, IC48179, IC39887, IC48025 and ALR showed resistance and another 10 lines were moderately resistant to purple blotch disease.

In garlic, cv. Blanco de Vallelado was most susceptible to SLB, while lines B4P 17 and B6P1 and cvs. Iberose and Golourose were less susceptible to the disease (Basallote-Ureba *et al.*, 1999).

Supe *et al.* (2008) conducted an experiment to evaluate the performance of 8 onion genotypes (S-1, S-2, S-3, M-9, M-11, N-53, AFDR and B-780) and recorded lowest PLB index (12%) on S-1 genotype.

Mishra *et al.* (2009) reported that out of 21 promising garlic lines which were screened for resistance against PLB and SLB, line G-54 was found moderately susceptible, line G-222 was moderately resistant and rest of lines were found to be resistant to PLB. Lines G-294, G-324, G-351, G-368, G369, G-176 and G-189 were found resistant against SLB, lines G-299, G-192, G-4 and G-323 14 were found moderately resistant, lines G-222, G-54, G-213, G-366 and G-264 were found susceptible, line G-52 was found moderately susceptible and line G-266 was found highly susceptible to SLB of garlic.

Chetana *et al.* (2011) screened different onion genotypes against purple blotch and revealed that the genotype Arka Kalyan was found moderately resistant while genotypes *viz.*, Rampur Rose, Agrifound Rose, Arka Pragati, Arka Niketan, Arka Pitamber and Arka Bindu was found moderately susceptible to the disease.

An experiment on evaluation of onion (*Allium cepa* L.) genotypes for tolerance to thrips (*Thrips tabaci* L.) and Purple Blotch [*Alternaria porri* (Ellis) Ciferri] was conducted by Tripathy *et al.* (2013). The results indicated that NRCRO-3, NRCWO-3, NRCWO-4 and VG-19 showed tolerance to both thrips (25.91 to 28.28 thrips plant-1) as well as purple blotch (PDI of 42.83 to 51.66%). The cultivars, Bhima Super, NRCWO-3, NRCRO-4 and the control, Arka Niketan produced significantly high total bulb yield (325.41 to 376.00 q/ha) having better tolerance to thrips (25.91 to 32.42 thrips plant-1) and Purple Blotch (42.83 to 56.50%). The cultivar with high yield potential having tolerance to both onion thrips and purple blotch disease identified for Odisha condition are Bhima Super, NRCRO-4, NRCWO-3 of DOGR, Pune and Arka Niketan of IIHR, Bangalore.

Behera *et al.* (2013) observed VG-18 cultivar as resistant and another 12 lines as moderately resistant to purple blotch. Cultivation practices comprised under TDTD viz., use of improved variety/season specific, Nursery Raising, Soil/Seed treatment, Transplanting, fertilizer application and control of Purple Blotch disease, showed that percentage increase in the yield of Onion ranged from (31.31 to 37.50%) over local check during the course of study from 2005-06 to 2009-10. (Ojha and Singh, 2013).

Kale and Ajjappalavara (2014) evaluated and screened Forty four genotypes of onion against purple blotch disease. The results revealed that the 5 genotypes viz., OG-4, OG-7, OG-14, OG-34 and OG-44 were found to be moderately resistant (Grade 2) and per cent of leaf area infection ranged from (11.00 to 20.00%). Under moderately susceptible (Grade 3) 31 genotypes were grouped with (21.00-40.00%) leaf area infection, five were susceptible (Grade 4) with leaf area infection from (41.00 - 60.00%) and the remaining two genotypes were highly susceptible with (Grade 5) and leaf area infection was more than (60%).

Tripathy *et al.* (2014) in a field investigation of onion for bulb yield and reactions to pests and diseases during kharif season reported the varieties like NRCWO-1, NRCWO-3. NRCWO-4, Bhima Super, Bhima Red and NRCRO-3 may be recommended for karif season cultivation to obtain maximum bulb yield. Similarly cultivars like Bhima Kiran, Bhima Super, NRCRO-2 and Col-652 may be recommended for better tolerance to both Purple Blotch and Thrips infestation.

Hasna and Begum (2014) conducted field experiment with 13 mutants of onion during the Rabi season in order to find out the reaction of these mutants to PLB. The mutant BP2/75/2 showed moderately susceptible reaction while rest of the mutants and the check variety (BARIpiaj-3) showed susceptible to highly susceptible reaction. Ul-Haq *et al* (2014) screened 21 varieties against PLB of onion and reported that only one variety Phulkara, exhibited resistant response while moderately resistant response was observed on five varieties viz., Desi Red, Early Red, Robina, Dark Red and Mirpurkhas.

Nanda *et al.* (2016) screened 43 *Allium* genotypes for purple blotch resistance under field conditions and observed *Allium cepa* accession 'CBT-Ac77' and cultivar 'Arka Kalyan' were highly resistant. *In vitro* inoculation of a selected set of genotypes with *A. porri*, revealed that 7 days after inoculation was suitable to observe the disease severity. *In vitro* screening of 43 genotypes for resistance to *A. porri* revealed two

resistant iines. An additional 14 genotypes showed consistent moderate resistance in the field as well as *in vitro* evaluations (Nanda *et al.,* 2016).

Veeraghanti *et al.* (2017) Screened different onion genotypes for purple blotch and found, the genotype Arka Kalyan was moderately resistant while the genotypes *viz.*, Arka Pragati, Arka kirthiman, Arka lalima and Arka Bindu were moderately susceptible and Arka Niketan, Arka Bhima, Satara gaurva and Bhima Super were susceptible to purple blotch of onion.

Mohapatra, (2017) observed lowest percent disease index (PDI) in Arka Kalyan, Arka Niketan, Indam Gulab and Red Diamond, whereas, highest PDI recorded in Light Red followed by Agrifound Rose. In the controlled condition after artificial inoculation of Purple Blotch almost every plant got infected but disease severity varied with the varieties; Agrifound Dark Red, Agrifound Rose got severe infection but varieties like Arka Niketan and Arka Bheem, Arka Kalyan, Indam Gulab, Indam Red Stone, Indam Hybrid-04, Arka Bindu showed less severity.

2.5. MOLECULAR MARKERS

Genetic analysis of *Alliums* germplasm by molecular markers will help in the understanding extent of genetic diversity and varietal identification. QTL detection and linkage mapping will help in marker-assisted selection for different qualitative traits in onion. Markers linked to colour, TSS, pungency and CMS will be useful in functional analysis of these traits and further improvement of the cultivars and recent sequencing efforts will definitely speedup the process.

Characterization and grouping based on phenotype are influenced by environmental variations; molecular markers are preferred because of polymorphic nature, co-dominance, selective neutral behaviour, easy and fast assay, high reproducibility and easy exchange of data between laboratories (Joshi *et al.,* 1999). A molecular marker is a DNA sequence that is readily detected and whose inheritance can easily be monitored. There are different marker systems available for crop plants such as restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), inter-simple sequence repeat (ISSR), microsatellite or simple sequence repeat (SSR), amplified fragment length polymorphism (AFLP), sequence characterized amplified region (SCAR), cleaved amplified polymorphic sequences (CAPS) and single nucleotide polymorphism (SNP) etc. (Semagn *et al.*,2006). In spite of advances in

sequencing technologies, sequencing of onion remains a huge challenge because of its 16.4 giga base genome size. It has one of the largest nuclear genomes among all diploid eukaryotes (Arumuganathan and Earle, 1991). The use of genetic markers in *Alliums* has increased several-fold with the development of different marker systems mentioned briefly above. Hanci and Gocke (2016) evaluated variability at 46 microsatellite loci and identified 308 alleles with these markers, out of which 303 were polymorphic. A dendrogram based on the UPGMA analysis grouped the 96 accessions into five main clusters. Dice's similarity coefficient ranged from 0.407 to 0.767 with an average of 0.587. The results showed that 44 out of the 46 SSR markers were convenient and polymorphic enough to distinguish all the studied accessions.

For diversity analysis, RAPD and ISSR markers are widely used by horticulturists due to their low cost, simplicity and no need of prior sequence information. AFLP is one of the powerful techniques used for diversity analysis. It combines both RFLP and PCR; therefore it is more specific, gives a large number of bands and allows higher genome coverage. The knowledge of genetic diversity helps in the efficient management of germplasm and selection of parents for crossing. The integrity of inbred lines was studied using RAPD (Bradeen and Havey, 1995). Diversity analysis of seven cultivars of *A. cepa* and single cultivar of Japanese bunching onion, chive, leek and a wild relative of onion (*A. roylei*) by RAPD showed *A. roylei* as the closest relative of *A. cepa*, questioning the current classification of *A. cepa* in the section *Rhizideum* (Susan *et al.*, 1993). 90 RAPD primers grouped 24 onion cultivars into northern and southern regions of India (Sangeeta *et al.*, 2006). Ten varieties of onion *(A. cepa* L.) were analysed, Bermis and India-2 were more dissimilar and Faridpuri and Bhati were the most genetically similar (Maniruzzaman *et al.*, 2010).

Xu *et al.* (2001) used five RAPD for classification and identification of thirty-one garlic (*A. sativum* L.) cultivars. Somaclonal variation in plants regenerated from long-term callus cultures of garlic was detected using RAPD (Al-Zahim *et al.*, 2005). Several locus-specific RAPD and AFLP markers were developed and used as a tool for the rapid characterization of garlic germplasm collections (Ipek *et al.*, 2008). Abdoli *et al.* (2009) found the paradox in genetic diversity detected by RAPD technique and geographical origins. This may be due to limited genome coverage and poor reproducibility of RAPDs; it showed the need of an alternative more efficient marker system.

The exotic cultivars Alisa Craig and Brigham Yellow Globe were different compared to the Indian cultivars and Nashik Red and Poona Red were indistinguishable, and similarly N-53 and Bombay Red were quite close (Mahajan *et al.*, 2009). The genetic fidelity of *A. ampeloprasum* L. and *A. sativum in vitro* regenerated clones was studied by Gantait *et al.* (2010) using 10 ISSR primers. Jakse (2005) identified 398 SNP, indels and SSRs which distinguished 35 elite onion populations. The diversity assessment of tropical Indian onion and cross amplification of genomic and expressed sequence tag (EST)-SSR markers in distantly related native wild species were estimated (Khar *et al.*, 2011). Oh *et al.* (2004) used microsatellite markers for characterization of an apomictic species *Allium senescens*.

Khosa *et al.* (2013) characterized twenty four *Allium* species available in India using twenty eight SSR markers. A total of 244 alleles with an average of 8.7 alleles per SSR marker were identified. Maximum numbers of alleles were detected in *A. cepa* var. *viviparum* (Pran) and minimum in *A. tuberosum*. The polymorphic information content varied from 0.290 to 0.904 with an average of 0.731. Cluster analysis grouped *Allium* species into three distinct clusters. The transferability of SSR markers was high with a mean value of 87.17 per cent and ranged between 35.71 to 100.0 per cent across the species under investigation. Four SSR markers, viz. ACM 018, ACM 065, ACM 301 and ACM 326 showed amplification in all the accessions of different species. The high transferability of bulb onion derived SSR markers in different *Allium* species available in India indicates its potential use for elucidating the genetic information from wild relatives for improvement of cultivated species.

Anandhan *et al.* (2014) evaluated seven open-pollinated varieties of onion for varietal identity using SSR primers. Pooled samples of 100 seeds were used to generate a representative profile of the population. Fifty EST-SSR primers were used and 21 were polymorphic among the varieties. Eight primers were found to have a PIC value more than 0.5 and are sufficient to discriminate all seven varieties. The genetic similarity (GS) value ranged from 0.77 to 0.92. The highest similarity was observed between varieties Bhima Raj and Bhima Red. As they are multi-allelic in nature, SSR primers have the ability to distinguish even within close groups. Amplification of unique alleles was also observed in six varieties and these could be of use in establishing varietal identity in close groups of varieties and for quality analysis during seed production.

González *et al.* (2015) evaluated seventeen onion landraces from North-West Spain using microsatellites markers and found eleven polymorphic markers identified 32 alleles in the whole collection, with an average of 2.9 alleles per locus. High values of observed (mean of 0.45) and expected heterozigosity (mean of 0.51) were detected for the majority of loci.

Abdou *et al.* (2016) analyzed variation among Niger onion landraces using simple sequence repeat (SSR) markers and observed mean heterozygosity (H_O) within onion landraces from Niger is 0.400, while the expected heterozygosity (H_S) is 0.452. Mohaptra (2017) studied genetic divergence of 16 varieties using 10 RAPD and 9 SSRs primers. Out of 10 RAPD primers seven primers showed 100% polymorphism (Oligo-01, Oligo-02, Oligo-03, Oligo-04, OPD-03, OPQ-06 and OPG-13) Oligo-05 had 89% moderate polymorphism and OPD-03 and OPA-09 had the lowest polymorphism (75%). Jaccard's Similarity Matrix generated by RAPD primer was found to be highest similarity between Arka Kalyan with Bhima Super and Arka Bheem (0.81) and lowest between Indam Gulab with N-53 and Arka Bindu (0.46), Light red with Agrifound Dark Red (0.46). Other varieties were moderately similar and grouped into four clusters. SSRs primers like; ACM-004, ACM-068, ACM-187, ACM-326, ACM-046 AND ACM-240 produced more bands and primer ACM-318, ACM-018 and ACM-300 amplified the fewest. The percentage of polymorphic was 88.89% and through SSRs primer similarity was noticed to be lowest between Agrifound Dark Red with Indam Red Stone, Indam Gulab, Indam Hybrid-04 (0.30) and highest between Bhima Super with Arka Niketan and Arka Bindu (0.95).

Karic *et al.* (2018) analysed five most common cultivars from the genus *Allium cepa* L. in Bosnia and Herzegovina (BiH) focusing on Konjic onion using seven SSR markers for genetic similarity analysis to address the genetic backgrounds. The total number of obtained SSR alleles was 30 bands, where 56.7% were polymorphic with the range of allele size of 130 to 650 base pairs (bp). The mean polymorphic information content (PIC) was 0.435 and the expected heterozygosity (H) values ranged from 0 to 0.785. Jaccard's coefficient of similarity values ranged from 0.14 to 0.55. The results in this study represent the first genetic diversity data on the onion cultivars in BiH and show significant dissimilarity among the onion cultivars. This study confirmed that the molecular SSR analysis represents an efficient tool for *Allium cepa* L. landrace genetic similarity analysis.

CHAPTER 3

Materials and Methods

MATERIALS AND METHODS

An investigation entitled "Characterization of onion genotypes using molecular markers and its field performance in the plains of West Bengal" was carried out at the research field of All India Network Research Project on Onion and Garlic (ICAR), C-Block Farm, Bidhan Chandra Krishi Viswavidyalaya, Kalyani, West Bengal during *Rabi* seasons of 2015-16 and 2016-17. The material used for this study and statistical methods adopted in the investigation are presented below in this chapter:

3.1. EXPERIMENTAL SITE

The field experiment were carried out in the research field of All India Network Research Project on Onion and Garlic (ICAR), C-Block Farm, Bidhan Chandra Krishi Viswavidyalaya, Kalyani in New Alluvial Zone, West Bengal during *Rabi* seasons of 2015-16 and 2016-17. Laboratory experiments were done in the Department of Vegetable Crops, Mohanpur and AICRP on Tuber Crops (ICAR), Kalyani, Nadia. The experimental site is situated at 23.5^0N latitude and 89^0 E longitudes at MSL of 9.75m.

3.2. CLIMATIC CONDITION

The "C" Block Farm is situated on warm sub-tropical humid climate within the torrid regions but in proximity of Bay of Bengal with a network of rivers, which protects the place from extreme conditions. The soil texture of the farm is sandy loam having neutral in reaction. The meteorological data pertaining to the period of investigation is presented in following table.

Table 3.1: Meteorological data pertaining to the investigation period during the year 2015 to 2017

Month, Year	Temperature (^{0}C)		Rainfall (mm)	Relative humidity (%)	
	Max.	Min.		Max.	Min.
September, 2015	33.10	26.10	227.30	96.00	71.00
October, 2015	33.40	23.70	42.10	94.00	63.00
November, 2015	31.30	18.80	0.00	93.00	53.00
December, 2015	26.30	15.00	6.60	93.00	56.00
January, 2016	24.90	9.90	0.00	98.00	54.00
February, 2016	29.80	16.10	3.70	98.00	57.00
March, 2016	33.20	19.60	0.90	93.00	52.00
April, 2016	38.20	23.70	0.10	90.00	48.00
May,2016	35.00	24.90	6.10	92.30	63.40
June, 2016	34.70	25.90	19.10	92.80	73.00
July, 2016	32.70	26.10	9.61	97.10	84.70
August, 2016	33.10	27.60	1.40	93.00	79.90
September, 2016	33.50	24.20	4.00	94.00	58.00
October, 2016	32.30	21.60	5.40	94.00	60.00
November, 2016	36.80	15.30	0.60	93.00	58.00
December, 2016	26.20	10.90	0.00	97.00	58.00
January, 2017	25.40	8.50	0.00	96.00	47.00
February, 2017	29.50	12.60	0.00	92.00	43.00
March, 2017	32.40	17.60	0.20	92.00	49.00
April, 2017	34.90	22.60	0.40	91.00	57.00

3.3 Properties of soil of experimental plot

Soil samples from the experimental plots were collected and analyzed before sowing to determine the Physico-chemical properties of the soil in experimental plots. The soil in the research station is sandy loam, fine in texture and having good water holding capacity.

Table 3.2: Physico-chemical properties of soil of the experimental field

Particulars	Status	Amount
Chemical composition		
pH	Neutral	7.20
EC	Normal	0.22 dS/m
Organic carbon	Low	0.52 (%)
Nitrogen	Low	206.43 kg/ha
Phosphorous	Low	07.77 kg/ha
Potassium	Medium	185.46 kg/ha

Sowing of onion seeds

Layout of main field of onion

Standing onion crop

Harvesting of onion bulbs

Plate 1: Photographs of field view of onion

3.4. FIELD EXPERIMENT

The Experiment was conducted with twenty genotypes in a Randomized Block Design with three replications maintaining row to row and plant to plant spacing as 15x10cm. Recommended fertilizer dose (N: P_2O_5: K_2O: S @ 120: 60:100: 50 kg per ha) was followed in the experiment with Farmyard manure (FYM) @ 20 tonnes per ha as basal application. Other cultural practices were followed in the schedule time. Sowing was done 2nd week of October in both the years and seedlings were transplanted first week of December and harvesting was done in last week of April.

Table 3.3: The experiment was laid out in a Randomised Block Design with three replications during the *Rabi* season, October-April (2015-16 and 2016-17)

Design of experiment	Randomized Block Design
Number of Genotypes	20
Number of replications	3
Plot size	2 m × 1.5 m
Spacing	15 × 10 cm
Transplanting	December 2015 and 2016
Harvesting	April 2016 and 2017
Fertilizer dose	100:50:100:40 (N:P_2O_5:K_2O:S) kg/ha
FYM	20 tones/ha

3.5. MATERIALS USED

Table: 3.4: Onion genotypes used and their sources

Sl. No	Genotypes	Source
1	IIHR-C-606	IIHR
2	DOGR-571-LR	DOGR
3	DOGR-546-DR	DOGR
4	JWO-08-09	JAU
5	LINE-355	NHRDF
6	BHIMA KIRAN	DOGR
7	BHIMA SWETA	DOGR
8	PKV WHITE	PKV AKOLA
9	RO-628	DURGAPURA
10	RO-636	DURGAPURA
11	BHIMA SHAKTI	DOGR
12	ARKA LALIMA	IIHR
13	ARKA KIRTIMAN	IIHR
14	DOGR-HY-1	DOGR
15	DOGR-HY-2	DOGR
16	JRDO-07-13	JAU
17	LUCIFER	BEJO SHEETAL
18	BSS-827	BEJO SHEETAL
19	PUNE RED	BEJO SHEETAL
20	ORIENT	BEJO SHEETAL

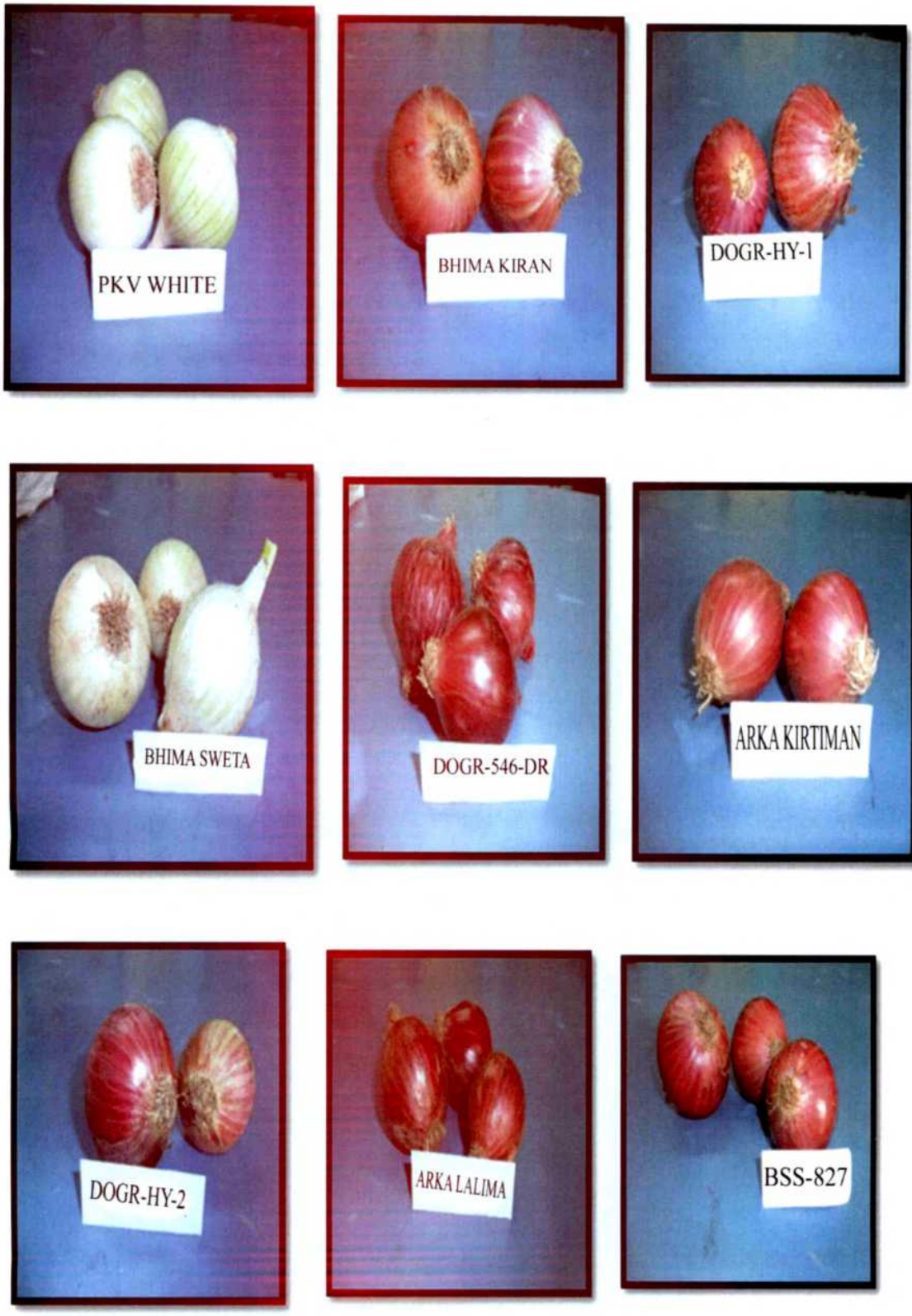


Plate 2.a: Onion genotypes

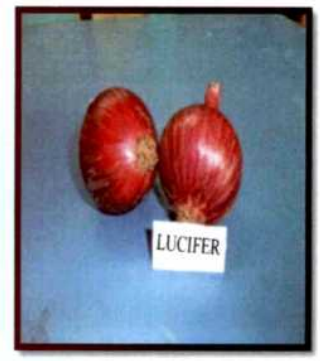


Plate 2.b: Onion genotypes

3.6 OBSERVATIONS TAKEN

Observations on different growth and bulb characters and yield were recorded from randomly selected ten plants per replication at 90-100 days after transplanting and bulb characters after harvest.

3.6.1 GROWTH PARAMETERS

Plant Height (cm)

Number of Leaves per Plant

Polar Diameter (mm)

Equatorial Diameter (mm)

Neck Thickness (mm)

Average Bulb Weight (g)

Doubles (%)

Number of Days to Maturity

Marketable Yield (q ha^{-1})

Total Yield (q/ha).

3.6.2. Quality Parameters

- Dry Matter (%).
- Total Soluble Solids (°Brix).
- Pyruvic Acid (µg/g).

The pyruvic acid content was estimated by Anthon and Barrett (2003) method with slight modification to the Schwimmer and Weston (1961) method.

Reagent used

Dinitro phenyl hydrazine (DNPH)

Procedure

- The selected bulbs were cut longitudinally into two pieces.
- Out of these, one half was immediately chopped and homogenized with water (1:1).

- The homogenate was filtered through cheese cloth and centrifuged at 10,000 rpm for 5 min.
- Clarified filtrate was taken and 1.0 ml of distilled water was added to it.
- After that 1.0 ml of 0.25 g/l DNPH (prepared in 1 N HCl – hydrochloric acid) was added to it.
- The reaction mixture was placed in a water bath at 37°C for 10 min.
- After removing the samples from the water bath, 1.0 ml of 1.5 N NaOH was added.
- The absorbance was recorded at 515 nm (Bio-spectrophotometer).

3.6.3 Observations on storage

The shelf life studies were conducted in the Laboratory of Department of Vegetable Crops, BCKV in ambient condition. After harvest, onion bulbs were kept for curing along with the top under shade. Then bulbs were randomly selected from each treatment and replications for storage study. Physiological weight loss (%) was recorded at 30, 60 and 90 days interval starting from 0, 30, 60 and 90 days after storage.

3.7. REACTION OF PURPLE BLOTCH DISEASE UNDER FIELD AND CONTROL CONDITIONS

Disease scoring: This experiment was deal with the pathogen *Alternaria porri*. Purple blotch infected leaves and bulbs were randomly collected from field and symptoms were studied.

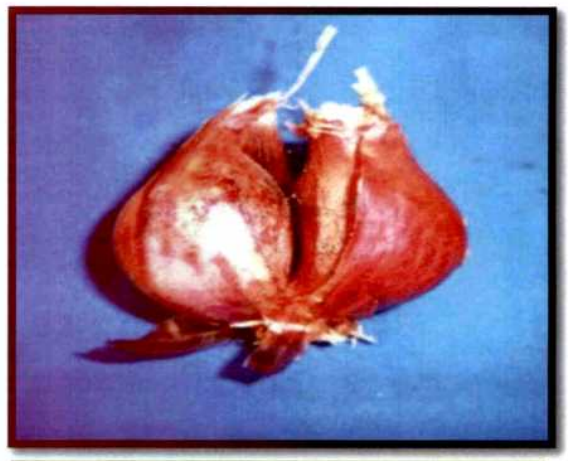
i) Field condition: Evaluation of the onion genotypes done against Purple leaf blotch under natural infection.

A disease infection index was then calculated according to Wheeler (1969) thus:

The scoring of the disease were done following scoring method

$$\text{P.D.I. (\%)} = \frac{\text{Sum of rating (0-9 scale)}}{\text{Maximum possible score x No. of leaves examined}} \times 100$$

Sprouted bulbs



Double bulbs

Rotted bulbs

Onion bulbs in storage

Plate 3: Onion bulbs in storage, sprouted bulbs, double bulbs & rotted bulbs

The rating of the following significance:

0: Absolutely free from infection

1: Small sized lesions on the leaf covering <1% area

2: Small sized lesions on the leaf covering <2-5% area

3: Small sized lesions on the leaf covering< 6-10% area

4: Small sized lesions on the leaf covering <11-15% area

5: Small sized lesions on the leaf covering < 16-25% area

6: 26-40% area covering

7: 41-60% area covering

8: 61-75% area covering

9: >75% area covered with spot, most of the leaves dried

ii) Control condition: Resistant reaction done in the varieties after challenged/artificial inoculation *in vitro* condition.

a) Plant material and growing conditions

Twenty onion genotypes were used in this study. Onion seeds of respective varieties were grown in clay pots containing soil: sand: compost mixture at a ratio of 4:4:1, respectively. Fifteen days old seedlings were transplanted in another earthen pots containing same soil mixture as above. The plants were kept in a growth chamber at 24°C for 16 h photoperiod for four weeks prior inoculation.

b) Fungal cultures and inoculum preparation

The *Alternaria porii* were isolated from onion plants showing purple blotch symptoms. The purple blotch infected leaves collected from onion fields of experimental field (C-Block Farm) of the present work, BCKV, Kalyani. Surface-sterilized with 0.1% mercuric chloride for one minute, rinsed thoroughly with sterilized distilled water, blotted to dry and then placed on Czapek-Dox (CD) agar medium. Cultural characteristics of the fungus isolated from the infected leaves were studied and tentatively identified it as *Alternaria porri* (Ellis, 1971) according to standard protocols. The pure culture was maintained on CD agar medium. Fungal isolates were grown on Czapek-Dox (CD) plates and incubated at 28°C for 7 to 10 days. Then, cultures were

grown for 6 days at 28°C on PDA with 16 h day-1 light to induce sporulation. Conidia were collected by washing plates with sterile water and the resulting spore suspension was adjusted to concentration of 1×10^5 spores ml-1 using the haemocytometer (Hadis *et al.*, 2011).

c) Plant infection

The onion verities were evaluated using spore suspension inoculation method under growth chamber conditions. Onion plants at the age of 45 days were inoculated by spraying conidial suspension (1×10^5 spores ml^{-1}) on plants. In the case of blank treatments, sterilized distilled water was used. At least three plants per pot in five replicates from each variety were used for inoculation. To ensure the adherence of conidial suspension at the inoculation site on the leaflet surfaces, the conidial suspension was supplemented with 0.1% tween 20. After inoculation, plants were kept for 24 h in a moist chamber at 25°C at 12 h photoperiod. Disease development was estimated by seeing the blotch symptom on the narrow leaves of the onion 7-10 days after inoculation. The sprayed varieties of the onion were judged for its susceptibility to purple blotch disease.

3.8. DNA FINGERPRINTING

Molecular characterization was done utilizing RAPD and SSR markers. RAPD helps to find the unknown polymorphic DNA fragments but SSRs are used to visualize known polymorphic DNA loci. High quality genomic DNA was required for molecular typing.

3.8.1 Plant materials

Twenty genotypes of onion were used to investigate the level of polymorphism detected by RAPD and SSRs. Fresh leaf samples were taken for genomic DNA isolation.

3.8.2 Reagents and chemicals

Extraction buffer consisting of 100m MTris-HCl (pH 8), 20m MEDTA (pH 8), 2M NaCl, 2% CTAB (w/v), 2% PVP (Mr.40,000), 2% β-mercaptoethanol (v/v) was prepared. In addition, phenol:chloroform:isoamylalcohol (25:24:1), 4% PEG solution, Wash solution [15m Mammonium acetate in 75% (v/v) of ethanol] and a TE buffer consisting of 10m MTris-HCl (pH 8) and 1m MEDTA (pH 8) also prepared and used.

3.8.3 DNA isolation

DNA was extracted from leaf samples following the methods developed by Sharma *et al.* (2008). Fresh leaf material (100 mg) washed in distilled water and rinsed with 80% ethanol. The surface sterilized leaves were kept at 20°C refrigerator for 30 minutes in motar-pestle. Chilled leaf sample was ground finely with 500µl extraction buffer and 20µl of β-mercaptoethanol. Then 500µl of extraction buffer was added and the sample was ground once more. Ground material was transferred to 2ml centrifuge tube. Then the mixture was vortexed and then incubated at 37°C for 30min. Again the mixture was incubated 70°C for 30min with frequent swirling after first incubation. Then mixture was centrifuged at 13000 rpm for 10min. Supernatant was collected in a new eppendrof and equal amount of phenol-chloroform-isoamylalcohol (25:24:1) was added to that followed by mixing by inversion 50-60times. The mixture was again centrifuged at 13000 rpm for 10 minutes and the supernatant was poured into a new eppendrof. Then 200µl of 4% PEG solution was added and mixed thoroughly and then the mixture was incubated at 0°C. After 20 minutes the mixture was again centrifuged at 13000 rpm for 10 minutes. The supernatant containing DNA was collected in a separate eppendrof followed by addition of pre-cooled isopropanol (65% volume of supernatant). It was then mixed thoroughly and waited for 5 min at room temperature. DNAwas precipitated after centrifugation at 13000 rpm. The supernatant was discarded and 500µl wash solution was added in the eppendrof. After centrifugation the DNA pellet was collected and air dried upto complete disappearance of alcoholic smell. DNA pellets were suspended in 100µl of TE buffer. Suspended DNA was treated with 1µl of RNase (1.43µg/µl, Sigma) and incubated at 37°C for 30-40 min. The extracted DNA samples were stored at -20°C until use.

3.8.4 DNA analysis

The quality of extracted DNA was analyzed by means of agarose gel electrophoresis (0.8%), followed by ethidium bromide staining. The purity of the DNA was estimated by spectrophotometry using A_{260}/A_{280} ratio, and the yield was estimated by measuring absorbance at 260 nm.

3.8.5 Detection of polymorphism utilizing dominant molecular marker system

RAPD was categorized under the molecular marker system. The methodologies are described below.

Table 3.5: Details of used RAPD primers

Sl. no.	Primer Id	Primer sequence (5′-3′)	Tm (°C)	Ta(°C)
1	Oligo-1	CTTCACCCGA	32	28
2	Oligo-2	TCGGCGATAG	32	28
3	Oligo-3	CAATCGCCGT	32	30
4	Oligo-4	CAAACGTCGG	32	27
5	Oligo-5	GTTGCGATCC	32	28
6	OPA-06	GGTCCCTGAC	34	28
7	OPA-09	GGGTAACGCC	34	27
8	OPA-11	CAATCGCCGT	32	27
9	OPA13	CAGCACCCAC	34	27
10	OPA-17	GACCGCTTGT	32	27
11	OPA-18	AGGTGACCGT	32	27
12	OPA-20	GTTGCGATCC	32	29
13	OPAD-05	ACCGCATGGG	34	27
14	OPC-04	CCGCATCTAC	32	27
15	OPC-08	TGGACCGGTG	34	28
16	OPC-10	TGTCTGGGTG	32	27
17	OPC-13	AAGCCTCGTC	32	27
18	OPD-03	GTCGCCGTCA	34	29
19	OPG-13	CTCTCCGCCA	34	28
20	OPO-1	GGCACGTAAG	32	28
21	OPO-5	CCCAGTCACT	32	28
22	OPO-18	CTCGCTATCC	32	25
23	OPO-19	GGTGCACGTT	32	25
24	OPQ-05	CCGCGTCTTG	34	27
25	OPQ-06	GAGCGCCTTG	34	30
26	OPQ-20	TCGCCCAGTC	34	27

A set of twenty six random decamer oligo nucleotide primers OPA, OPC, OPD, OPG, OPAD, OPO selected from Operon kit and selection of primers like Oligo-1, Oligo-2, Oligo-3, Oligo-4,

[Tm =Primer melting temperature; Ta = Primer annealing temperature]

Oligo-5 was performed on the basis of previous study by Wilkie *et al.* (1993). All the primers were used for RAPD analysis (Table3.5). Each 25µl of PCR mixture consisted of 20 ng of template DNA, 100µ Meachdeoxy nucleotide triphosphate, 20ng of

deca nucleotide primers (Sigma), 1.5mM $MgCl_2$, 1XT aqbuffer (supplied with the TaqPolymerase enzyme), 1U of Taq DNA polymerase (Xcelris). Amplifications were performed in Veriti Thermal Cycler (Applied Biosystems). The PCR mixtures were heated at an initial step of 94°C for 3 minutes and then subjected to 35 cycles of following programme: 94°C for 30 s, 27-30°C for 45s (depends upon primers), 72°C for 1 min. After the last cycle temperature was maintained at 72°C for 5 mins. Amplified products were resolved on a 1.5% agarose gel containing 0.5 mg/ml ethidium bromide and visualized under UV light. Gel photographs were scanned through Gel Doc System (QUANTUM-ST4, LED" s bar Epi-illumination, France). Clear bands were revealed and were scored for their presence (1) or absence (0). All profiles were reproducible and gave clear and easy to score bands.

- **Detection of polymorphism as revealed by codominant markers (SSRs)**

Nine SSR primer sets developed by McCallum *et al.* (2008) were used for this study (Table3.6). All SSR primers were synthesized by Sigma-Aldrich.

Table 3.6: Details of used SSR primers

Sl. no.	Primer Id	Primer sequence (5′-3′)	Tm (°C)	Ta (°C)
1	ACM004	F-TCGTTCTTTAGAACACGTTAGG	59.9	58
		R-GTCGGCGGATATAGTGACA	61.2	
2	ACM018	F-GGGGAATGGTGGAGAATAGA	62.6	57
		R-AACAGAGGCAAGAGGAGCG	64.7	
3	ACM046	F-TCCTCGTCACCACCACAG	63.8	57
		R-CTGAAAGGGAGTAGCGGAG	61.6	
4	ACM068	F-GAAGGTGAAGGTGTACGGT	59.0	57
		R-CAAATGGCTGCAATAAGCAA	63.6	
5	ACM187	F-GTACTCGGGCAGTGGAGGTA	64.0	59
		R-GGAGCTGTCCAAATGCTAGG	63.7	
6	ACM240	F-GTGCAACTCCAAGAGAAGGG	63.8	58
		R-AATATAAAGGCGTTGGCCTG	62.6	
7	ACM300	F-AGGTGCAGTTTCGTGGTAGG	64.0	58
		R-TTAGCCCCTGGTAAGTGTGG	63.8	
8	ACM318	F-TCCTCCTTCCAAACCACATC	63.9	57
		R-GATCAGAAACAGCAGCGTC	61.2	
9.	ACM326	F-AAACCAGCAACAACCAATG	61.0	57
		R-AAAATTGGAGAGCAGGCAAA	63.5	

[Tm =Primer melting temperature; Ta = Primer annealing temperature]

PCR amplification was carried out in a volume of 25 µl containing 2 µl of 10 ng/µl DNA, 1 µl of each primer (100ng/µl), 1 µl of 2.5 mM dNTPs, 1 µl of 25 mM $MgCl_2$ 2.5 µl of 10X reaction buffer (Xcelris), 0.2 µl of 5 U/µl Taq polymerase (Xcelris) and 16.3 µl distilled water. PCR reactions were performed in Veriti Thermal Cycler (Applied Biosystems), an initial step of 3mins at 94°C, 35 cycles of 30 s at 94°C, 45 s at 55-60°C and 1 min at 72°C, and a final step of 10min at 72°C. The PCR products were separated and visualized on 2% agarose gel by ethidium bromide staining.

3.8.6 Molecular data analysis

Presence or absence of the band was scored as 1 or 0, respectively, obtaining the molecular identification profile for each individual. The binary matrix was used to calculate the Simple Matching coefficient: SM=(a+d)/(a+b+c +d), where: "a" is the number of bands present in both individuals,"b" and "c" are the number of bands present in only one individual, and "d" is the number of bands absent in both individuals. Cluster analyses were implemented by UPGMA method, and the corresponding dendrogram was constructed. The capacity of each primer to distinguish among the genotypes studied was evaluated by the Resolving power (RP) (Prevost and Wilkinson, 1999), Marker index (MI) (Powel *et al.,* 1996), and the polymorphic information content (PIC) (Weising *et al.,* 2005). PIC of dominant bi-allelic data was estimated by the formula: $PIC = 1-p_i^2-q_i^2$, were *"p"* is frequency of visual alleles and *"q"* is the frequency of null alleles. PIC for the SSRs marker was estimated by using the formula $PIC_i = 2f_i(1-f_i)$. Where, f_i is the frequency of the marker fragments that were present ($1-f_i$) is the frequency of the marker fragments that were absent. MI was calculated as: MI = PIC x number of polymorphic loci. RP is defined per primer as: $RP = \sum Ib$, were "Ib" is the band informativeness, that takes the values of 1-(2x [0.5-p]), being "p" the proportion of each genotype containing the band. The diversity index, which indicates the genetic diversity of the germplasm, was calculated using the formula DI = 1 - 1/L ΣPi2, in which Pi is the allele frequency (each individual allele is considered a unique fragment amplification) and L is the number ofloci. Each primer received a score (1 for presence and 0 for absence of bands in each accession), and a binary matrix was generated (Saini *et al.*, 2013).

3.9. STATISTICAL ANALYSIS

Replication wise mean values of each of the twenty genotypes were computed for respective characters and subjected to statistical analysis. All the statistical analysis was carried out following OPSTAT (www.hau.ernet.in) and GENRUS statistical analysis tool.

The genotypic and phenotypic variances and coefficients of variation were worked out as per the method of Burton (1952) and heritability and genetic advance by following the method suggested by Lush (1949), Robinson *et al.* (1949) and Burton and De Vane 1953), and the correlations among various variables. The following methods were followed for the analysis of data:

3.9.1 ANALYSIS OF VARIANCE

Analysis of variance of the observations recorded on different characteristics was carried out as per the standard procedure suggested by Panse and Sukhatme (1978). The following model was adopted for the analysis of variance of various characters:

$Y_{ij} = \mu + \alpha j + \beta j + e\,ij$

Where,

Y_{ij}=observation of i^{th} treatment and j^{th} block

μ = General mean

α_j= i^{th} treatment effect

β_j= j^{th} block effect

e_{ij} = random error associated with the i^{th} treatment and j^{th} block

The assumptions of the model adopted were:

i. All the observations should be independent.

ii. The different effects in the model should be additive.

iii. Error involved in the population should be distributed normally and independently with mean zero and variance.

Analysis of variance tables for all characters under study were constructed as follows:

Analysis of variance

Sources of variation	Degrees of freedom	Sum of squares	Mean sum of squares	Expected mean squares	F calculated value
Replication	r-1	SSr	MSr	$\sigma^2 g + g\sigma^2 r$	MSr/MSe
Genotypes	g-1	SSg	MSg	$r\sigma^2 g + \sigma^2 e$	Msg/MSe
Error	(r-1) (g-1)	SSe	Mse	$\sigma^2 g$	
Total	rg-1				

Where,

r = Number of replications

g = Number of genotypes

MSr, MSg and MSe stood for means squares due to replications, genotypes, error, respectively.

$\sigma^2 g$ = Genotypic variance of the character

$\sigma^2 r$ = Variance due to replication

$\sigma^2 e$ = Error variance of character

The genotypic and phenotypic variances were calculated by adopting the following formulae:

Genotypic variances of a character $\sigma^2 g = \frac{MSg - MSe}{r}$

Phenotypic variances of a character = $r\sigma^2 g + \sigma^2 e$

3.9.2 PARAMETERS OF VARIABILITY

i) Mean

The mean value of each character was worked out by dividing the total sum of values with corresponding number of observations.

$$\bar{X} = \frac{1}{n}\sum_{i=1}^{n} X_i$$

Where,

$\bar{X}$ = Sample mean

X_i = Individual value

n = Number of observations

ii) Range

The lowest and highest values of each character were recorded.

iii) Standard error of mean (S.E.m)

Standard error of mean was calculated with the help of error mean squares from the analysis of variance table given as under:

$$S.E.(m) = \frac{\sqrt{MSe}}{r}$$

S.E. = Standard error

MSe = Error mean sum of squares

R = Number of replications

iv) Critical difference (CD)

For every character, the critical difference as the difference of any two mean values in order to compare the treatment means was calculated using the following formula:

$$S.E.(d) \pm = \frac{\sqrt{2MSe}}{r}$$

$$CD \text{ at } 5 \text{ or} 1\% = \frac{\sqrt{2MSe}}{r} \times \text{'t'}$$

Where,

't' is the tabulated value at error degree of freedom at 5 and 1% level of significance.

3.9.3. ESTIMATION OF GENETIC VARIABILITY PARAMETERS

i. Estimation of genotypic, phenotypic and environmental variance

The variances due to genotype, phenotype and environmental were computed by using the following formulae:

$$\textbf{Genotypic variance } (\sigma^2 g) = \frac{\text{Treatment MSS} - \text{Error MSS}}{r}$$

$$\textbf{Phenotypic variance } (\sigma^2 p) = \sigma^2 g + \sigma^2 e$$

Where,

'r' is number of replications.

ii. Coefficient of variability

The coefficient of variation being a standardized form of variance is useful for comparing the extent of variance between different characters with different scales (Singh and Choudhary, 1979). Genotypic and phenotypic coefficients of variation were estimated according to Burton and Devane (1953) based on the estimate of genotypic and phenotypic variance.

$$\text{Genotypic coefficient of variability (GCV \%)} = \frac{\sigma^2 g \times 100}{\bar{X}}$$

$$\text{Phenotypic coefficient of variability (PCV \%)} = \frac{\sigma^2 p \times 100}{\bar{X}}$$

Where,

$\bar{X}$= General mean

$\sigma^2 g$ = Genotypic variance

$\sigma^2 p$ = Phenotypic variance

The genotypic and phenotypic coefficients of variation were categorized as per the method suggested by Shivasubramanian and Menon (1973):

0-10% = Low

10-20% = Moderate

>20% = High

iii. Heritability

Heritability in broad sense was calculated as the ratio of genotypic variance to the phenotypic variance and expressed in percentage (Falconer, 1981).

$$\text{Heritability } (h^2) = \frac{\sigma^2 g}{\sigma^2 p} X\ 100$$

The calculated heritability was classified into three groups as suggested by Johnson *et al.* (1955).

0-30% = Low

30-60% = Moderate

> 60% = High

iv. Genetic advance (GA)

Genetic advance as percent mean of each character was worked out by adopting the following formula given by Johnson *et al.* (1955).

$GA = k \times h^2 \times \sqrt{\sigma^2 p}$

Where,

h^2 = Heritability in broad sense

k = Selection differential, which is equal to 2.06 at 5% intensity of selection (Lush, 1949).

$\sqrt{\sigma^2 p}$ = Phenotypic standard deviation

v. Genetic advance as per cent of mean (GAM)

Genetic advance as percentage over mean was worked as suggested by Johnson *et al.* (1955). $GAM = \frac{GA}{\bar{X}} \times 100$

Where,

GA = Genetic advance

$\bar{X}$ = General mean

Genetic advance as percent of mean was categorized as per the formula suggested by Johansone*t al.* (1955).

0-10 % = Low

10-20 % = Moderate

>20 % = High

3.9.4 CORRELATION STUDIES

The correlation coefficients among all possible character combinations at phenotypic (rp) and genotypic (rg) level were estimated by employing the formulae given by Al-Jibourie*et al.* (1958).

$$\text{Genotypic correlation } r_{xy}(G) = \frac{Cov_{xy}(G)}{V_x(G) \times V_y(G)}$$

$$\text{Phenotypic correlation } r_{xy}(P) = \frac{Cov_{xy}(P)}{V_x(P) \times V_y(P)}$$

Where,

$Cov_{xy}(G)$ = Genotypic coefficient of variance between 'x' and 'y'

$Cov_{xy}(P)$ = Phenotypic coefficient of variance between 'x' and 'y'

V_x (G) = Genotypic variance of character 'x'

V_x (P) = Phenotypic variance of character 'x'

V_y (G) = Genotypic variance of character 'y'

V_y (P) = Phenotypic variance of character 'y'

The significance of correlation was tested by comparing 'r' value with obtained value.

3.9.5. GENETIC DIVERSITY

Genetic diversity between genotypes can be well estimated by using D^2 analysis given by Mahalanobis (1936). The D^2 value between ith and jth genotypes for P characters was calculated as $\overline{D_{ij}^2} = \sum_{t-1}(Y_{it} - Y_{jt})$

Where,

Yit = Uncorrelated mean value of ith genotype for 't' character.

Yjt = Uncorrelated mean value of jth genotype for 't' character

D_{ij}^2=D^2 value betweenith and jth genotypes.

Test of Significance

Variances were calculated for all the twelve characters and test of significance was done. Analysis of covariance for the character pairs was estimated on the basis of mean values. A dispersion table was prepared from the estimates. After testing the differences between genotypes for each of the character a simultaneous test of significance of difference between the mean values of a number of correlated variables was done (Rao, 1952) by using 'V' statistic, which in turn utilizes Wilk's criterion (Wilks, 1932). The sum of squares and sum of products of error and error plus variety variance - covariance matrix were used for this purpose (Panse and Sukhatme, 1961).

The estimate of 'λ' (Wilk's Criterion) was done using the following formula.

$$\lambda = \frac{(E)}{(E+V)}$$

Where,

(E) = Determinant of error matrix

(E+V) = Determinant of error + varieties matrix.

The significance of 'λ' was tested by

$$X^2_{pq} = -m \log_e \lambda$$

Where

m = n-(p+q+1)/2 with pq degree of freedom

n = Degree of freedom of error + varieties

p = Number of characters

q = Number of genotypes - 1

log λ = 2.3026 log10 λ

Transformation of correlated variables

In the present model, computation of D^2 value was reduced to simple summation of differences in mean values of various characters of two genotypes i.e. $\sum d_i^2$

Therefore, transformation of correlated variables into standardized uncorrelated ones was done before working out the D^2 values. Transformation was done using pivotal condensation method in computation of D^2 values.

Computation of D^2 values

The D^2 value between i^{th} and j^{th} genotypes for p characters was calculated as:

$$D^2_{ij} = \sum_{t=1}^{p} (\overline{Y_{it}} - \overline{Y_{jt}})$$

Where

Y_{it} = Uncorrelated mean value of i^{th} genotype for 't' character.

Y_{jt} = Uncorrelated mean value of j^{th} genotype for 't' character

D^2_{ij}= D^2 value between i^{th} and j^{th} genotype.

Testing the significance of D^2 values

The D^2 values obtained for a pair of genotypes was taken as the values of 'x^2' and tested against tabulated 'x^2' at 'p' degree of freedom where 'p' is the number of characters considered.

Grouping of genotypes into various clusters

Grouping of the genotypes into various clusters was done by using Tocher's method as described by Rao (1952). The criterion used in clustering by this method is that any two variables belonging to the same cluster should at least on an average show a smaller D^2 value. The combinations of each genotype were arranged in increasing (ascending) order of their magnitude in a tabular form as described by Singh and Chaudhary (1977). To start with, two populations having the smallest distance with each other were considered, to which a third population having smaller D^2 value from the first two populations was added. Similarly, the next nearest fourth population was considered and this procedure was continued. At certain stage when it was felt that after adding a particular population if there was rise in the average D^2, then, that population was not considered for inclusion in that cluster. The groups of the first cluster were then omitted and the rest were treated in a similar way. This process was continued till all the populations were included into one (or) the other cluster. After the formation of the cluster, the averages inter and intra cluster distances (divergence) were calculated.

Average intra - cluster distance

For the measurement of intra - cluster distances the formula used is $\sum D_i^2$ /n.Where, $\sum D_i^2$ /n was the sum of distances between all possible combinations (n) of the populations included in a cluster.

Average inter-cluster distance

Clusters were taken one by one and their distances from other clusters were calculated. The distance between two clusters was the sum of the D^2 values between the members of the other clusters divided by the product of number of genotypes in both the clusters under consideration.

Contribution of individual characters towards divergence

In all the combinations, each character was ranked on the basis of their contribution towards divergence between two entries ($d_i = y_{it} - y_{ij}$). Rank I was given to the highest mean difference and rank 'P' to the lowest mean difference, where 'P' is the total number of characters considered. The number of cases where a particular character ranked first was continued, the proportion of this to the total number of combinations expressed in percentage was quantified as the contribution of character to the overall genetic divergence between the genotypes.

$X = (N \times 100)/M$

Where,

X = Per cent contribution of character.

N = Number of genotypes combinations where the character ranked first.

M = All possible combinations of the genotypes concerned.

Principal Component Analysis (PCA)

In statistics, principal component analysis (PCA) is a tool for simplifying a multivariate data set by reducing the dimensions for analysis while retaining those characteristics of the data set that contribute to most of its variance.

Technically PCA is a linear transformation that transforms the data into a new coordinate system such that the greatest variance by any projection of the data comes to lie on the first co-ordinate (called the first principal component), the second greatest variance on the second coordinate and so on. PCA is a "rigid rotation" of the data matrix, it does not change the positions of points relative to each other; it just changes the coordinate systems.

In principal component analysis, axes are created such that the perpendicular distance from each object to the ordination axes is minimized. Axes are linear combination of variables. The weights in each axes are known as "loadings". Traditionally, principal component analysis is performed on the symmetric covariance matrix (PCA-Q) or on the symmetric correlation matrix (PCA-R). The covariance matrix contains scaled sum of squares and cross products. A correlation matrix is like a covariance matrix but first the variable *i.e.*, the columns have been standardized.

Principal components were computed from the correlation matrix and genotypic scores obtained for the first component and succeeding components with latent roots greater than unity (Jager *et al.*, 1983).

Eigen values (Latent root) and Eigen vector (Loading)

Principal component consists of a series of eigen values and eigen vectors. Each principal component has an eigen value and eigen vector. There are as many eigen vectors and eigen values as there are columns in the initial matrix. Eigen value is a measure of the strength of an axis, the amount of variation along an axis. Eigen values are usually ranked from the greatest to the least. If PCA performed on a correlation

matrix, the sum of the eigen values will be equal to the number of variables. Eigen vectors determined the ordination of principal components or axis in space. The values in an eigen vector are not unique because any coordinates that describe the same orientation would be acceptable because they are standardized in same way *eg.* their square values sum to one. The eigen vectors are normally used to aid in the interpretation of a multivariate analysis.

CHAPTER 4

Results and Discussion

RESULTS AND DISCUSSION

The observational data were recorded as per the materials and methods discussed in the earlier chapter. The experimental results for various characters were compiled, tabulated and subjected to statistical analysis. The results of investigation entitled "Characterization of onion genotypes using molecular markers and its field performance in the plains of West Bengal" are presented in this chapter with the help of data given in respective tables under the following heads:

4.1 Analysis of variance

4.2 Mean performances of genotypes

4.3 Components of variation and estimates of genetic parameters

4.4 Study on character association

4.5 Study on genetic divergence using D^2 analysis

4.6 Principal component analysis

4.7 Reaction of purple blotch disease to onion genotypes under field and controlled condition

4.8 Molecular characterization using RAPD and SSR marker

4.1. ANALYSIS OF VARIANCE

The analysis of variance investigated during the study indicated significantly higher amount of variability among the genotypes for all the characters studied *viz.*, plant height, number of leaves, leaf length, double bulb (%), neck thickness, days to maturity, polar and equatorial diameter of bulb, bulb weight, grade A, B and C bulb, marketable bulb per plot, total yield, marketable yield, Percent disease index, dry matter, pyruvic acid, total soluble solid percent weight loss after one, two, three and four month. Therefore, there is a lot of scope for selecting majority of the traits in the genotypes. Sufficient genetic variability for many traits had also been reported by Hosamani *et al.* (2010) and Ibrahim *et al.* (2013) for bulb yield in onion. In view of high magnitude of genotypic variances, the significance of replication variances for these characters was not of much consideration and importance.

Table 4.1: Analysis of variance for onion genotypes pooled data over two years

Sl. no.	Characters	Source of variation with degrees of freedom		
		Replication(2)	Treatments(19)	Error(38)
1	Plant height	1.46	16.75**	1.48
2	Number of leaves	0.08	2.52**	0.22
3	Double Bulb (%)	0.28	1.16**	0.50
4	Neck thickness	0.27	0.47**	0.19
5	Days to maturity	0.35	11.33**	2.58
6	Polar diameter (mm)	1.01	12.29**	0.98
7	Equitorial diameter (mm)	5.35	14.64**	1.04
8	Individual bulb weight(g)	0.30	27.27**	1.98
9	Grade (A) bulb number	7.27	21.89**	5.11
10	Grade (B) bulb number	3.27	28.21**	6.37
11	Grade (C) bulb number	0.87	28.15**	5.52
12	Marketable bulb/plot	26.72	218.66**	37.23
13	Marketable yield (kg/plot)	0.09	0.69*	0.13
14	Marketable yield (q/ha)	99.22	763.50**	143.75
15	Total yield (kg/plot)	0.03	0.60**	0.11
16	Total yield (q/ha)	28.73	667.49**	125.40
17	Percent disease index (%)	1.40	142.39**	4.14
18	Total Soluble Solids (^{0}Brix)	0.16	3.01*	0.69
19	Dry matter (%)	0.73	15.19**	0.08
20	Pyruvic Acid (µg/g)	0.02	1.53*	0.10
21	PWL (%) after one month	0.09	0.03**	0.02
22	PWL (%) after two months	0.07	1.58**	0.04
23	PWL (%) after three months	0.07	5.05**	0.06
24	PWL (%) after four months	0.00	4.08**	0.03

Significant at 5 per cent level, **Significant at 1 per cent level

4.2. MEAN PERFORMANCE OF GENOTYPES

Evaluation of onion genotypes on the basis of morphological, biochemical characters and yield performance were presented in (Table 4.2). The details of different characters recorded during the year 2015-16 and 2016-17 and pooled data of two years of experiment are given below.

4.2.1. Plant height (cm)

In the first year 2015-16, the plant height ranged from 48.09 to 55.73 cm. with overall mean value of 52.67 cm. RO-636 (55.73 cm) was found tallest followed by RO-628 (55.63 cm) and Bhima Sweta (55.00 cm) where as Orient (48.09 cm) was the shortest genotype followed by BSS-827 (49.03 cm). In the second year 2016-17, the plant height ranged from 50.39 to 56.51 cm. with overall mean value of 63.97cm. RO-628 (56.51 cm) was found tallest followed by Bhima Sweta (55.79 cm) and RO-636 (55.11 cm) where as DOGR-HY-2 (50.39 cm) was the shortest genotype followed by Orient (50.47 cm).

In the pooled of two years data, the plant height ranged from 49.28 to 56.07 cm. with overall mean value of 52.15 cm. RO-628 (56.07 cm) was found tallest followed by RO-636 (55.42 cm) and Bhima Sweta (55.39 cm) whereas Orient (49.28 cm) was the shortest genotype followed by DOGR-HY-2 (49.73 cm).

4.2.2. Number of leaves per plant

The maximum number of leaves per plant was observed in JRDO-07-13 (11.33) followed by Arka Lalima (10.44) and RO-628 (10.29) where as minimum number of leaves per plant recorded in Bhima Kiran (7.55) in the first year 2015-16. Whereas LINE-355 (11.33) got maximum number of leaves per plant followed by Orient (11.18) and minimum in Bhima Kiran (8.73) followed by RO-628 (8.78) in the 2nd year 2016-17. But observation recorded in the pooled data maximum number of leaves found in JRDO-07-13 (10.91) followed by Arka Lalima (10.70), DOGR-HY-1 (10.61) and minimum leaves number recorded in Bhima Kiran (8.14) followed by DOGR-571-LR (8.57).

Table 4.2: Mean performance of onion genotypes based on different characters

Sl. no	Characters / Genotypes	Plant height (cm)			Number of leaves/plant			Double bulb (%)		
		First year	Second year	Pooled	First year	Second year	Pooled	First year	Second year	Pooled
1	IIHR-C-606	49.77	51.44	50.60	8.67	10.26	9.47	1.54	3.20	2.37
2	DOGR-571-LR	50.37	51.20	50.79	8.22	8.91	8.57	0.81	2.94	1.87
3	DOGR-546-DR	49.79	51.30	50.55	8.67	10.33	9.50	1.77	2.28	2.02
4	JWO-08-09	51.49	52.27	51.88	9.11	9.65	9.38	1.07	4.42	2.75
5	LINE-355	52.41	52.61	52.51	8.45	11.33	9.89	1.20	2.86	2.03
6	BHIMA KIRAN	49.89	51.34	50.62	7.55	8.73	8.14	0.96	2.86	1.91
7	BHIMA SWETA	55.00	55.79	55.39	9.89	9.61	9.75	1.66	1.92	1.79
8	PKV WHITE	53.35	53.58	53.46	9.78	9.67	9.72	0.49	1.71	1.10
9	RO-628	55.63	56.51	56.07	10.29	8.78	9.54	2.49	3.27	2.88
10	RO-636	55.73	55.11	55.42	9.28	9.90	9.59	2.66	4.60	3.63
11	BHIMA SHAKTI	50.01	51.21	50.61	8.55	9.31	8.93	1.20	2.62	1.91
12	ARKA LALIMA	49.62	51.83	50.72	10.44	10.96	10.70	1.02	2.95	1.99
13	ARKA	51.19	54.48	52.84	8.78	10.10	9.44	0.71	2.11	1.41
14	DOGR-HY-1	51.12	52.62	51.87	10.22	11.00	10.61	0.95	2.34	1.65
15	DOGR-HY-2	49.08	50.39	49.73	9.22	10.75	9.99	1.77	2.23	2.00
16	JRDO-07-13	53.07	52.57	52.82	11.33	10.48	10.91	0.53	2.79	1.66
17	LUCIFER	54.84	54.87	54.85	8.45	10.17	9.31	1.80	3.24	2.52
18	BSS-827	49.03	50.48	49.76	9.44	11.02	10.23	1.94	3.01	2.47
19	PUNE RED	52.85	53.41	53.13	9.00	10.00	9.50	1.33	3.64	2.49
20	ORIENT	48.09	50.47	49.28	8.33	11.18	9.76	2.16	3.79	2.97
	Mean	**51.62**	**52.67**	**52.15**	**9.18**	**10.11**	**9.65**	**1.40**	**2.94**	**2.14**
	S.E(M)	0.70	0.65	0.60	0.27	0.20	0.19	0.41	0.26	0.25
	CD(5%)	2.01	1.86	1.72	0.78	0.57	0.55	1.16	0.75	0.73

4.2.3. Double bulb (%)

The genotypes with highest double bulb recorded in RO-636 (2.66 %) followed by RO-628 (2.49 %) and Orient (2.16 %) and minimum double bulb recorded in PKV White (0.49 %) in the year 2015-16. In the next year 2016-17, RO-636 (4.60 %) followed by JWO-08-09 (4.42 %) got maximum double bulbs and least double bulbs recorded in PKV White (1.71 %) and Bhima Sweta (1.92 %). In the Pooled data observation highest double bulb recorded in RO-636 (3.63 %) followed by Orient (2.97 %) and RO-628 (2.88 %). Minimum double bulb found in PKV White (1.10 %), Arka Kirtiman (1.41 %) and DOGR-HY-1 (1.65 %).

4.2.4. Neck thickness (mm)

In the first year 2015-16, the range for Neck thickness was (4.70 - 6.34 mm) with general mean (5.81 mm). Desirable minimum neck thickness was found in Bhima Shakti

(4.70 mm) followed by Orient (5.09 mm), Arka Kirtiman (5.55 mm) and it was highest in DOGR-HY-2 (6.34 mm) followed by RO-636 (6.29 mm).

In the second year 2016-17, the range for Neck thichness was 4.97-6.91 mm with general mean 5.95 mm. Desirable minimum neck thickness was found in Bhima Shakti (4.97 mm) followed by Orient (5.09 mm), Bhima Sweta (5.37 mm) and it was highest in DOGR-HY-2 (6.91 mm) followed by Bhima Kiran (6.64 mm).

In the pooled year data showed that the range for Neck thichness was 4.84- 6.63 mm with general mean 5.88 mm. Desirable minimum neck thickness was found in Bhima Shakti (4.84 mm) followed by Orient (5.09 mm), Bhima Sweta (5.50 mm) and it was highest in DOGR-HY-2 (6.33 mm) followed by RO-636 (6.44 mm).

Table 4.2: Mean performance of onion genotypes based on different characters (Continued)

Sl. no	Characters	Neck thickness(mm)			Days to maturity			Polar diameter(mm)		
	Genotypes	First year	Second year	Pooled	First year	Second year	Pooled	First year	Second year	Pooled
1	IIHR-C-606	5.68	5.55	5.62	110.67	109.00	109.83	48.89	47.53	48.21
2	DOGR-571-LR	5.64	5.61	5.63	109.33	111.00	110.17	50.07	50.61	50.34
3	DOGR-546-DR	6.13	6.37	6.25	111.00	110.33	110.67	46.66	46.20	46.43
4	JWO-08-09	5.83	6.42	6.12	110.00	110.33	110.17	47.98	48.75	48.37
5	LINE-355	5.94	6.39	6.17	108.67	108.67	108.67	50.44	51.16	50.80
6	BHIMA KIRAN	6.16	6.64	6.40	108.33	108.33	108.33	47.90	47.43	47.67
7	BHIMA SWETA	5.62	5.37	5.50	109.33	108.00	108.67	49.51	50.02	49.76
8	PKV WHITE	5.76	5.68	5.72	112.00	111.67	111.83	51.15	51.53	51.34
9	RO-628	5.76	5.47	5.61	108.67	108.00	108.33	53.71	54.00	53.85
10	RO-636	6.29	6.59	6.44	107.67	107.00	107.33	52.20	52.46	52.33
11	BHIMA SHAKTI	4.70	4.97	4.84	109.00	109.00	109.00	48.01	48.25	48.13
12	ARKA LALIMA	6.02	5.61	5.81	106.00	105.67	105.83	49.97	50.33	50.15
13	ARKA KIRTIMAN	5.55	6.10	5.83	111.67	112.00	111.83	49.75	50.21	49.88
14	DOGR-HY-1	5.89	5.99	5.94	106.33	106.67	106.50	53.05	53.15	53.10
15	DOGR-HY-2	6.34	6.91	6.63	108.00	108.33	108.17	48.10	48.63	48.36
16	JRDO-07-13	5.78	6.02	5.90	108.00	108.00	108.00	48.73	49.63	49.18
17	LUCIFER	6.02	6.15	6.09	106.33	105.00	105.67	51.91	53.33	52.62
18	BSS-827	6.24	5.97	6.10	112.33	112.67	112.50	46.29	46.56	46.43
19	PUNE RED	5.68	6.13	5.91	106.33	105.00	105.67	51.05	51.37	51.21
20	ORIENT	5.09	5.09	5.09	110.33	109.00	109.67	48.86	49.44	49.15
	Mean	**5.81**	**5.95**	**5.88**	**109**	**108.68**	**108.84**	**49.71**	**50.03**	**49.87**
	S.E(M)	0.25	0.12	0.15	0.93	0.51	0.57	0.57	0.57	0.57
	CD(5%)	0.72	0.33	0.44	2.65	1.47	1.63	1.63	1.64	1.64

4.2.5. Days to maturity

For the first year the general mean for days to maturity was (109) and it ranged 106-112.33 days. Arka Lalima requires minimum number of days to maturity followed by DOGR-HY-1, Lucifer and Pune Red while BSS-827 required maximum number of days to maturity. In the second year the general mean for days to maturity was 108.68 and it ranged 105.00-112.67 days. Lucifer and Pune Red require minimum number of days to maturity while BSS-827 required maximum number of days to maturity. In the pooled data the mean was 108.84 and range was 105.67-112.50. Lucifer and Pune Red requires minimum (105.00) number of days to maturity followed by Arka Lalima (105.67) and while maximum days requires BSS-827 (112.67).

4.2.6. Polar diameter of bulb (mm)

There was significant difference among the genotypes for polar diameter of the bulb, which ranged from 46.29- 53.71 mm with a mean value of (49.71 mm). The minimum polar diameter of the bulb was recorded with BSS-827 and maximum polar diameter of bulb with RO-628 in the year 2015-16. In the year 2016-17 polar diameter of the bulb which ranged from 46.20-54.00 mm with a mean value 50.03 mm, maximum polar diameter was found in RO-628 and minimum was in DOGR-546-DR. The pooled data ranged from 46.43-53.85 mm with mean value of 49.87 mm. Maximum polar diameter found in RO-628 and minimum in DOGR-546-DR.

4.2.7. Equatorial diameter of bulb (mm)

In the year 2015-16, the equatorial diameter of bulb ranged from 49.26-57.39 mm and the general mean for equatorial diameter of bulb was 53.23 mm. The highest equatorial diameter of bulb was recorded with DOGR-HY-1, followed by RO-628 whereas, the minimum equatorial diameter of bulb was observed in BSS-827 followed by DOGR-546-DR. In the year 2016-17, the equatorial diameter of bulb ranged from 49.81-57.62 mm and the general mean for equatorial diameter of bulb was 53.30 mm. The highest equatorial diameter of bulb was recorded with RO-628, followed by RO-636 Whereas, the minimum equatorial diameter of bulb was observed in BSS-827 followed by JWO-08-09. The pooled data ranged from 49.54-57.34 mm with mean value of 53.27 mm. Maximum equatorial diameter found in RO-628 and minimum equatorial diameter found in BSS-827.

Table 4.2: Mean performance of onion genotypes based on different characters (Continued)

Sl. no	Characters	Equitorial diameter			Individual bulb weight			Grade A bulb no		
	Genotypes	First year	Second year	Pooled	First year	Second year	Pooled	First year	Second year	Pooled
1	IIHR-C-606	52.35	51.84	52.09	56.71	57.71	57.21	41.33	42.00	43.67
2	DOGR-571-LR	53.90	54.06	53.98	56.39	56.71	56.55	45.00	45.00	45.50
3	DOGR-546-DR	50.50	50.72	50.61	50.54	50.55	50.54	45.33	45.67	42.83
4	JWO-08-09	51.43	50.64	51.04	50.99	50.85	50.92	41.33	41.67	40.17
5	LINE-355	54.15	54.19	54.17	51.68	52.37	52.03	46.33	47.00	46.00
6	BHIMA KIRAN	51.29	51.27	51.28	58.76	59.84	59.30	45.67	45.67	45.17
7	BHIMA SWETA	52.98	53.39	53.19	49.69	49.89	49.79	45.00	45.00	46.00
8	PKV WHITE	54.82	54.88	54.85	52.34	52.47	52.41	45.67	45.33	44.33
9	RO-628	57.06	57.62	57.34	52.85	53.68	53.26	39.67	42.33	40.17
10	RO-636	56.50	56.73	56.62	57.36	57.63	57.49	37.00	37.00	36.67
11	BHIMA SHAKTI	51.47	51.68	51.57	47.56	49.91	48.74	44.67	46.00	45.83
12	ARKA LALIMA	52.93	52.93	52.93	49.80	50.33	50.06	43.33	43.33	45.17
13	ARKA KIRTIMAN	53.06	52.79	52.93	50.77	51.82	51.30	46.00	47.00	46.50
14	DOGR-HY-1	57.39	56.28	56.84	52.55	53.49	53.02	46.33	47.00	46.67
15	DOGR-HY-2	51.02	51.48	51.25	52.14	51.37	51.76	43.00	43.33	44.00
16	JRDO-07-13	52.26	52.79	52.53	56.92	57.15	57.04	42.33	43.00	41.67
17	LUCIFER	55.50	55.70	55.60	51.79	52.19	51.99	42.33	44.33	42.67
18	BSS-827	49.26	49.81	49.54	53.90	54.09	54.00	39.33	40.67	38.33
19	PUNE RED	54.02	54.09	54.06	51.38	51.90	51.64	42.00	42.33	40.67
20	ORIENT	52.68	53.16	52.92	55.43	55.77	55.60	40.00	41.00	39.33
	Mean	**53.23**	**53.30**	**53.27**	**52.98**	**53.49**	**53.23**	**43.08**	**43.73**	**43.41**
	S.E(M)	0.59	0.44	0.45	0.81	0.68	0.67	1.30	1.25	1.21
	CD(5%)	1.68	1.27	1.29	2.33	1.96	1.91	3.74	3.58	3.47

4.2.8. Individual bulb weight (g)

In the first year, the range and general mean for individual bulb weight was recorded (47.56-58.76 g) and (52.98 g), respectively. Highest individual bulb weight found in the variety Bhima Kiran (58.76 g) followed by RO-636 (57.36 g) and JRDO-07-13 (56.92 g) and lowest in Bhima Shakti (47.56 g).

In the second year, the range and general mean for individual bulb weight was recorded (49.89- 59.84 g) and (53.49 g), respectively. Highest individual bulb weight found in the variety Bhima Kiran (59.84 g) followed by IIHR-C-606 (57.71 g) and RO-636 (57.63 g) and lowest in Bhima Sweta (49.89 g).

In the pooled data ranged from (48.74-59.30 g) and mean was (53.23 g). Highest individual bulb weight found in the variety Bhima Kiran (59.30 g) followed by RO-636 (57.49 g) and IIHR-C-606 (57.21 g) and lowest in Arka Bindu (48.74 g).

4.2.9. Grade A bulb number

In the first year 2015-16, Grade A bulb ranged from (37.0 – 46.33) and mean was (43.08). Highest Grade A bulb found in DOGR-HY-1 followed by Bhima Kiran and PKV White (45.67). Lowest number found in RO-636 (37.00).

In the next year 2016-17, Grade A bulb ranged from (37.00-47.00) and mean was (43.73). Highest Grade A bulb found in Arka Kirtiman, Line-355, DOGR-HY-1 followed by Bhima Shakti (46.00). Lowest number found in RO-636 (37.00

In the pooled data ranged from (36.67-46.67) and mean was (43.41). Highest Grade A bulb found in DOGR-HY-1 followed by Arka Kirtiman (46.50). Lowest number found in RO-636 (36.67).

4.2.10. Grade B bulb number

In the first year 2015-16, Grade B bulb ranged from (36.00-46.33) and mean was (42.57). Highest Grade B bulb found in Arka Kirtiman and DOGR-HY-1 followed by Line-355 (46.00) and Bhima Sweta (45.67 Lowest number found in RO-636 (36.00).

In the next year 2016-17, Grade B bulb ranged from (37.33-47.00) and mean was (43.37). Highest Grade B bulb found in DOGR-HY-1 followed by Arka Kirtiman (46.67) and Bhima Shakti (46.00). Lowest number found in RO-636 (37.33).

In the pooled data ranged from (36.67-46.67) and mean was (42.97). Highest Grade B bulb found in DOGR-HY-1 (46.67) followed by Arka Kirtiman (46.50) and Bhima Sweta (46.00). Lowest number found in RO-636 (36.67).

Table 4.2: Mean performance of onion genotypes based on different characters (Continued)

Sl. no	Characters / Genotypes	Grade B bulb no			Grade C bulb no			Marketable bulb/plot		
		First year	Second year	Pooled	First year	Second year	Pooled	First year	Second year	Pooled
1	IIHR-C-606	42.33	45.00	43.67	44.67	44.00	44.33	128.25	131.00	129.63
2	DOGR-571-LR	43.00	44.00	43.50	42.67	43.33	43.00	130.67	132.33	131.50
3	DOGR-546-DR	43.00	42.67	42.83	40.33	40.67	40.50	128.69	129.00	128.85
4	JWO-08-09	40.33	40.00	40.17	39.67	40.67	40.17	121.62	122.33	121.98
5	LINE-355	46.00	46.00	46.00	43.00	43.33	43.17	135.46	136.33	135.90
6	BHIMA KIRAN	44.67	45.67	45.17	44.67	44.33	44.50	135.05	135.67	135.36
7	BHIMA SWETA	45.67	46.33	46.00	45.00	45.00	45.00	135.68	136.33	136.01
8	PKV WHITE	44.33	44.33	44.33	44.33	44.00	44.17	134.39	133.67	134.03
9	RO-628	38.00	42.33	40.17	38.00	43.67	40.83	115.39	128.33	121.86
10	RO-636	36.00	37.33	36.67	35.67	36.00	35.83	108.77	110.33	109.55
11	BHIMA SHAKTI	45.67	46.00	45.83	45.00	44.33	44.67	135.23	136.33	135.78
12	ARKA LALIMA	44.67	45.67	45.17	42.67	42.67	42.67	130.42	131.67	131.04
13	ARKA KIRTIMAN	46.33	46.67	46.50	45.33	45.33	45.33	137.68	139.00	138.34
14	DOGR-HY-1	46.33	47.00	46.67	45.33	45.00	45.17	138.12	139.00	138.56
15	DOGR-HY-2	43.67	44.33	44.00	42.33	43.67	43.00	128.84	131.33	130.08
16	JRDO-07-13	41.67	41.67	41.67	41.33	42.67	42.00	125.34	127.33	126.33
17	LUCIFER	42.00	43.33	42.67	41.33	41.67	41.50	125.61	129.33	127.47
18	BSS-827	38.33	38.33	38.33	38.00	39.33	38.67	115.43	118.33	116.88
19	PUNE RED	40.33	41.00	40.67	40.00	40.33	40.17	122.36	123.67	123.01
20	ORIENT	39.00	39.67	39.33	36.33	38.33	37.33	115.08	119.00	117.04
	Mean	**42.57**	**43.37**	**42.97**	**41.78**	**42.42**	**42.10**	**127.40**	**129.52**	**128.46**
	S.E(M)	1.46	1.15	1.21	1.36	0.93	1.04	3.58	2.18	2.67
	CD(5%)	4.17	3.30	3.47	3.88	2.67	2.99	10.25	6.24	7.64

4.2.11. Grade C bulb number

In the first year 2015-16, Grade C bulb ranged from (35.67-45.33) and mean was (41.78). Highest Grade B bulb found in Arka Kirtiman and DOGR-HY-1 followed by Bhima ShaktI and Bhima Sweta (45.00). Lowest number found in RO-636 (35.67).

In the next year 2016-17, Grade C bulb ranged from (36.00-19.67) and mean was (42.42). Highest Grade C bulb found in Arka Kirtiman followed by DOGR-HY-1 (45.00) while, lowest number found in RO-636 (36.00).

In the pooled data, ranged from (35.83-15.00) and mean was (42.10). Highest Grade C bulb found in Arka Kirtiman followed by DOGR-HY-1 (45.17) and Bhima Sweta (45.00). Lowest number found in ro-636 (35.83).

4.2.12. Number of Marketable bulb/plot

There was significant variation among the genotypes for marketable bulb. The general mean for marketable bulb/plot was (127.40) and ranged from (108.77-138.12). The highest marketable bulb/plot was recorded in DOGR-HY-1 (138.12) followed by Arka Kirtiman (137.68) while the minimum was observed in RO-636 (108.77) in the first year. In the second year general mean for marketable bulb/plot was (129.52) and ranged from (110.33-139.00). The highest marketable bulb/plot was recorded in Arka Kirtiman and DOGR-HY-1 followed by Line-355 and Bhima Sweta (136.33) while the minimum was observed in RO-636 (110.33).

In pooled of two years, general mean for marketable bulb/plot was (128.46) and ranged from (109.55-138.56). The highest marketable bulb/plot was recorded in DOGR-HY-1 followed by Arka Kirtiman (138.34) and Bhima Sweta (136.01) while the minimum yield was observed in RO-636.

4.2.13. Marketable yield/plot (kg)

There was significant variation among the genotypes for marketable yield. In the year 2015-16, the general mean for marketable yield (kg/plot) was (6.75 kg/plot) and ranged was from (6.10-7.93 kg/plot). The highest marketable yield (kg/plot) was recorded in Bhima Kiran (7.93 kg/plot) followed by DOGR-571-LR (7.37 kg/plot) and IIHR-C-606 (7.28 kg/plot) while the minimum yield was observed in RO-628 (6.10 kg/plot).

In the year 2016-17, the general mean for marketable yield (kg/plot) was (6.92 kg/plot) and ranged was from (6.22-8.12 kg/plot). The highest marketable yield (kg/plot) was recorded in Bhima Kiran followed by IIHR-C-606 (7.56 kg/plot) and DOGR-571-LR (7.50 kg/plot) while the minimum yield was observed in JWO-08-09 (6.22 kg/plot).

In the pooled data, the general mean for marketable yield (kg/plot) was (6.84 kg/plot) and ranged was from (6.21-8.02 kg/plot). The highest marketable yield (kg/plot) was recorded in Bhima Kiran followed by DOGR-571-LR (7.44 kg/plot) and IIHR-C-606 (7.42 kg/plot) while the minimum yield was observed in JWO-08-09.

Table 4.2: Mean performance of onion genotypes based on different characters (Continued)

Sl. no	Characters	Marketable yield (kg/plot)			Marketable yield (q/ha)			Total yield (kg/plot)		
	Genotypes	First year	Second year	Pooled	First year	Second year	Pooled	First year	Second year	Pooled
1	IIHR-C-606	7.28	7.56	7.42	242.55	251.97	247.26	7.13	7.81	7.47
2	DOGR-571-LR	7.37	7.50	7.44	245.64	250.14	247.89	7.56	7.73	7.64
3	DOGR-546-DR	6.50	6.52	6.51	216.74	217.43	217.08	6.64	6.67	6.66
4	JWO-08-09	6.20	6.22	6.21	206.59	207.33	206.96	6.39	6.51	6.45
5	LINE-355	7.00	7.14	7.07	233.21	237.87	235.54	7.12	7.35	7.23
6	BHIMA KIRAN	7.93	8.12	8.02	264.35	270.58	267.46	8.11	8.36	8.24
7	BHIMA SWETA	6.74	6.80	6.77	224.71	226.74	225.73	6.99	6.93	6.96
8	PKV WHITE	7.03	7.01	7.02	234.36	233.78	234.07	7.08	7.13	7.11
9	RO-628	6.10	6.89	6.49	203.22	229.66	216.44	6.32	7.12	6.72
10	RO-636	6.24	6.36	6.30	208.15	212.12	210.13	6.59	6.67	6.63
11	BHIMA SHAKTI	6.43	6.80	6.62	214.24	226.82	220.53	6.57	6.99	6.78
12	ARKA LALIMA	6.49	6.63	6.56	216.45	220.92	218.68	6.63	6.83	6.73
13	ARKA KIRTIMAN	6.99	7.20	7.10	233.02	240.11	236.57	7.13	7.36	7.24
14	DOGR-HY-1	7.26	7.44	7.35	241.87	247.85	244.86	7.40	7.61	7.51
15	DOGR-HY-2	6.74	6.75	6.74	224.55	225.01	224.78	6.88	6.90	6.89
16	JRDO-07-13	7.13	7.27	7.20	237.61	242.48	240.04	7.22	7.48	7.35
17	LUCIFER	6.50	6.75	6.63	216.83	225.07	220.95	6.70	6.98	6.84
18	BSS-827	6.48	6.40	6.44	215.87	213.47	214.67	6.64	6.60	6.62
19	PUNE RED	6.29	6.42	6.35	209.67	213.97	211.82	6.46	6.66	6.56
20	ORIENT	6.38	6.64	6.51	212.72	221.37	217.05	6.55	6.90	6.72
	Mean	**6.75**	**6.92**	**6.84**	**225.12**	**230.73**	**227.93**	**6.91**	**7.13**	**7.02**
	S.E(M)	0.21	0.16	0.17	6.92	5.37	5.77	0.19	0.16	0.17
	CD(5%)	0.59	0.46	0.50	19.82	15.38	16.51	0.56	0.46	0.48

4.2.14. Marketable yield (q/ha)

In the first year, the general mean for marketable yield was (225.12 q/ha). The highest marketable yield was recorded in Bhima Kiran (264.35 q/ha) followed by DOGR-571-LR (245.64 q/ha) and IIHR-C-606 (242.55 q/ha) while the minimum yield was observed in RO-628 (203.22 q/ha).

In the second year, the general mean for marketable yield was (230.73 q/ha). The highest marketable yield was recorded in Bhima Kiran (270.58 q/ha) followed by IIHR-C-606 (251.97 q/ha) and DOGR-571-LR (250.14 q/ha) and while the minimum yield was observed in JWO-08-09 (207.33 q/ha).

In the pooled year's data, the general mean for marketable yield was (227.93 q/ha). The highest marketable yield was recorded in Bhima Kiran (267.46 q/ha) followed

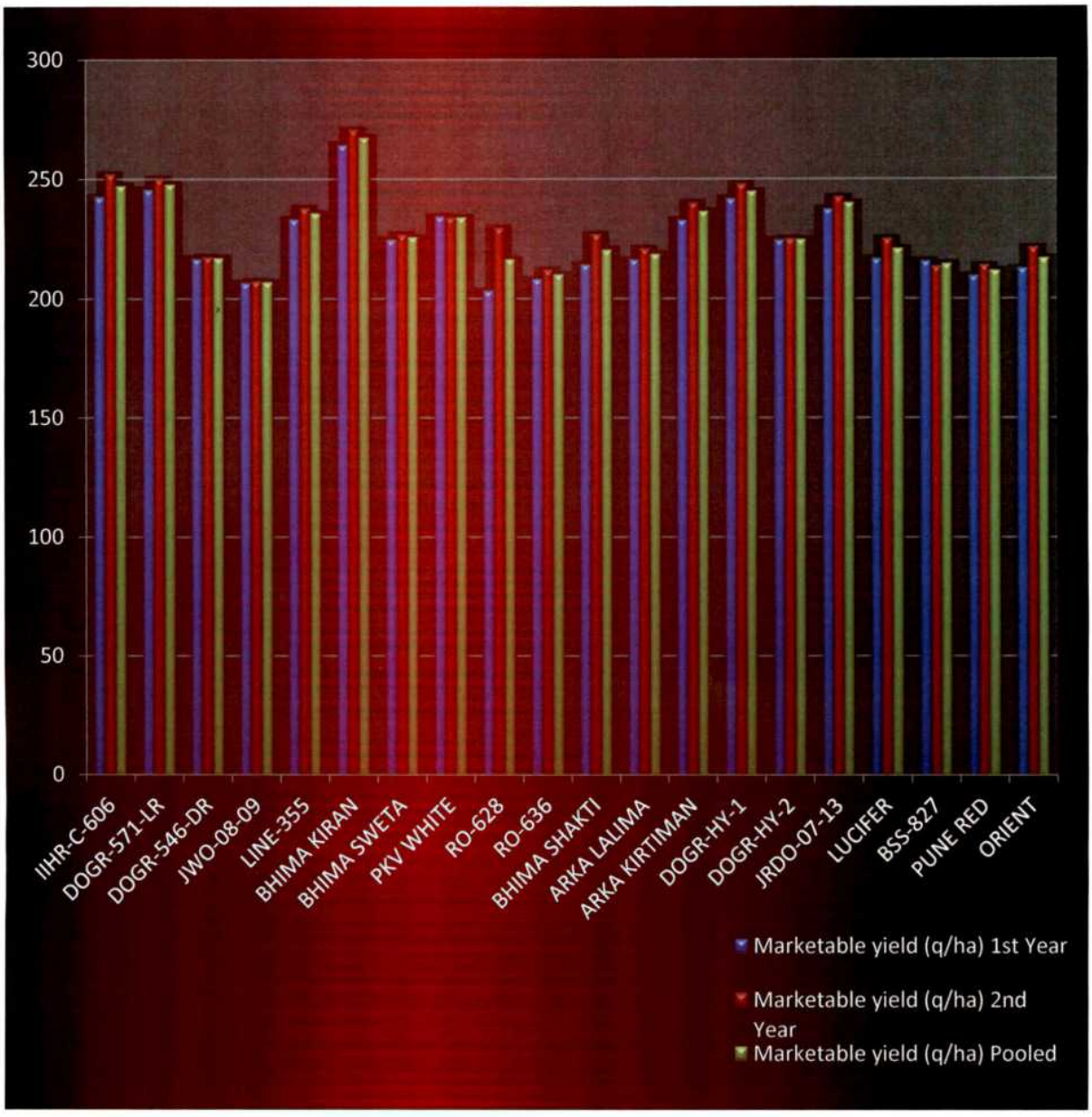


Figure 1: Marketable yield (q/ha) of 20 onion genotypes

by DOGR-571-LR (247.89 q/ha) and IIHR-C-606 (247.26 q/ha) while the minimum yield was observed in JWO-08-09 (206.96 q/ha).

4.2.15. Total yield/plot (kg)

There was significant variation among the genotypes for total yield (kg/plot). In the year 2015-16, the general mean for total yield (kg/plot) was (6.91 kg/plot) and ranged was from (6.32-8.11 kg/plot). The highest total yield (kg/plot) was recorded in Bhima Kiran followed by DOGR-571-LR (7.56 kg/plot) and DOGR-HY-1 (7.40 kg/plot) while the minimum total yield was observed in RO-628.

In the year 2016-17, the general mean for total yield (kg/plot) was 7.13 and ranged was from (6.51-2.67 kg/plot). The highest total yield (kg/plot) was recorded in Bhima Kiran (8.36 kg/plot) followed by IIHR-C-606 (7.81 kg/plot) and DOGR-571-LR (7.73 kg/plot) while the minimum yield was observed in JWO-08-09 (6.51 kg/plot).

In the pooled data, the general mean for total yield (kg/plot) was (7.02 kg/plot) and ranged was from (6.45-8.24 kg/plot). The highest total yield (kg/plot) was recorded in Bhima Kiran followed by DOGR-571-LR (7.64 kg/plot) and DOGR-HY-1 (7.51 kg/plot) while the minimum yield was observed in JWO-08-09.

4.2.16. Total yield (q/ha)

There was significant variation among the genotypes for total yield. The general mean for total yield was (230.13 q/ha) and it ranged from (210.83 to 270.22 q/ha). The highest yield was recorded in Bhima Kiran (270.22 q/ha) followed by DOGR-571-LR (251.90 q/ha) and DOGR-HY-1 (246.55 q/ha) while the minimum yield was observed in JWO-08-09 (210.83 q/ha) in the first year.

In the second year total yield mean was (237.68 q/ha) and ranged was from (216.93-278.56 q/ha). The highest yield was recorded in Bhima Kiran followed by IIHR-C-606 (260.30 q/ha) and DOGR-571-LR (257.70 q/ha) and the lowest total yield found in JWO-08-09 (216.93 q/ha).

In the pooled data, the general mean for total yield was (233.90 q/ha) and ranged was from (214.89-274.39 q/ha). The highest total yield (q/ha) was recorded in Bhima Kiran followed by DOGR-571-LR (254.80 q/ha) and DOGR-HY-1 (250.17 q/ha) while the minimum yield was observed in JWO-08-09 (214.89 q/ha).

Table 4.2: Mean performance of onion genotypes based on different characters (Continued)

Sl. no	Characters	Total yield (q/ha)			Total soluble solids (^{0}Brix)			Dry matter (%)		
	Genotypes	First year	Second year	Pooled	First year	Second year	Pooled	First year	Second year	Pooled
1	IIHR-C-606	237.56	260.30	248.93	10.93	11.71	11.32	19.08	16.14	17.61
2	DOGR-571-LR	251.90	257.70	254.80	9.94	11.41	10.68	14.87	15.71	15.29
3	DOGR-546-DR	221.22	222.47	221.85	11.36	10.61	10.99	15.29	18.14	16.71
4	JWO-08-09	212.84	216.93	214.89	10.13	11.49	10.81	15.10	12.48	13.79
5	LINE-355	237.22	244.87	241.05	13.32	10.21	11.77	11.34	15.20	13.27
6	BHIMA KIRAN	270.22	278.56	274.39	10.42	12.18	11.30	15.03	14.14	14.59
7	BHIMA SWETA	233.00	231.17	232.08	11.01	11.64	11.33	18.78	15.83	17.30
8	PKV WHITE	236.11	237.83	236.97	11.44	11.55	11.50	15.36	12.63	13.99
9	RO-628	210.83	237.41	224.12	12.99	13.55	13.27	15.19	12.26	13.72
10	RO-636	219.58	222.37	220.97	11.92	14.28	13.10	17.84	14.56	16.20
11	BHIMA SHAKTI	219.00	232.92	225.96	10.84	12.19	11.51	14.93	16.21	15.57
12	ARKA LALIMA	220.89	227.63	224.26	10.95	11.98	11.47	14.02	15.86	14.94
13	ARKA KIRTIMAN	237.56	245.29	241.42	11.40	11.60	11.50	12.07	19.45	15.76
14	DOGR-HY-1	246.56	253.78	250.17	10.05	11.75	10.90	19.12	11.95	15.53
15	DOGR-HY-2	229.17	230.13	229.65	13.36	11.53	12.45	18.39	13.96	16.18
16	JRDO-07-13	240.80	249.46	245.13	10.81	10.65	10.73	13.30	12.45	12.88
17	LUCIFER	223.18	232.60	227.89	10.55	10.61	10.58	16.09	14.98	15.54
18	BSS-827	221.26	220.12	220.69	10.49	11.04	10.76	14.90	14.88	14.89
19	PUNE RED	215.36	222.04	218.70	11.03	11.51	11.27	17.24	14.84	16.04
20	ORIENT	218.27	230.03	224.15	11.06	11.75	11.41	13.35	12.84	13.09
	Mean	**230.13**	**237.68**	**233.90**	**11.20**	**11.66**	**11.43**	**15.56**	**14.73**	**15.14**
	S.E(M)	**6.47**	**5.41**	**5.58**	**0.48**	**0.15**	**0.25**	**0.16**	**0.36**	**0.19**
	CD(5%)	**18.51**	**15.49**	**15.97**	**1.38**	**0.42**	**0.72**	**0.47**	**1.03**	**0.56**

4.2.17. Total soluble solids (^{0} Brix)

First year data showed that the total soluble solids ranged from (9.94 to 13.36 ^{0}Brix) and mean was (11.20 ^{0}Brix). Highest total soluble solids (TSS) were found in the variety DOGR-HY-2 followed by Line-355 (13.32 ^{0}Brix) and RO-628 (12.99 ^{0}Brix). The minimum total soluble solid was recorded with DOGR-571-LR.

Second year data showed that the total soluble solids ranged from (10.21-14.28 ^{0} Brix) and mean was (11.66 ^{0}Brix). Highest total soluble solids (TSS) were found in the variety RO-636 followed by RO-628 (13.55 ^{0}Brix) and Bhima Shakti (12.19 ^{0}Brix). The minimum total soluble solid was recorded with Line-355.

Pooled data showed that the total soluble solids ranged from (10.58 to 13.27^0 Brix) and mean was (11.43^0 Brix). Highest total soluble solids (TSS) were found in the

variety RO-628 followed by and RO-636 (13.10^0 Brix) and DOGR-HY-2 (12.45^0 Brix). The minimum total soluble solid was recorded with Lucifer.

4.2.18. Dry matter (%)

In the first year, dry matter ranged from (11.34 to 19.08 %) and mean was (15.56 %) and highest found in IIHR-C-606 followed by Bhima Sweta (18.78 %) while Line-355 had lowest.

In the second year dry matter ranged from (11.95-19.45 %) and mean was (14.73 %) and highest was in Arka Kirtiman followed by DOGR-546-DR (18.14 %) while DOGR-HY-1 had lowest.

In the pooled of two years dry matter ranged from (12.88-17.61 %) and mean (15.14 %) and highest was in IIHR-C-606 followed by Bhima Sweta (17.30 %) while lowest was in JRDO-07-13.

4.2.19. Pyruvic acid (µg/g)

In the first year, Pyruvic acid content in these cultivars ranged from (0.93-2.92 µg/g) and mean was (2.13 µg/g). Orient had highest pyruvic acid content followed by Bhima Shakti (2.89 µg/g) and DOGR-HY-1 (2.84 µg/g) and it was found lowest in RO-628.

In the second year, Pyruvic acid was ranged from (0.94-3.00 µg/g) and mean was (2.07 µg/g). PKV White had highest pyruvic acid content followed by DOGR-HY-1 (2.83 µg/g) and Line-355 (2.59 µg/g) and it was found lowest in DOGR-546-DR.

The pooled data showed the range (0.96-3.15 µg/g) and the mean was (2.10 µg/g). The highest pyruvic acid content in the genotype PKV White followed by DOGR-HY-1 (2.84 µg/g) and Line-355 (2.59 µg/g). Lowest Pyruvic acid content was found in DOGR-546-DR.

Table 4.2: Mean performance of onion genotypes based on different characters (Continued)

Sl. no	Characters	Pyruvic acid (µg/g)			Physiological weight loss (%) after 1 month		
	Genotypes	First year	Second year	Pooled	First year	Second year	Pooled
1	IIHR-C-606	2.11	2.46	2.28	4.07	4.53	4.30
2	DOGR-571-LR	1.40	1.63	1.52	3.92	3.99	3.96
3	DOGR-546-DR	0.98	0.94	0.96	3.64	5.62	4.63
4	JWO-08-09	2.77	2.31	2.54	3.83	4.63	4.23
5	LINE-355	2.59	2.59	2.59	3.71	3.68	3.70
6	BHIMA KIRAN	1.40	2.31	1.86	3.82	4.66	4.24
7	BHIMA SWETA	2.46	1.25	1.86	3.85	3.34	3.59
8	PKV WHITE	3.30	3.00	3.15	3.92	5.48	4.70
9	RO-628	0.93	1.21	1.07	3.76	3.66	3.71
10	RO-636	2.31	2.44	2.38	3.93	4.60	4.27
11	BHIMA SHAKTI	2.89	2.18	2.54	3.80	4.54	4.17
12	ARKA LALIMA	2.29	2.40	2.34	3.92	5.75	4.84
13	ARKA KIRTIMAN	1.17	2.25	1.71	3.94	4.75	4.34
14	DOGR-HY-1	2.84	2.83	2.84	3.91	5.18	4.55
15	DOGR-HY-2	1.67	2.12	1.90	3.77	5.50	4.63
16	JRDO-07-13	2.51	1.73	2.12	3.91	5.69	4.80
17	LUCIFER	1.38	1.04	1.21	3.92	5.77	4.85
18	BSS-827	2.41	2.25	2.33	3.82	4.88	4.35
19	PUNE RED	2.33	2.55	2.44	3.79	5.42	4.60
20	ORIENT	2.92	1.84	2.38	3.87	5.65	4.76
	Mean	**2.13**	**2.07**	**2.10**	**3.86**	**4.87**	**4.36**
	S.E(M)	0.19	0.21	0.16	0.08	0.08	0.08
	CD(5%)	0.53	0.61	0.45	0.23	0.23	0.23

4.2.20. Physiological weight loss (%) after one month

First year data showed the range (3.64-4.07 %) and mean was (3.86 %). Highest Physiological weight loss observed in IIHR-C-606 followed by RO-636 (3.94 %), Arka Kirtiman (3.93 %) and lowest Physiological weight loss observed in DOGR-546-DR. In the second year mean was (4.87 %) and range was (3.34-5.77 %). Highest Physiological weight loss occurred in Lucifer followed by Arka Lalima (5.75 %) and JRDO-07-13 (5.69 %). And Physiological weight loss was lowest in Bhima Sweta. Pooled data showed that the mean was (4.36 %) and the range was (03.59-4.85 %). The highest Physiological weight loss found in Lucifer followed by Arka Lalima (4.84 %) and JRDO-07-13 (4.80 Lowest Physiological weight loss occurred in Bhima Sweta. Mahanthesh *et al.* (2009) found similar type of result when Arka Pragathi stored in ventilated room for 30 days recorded reduced storage loss in weight (10.13%).

4.2.21. Physiological weight loss (%) after two months

First year data showed the range (4.68-7.45 %) and mean was (6.14 %). Highest Physiological weight losses were observed in Arka Kirtiman followed by Pune Red (7.34 %) and JWO-08-09 (7.10 %). And lowest physiological weight loss observed in BSS-827. In the second year mean was (9.65 %) and range was (7.99-10.64 %). Highest physiological weight loss occurred in JRDO-07-13 followed by Pune Red (10.42 %) and Orient (10.40 %). And physiological weight loss was lowest in RO-628. Pooled data showed that the mean was (11.89 %) and the range was (4.69-28.02 %). Highest physiological weight loss found in Pune Red followed by PKV White (8.58 %) and Arka Kirtiman (8.44 %). Lowest physiological weight loss occurred in Arka Kalyan.

4.2.22. Physiological weight loss (%) after three months

First year data showed the range (8.63-13.64 %) and mean was (10.98 %). Highest Physiological weight loss observed in Pune Red followed by Bhima Kiran (12.96 %), PKV White (12.55 %) and lowest Physiological weight loss observed in BSS-827. In the second year mean was (14.35 %) and range was (13.12-16.00 %). Highest Physiological weight loss occurred in PKV White followed by Lucifer (15.99 %) and DOGR-HY-1 (15.80 %). And Physiological weight loss was lowest in RO-628. Pooled data showed that the mean was (12.67 %) and the range was (11.21-14.70 %). Highest Physiological weight loss was found in Pune Red followed by PKV White (14.27 %) and Bhima Kiran (13.62 %). Lowest Physiological weight loss occurred in IIHR-C606 (11.21 %). Similar type result got in Arka Pragathi stored in ventilated room for 30 days recorded reduced storage loss in weight (10.13%) by Mahanthesh *et al.* (2009).

4.2.23. Physiological weight loss (%) after four months

First year data showed the range (14.15-18.53 %) and mean was (15.96 %). Highest Physiological weight losses were observed in Pune Red followed by JWO-08-09 (17.60 %) and PKV White (17.21 %). And lowest physiological weight loss observed in Lucifer. In the second year mean was (19.11 %) and range was (16.41-21.41 %). Highest physiological weight loss occurred in PKV White followed by DOGR-HY-1 (21.16 %) and Arka Lalima, Orient (20.90 %). And physiological weight loss was lowest in DOGR-HY-2 (16.41 %). Pooled data showed that the mean was (17.54 %) and the range was (16.14-19.31 %). The highest physiological weight loss found in PKV White followed by Agrifound Dark Red (19.10 %) and Arka Lalima (18.64 %). Lowest physiological weight loss occurred in Bhima Shakti.

Table 4.2: Mean performance of onion genotypes based on different characters (Continued)

Sl. no	Characters	Physiological weight loss (%) after 2 months			Physiological weight loss (%) after 3 months			Physiological weight loss (%) after 4 months		
	Genotypes	First year	Second year	Pooled	First year	Second year	Pooled	First year	Second year	Pooled
1	IIHR-C-606	5.58	9.46	7.52	9.26	13.15	11.21	14.63	17.77	16.20
2	DOGR-571-LR	6.07	8.10	7.08	10.49	13.37	11.93	15.52	18.45	16.99
3	DOGR-546-DR	6.56	10.24	8.40	10.97	15.18	13.08	16.40	20.86	18.63
4	JWO-08-09	7.10	9.30	8.20	12.00	13.99	13.00	17.60	18.31	17.95
5	LINE-355	5.91	8.31	7.11	9.98	13.46	11.72	14.51	18.57	16.54
6	BHIMA KIRAN	5.89	10.10	8.00	12.96	14.27	13.62	16.66	19.41	18.04
7	BHIMA SWETA	6.17	8.20	7.19	11.22	13.16	12.19	15.08	18.12	16.60
8	PKV WHITE	6.88	10.28	8.58	12.55	16.00	14.27	17.21	21.41	19.31
9	RO-628	5.93	7.99	6.96	10.57	13.12	11.85	15.72	18.63	17.18
10	RO-636	6.51	9.50	8.00	11.74	13.60	12.67	17.11	18.84	17.97
11	BHIMA SHAKTI	5.74	9.84	7.79	10.35	10.84	10.60	15.54	16.73	16.14
12	ARKA LALIMA	6.47	10.31	8.39	11.52	15.67	13.60	16.38	20.90	18.64
13	ARKA KIRTIMAN	7.45	9.43	8.44	11.92	14.25	13.09	17.05	19.43	18.24
14	DOGR-HY-1	5.54	10.36	7.95	10.55	15.80	13.17	15.75	21.16	18.45
15	DOGR-HY-2	6.22	10.33	8.27	11.66	14.55	13.11	16.45	16.41	16.43
16	JRDO-07-13	5.29	10.64	7.97	10.51	14.63	12.57	15.10	17.42	16.26
17	LUCIFER	5.13	10.38	7.76	9.96	15.99	12.98	14.15	20.89	17.52
18	BSS-827	4.68	9.43	7.06	8.63	14.88	11.75	14.68	18.36	16.52
19	PUNE RED	7.34	10.40	8.87	13.64	15.76	14.70	18.53	19.67	19.10
20	ORIENT	6.41	10.42	8.42	9.13	15.35	12.24	15.12	20.90	18.01
	Mean	**6.14**	**9.65**	**7.90**	**10.98**	**14.35**	**12.67**	**15.96**	**19.11**	**17.54**
	S.E(M)	0.12	0.10	0.07	0.15	0.47	0.24	0.11	0.39	0.20
	CD(5%)	0.33	0.29	0.20	0.42	1.35	0.67	0.31	1.11	0.57

4.3. COMPONENTS OF VARIATION AND ESTIMATES OF GENETIC PARAMETERS

The different parameters for estimation of genetic component of variation to study variability i.e., mean, range, phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability (broad sense) and genetic advance expressed as per cent of mean values are presented in the Table 4.3, 4.4 and 4.5.

Highly significant variation was observed in all the characters viz., Plant height (cm), Number of leaves, Double bulb percentage, Neck thickness (mm), Days to maturity, Polar diameter (mm), Equitorial diameter (mm), Individual bulb weight (g), Grade A bulb number, Grade B bulb number, Grade C bulb number, Marketable bulb

number/plot, Marketable yield kg/plot, Marketable yield (q/ha), Total yield (kg/plot), Total yield (q/ha), Percent disease index, Total soluble solids (^{0}Brix), Dry matter (%), Pyruvic acid (µg/g), Physiological weight loss percentage after one month, Physiological weight loss percentage after two month content, Physiological weight loss percentage after three month content and Physiological weight loss percentage after four month content; indicated variability present in the experimental materials. Means with their ranges of different traits considered were almost similar for two consecutive years which was also reflected from the pooled data (Table 4.3, 4.4 and 4.5).

Table 4.3: Genetic components of different growth parameters in onion genotypes during 2015-16

Sl. No.	Characters	General Mean	Range	PCV	GCV	h^2	GA	GA as % of Mean
1	Plant height (cm)	51.62	48.09-55.75	4.97	4.37	77.45	4.09	7.92
2	Number of leaves	9.18	7.55-11.33	10.83	9.53	77.38	1.59	17.26
3	Double bulb (%)	1.40	0.98-1.76	24.26	12.81	27.87	0.19	13.93
4	Neck thickness (mm)	5.81	4.7-6.34	9.13	5.25	33.03	0.36	6.21
5	Days to maturity	109.00	106-112.33	2.15	1.57	53.10	2.56	2.35
6	Polar diameter (mm)	49.71	46.29-53.71	4.38	3.91	79.44	3.57	7.17
7	Equitorial diameter (mm)	53.23	49.26-57.39	4.43	4.00	81.36	3.96	7.43
8	Individual bulb weight(g)	52.98	47.56-58.76	6.09	5.48	80.97	5.38	10.16
9	Grade A bulb number	43.08	37-46.33	7.59	5.49	52.27	3.52	8.18
10	Grade B bulb number	42.57	36-46.33	8.68	6.34	53.33	4.06	9.54
11	Grade C bulb number	41.78	35.67-45.33	8.65	6.57	57.77	4.30	10.29
12	Marketable bulb/Plot	127.40	108.77-138.12	7.82	6.12	61.28	12.58	9.87
13	Marketable yield (kg/plot)	6.75	6.1-7.93	8.31	6.39	59.06	0.68	10.12
14	Marketable yield (q/ha)	225.12	203.22-264.35	8.31	6.38	58.97	22.74	10.10
15	Total yield (kg/plot)	6.90	6.33-8.11	7.60	5.84	59.05	0.64	9.24
16	Total yield (q/ha)	230.13	210.83-270.22	7.60	5.84	59.03	21.28	9.25
17	Percent disease index	3.56	2.46-5.5	26.50	24.97	88.75	1.72	48.45
18	Total soluble solids (^{0}Brix)	11.20	9.94-13.36	10.81	7.85	52.67	1.31	11.73
19	Dry matter (%)	15.56	11.34-19.12	14.53	14.42	98.45	4.59	29.47
20	Pyruvic acid(µg/g)	2.13	0.93-3.3	35.62	32.28	82.09	1.28	60.24
21	PWL (%) after one month	3.86	3.64-4.07	3.91	1.37	12.18	0.04	0.98
22	PWL (%) after two months	6.14	4.68-7.45	12.11	11.67	92.78	1.42	23.15
23	PWL (%) after third months	10.98	8.63-13.64	11.96	11.74	96.25	2.61	23.72
24	PWL (%) after fourth months	15.96	14.15-18.53	7.37	7.28	97.53	2.36	14.81

Higher values for phenotypic coefficient of variability were obtained than that of genotypic coefficients of variability values, indicating the influence of environment on these traits. The results indicated that estimation of PCV were greater than the corresponding GCV for all the traits in the both the years as well as over pooled analysis. The results are similar to the findings of Chattopadhyay *et al* (2013). Difference between

GCV and PCV were found minimum for most of the characters except double bulb (%) exhibited high difference between GCV and PCV. Gurjar and Singhania (2006) reported low GCV and PCV for plant height and days to maturity.The narrow difference between GCV and PCV indicated that these characters were least influenced by the environment. On the other hand, the wide difference between GCV and PCV designated that environment had major role for phenotypic expression of these traits. Similar results were highlighted by Mohanty (2004) and Yaso (2007); while they were studying yield attributing traits of onion. Hosmani *et al.* (2010) reported high value of GCV and PCV for yield per hectare and for average bulb weight. Exactly not equal but somewhat similar magnitude of GCV and PCV was recorded for the characters *viz* plant height, days to harvest, number of marketable bulbs per plot, TSS and yield per plot which indicates the less influence of environment and that the character expressions are totally due to genetic makeup.

Table 4.4: Genetic components of different growth parameters in onion genotypes during 2016-17

Sl. No.	Characters	General Mean	Range	PCV	GCV	h2	GA	GA as % of Mean
1	Plant height (cm)	52.67	50.39-56.51	3.92	3.29	70.43	3.00	5.69
2	Number of leaves	10.11	8.73-11.33	8.37	7.64	83.12	1.45	14.34
3	Double bulb (%)	2.94	1.7-4.61	28.98	24.48	71.34	1.25	42.59
4	Neck thickness (mm)	5.95	4.97-6.91	9.25	8.60	86.49	0.98	16.48
5	Days to maturity	108.68	105-112.67	2.13	1.97	85.26	4.06	3.74
6	Polar diameter (mm)	50.03	46.2-54	4.75	4.32	82.67	4.05	8.10
7	Equatorial diameter (mm)	53.30	49.81-57.62	4.21	3.96	88.23	4.08	7.66
8	Individual bulb weight(g)	53.49	49.89-59.84	5.88	5.44	85.78	5.55	10.38
9	Grade A bulb number	43.73	37-47	7.13	5.12	51.64	3.32	7.58
10	Grade B bulb number	43.37	37.33-47	7.66	6.12	63.87	4.37	10.08
11	Grade C bulb number	42.42	36-45.33	6.64	5.43	66.99	3.89	9.16
12	Marketable bulb/Plot	129.52	110.33-139	6.31	5.60	78.67	13.24	10.22
13	Marketable yield (kg/plot)	6.92	6.22-8.12	7.71	6.57	72.61	0.80	11.54
14	Marketable yield (q/ha)	230.73	207.33-270.58	7.72	6.58	72.68	26.66	11.55
15	Total yield (kg/plot)	7.13	6.51-8.36	7.45	6.33	72.11	0.79	11.07
16	Total yield (q/ha)	237.68	216.93-278.56	7.45	6.32	71.99	26.27	11.05
17	Percent disease index	11.30	1.81-22.56	59.20	54.69	85.33	11.76	104.07
18	Total soluble solids (°Brix)	11.66	10.21-14.28	8.29	8.00	93.09	1.85	15.90
19	Dry matter (%)	14.73	11.95-19.45	13.92	13.26	90.77	3.83	26.03
20	Pyruvic acid(µg/g)	2.07	0.94-3	32.14	26.67	68.88	0.94	45.60
21	PWL (%) after 1 month	4.87	3.34-5.77	15.79	15.54	96.85	1.53	31.50
22	PWL (%) after 2 months	9.65	7.99-10.64	9.14	8.96	96.09	1.75	18.09
23	PWL (%) after 3 months	14.35	10.84-16	10.23	8.49	68.94	2.09	14.53
24	PWL (%) after 4 months	19.11	16.41-21.41	8.42	7.65	82.55	2.74	14.32

Heritability estimates aim at determining the relative amount of heritable portion of variation. Knowledge of heritability along with genetic advance is more useful. Genetic advance (GA) under selection refers to the improvement of characters in genotypic value for the new population compared with the base population under one cycle of selection at a given selection intensity (Hamdi *et al.* 2003). Singh (1990) suggested that heritability values greater than 80% were very high, values from 60-79% were moderately high, 40-59% were medium and less than 40% low. In the present study, low to moderate heritability accompanied by low genetic advance was estimated in Grade A bulb number in both the years along with pooled analysis and therefore it can be concluded that this character was high influence of the environmental factors. As a result, selection of this character was not to be effective. The characters like Double bulb (%), Neck thickness (mm), Days to maturity, Grade B bulb, Grade C bulb, Marketable yield (kg/plot), Total yield (kg/plot), Total soluble solids (^{0}Brix) and Physiological weight loss percentage after one month showed low to moderate heritability with low genetic advance, whereas Marketable yield (q/ha) and Total yield (q/ha) showed moderate heritability with high genetic advance in the year 2015-16. On the contrary, high heritability concomitant with low genetic advance had exhibited by most of the characters except Marketable (bulb/plot), Marketable yield (q/ha), Total yield (q/ha), Percent disease index, which showed high heritability with moderate to high genetic advance. Similar to our findings, low genetic advance for neck thickness, bulb diameter, plant height, and number of leavers/plant while, high genetic advance for bulb weight, yield and number of marketable bulb was reported by Trivedi *et al.* (2006), Bharti *et al.* (2011) and Aditika *et al.* (2017). In agreement with our findings, very high heritability was recorded for plant height, number of leaves, total phenols and pyruvic acid (Chattopadhyay *et al.*, 2013) and plant height (Khosa and Dhatt 2015, Santra *et al.*, 2017). Chattopadhyay *et al.* (2013) reported that all the traits except polar diameter had high heritability and high genetic advance as percent of mean. Traits with high values of heritability coupled with moderate genetic advance as percent of mean, *viz.* plant height suggest that selection for improvement of these characters may be rewarding. It also indicates greater role of non-additive gene action in their inheritance. The traits showed moderate to high heritability coupled with high genetic advance, additive genes controlled for such traits. Similar speculation was drawn by Bharti *et al.* (2011).

Table 4.5: Genetic components of different growth parameters in onion genotypes pooled of two years

Sl. No.	Characters	General Mean	Range	PCV	GCV	h2	GA	GA as % of Mean
1	Plant height (cm)	52.15	49.28-56.07	4.29	3.79	78.30	3.61	6.91
2	Number of leaves	9.65	8.14-10.91	7.47	6.62	78.69	1.17	12.10
3	Double bulb (%)	2.14	1.34-3.19	23.33	19.36	68.85	0.71	33.08
4	Neck thickness (mm)	5.88	4.84-6.63	8.31	6.95	69.91	0.70	11.97
5	Days to maturity	108.84	105.67-112.5	2.01	1.79	79.56	3.59	3.29
6	Polar diameter (mm)	49.87	46.43-53.85	4.47	4.12	84.71	3.89	7.80
7	Equatorial diameter (mm)	53.27	49.54-57.34	4.25	3.99	88.13	4.11	7.71
8	Individual bulb weight(g)	53.23	48.74-59.3	5.88	5.47	86.41	5.57	10.47
9	Grade A bulb number	43.41	36.67-46.67	6.87	5.53	64.82	3.98	9.17
10	Grade B bulb number	42.97	36.67-46.67	7.90	6.21	61.85	4.33	10.07
11	Grade C bulb number	42.10	35.83-45.33	7.31	5.92	65.58	4.16	9.88
12	Marketable bulb/Plot	128.46	109.55-138.56	6.86	5.85	72.55	13.18	10.26
13	Marketable yield (kg/plot)	6.84	6.21-8.03	7.77	6.42	68.20	0.75	10.92
14	Marketable yield (q/ha)	227.93	206.96-267.46	7.77	6.42	68.23	24.91	10.93
15	Total yield (kg/plot)	7.02	6.45-8.24	7.26	5.97	67.62	0.71	10.12
16	Total yield (q/ha)	233.91	214.89-274.39	7.26	5.97	67.62	23.65	10.11
17	Percent disease index	7.43	2.93-12.73	45.47	41.91	84.94	5.91	79.56
18	Total soluble solids (oBrix)	11.43	10.59-13.27	7.21	6.11	71.96	1.22	10.68
19	Dry matter (%)	15.15	12.88-17.61	9.21	8.94	94.21	2.71	17.87
20	Pyruvic acid(µg/g)	2.10	0.96-3.15	29.79	26.79	80.84	1.04	49.61
21	PWL (%) after 1 month	4.36	3.59-4.85	9.09	8.82	94.15	0.77	17.63
22	PWL (%) after 2 months	7.90	6.96-8.87	7.39	7.23	95.70	1.15	14.57
23	PWL (%) after 3 months	12.67	10.6-14.71	8.38	7.73	85.22	1.86	14.71
24	PWL (%) after 4 months	17.54	16.14-19.31	6.08	5.75	89.40	1.97	11.21

4.4. STUDY ON CHARACTER ASSOCIATION

The results showed that, in most cases, the genotypic correlation coefficients were higher than the phenotypic correlation coefficients which indicated the inherent association among various characters independent of environmental influence. The results are consistent with the reports of Kalloo *et al.* (1982) and Hosamani *et al.* (2010) in onion.

Plant height is positively correlated with Polar diameter and Equatorial diameter, at phenotypic levels both the years and over pooled data analysis. Similarly, Days to maturity is significantly negatively correlated with Polar diameter (mm), Equatorial diameter (mm) at phenotypic level for the both the years and over pooled data analysis. Individual bulb weight (g) consistently significantly positive associations with a number of characters like Marketable yield (kg/plot), Marketable yield (q/ha), Total yield (kg/plot) and Total yield (q/ha) at phenotypic level, therefore, this trait may be considered important and undeviating relationship with yield. These results are in conformity with the findings of Hosamani *et al.* (2010) who reported the interrelationship of bulb yield significantly positive with bulb diameter, bulb neck thickness and average bulb weight. In his findings Bulb yield was positively and significantly associated with plant height, leaf length, leaf sheath length, leaf sheath diameter, bulb length, bulb diameter, and bulb dry weight, biological yield per plant and marketable yield per plant at both phenotypic and genotypic levels. Significant association of growth attributes such as plant height, dry matter and yield attributes like equatorial diameter and bulb weight, A grade bulb and marketable yield increase the yield potential. Any improvement in these characters will directly increase the yield potential of the genotypes. Hence, successful exploitation of selection pressure on these attributes will help in improving genotypes (Trivedi *et al.,* 2006). Workers like Patil *et al.* (1987) and Netra Pal, *et al.* (1988) indicated the same trend. Degewione *et al.* (2011) also reported bulb dry weight had positive and significant association with biological yield per plant, marketable yield per plant, percentage of bulb sprouting and percentage of bulb weight loss, plant height, leaf length, leaf sheath length, leaf sheath diameter, bulb length and bulb diameter at phenotypic and genotypic levels. Physiological weight loss after one month is significantly positive correlated with physiological weight loss after two months, physiological weight loss after 3 months and physiological weight loss after 4 months at both levels in second year and pooled data except first year. These results are in conformity with the findings of Barman *et al.* (1996).

Table 4.6: Phenotypic (above diagonal) and genotypic (below diagonal) Correlation Matrix of twenty onion genotypes in the year 2015-16

	PH	NOL	DB	NT	DTM	PD	ED	IBW	G(A)	G(B)	G(C)	MB/P	MY	MY	TY	TY	PDI	TSS	DM	PA	PWL1	PWL2	PWL3	PWL4
PH	1	0.287	0.082	-0.204	-0.328	0.408	.583**	-0.171	-0.204	-0.287	-0.287	-0.375	-0.042	-0.042	0.082	0.082	-0.328	0.257	0.257	0.25	0	0	0.082	-0.123
NOL	0.354	1	0.192	0.101	-0.212	0.101	0.082	-0.032	-0.101	0.01	0.01	-0.082	-0.123	-0.123	-0.01	-0.01	-0.212	0.179	0.179	0.328	0.101	0.101	0.192	-0.01
DB	0.209	-0.094	1	0.101	0.192	-0.302	-0.123	0.179	-.503*	-0.394	-0.394	-0.287	-.533*	-.533*	-0.414	-0.414	-0.212	0.179	0.39	-0.287	-0.101	0.101	-0.212	-0.212
NT	0.136	0.135	0.182	1	-0.302	0	0	-0.105	0	0.101	-0.101	0	-0.204	-0.204	-0.302	-0.302	0.101	0.105	0.105	0	-0.2	0	0.101	0.302
DTM	-0.284	-0.208	-0.041	-0.172	1	-0.302	-0.328	0.179	0.101	0.01	0.01	0.123	0.082	0.082	0.192	0.192	-0.01	-0.032	-0.242	-0.082	0.101	0.302	-0.01	-0.01
PD	.687**	0.291	0.132	0.017	-.501*	1	.816**	-0.314	0.2	0.101	0.101	0	0.204	0.204	0.101	0.101	0.101	0.314	0.105	0	0.4	0	0.101	0.101
ED	.676**	0.254	0.136	0.004	-.466*	.986**	1	-0.171	0	-0.082	-0.082	-0.167	0.167	0.167	0.082	0.082	-0.123	0.257	0.257	0.042	0.204	-0.204	-0.123	-0.123
IBW	-0.037	-0.135	0.094	0.294	0.033	0.018	0.068	1	-0.314	-0.39	-0.179	-0.257	0.257	0.257	0.179	0.179	0.179	-0.319	-0.099	-0.043	0.314	-0.314	-0.242	-0.242
G(A)	-0.187	-0.165	-.718**	-0.147	0.028	-0.076	-0.057	-0.349	1	.905**	.704**	.816**	0.408	0.408	.503*	.503*	0.302	0.105	-0.314	0	0	0	0.101	0.101
G(B)	-0.226	-0.09	-.682**	-0.197	-0.048	-0.091	-0.089	-0.422	.938**	1	.798**	.903**	0.328	0.328	0.414	0.414	0.212	0.242	-0.179	-0.123	-0.101	0.101	0.212	0.212
G(C)	-0.142	-0.062	-.705**	-0.202	-0.007	-0.043	-0.053	-0.269	.840**	.926**	1	.903**	.533*	.533*	.616**	.616**	0.212	0.032	0.032	-0.123	0.101	-0.101	0.212	0.01
MB/P	-0.185	-0.108	-.728**	-0.186	-0.012	-0.071	-0.066	-0.356	.956**	.987**	.956**	1	.458*	.458*	.533*	.533*	0.328	0.171	-0.043	-0.25	0	0	0.123	0.123
MY	-0.253	-0.214	-.610**	0.105	0.062	-0.109	-0.071	.481*	.605**	.571**	.673**	.639**	1	1.000**	.903**	.903**	.492*	0.043	-0.171	-0.167	0.408	-0.408	-0.123	-0.123
MY	-0.251	-0.215	-.609**	0.106	0.06	-0.108	-0.07	.481*	.606**	.571**	.673**	.639**	1.000**	1	.903**	.903**	.492*	0.043	-0.171	-0.167	0.408	-0.408	-0.123	-0.123
TY	-0.182	-0.228	-.565**	0.148	0.002	-0.064	-0.021	.500*	.594**	.541*	.622**	.607**	.982**	.982**	1	1.000**	0.394	-0.032	-0.032	-0.082	0.302	-0.302	-0.01	-0.212
TY	-0.179	-0.225	-.566**	0.146	0.002	-0.062	-0.019	.500*	.594**	.541*	.622**	.607**	.982**	.982**	1.000**	1	0.394	-0.032	-0.032	-0.082	0.302	-0.302	-0.01	-0.212
PDI	-0.288	-0.181	-0.176	0.138	-0.01	-0.179	-0.138	0.181	0.294	0.199	0.171	0.223	0.349	0.351	0.321	0.319	1	-0.242	-0.032	-.492*	.503*	-0.302	-0.212	-0.01
TSS	0.231	0.083	0.387	0.213	-0.012	0.206	0.161	-0.138	-0.097	-0.063	-0.172	-0.118	-0.249	-0.25	-0.255	-0.254	-0.253	1	-0.099	-0.257	-0.105	0.314	0.179	0.39
DM	0.174	0.122	0.306	0.218	-0.263	0.199	0.205	0.064	-0.18	-0.096	0.094	-0.056	-0.007	-0.008	-0.012	-0.013	-0.114	-0.114	1	-0.043	0.105	0.105	0.179	-0.032
PA	-0.056	0.265	-0.235	-0.335	0.029	-0.018	0.014	-0.12	-0.039	0.05	0.035	0.021	-0.083	-0.084	-0.121	-0.12	-.638**	-0.186	0.052	1	0	0.204	0.123	-0.082
PWL1	0.056	0.141	-0.227	-0.131	-0.017	0.256	0.262	0.381	-0.162	-0.007	0.225	0.027	0.313	0.314	0.264	0.264	0.05	-0.427	0.236	0.205	1	0	-0.101	-0.101
PWL2	0.058	-0.117	-0.17	-0.134	0.103	0.093	0.074	-0.281	0.14	0.067	-0.011	0.07	-0.219	-0.218	-0.203	-0.204	-0.141	0.146	-0.112	-0.003	-0.092	1	.704**	.704**
PWL3	0.248	0.001	-0.325	0.187	-0.25	0.156	0.111	-0.109	0.25	0.165	0.188	0.212	0.073	0.075	0.13	0.131	-0.185	0.042	0.12	-0.069	-0.124	.715**	1	.798**
PWL4	0.061	0.01	-0.184	0.117	-0.062	0.068	0.038	-0.121	0.01	-0.087	-0.079	-0.05	-0.171	-0.17	-0.135	-0.135	-0.231	0.006	0.092	0.015	-0.131	.823**	.880**	1

**Significant at 1% level and *Significant at 5% level

(PH = Plant height, NOL= Number of leaves, DB = Double bulb (%), NT = Neck thickness, DTM = Days to maturity, PD = Polar diameter, ED = Equatorial diameter, IBW = Individual bulb weight, G(A)= Grade A, G(B)= Grade B, G(C)= Grade C, MB/P= Marketable bulb/plot, MY (kg/p) = Marketable yield (kg/plot), MY(q/ha) = Marketable yield (q/ha), TY (kg/p) = Total yield (kg/plot), TY (q/ha) = Total yield (q/ha), PDI=Percent disease index, DM = Dry matter , TSS = Total Soluble salts, PA = Pyruvic acid, PWL1=Physiological weight loss after one month, PWL2=Physiological weight loss after two months, PWL3=Physiological weight loss after three months, PWL4=Physiological weight loss after four months)

Table 4.7: Phenotypic (above diagonal) and genotypic (below diagonal) Correlation Matrix of twenty onion genotypes in the year 2016-17

	PH	NOL	DB	NT	DTM	PD	ED	IBW	G(A)	G(B)	G(C)	MB/P	MY	MY	TY	TY	PDI	TSS	DM	PA	PWL1	PWL2	PWL3	PWL4
PH	1	-.524*	0.179	-0.043	-0.242	.524*	.601**	-0.242	0.105	-0.179	-0.121	-0.179	-0.171	-0.171	-0.171	-0.171	-.664**	-0.171	0.032	-0.121	-0.105	-0.179	-0.105	0.179
NOL	-0.348	1	0.101	0.204	-0.101	-0.2	-0.302	0.101	-0.2	-0.101	-0.105	-0.101	0	0	0	0	.503*	0	0.101	-0.105	.600**	0.302	.600**	0.101
DB	0.033	-0.032	1	-0.082	-0.01	0.101	-0.01	0.192	-.704**	-.596**	-.601**	-.596**	-.533*	-.533*	-.533*	-.533*	-0.212	0.287	0.01	0.032	0.101	-0.192	0.101	-0.01
NT	-0.046	0.124	0.128	1	-0.287	0	-0.082	-0.082	0	-0.328	-0.385	-0.328	0.042	0.042	0.042	0.042	0.328	-0.375	-0.123	0.257	0.204	0.082	0.204	0.123
DTM	-0.305	-0.1	-0.282	-0.078	1	-0.302	-0.414	-0.01	0.101	0.01	-0.179	0.01	0.082	0.082	0.082	0.082	0.192	-0.123	0.212	0.032	-0.101	-0.192	-0.101	-0.01
PD	.722**	-0.092	0.135	-0.076	-.501*	1	.704**	-0.101	0.200	0.101	0.105	0.101	0.204	0.204	0.204	0.204	-0.302	0	0.101	0.105	0	-0.101	0	0.302
ED	.715**	-0.146	0.127	-0.127	-.472*	.954**	1	-0.01	0.302	0.01	0.032	0.01	0.082	0.082	0.082	0.082	-0.212	-0.123	0.01	-0.179	-0.101	-0.192	-0.101	-0.01
IBW	-0.148	-0.185	0.334	0.121	0.036	-0.045	0.107	1	-0.302	-0.192	0.032	-0.192	0.287	0.287	0.287	0.287	0.192	.492*	-0.394	-0.179	-0.101	-0.192	-0.101	-0.212
G(A)	-0.044	-0.064	-.763**	-0.065	0.134	-0.007	-0.062	-0.343	1	.503*	0.314	.503*	0.408	0.408	0.408	0.408	-0.101	-0.204	0.302	-0.105	-0.2	0.101	-0.2	0.302
G(B)	0.028	-0.082	-.723**	-0.16	-0.065	0.053	0.02	-0.264	.861**	1	.811**	1.000**	.533*	.533*	.533*	.533*	0.01	0.123	0.192	0.39	-0.302	-0.01	-0.302	0.01
G(C)	0.088	-0.25	-.746**	-0.189	0.093	0.043	-0.009	-0.197	.802**	.912**	1	.811**	.599**	.599**	.599**	.599**	0.032	0.171	-0.032	0.121	-0.314	-0.032	-0.314	-0.179
MB/P	0.025	-0.135	-.780**	-0.145	0.051	0.032	-0.016	-0.282	.931**	.974**	.949**	1	.533*	.533*	.533*	.533*	0.01	0.123	0.192	0.39	-0.302	-0.01	-0.302	0.01
MY	-0.114	-0.269	-0.393	-0.016	0.073	-0.02	0.057	.566**	.525*	.618**	.656**	.630**	1	1.000**	1.000**	1.000**	0.287	-0.042	-0.082	0.171	-0.204	-0.082	-0.204	0.082
MY	-0.113	-0.271	-0.394	-0.017	0.076	-0.021	0.056	.567**	.524*	.617**	.656**	.629**	1.000**	1	1.000**	1.000**	0.287	-0.042	-0.082	0.171	-0.204	-0.082	-0.204	0.082
TY	-0.115	-0.286	-0.294	-0.004	0.045	-0.008	0.071	.627**	.458*	.560*	.596**	.565**	.994**	.994**	1	1.000**	0.287	-0.042	-0.082	0.171	-0.204	-0.082	-0.204	0.082
TY	-0.115	-0.285	-0.297	-0.005	0.046	-0.008	0.072	.628**	.458*	.559*	.597**	.565**	.995**	.995**	1.000**	1	0.287	-0.042	-0.082	0.171	-0.204	-0.082	-0.204	0.082
PDI	-.514*	.446*	0.085	0.345	0.197	-0.281	-0.29	0.098	0.019	-0.125	-0.092	-0.072	0.029	0.027	0.037	0.038	1	-0.328	0.01	0.032	0.101	-0.192	0.101	-0.414
TSS	0.381	-.446*	0.389	-0.115	-0.183	0.327	0.442	0.269	-.502*	-0.237	-0.216	-0.331	-0.081	-0.081	-0.041	-0.041	-0.42	1	-0.287	0.171	-0.204	0.123	-0.204	0.082
DM	-0.037	-0.002	-0.281	-0.008	0.224	-0.368	-0.338	-0.27	0.3	0.311	0.138	0.266	-0.001	0.001	-0.029	-0.03	-0.306	-0.191	1	-0.032	-0.101	-0.212	-0.101	0.01
PA	-0.234	0.243	0.045	0.143	0.075	0.031	-0.018	0.128	-0.013	0.1	0.054	0.052	0.147	0.145	0.154	0.153	0.27	0.126	-0.211	1	-0.105	-0.032	-0.105	0.032
PWL1	-0.364	.444*	-0.008	0.183	-0.21	-0.116	-0.172	-0.053	-0.109	-0.207	-0.292	-0.212	-0.215	-0.215	-0.226	-0.225	-0.026	-0.27	-0.107	0.096	1	.704**	1.000**	.503*
PWL2	-0.37	0.378	-0.041	0.196	-0.282	-0.176	-0.21	0.013	-0.048	-0.1	-0.197	-0.119	-0.085	-0.085	-0.094	-0.094	-0.014	-0.189	-0.142	0.229	.936**	1	.704**	.616**
PWL3	-0.046	.454*	-0.066	0.298	-0.209	0.186	0.111	-0.035	-0.027	-0.166	-0.231	-0.148	-0.139	-0.141	-0.156	-0.154	0.028	-0.324	-0.228	0.112	.727**	.602**	1	.503*
PWL4	0.141	0.202	-0.057	-0.057	-0.163	0.321	0.331	-0.07	0.161	0.023	-0.158	0.012	-0.04	-0.041	-0.051	-0.05	-0.234	-0.085	-0.046	0.054	.451*	0.361	.744**	1

**Significant at 1% level and *Significant at 5% level

(PH = Plant height, NOL= Number of leaves, DB = Double bulb (%), NT = Neck thickness, DTM = Days to maturity, PD = Polar diameter, ED = Equatorial diameter, IBW = Individual bulb weight, G(A)= Grade A, G(B)= Grade B, G(C)= Grade C, MB/P= Marketable bulb/plot, MY (kg/p) = Marketable yield (kg/plot), MY(q/ha) = Marketable yield (q/ha), TY (kg/p) = Total yield (kg/plot), TY (q/ha) = Total yield (q/ha), PDI=Percent disease index, DM = Dry matter , TSS = Total Soluble salts, PA = Pyruvic acid, PWL1=Physiological weight loss after one month, PWL2=Physiological weight loss after two months, PWL3=Physiological weight loss after three months, PWL4=Physiological weight loss after four months)

Table 4.8: Phenotypic (above diagonal) and genotypic (below diagonal) Correlation Matrix of sixteen onion genotypes pooled over two years

	PH	NOL	DB	NT	DTM	PD	ED	IBW	G(A)	G(B)	G(C)	MB/P	MY	MY	TY	TY	PDI	TSS	DM	PA	PWL1	PWL2	PWL3	PWL4
PH	1	-0.01	0.082	0.01	-0.414	.503*	.492*	-0.123	-0.01	-0.192	-0.192	-0.287	0.082	0.082	0.082	0.082	-0.328	0.287	0.01	-0.082	-0.01	-0.082	0.01	-0.101
NOL	0.027	1	-0.328	0.01	-0.212	-0.101	-0.123	-0.123	-0.01	0.212	0.212	0.123	0.082	0.082	0.082	0.082	0.082	0.082	-0.394	0.328	0.394	0.123	-0.192	-0.101
DB	0.174	-0.095	1	0.123	0.082	0	0.167	0.375	-.739**	-.698**	-.698**	-.792**	-.458*	-.458*	-.458*	-.458*	-0.25	-0.25	-0.082	0.25	-0.123	-0.167	-0.082	0
NT	0.053	0.075	0.11	1	-0.394	-0.101	0.123	-0.082	-0.192	-0.414	-0.414	-0.328	-0.082	-0.082	-0.082	-0.082	0.328	-0.287	-0.01	0.082	0.212	0.287	0.394	0.101
DTM	-0.306	-0.188	-0.245	-0.143	1	-0.302	-0.328	0.082	0.192	0.01	0.01	0.123	0.082	0.082	0.082	0.082	0.082	-0.123	0.01	0.123	-0.212	-0.082	-0.192	0.101
PD	.728**	0.142	0.148	-0.047	-.522*	1	.816**	-0.204	0.101	0.101	0.101	0	0.204	0.204	0.204	0.204	-0.204	0.408	0.101	0	0.101	0	0.302	0.2
ED	.716**	0.1	0.182	-0.064	-.478*	.978**	1	-0.042	0.082	-0.082	-0.082	-0.167	0.167	0.167	0.167	0.167	-0.042	0.167	0.123	0.042	0.082	-0.167	0.123	0
IBW	-0.093	-0.201	0.258	0.208	0.037	-0.011	0.09	1	-0.328	-0.287	-0.287	-0.375	0.167	0.167	0.167	0.167	-0.042	-0.25	-0.287	0.042	-0.328	-0.167	-.492*	-0.204
G(A)	-0.133	-0.15	-.853**	-0.094	0.09	-0.046	-0.064	-0.355	1	.616**	.616**	.739**	.492*	.492*	.492*	.492*	0.082	0.082	0.212	-0.287	-0.212	-0.082	0.01	0.101
G(B)	-0.123	-0.062	-.811**	-0.155	-0.047	-0.01	-0.039	-0.357	.913**	1	1.000**	.903**	.533*	.533*	.533*	.533*	0.123	0.328	0.192	-0.123	-0.192	-0.123	-0.01	-0.101
G(C)	-0.052	-0.113	-.835**	-0.183	0.043	-0.012	-0.047	-0.239	.834**	.938**	1	.903**	.533*	.533*	.533*	.533*	0.123	0.328	0.192	-0.123	-0.192	-0.123	-0.01	-0.101
MB/P	-0.104	-0.112	-.862**	-0.147	0.027	-0.022	-0.05	-0.328	.949**	.986**	.958**	1	.458*	.458*	.458*	.458*	0.25	0.25	0.287	-0.25	-0.082	-0.042	0.082	0
MY	-0.202	-0.265	-.574**	0.053	0.081	-0.062	-0.01	.524*	.564**	.588**	.668**	.629**	1	1.000**	1.000**	1.000**	0.375	-0.042	-0.082	0.042	-0.123	0.042	-0.082	0
MY	-0.201	-0.266	-.573**	0.055	0.08	-0.062	-0.01	.526*	.562**	.586**	.667**	.627**	1.000**	1	1.000**	1.000**	0.375	-0.042	-0.082	0.042	-0.123	0.042	-0.082	0
TY	-0.164	-0.304	-.511*	0.084	0.04	-0.025	0.03	.569**	.525*	.542*	.618**	.582**	.993**	.994**	1	1.000**	0.375	-0.042	-0.082	0.042	-0.123	0.042	-0.082	0
TY	-0.163	-0.304	-.510*	0.082	0.041	-0.025	0.03	.571**	.524*	.540*	.616**	.581**	.993**	.993**	1.000**	1	0.375	-0.042	-0.082	0.042	-0.123	0.042	-0.082	0
PDI	-.486*	0.083	-0.174	0.289	0.112	-0.339	-0.279	0.218	0.256	0.125	0.079	0.158	0.339	0.337	0.317	0.315	1	-0.25	0.123	0.042	0.082	0.042	-0.082	-0.204
TSS	0.381	-0.013	0.408	0.116	-0.139	0.379	0.414	0.048	-0.368	-0.228	-0.233	-0.286	-0.252	-0.25	-0.223	-0.221	-.454*	1	-0.082	0.042	-0.123	0.042	0.123	0
DM	0.032	-0.189	-0.018	0.093	-0.081	-0.141	-0.096	-0.175	0.048	0.205	0.22	0.166	0	0	-0.02	-0.023	0.034	0.015	1	-0.328	0.01	-0.123	0.192	-0.101
PA	-0.183	0.321	-0.13	-0.155	0.027	0.033	0.014	-0.013	-0.027	0.056	0.056	0.033	0.014	0.013	-0.007	-0.008	-0.255	-0.065	-0.232	1	0.123	0.167	-0.123	0.204
PWL1	-0.334	0.364	-0.085	0.147	-0.202	-0.104	-0.134	0.027	-0.115	-0.135	-0.197	-0.156	-0.115	-0.114	-0.146	-0.148	0.141	-0.323	-0.076	0.148	1	.533*	0.414	0.302
PWL2	-0.191	0.102	-0.123	0.125	-0.142	-0.083	-0.118	-0.172	0.035	-0.002	-0.096	-0.02	-0.179	-0.177	-0.197	-0.198	-0.115	-0.075	0.018	0.261	.731**	1	.698**	.816**
PWL3	0.131	0.102	-0.199	.462*	-0.275	0.206	0.139	-0.067	0.118	0.017	-0.027	0.038	-0.021	-0.018	-0.012	-0.013	-0.122	-0.09	-0.033	0.116	.523*	.735**	1	.704**
PWL4	0.107	-0.02	-0.053	0.159	-0.116	0.277	0.268	-0.125	0.096	-0.042	-0.146	-0.031	-0.141	-0.139	-0.128	-0.13	-0.156	-0.041	-0.05	0.146	0.437	.708**	.821**	1

**Significant at 1% level and *Significant at 5% level

(PH = Plant height, NOL= Number of leaves, DB = Double bulb (%), NT = Neck thickness, DTM = Days to maturity, PD = Polar diameter, ED = Equatorial diameter, IBW = Individual bulb weight, G(A)= Grade A, G(B)= Grade B, G(C)= Grade C, MB/P= Marketable bulb/plot, MY (kg/p) = Marketable yield (kg/plot), MY(q/ha) = Marketable yield (q/ha), TY (kg/p) = Total yield (kg/plot), TY (q/ha) = Total yield (q/ha), PDI=Percent disease index, DM = Dry matter , TSS = Total Soluble salts, PA = Pyruvic acid, PWL1=Physiological weight loss after one month, PWL2=Physiological weight loss after two months, PWL3=Physiological weight loss after three months, PWL4=Physiological weight loss after four months)

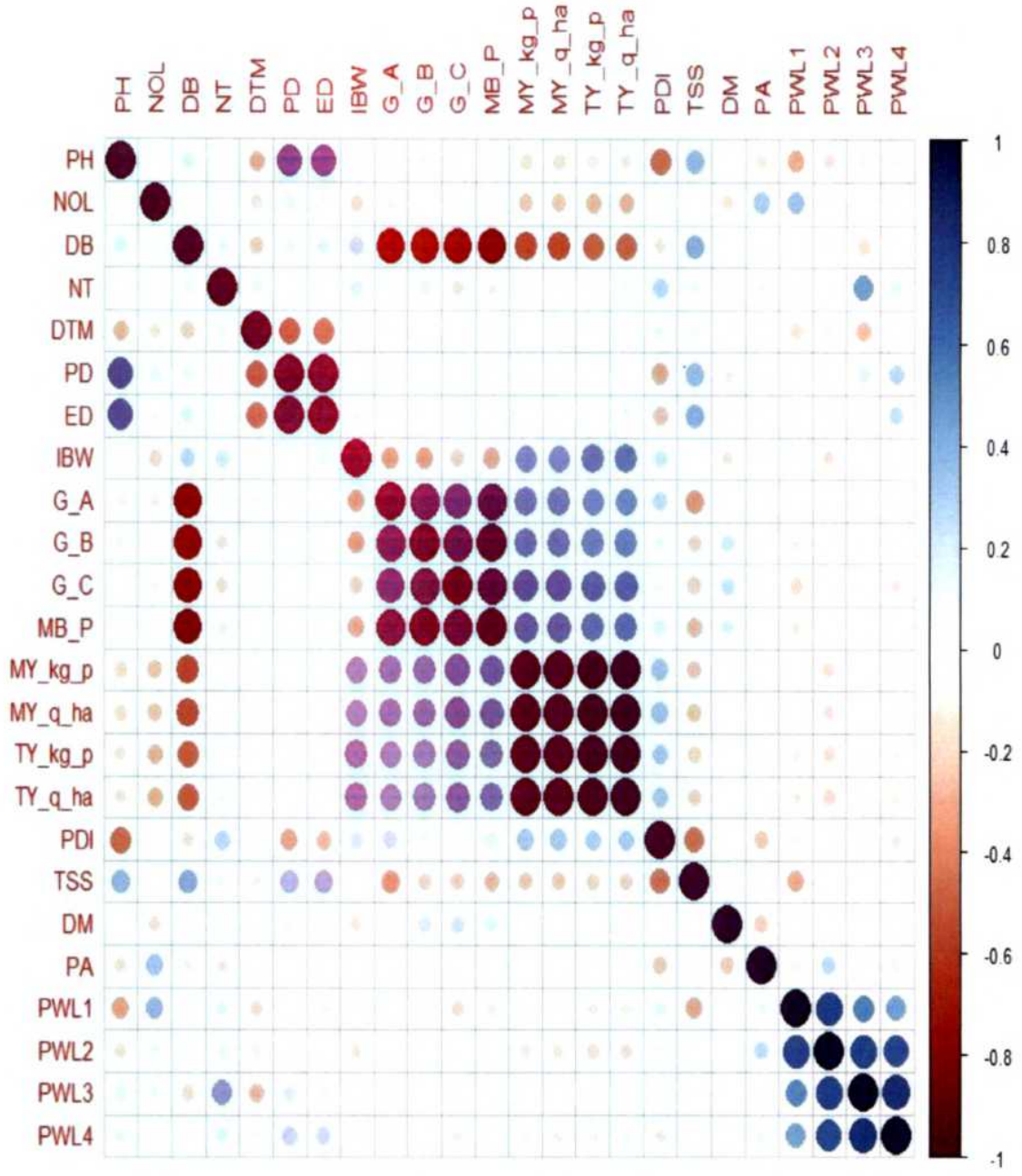


Plate 4: Pictorial diagram showing phenotypical correlations matrix

4.5. STUDY ON GENETIC DIVERGENCE USING D^2 ANALYSIS

Genetic diversity present in available germplasm plays an important role in crop improvement for characters of interest. For the selection of parents in hybridization, diversity among parents for the character of interest, estimation of genetic distance is most important. In present study, the genetic divergence is estimated by using Mahalanobis D^2 statistics, which gives clear idea about diverse nature of the population. D^2 analysis revealed high magnitude of diversity present in the present population. The results indicated contribution of each character towards divergence in table 4.9. Total yield contributed highest amount towards divergence followed by number of marketable bulb and individual bulb weight. Singh and Dubey, (2011) found significant differences among twenty-six Onion lines for all the traits indicating sufficient genetic diversity among the cultivars.

Table 4.9: Contribution of each character to Divergence

Characters	% Contribution	Rank
plant height	3.7447	17
no of leaves per plant	3.7354	19
double bulb	4.2880	13
neck thickness	2.8475	22
days to maturity	2.3399	25
polar diameter	4.5561	9
equitorial diameter	4.4264	11
individual bulb weight	4.6929	6
grade a bulb no	4.4341	10
grade b bulb no	4.6484	7
grade c bulb no	4.5652	8
no of marketable bulbs	4.8370	3
marketable yield kg/plot	4.8340	4
marketable yield q/ha	4.8337	5
total yield kg/plot	4.8426	1
total yield q/ha	4.8422	2
PDI	3.7959	16
total soluable solids	2.3457	24
dry matter	2.4032	23
pyruvic acid	3.1055	21
Total	100.00	

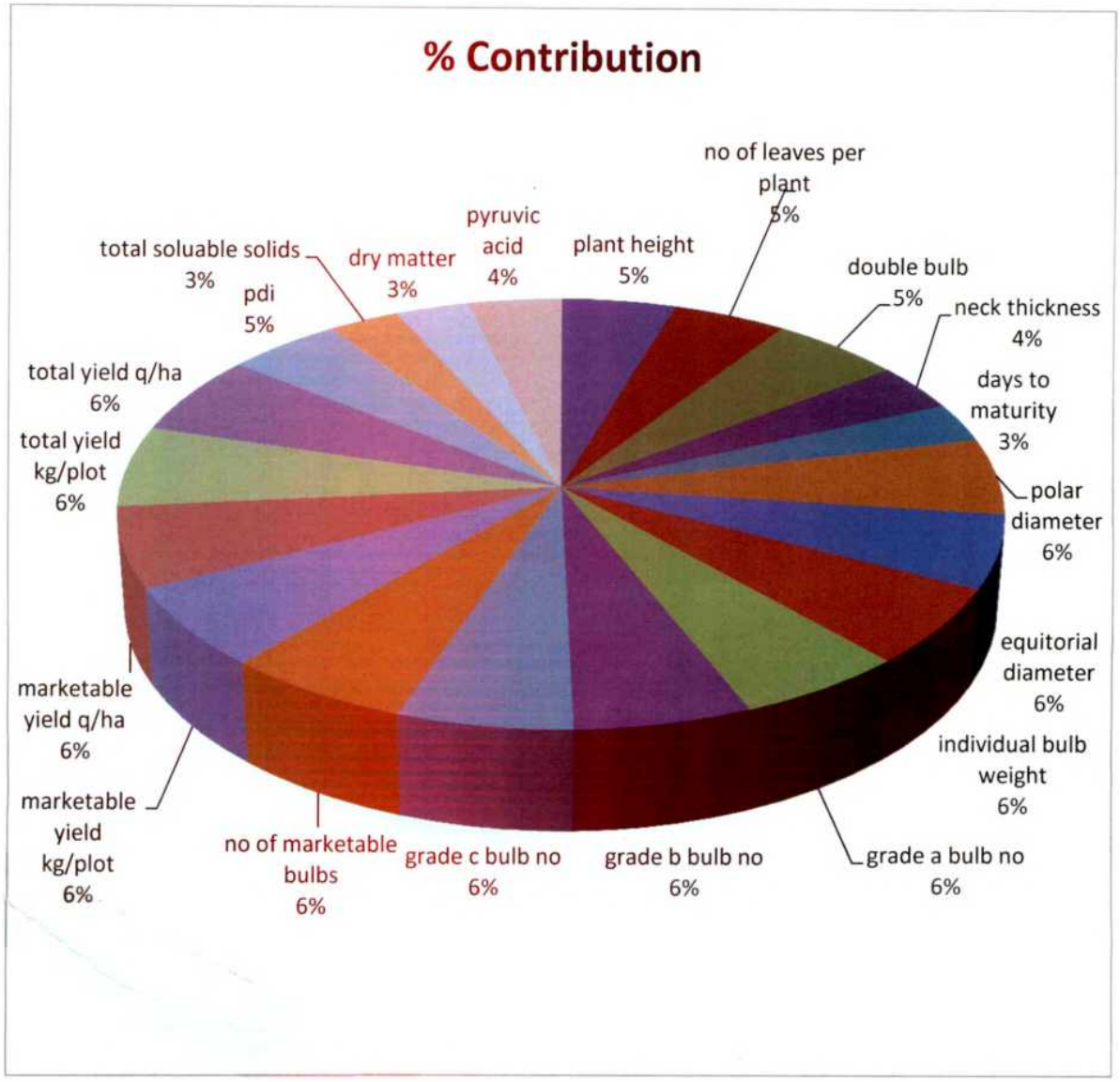


Figure 2 : Contribution of each character to Divergence

Table 4.10: Different cluster means of twenty onion genotypes for twenty four characters

Sl. No.	Character	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Mean
1	Plant height (cm)	50.67	51.80	50.30	52.78	55.75	52.26
2	Number of leaves	8.72	9.80	9.79	9.89	9.56	9.55
3	Double Bulb (%)	2.05	2.20	2.73	1.65	3.26	2.38
4	Neck thickness	5.88	6.14	5.77	5.70	6.03	5.90
5	Days to maturity	109.44	107.20	110.78	109.21	107.83	108.89
6	Polar diameter (mm)	48.74	49.75	47.98	50.33	53.09	49.98
7	Equatorial diameter (mm)	52.45	52.89	51.16	53.72	56.98	53.44
8	Individual bulb weight(g)	57.69	51.20	53.51	52.04	55.38	53.96
9	Grade (A) bulb number	44.11	43.50	40.67	45.48	39.00	42.55
10	Grade (B) bulb number	44.11	43.07	39.28	45.29	38.42	42.03
11	Grade (C) bulb number	43.94	41.57	38.72	44.21	38.33	41.36
12	Marketable bulb/plot	132.16	128.09	118.63	134.99	115.70	125.92
13	Marketable yield (kg/plot)	7.63	6.56	6.39	7.02	6.40	6.80
14	Marketable yield (q/ha)	254.20	218.66	212.89	233.90	213.28	226.59
15	Total yield (kg/plot)	7.78	6.73	6.60	7.17	6.68	6.99
16	Total yield (q/ha)	259.37	224.47	219.91	238.97	222.55	233.05
17	Percent disease index (%)	13.72	11.02	9.75	8.94	5.04	9.69
18	Total Soluble Solids (^{0}Brix)	11.10	11.35	10.99	11.32	13.18	11.59
19	Dry matter (%)	15.83	15.88	13.92	14.90	14.96	15.10
20	Pyruvic Acid (µg/g)	1.89	1.77	2.42	2.40	1.72	2.04
21	PWL (%) after 1 month	4.17	4.71	4.45	4.26	3.99	4.32
22	PWL (%) after 2 months	7.53	8.34	7.89	7.86	7.48	7.82
23	PWL (%) after 3 months	12.25	13.49	12.33	12.52	12.26	12.57
24	PWL (%) after 4 months	17.07	18.06	17.49	17.36	17.57	17.51

Cluster means for different characters showed considerable amount of differences between the clusters for all the characters (table 4.9). The genotypes of cluster I recorded the superior performance for total yield (259.37 q/ha), marketable yield (254.20 q/ha), days to maturity (109.44), individual bulb weight (57.69), minimum double bulb percentage (2.05). Physiological weight loss percentage after one month (4.17), whereas the genotypes of cluster – II had higher days to maturity (114.22), total yield (224.47 q/ha), marketable yield (218.66 q/ha), and desirable minimum values for PWL after one month (4.71 %), double bulb percentage (2.20 %). Similarly, the genotypes of clusters-III, clusters-IV cluster-V showed maximum values for days to maturity *viz*; 110.78, 109.21 and 107.83 respectively. The results are in agreement with the findings of Dangi *et al.* (2018) who grouped 58 accessions into four clusters. Mallor *et al.* (2011) grouped

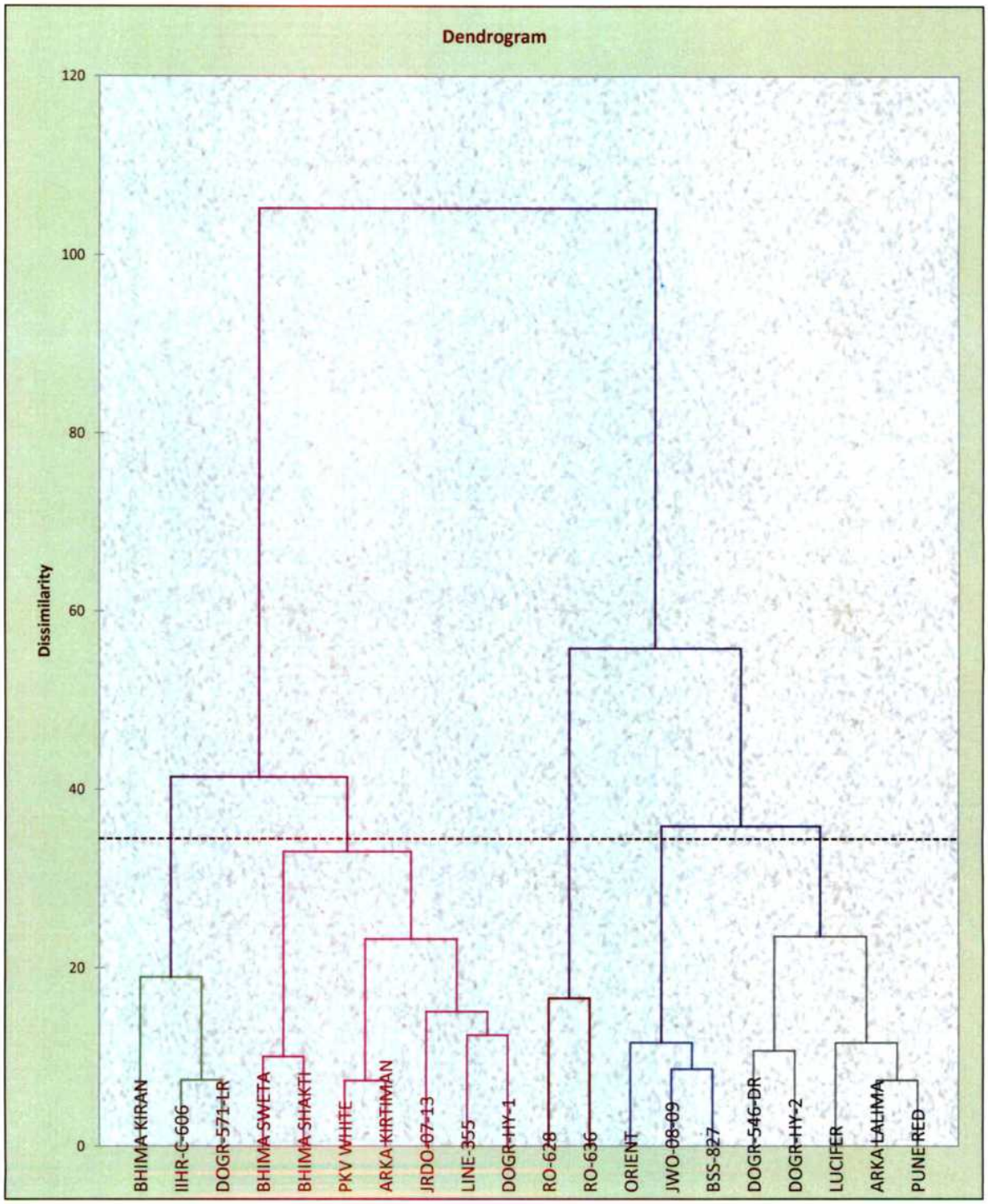


Plate 5: Dendrogram of onion genotypes

86 Spanish onion into four distinct clusters based on bulb size, pungency, dry matter and TSS. Khosa and Dhatt (2015) clustered 43 Indian onion accessions into seven clusters and Arya *et al.* (2017) classified 26 onion accessions into four clusters and they reported that clustering was not on the basis of geographical origin which is in confirmation with our findings.

All the genotypes were grouped into 5 clusters showed the considerable amount of genetic diversity present in the genotypes. The maximum number of genotypes (7) was included in cluster IV followed by cluster II (5) and (5) in cluster I and cluster III whereas, minimum number of genotypes was recorded in cluster V (2) (Table 4.10).

Table 4.11: Clustering of the Genotypes based on different characters

Clusters	Genotypes
Cluster I (3)	IIHR-C-606, DOGR-571-LR, Bhima Kiran
Cluster II (5)	DOGR-546-DR, Arka Lalima, DOGR-HY-2, Lucifer, Pune Red
Cluster III (3)	JWO-08-09, BSS-827, Orient
Cluster IV (7)	LINE-355, Bhima Sweta, PKV White, Bhima Shakti, Arka Kirtiman, DOGR-HY-1, JRDO-07-13
Cluster V (2)	RO-628, RO-636

Table 4.12: Distances between clusters of twenty onion genotypes

	1 Cluster	2 Cluster	3 Cluster	4 Cluster	5 Cluster
1 Cluster		44.19	51.65	20.57	58.60
2 Cluster			20.05	25.64	28.74
3 Cluster				37.68	16.94
4 Cluster					45.99
5 Cluster					

The highest inter-cluster distance was observed between cluster I and cluster V (58.60) followed by cluster I and III (51.65), cluster IV and V (45.99) and cluster I and cluster II (44.19) respectively. In contrast, minimum inter-cluster distance was recorded between cluster III and cluster V (16.94). According to Ghaderi *et al.* (1984), increasing parental distance implies a great number of contrasting alleles at the desired loci, and to

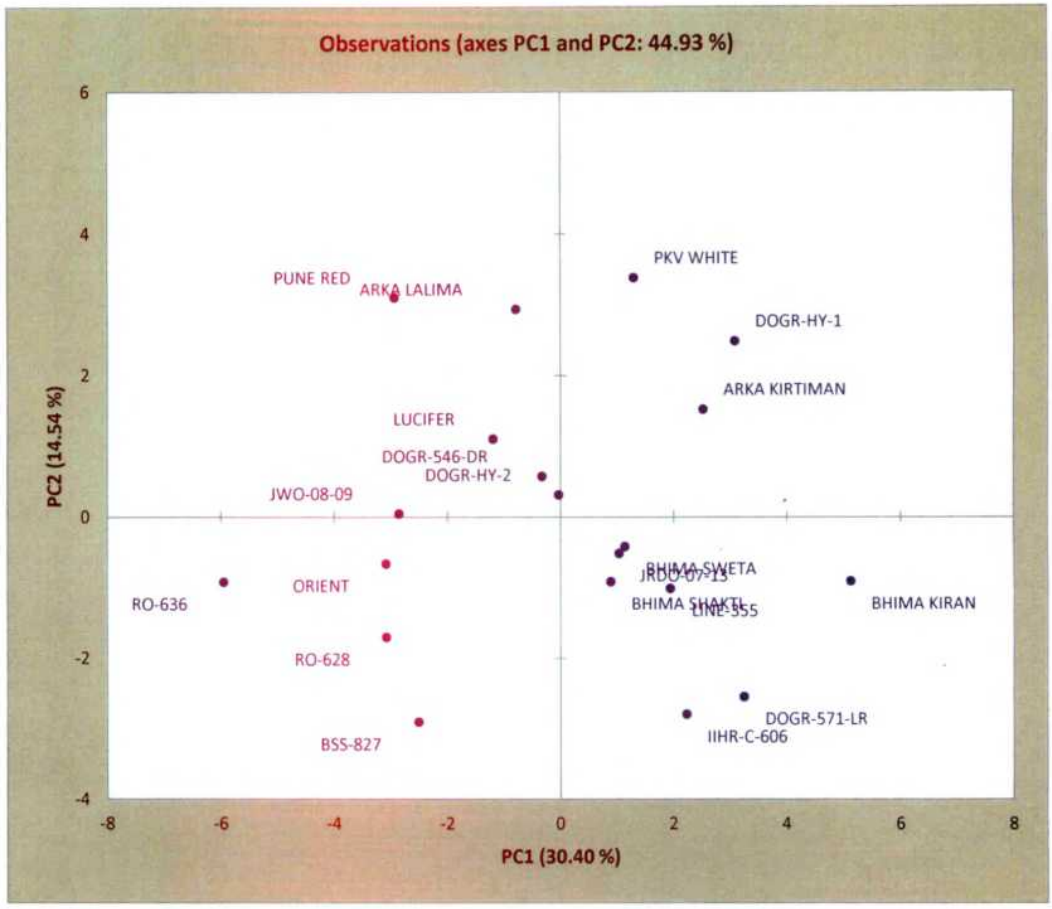
the extent that these loci recombine in the F2 and F3 generation following a cross of distantly related parents, the greater will be the opportunities for the effective selection for yield factors. Similar results have been reported by Mohanty (2001) and Khar *et al.* (2006) in onion. Study on genetic divergence existing germplasm for selecting the parent for hybridization is realistic (Singh and Dubey, 2011). Therefore, the genotypes grouped under different clusters showed highest distance and from the above results a generalized conclusion may be drawn that genotypes under those clusters might have originated from different geographical origin with maximum heterogeneity within the population. Therefore, crossing between two genotype selected from these different clusters might have produce more heterotic lines due to combination of diverse gene complexes. Kale *et al.* (2014) concluded similar kind of inference while studying genetic divergence among group of onion genotypes.

4.6 PRINCIPAL COMPONENT ANALYSIS

PCA reduces the dimensionality of the data by transforming initial variables into a new small set of variables without losing the important information in the original. In the present study, principal component analysis (PCA) was used to evaluate the variation among Onion genotypes. Onion germplasm covering 20 genotypes collected from different sources were evaluated for twenty-four traits through principal component analysis. According to PCA results, eigenvalue was maximum in PC-I (7.38) followed by PC-II (3.72) and PC-III (3.38). Total variance percentage was maximum in PC-I (29.53) followed by PC-II (14.89) and PC-III (13.50). The first six PCs with Eigen values >1 contributed (81.96 %) of the variability amongst genotypes.

Table 4.13: Eigen values and percentage of variation in twenty onion genotypes

Component	Eigene Value (Root)	% Var. Exp.	Cum. Var. Exp.
PC-I	7.38	29.53	29.53
PC-II	3.72	14.89	44.42
PC-III	3.38	13.50	57.92
PC-IV	2.91	11.66	69.58
PC-V	1.65	6.62	76.19
PC-VI	1.44	5.76	81.96



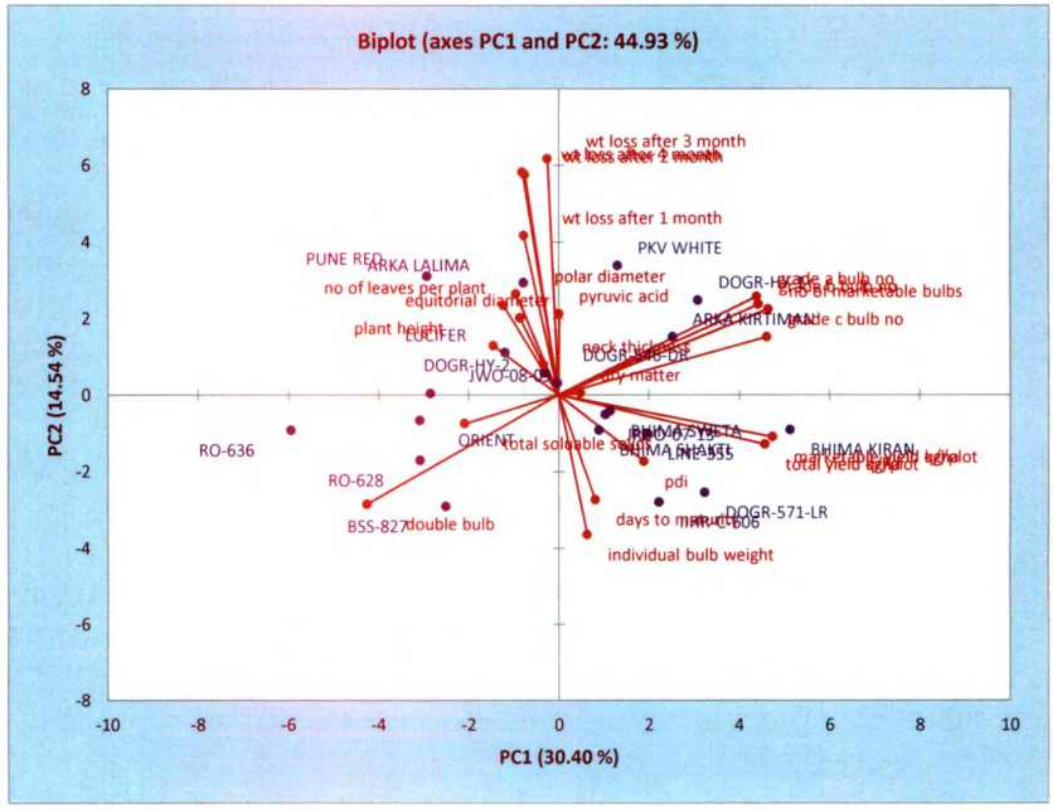


Plate 6: PCA 2D Plot of onion genotypes

Table 4.14: Eigen vectors of the first six principal components

	1	2	3	4	5	6
Plant height (cm)	-0.290	**0.349**	**0.731**	0.015	-0.160	0.034
Number of leaves	-0.256	**0.333**	-0.170	-0.058	**0.588**	**0.459**
Double bulb (%)	**-0.785**	**-0.416**	0.218	0.179	-0.102	0.019
Neck thickness (mm)	-0.055	-0.012	-0.104	**0.487**	-0.299	**0.492**
Days to maturity	0.164	**-0.373**	**-0.317**	**-0.307**	0.061	**-0.339**
Polar diameter (mm)	-0.202	**0.514**	**0.745**	0.222	0.135	0.076
Equatorial diameter (mm)	-0.181	**0.427**	**0.777**	0.259	0.124	0.068
Individual bulb weight(g)	0.158	**-0.601**	0.216	**0.699**	0.197	-0.046
Grade A bulb number	**0.811**	**0.425**	-0.069	-0.235	-0.095	0.041
Grade B bulb number	**0.817**	**0.434**	0.028	**-0.300**	-0.065	0.038
Grade C bulb number	**0.859**	**0.323**	0.103	-0.279	-0.052	-0.034
Marketable bulb/Plot	**0.859**	**0.410**	0.023	-0.280	-0.074	0.016
Marketable yield (kg/plot)	**0.912**	-0.147	0.168	**0.315**	0.096	-0.016
Marketable yield (q/ha)	**0.911**	-0.148	0.168	**0.318**	0.094	-0.017
Total yield (kg/plot)	**0.881**	-0.172	0.219	**0.363**	0.075	-0.022
Total yield (q/ha)	**0.880**	-0.174	0.221	**0.363**	0.076	-0.024
Percent disease index	**0.369**	**-0.306**	**-0.340**	0.123	0.041	**0.644**
Total soluble solids (^{0}Brix)	**-0.394**	0.012	**0.536**	0.011	-0.128	-0.145
Dry matter (%)	0.096	0.014	-0.018	-0.179	**-0.654**	0.151
Pyruvic acid(µg/g)	-0.007	0.249	-0.171	0.060	**0.619**	**-0.398**
PWL (%) after 1 month	-0.153	0.324	**-0.620**	**0.455**	0.165	0.142
PWL (%) after 2 months	-0.149	0.538	**-0.572**	**0.404**	-0.125	-0.238
PWL (%) after 3 months	-0.052	0.613	-0.277	**0.622**	-.0245	-0.069
PWL (%) after 4 months	-.0161	0.606	-0.227	**0.503**	-0.211	-0.267

In each principal component, coefficients equal or greater than |0.3| were chosen to determine the cut off limit for the coefficients of the proper vectors (Raji, 2002). The characters contributing more positively with (PC-I) were the Grade A bulb (0.811), Grade B bulb (0.817), Grade C bulb (0.859), Marketable bulb/plot (0.859), Marketable yield (kg/plot) (0.912), Marketable yield (q/ha) (0.911), Total yield (kg/plot) (0.881), Total yield (q/ha) (0.880), Percent disease index (0.369) and negatively with Double bulb (-0.785), Total soluble solids (-0.394). The (PC-II) had high positive component value for Plant height (0.349), Number of leaves (0.333), Polar diameter (0.514), Equatorial diameter (0.427), Grade A bulb (0.425), Grade B bulb (0.434), Grade C bulb (0.323) and Marketable bulb/plot (0.410) and high negatively with Double bulb (%), Days to maturity, Individual bulb weight and Percent disease index. The characters contributing more positively with (PC-III) were Plant height (0.731), Polar diameter (0.745), Equatorial diameter (0.777), Total soluble solids (0.536) and negatively with Days to maturity (-0.317), Percent disease index (-0.340), PWL (%) 1 (-0.620), PWL (%) 2 (-

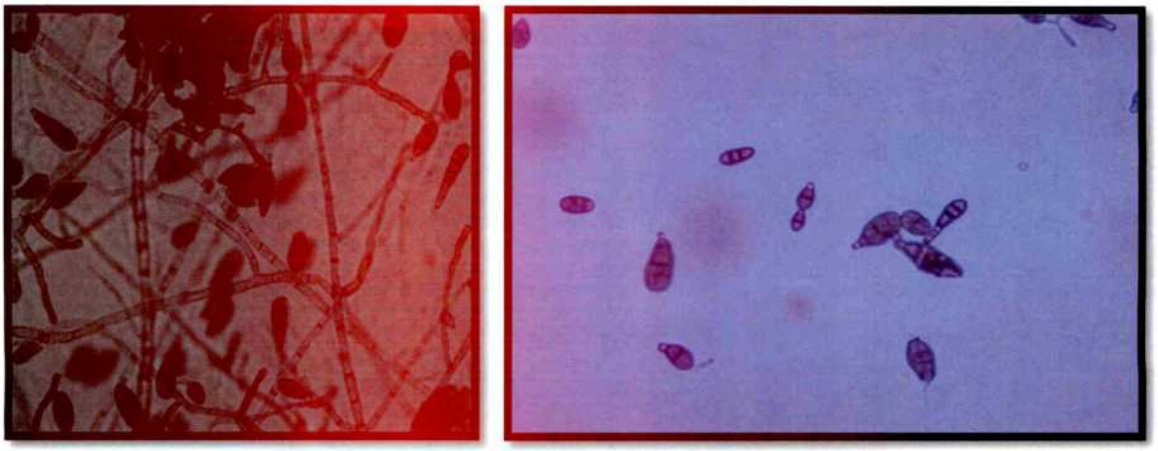
0.572). The characters contributing more positively with (PC-IV) were Neck thickness (0.487), Individual bulb weight (0.699), Marketable yield (kg/plot) (0.315), Marketable yield (q/ha) (0.318), Total yield (kg/plot) (0.3631), Total yield (q/ha) (0.363), PWL (%) 1 (0.455), PWL (%) 2 (0.404), PWL (%) 3 (0.622), PWL (%) 4 (0.503) and negatively with days to maturity, Grade B bulb. The characters like number of leaves (0.588), Pyruvic acid (0.619) were positively contributed to (PC-V), whereas Dry matter (%) (-0.654) was negatively contributed. The character like Number of leaves (0.459), Neck thickness (0.492) and Percent disease index (0.644) positively contributed to (PC-VI) and negative for Days to maturity (-0.339) and Pyruvic acid (-0.398). These traits having high positive or negative component value reveal more genetic diversity (Plate-6). Similar kind of inference concluded by Arya *et al.* (2017) whose observation that three principal components contributed to 95.61% of the variation. They observed that high positive loading from average bulb weight, bulb yield and high negative loading from leaf length, double/deformed bulb in PC1 contributed more towards differentiating the clusters which is in agreement with our studies. Similarly, Singh *et al.* (2013) observed 3 principal components having 71.03% of total variation and in first principal component plant height, marketable yield, bulb polar diameter and bulb neck thickness were the major contributor which is in agreement with our findings. In contrast, Hanci and Gokce (2016) observed that nine PCs with eigen values >1 contributed 71.84% of the variability among 96 Turkish onion accessions. But in agreement with our findings, the characters contributing more positively with PC1 were marketable yield and total yield. The first principal component accounts for maximum variability in the data with respect to succeeding components (Leilah and Al-Khateeb, 2005).

4.7 REACTION OF PURPLE BLOTCH (*Alternaria porri* L.) DISEASE TO THE ONION GENOTYPES UNDER FIELD AND CONTROLLED CONDITIONS.

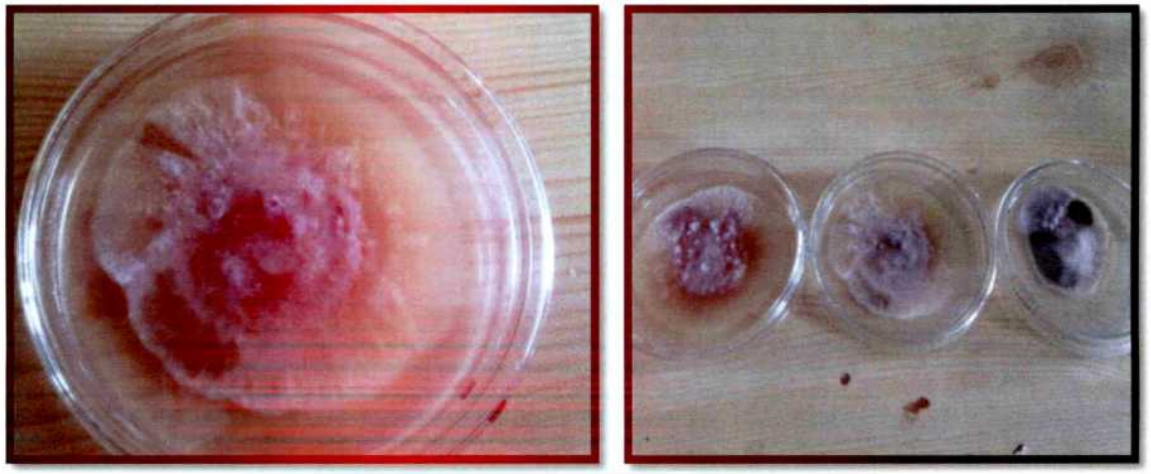
4.7.1. Percent disease index (%)

In field conditions 2015-16 year, Percent disease index range was from (1.12-25.33 %) and mean was (8.52 %). Lowest PDI observed in RO-636 followed by PKV White and highest PDI observed in DOGR-546-DR (25.33) followed by DOGR-571-LR (21.26). In the second year, percent disease index range was from (1.81-20.31 %) and mean was (11.30 %). Lowest PDI observed in Arka Kirtiman, and highest PDI observed in Line-355 (22.56 %) followed by DOGR-HY-1 (21.16 %). In the pooled over years, percent disease index range was from (4.06-20.31 %) and mean was (9.91 %). Lowest

Symptoms of *Alternaria porri* L.



Spores of *Alternaria porri* L.



Culture of *Alternaria porri* L.

Plate 11: Symptoms, Spores and culture of *Alternaria porri* L

Plate 12: Onion plants under controlled conditions

PDI observed in Bhima Sweta (4.06 %) followed by PKV White (4.30 %) and highest PDI observed in DOGR-546-DR (20.31 %) followed by DOGR-571-LR (17.39 %) indicating very high disease pressure. The results of present study are quite similar with research of Singh (1999) and Ahmad and Iqbal (2001) who evaluated onion germ plasm under field conditions and concluded that use of resistant varieties against purple blotch disease is the most economical and best method. These results were in conformity with the results obtained by Chetana *et al.* (2011) who screened different onion genotypes against purple blotch and revealed that the genotype Arka Kalyan was found moderately resistant while genotypes *viz.*, Rampur Rose, Agrifound Rose, Arka Pragati, Arka Niketan, Arka Pitamber and Arka Bindu was found moderately susceptible to the disease. Our results are also in agreement with previous reports which demonstrated that, only a few lines are resistant while the majority have moderate resistance to purple blotch under natural infestation in open field condition (Pathak *et al.*, 1986). Sugha *et al.* (1992) evaluated 94 onion genotypes under natural conditions and designated just two varieties, IC39178 and IC49371 as resistant to purple blotch. In the same context, Behera *et al.* (2013) observed VG-18 cultivar as resistant and another 12 lines as moderately resistant to purple blotch. Kale and Ajjappalavara (2014) evaluated and screened forty four genotypes of Onion against purple blotch disease. Their results revealed that the 5 genotypes were found to be moderately resistant and percent of leaf area infection ranged from (11.00 to 20.00%), whereas, moderately susceptible 31 genotypes were grouped with (21.00-40.00%) leaf area infection, five were susceptible with leaf area infection from (41.00 - 60.00%) and the remaining two genotypes were highly susceptible with and leaf area infection was more than (60%). The specific disease reaction of different genotypes against purple blotch as evident from this survey could be highly useful for researchers in disease forecasting and pest management programs. Similar results have been reported by Mohapatra (2017) who observed lowest percent disease index (PDI) in Arka Kalyan, Arka Niketan, Indam Gulab and Red Diamond and highest PDI observed in Light Red followed by Agrifound Rose.

Table 4.15: Mean performance of onion genotypes based on Percent disease index

Sl. No.	Characters	*Percent disease index (%)		
	Genotypes	First year	Second year	Pooled
1	IIHR-C-606	14.14 (**22.05**)	14.65 (**22.48**)	14.39 (**22.29**)
2	DOGR-571-LR	21.26 (**27.41**)	13.52 (**21.54**)	17.39 (**24.51**)
3	DOGR-546-DR	25.33 (**30.17**)	15.30 (**22.98**)	20.31 (**26.62**)
4	JWO-08-09	2.81 (**9.12**)	19.92 (**26.39**)	11.37 (**18.08**)
5	LINE-355	3.70 (**8.96**)	22.56 (**28.32**)	13.13 (**19.72**)
6	BHIMA KIRAN	11.05 (**19.41**)	7.71 (**15.77**)	9.38 (**17.76**)
7	BHIMA SWETA	2.11 (**6.81**)	6.01 (**13.99**)	4.06 (**11.27**)
8	PKV WHITE	1.24 (**3.71**)	7.36 (**15.45**)	4.30 (**11.07**)
9	RO-628	6.55 (**14.81**)	4.33 (**11.95**)	5.44 (**13.41**)
10	RO-636	1.12 (**3.52**)	8.17 (**16.21**)	4.65 (**11.34**)
11	BHIMA SHAKTI	3.09 (**9.90**)	6.89 (**15.09**)	4.99 (**12.67**)
12	ARKA LALIMA	12.43 (**20.63**)	4.01 (**11.52**)	8.22 (**16.09**)
13	ARKA KIRTIMAN	11.37 (**19.69**)	1.81 (**6.32**)	6.59 (**13.72**)
14	DOGR-HY-1	8.68 (**17.13**)	21.16 (**27.35**)	14.92 (**22.26**)
15	DOGR-HY-2	6.75 (**15.05**)	16.87 (**24.23**)	11.81 (**19.65**)
16	JRDO-07-13	13.15 (**21.26**)	16.05 (**23.60**)	14.60 (**22.43**)
17	LUCIFER	15.20 (**22.94**)	2.68 (**9.26**)	8.94 (**16.18**)
18	BSS-827	2.41 (**7.23**)	16.63 (**24.06**)	9.52 (**16.49**)
19	PUNE RED	1.75 (**4.41**)	9.84 (**18.25**)	5.79 (**12.93**)
20	ORIENT	6.23 (**14.38**)	10.47 (**18.82**)	8.35 (**16.66**)
	Mean	**8.52**	**11.30**	**9.91**
	S.E(M)	1.17	1.48	0.98
	CD(5%)	3.36	4.23	2.80

*Values were arc sine transformed before analysis.

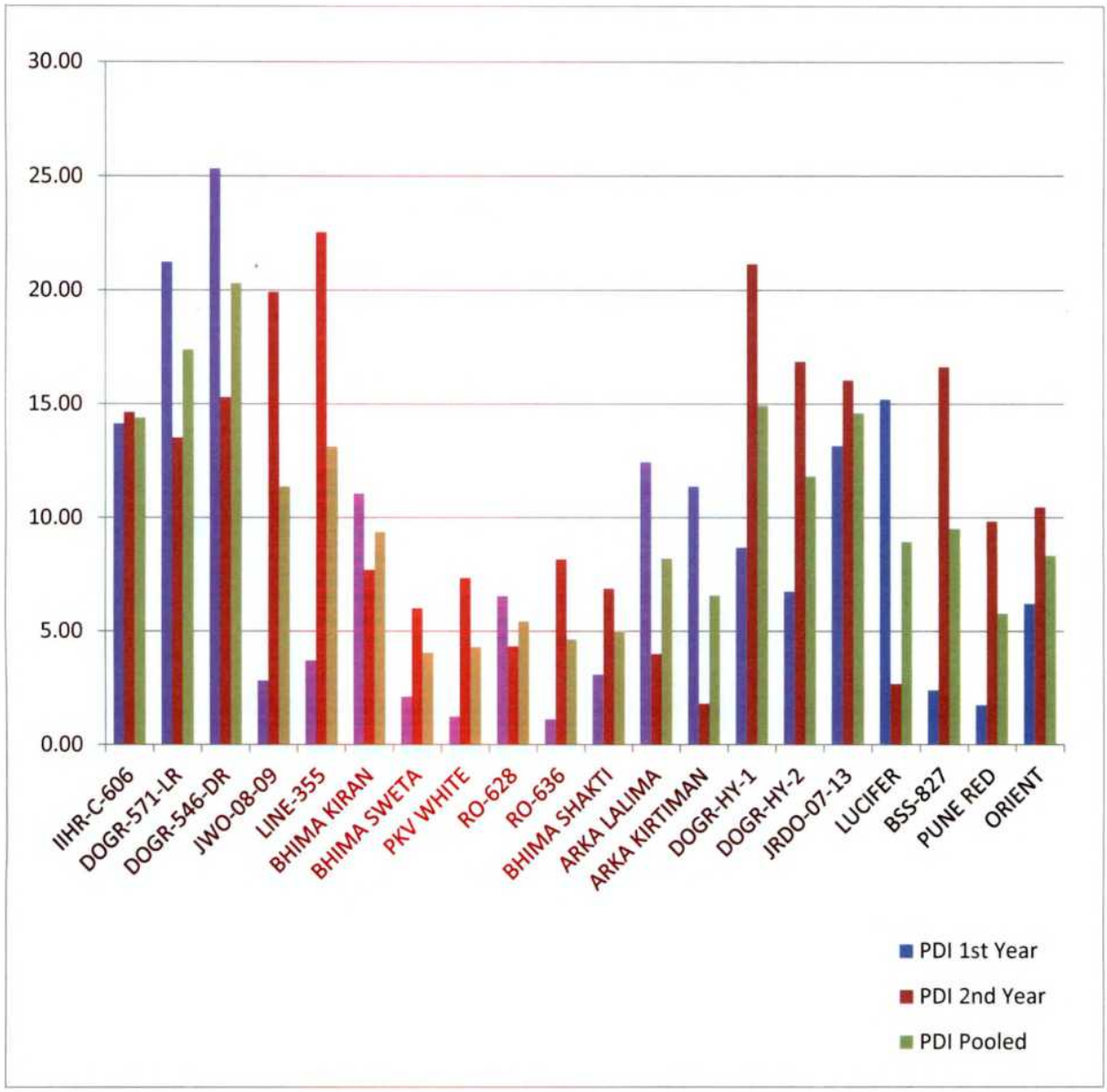


Figure 3: Percent disease index of onion genotypes

In the controlled condition after artificial inoculation of purple blotch almost every plant got infected but disease severity varied with the genotypes. This indicates that, the field resistance category may break in controlled condition under heavy disease pressure. Therefore, screening should be carried out under both artificial and natural conditions before assigning disease categories to the genotypes. DOGR-546-DR, DOGR-571-LR and Line-355 got severe infection but genotype like Arka Kirtiman and Arka Sweta, PKV White and RO-636 showed less severity. Inoculation with a virulent isolate or high concentration of the inoculums often result in the shifting of disease severity from resistant to susceptible category during artificial inoculation (Garg *et al.*, 2013). However, some genotypes (PKV White, Arka Kirtiman and RO-636) showed tolerant reaction for both field and *in vitro* inoculation. So, based on field and controlled conditions, we can suggest some of the varieties which are less susceptible to purple blotch disease. Similar kind of inference concluded by Mohaptra (2017) who observed that Agrifound Dark Red, Agrifound Rose got severe infection but varieties like Arka Niketan and Arka Bheem, Arka Kalyan, Indam Gulab, Indam Red Stone, Indam Hybrid-04, Arka Bindu showed less severity. *In vitro* screening of 43 genotypes for resistance to *A. porri* revealed two resistant lines. An additional 14 genotypes showed consistent moderate resistance in the field as well as *in vitro* evaluations (Nanda *et al.*, 2016).

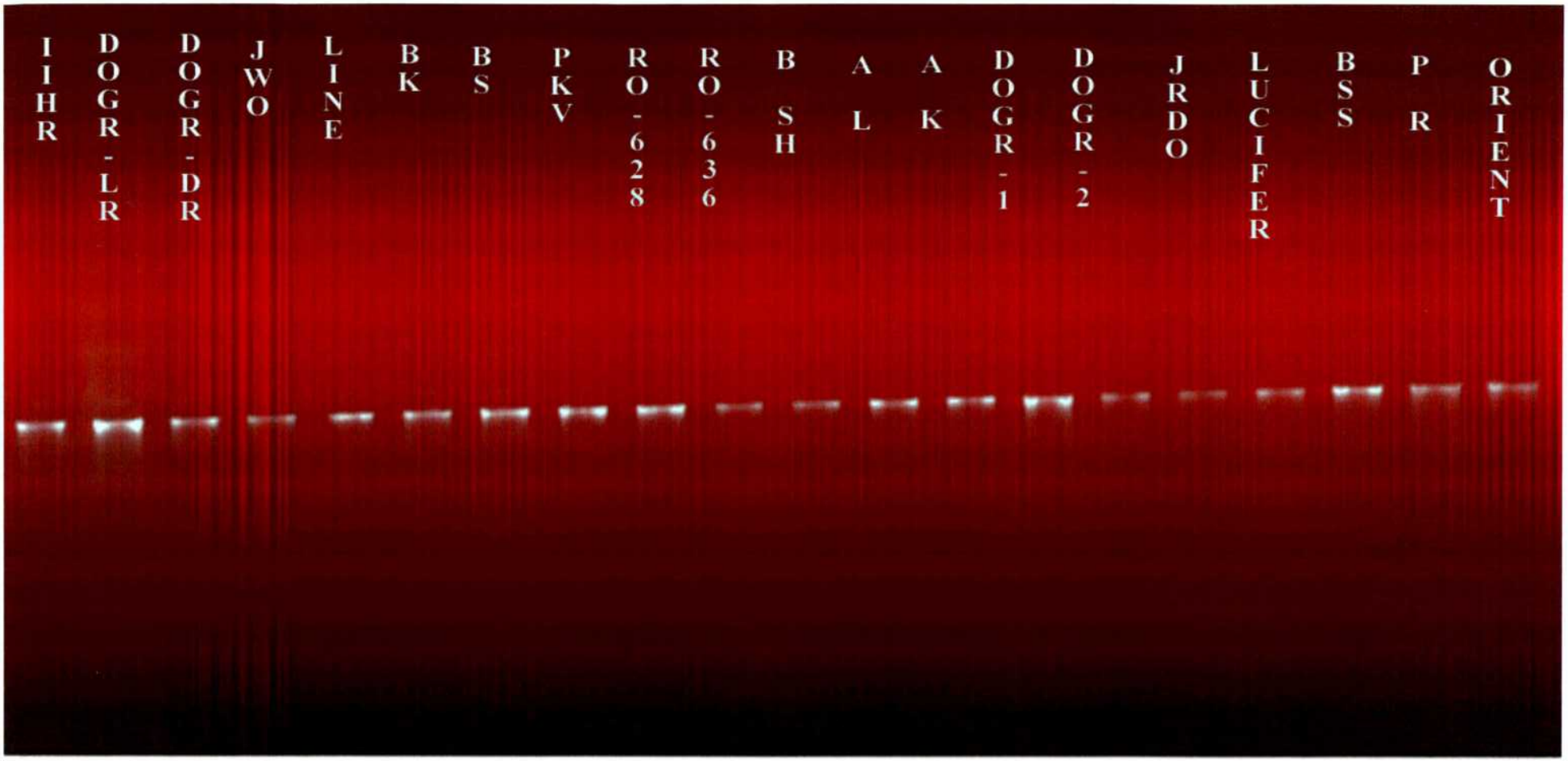
4.8. MOLECULAR CHARACTERIZATION USING RAPD AND SSR MARKER SYSTEM

4.8.1. DNA quantification

Genomic DNA was successfully isolated from all the twenty genotypes and quality of isolated DNA was studied by 0.8% agarose gel (Plate-7). The concentrated genomic DNA samples were diluted to 10ng/µl based on stock DNA concentration for the RAPD and SSR profiling.

4.8.2. RAPD profiling and polymorphism

By the 10 RAPD primers, a total of 55 loci were observed with 503 amplicons. Out of the 55 loci produced, 52 were polymorphic, amounting to a total polymorphism percentage of 94.81 (Table 4.15). Six primers (Oligo-02, Oligo-04, Oligo-05, OPC-04, OPQ-06 and OPG-13) analyzed produced 100% polymorphism in the population. Oligo-01 had 94.9% moderate polymorphism and OPA-09 had the lowest polymorphism (78.4 %). The amplified products of RAPD primers showed a distribution of amplified



IIHR: IIHR-C-606, DOGR-LR: DOGR-571-LR, DOGR-DR: DOGR-546-DR, JWO: JWO-08-09, LINE: LINE-355, BK: BHIMA KIRAN, BS: BHIMA SWETA, PKV: PKV WHITE, RO-628, RO-636, BSH: BHIMA SHAKTI, AL: ARKA LALIMA, AK: ARKA KIRTIMAN, DOGR-1: DOGR-HY-1, DOGR-2: DOGR-HY-2, JRDO: JRDO-07-13, LUCIFER, BSS: BSS-827, PR: PUNE RED, ORIENT

Plate 7: Gel electrophoresis of twenty onion genomic DNA

fragment unique for each primer. The population specific bands could be discerned from the fragment patterns generated. Ten primers produced bands ranging in size from 100 bp to 1.5 kb. The polymorphic bands produced were used in assessing the genetic diversity among the cultivars. The number of alleles ranged from 2 (Oligo-01) to 11 (OPC-04). The average number of alleles was 3.56 per locus. The agarose gel picture of six RAPD primes are presented in the (Plate-8).

Table 4.16: Primer sequences and number of scored polymorphic bands generated by RAPD conferring genetic diversity in onion

Sl.	Marker	Sequence	TSB	NTB	NPB	PPB	PIC	MI	DI	RP
1	**Oligo-01**	CTTGATTCGA	24	5	2	94.9	0.37	0.65	0.82	0.61
2	**Oligo-02**	TAGGTGATGG	28	7	3	100	0.41	0.98	0.84	0.67
3	**Oligo-03**	GAACCGACGT	31	3	4	88.9	0.29	0.29	0.83	0.53
4	**Oligo-04**	CAGACGCTGG	41	5	6	100	0.34	1.42	0.84	0.61
5	**Oligo-05**	GATGCTATAC	81	8	9	100	0.4	4.07	0.87	0.51
6	**OPC-04**	TGACAACTAC	95	10	11	100	0.44	9.58	0.82	0.66
7	**OPD-03**	CTGGCCTGCA	49	5	6	85.9	0.21	0.32	0.85	0.27
8	**OPA-09**	TAGTAACGCC	48	3	5	78.4	0.31	0.97	0.81	0.49
9	**OPQ-06**	CTGCGACTCG	51	8	4	100	0.38	2.73	0.92	0.31
10	**OPG-13**	GTATCCAGCA	43	4	6	100	0.39	3.81	0.91	0.42
		Mean	49.1	5.8	5.6	94.81	0.354	2.482	0.851	0.508

[**TSB:** Total scorable bands; **NTB**: Number of Total Bands; **NPB**: Number of Polymorphic Band; **PPB**: Percentage of Polymorphic bands; **PIC**: Polymorphic Information Content; **MI**: Marker Index; **DI**: Diversity Index; **RP**: Resolving Power:]

For all the genotypes, the PIC values denote allelic diversity and frequency. The range of PIC value was (0.21) in OPD-03 to (0.44) in OPC-04. The highest PIC value which indicated its usefulness in differentiating individuals and presented high information content compared to other primers. The maximum DI was (0.92), found for the primer OPQ-03 followed by OPG-13 (0.88), OPQ-06 (0.91), Oligo-05 (0.87). The minimum diversity index was found for the primer OPC-09 (0.81). The Marker Index value was ranged from (0.29) Oligo-03 to (9.58) OPC-04. The MI value for other primers were intermediate like (4.07) Oligo-05, (3.81) OPG-13, (2.73) OPQ-06, (1.42) Oligo-04, (0.98) Oligo-02, (0.97) OPA-09, (0.65) Oligo-01 and (0.32) OPD-03. The estimated RP value found to be the highest for the primer Oligo-02 (0.67), followed by OPC-04 (0.66) and was lowest for the primer OPD-03 (0.27). The primers Oligo-05, OPQ-06, OPA-09, Oligo-03, Oligo-04, OPG-13 and Oligo-01 also exhibited medium resolving power (0.31-0.61). These values were below those found by Leite and

Anthonisen (2009) and Maniruzzaman *et al.* (2010) with 15 and 14 bands per primer respectively, both on onion. On the other hand, Vieira and Nodari (2007), working with genetic divergence in garlic obtained 5.7 bands per primer. The result of present investigation are also in agreement with previous studies carried out in onion by Mohapatra (2017) who studied genetic divergence of 16 varieties using 10 RAPD primers and observed seven primers showing 100% polymorphism (Oligo-01, Oligo-02, Oligo-03, Oligo-04, OPD-03, OPQ-06 and OPG-13) Oligo-05 had 89% moderate polymorphism and OPD-03 and OPA-09 had the lowest polymorphism (75%). Pavlovic *et al.* (2012) analyzed onion genotypes using five RAPD primers and Wilkie *et al.* (1993) found high degree of polymorphism. Amplification of DNA segments was found in all five cases and polymorphism observed in all five analyzed markers. A total of 50 bands were read and the length of fragments was 8 to 13. The highest polymorphism was found in the primer Oligo-02 and the lowest in Oligo03.

Jaccard's Similarity Matrix generated by RAPD primer was found to be highest between Orient with BSS-827 (0.80) followed by BSS-827 with JWO-08-09, Orient with JWO-08-09 (0.72) and Lucifer with RO-628, Pune Red with JWO-08-09, Lucifer with RO-628 (0.70) while it was lowest between RO-636 with DOGR-571-LR and Arka Kirtiman with RO-636 (0.00), PKV White with Bhima Kiran, Lucifer with PKV White (0.05). Other genotypes were moderately similar. Mohaptra (2017) found Jaccard's Similarity Matrix generated by RAPD primer to be highest similarity between Arka Kalyan with Bhima Super and Arka Bheem (0.81) and lowest between Indam Gulab with N-53 and Arka Bindu (0.46), Light red with Agrifound Dark Red (0.46). Maniruzzaman *et al* (2010) reported 12 of the primers which revealed scorable (168 bands) polymorphisms between cultivars of *A. cepa* and found that Bermis and India-2 were more dissimilar, whereas, Faridpuri and Bhati were the most similar in their genetic level.

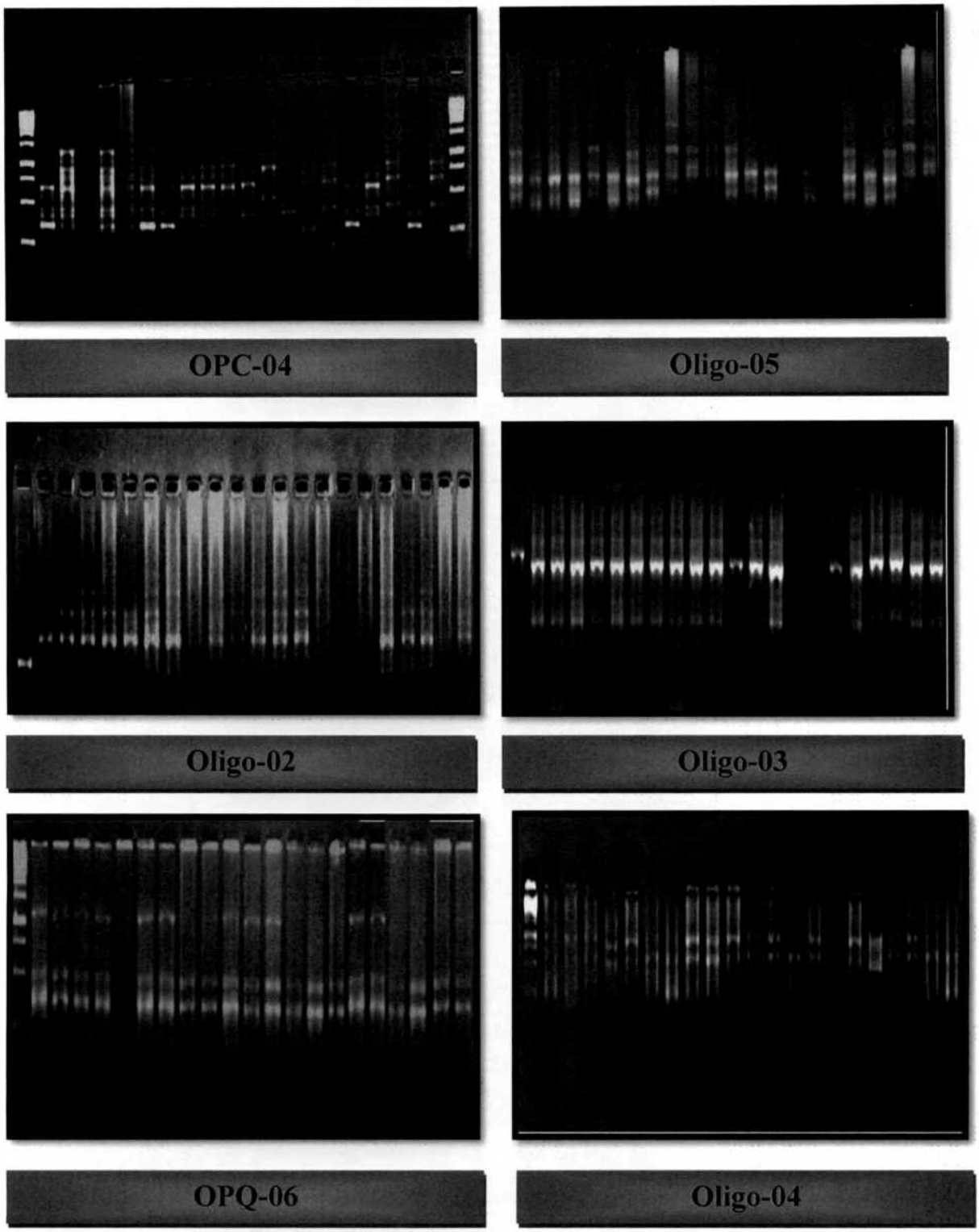
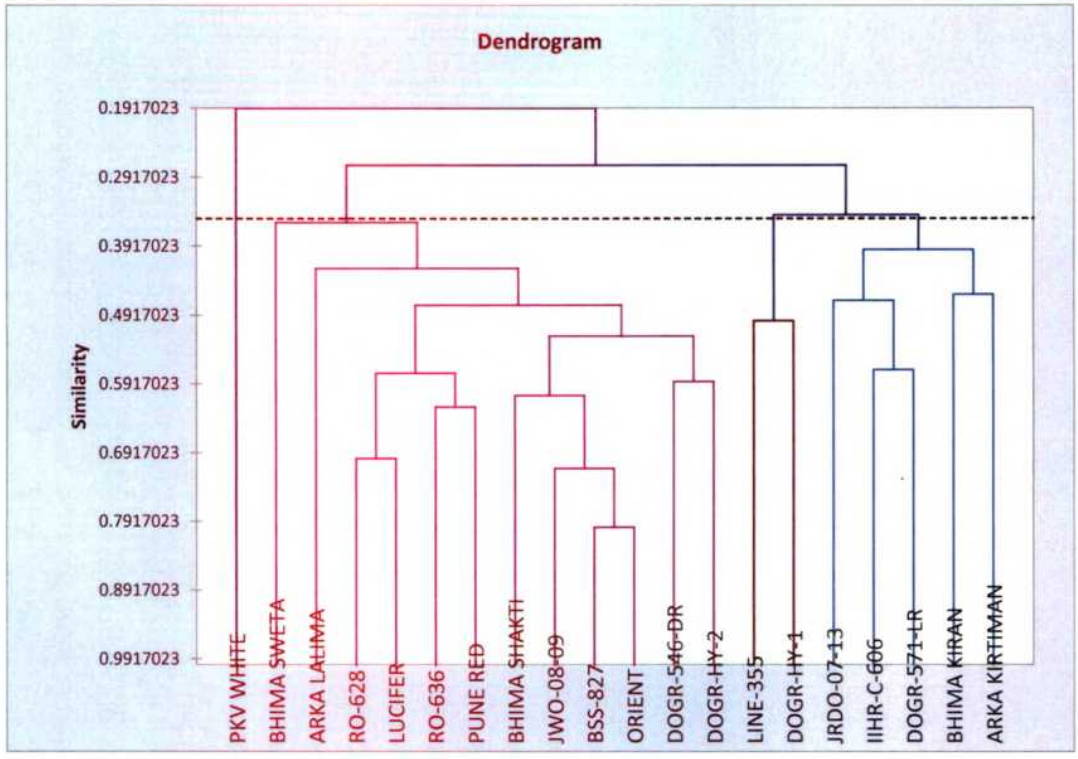


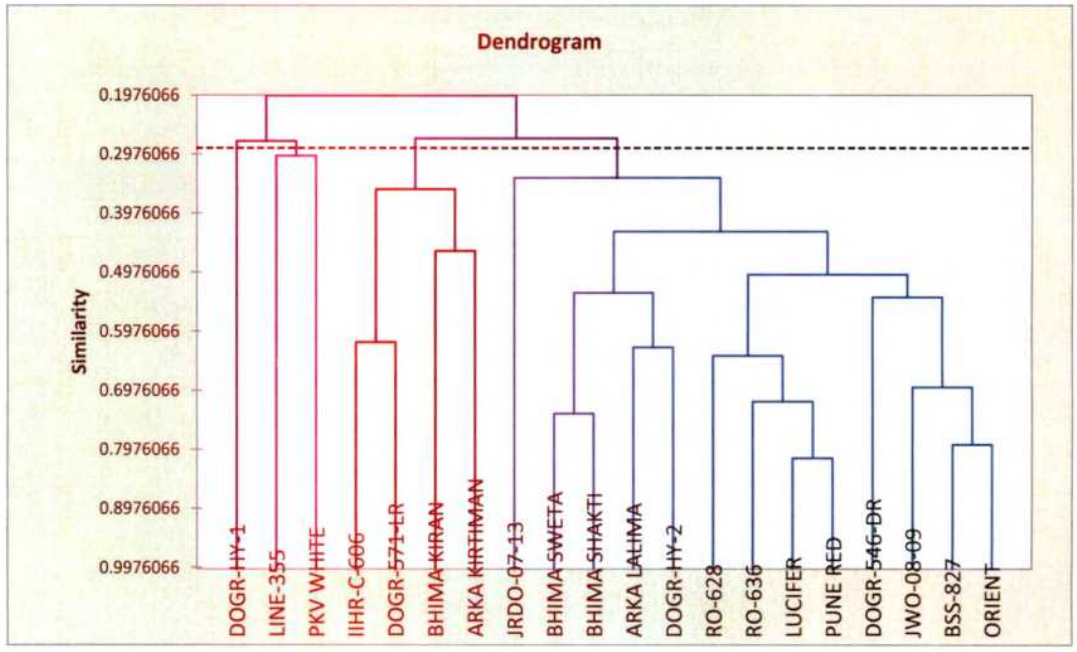
Plate 8: RAPD Profile of 20 Onion genotypes using primers OPC-04, Oligo-05, Oligo-02, Oligo-03, OPQ-06 and Oligo-04

Table: 4.17: RAPD Similarity Matrix

	IIHR-C-606	DOGR-571-LR	DOGR-546-DR	JWO-08-09	LINE-355	BHIMA KIRAN	BHIMA SWETA	PKV WHITE	RO-628	RO-636	BHIMA SHAKTI	ARKA LALIMA	ARKA KIRTIMAN	DOGR-HY-1	DOGR-HY-2	JRDO-07-13	LUCIFER	BSS-827	PUNE RED	ORIENT
IIHR-C-606	1																			
DOGR-571-LR	0.57	1																		
DOGR-546-DR	0.30	0.26	1																	
JWO-08-09	0.30	0.22	0.60	1																
LINE-355	0.22	0.43	0.26	0.27	1															
BHIMA KIRAN	0.44	0.50	0.39	0.38	0.40	1														
BHIMA SWETA	0.29	0.18	0.33	0.40	0.25	0.24	1													
PKV WHITE	0.06	0.25	0.12	0.21	0.36	0.14	0.25	1												
RO-628	0.30	0.27	0.46	0.50	0.40	0.21	0.33	0.21	1											
RO-636	0.10	0.00	0.26	0.48	0.11	0.05	0.18	0.07	0.56	1										
BHIMA SHAKTI	0.41	0.45	0.57	0.61	0.53	0.50	0.53	0.26	0.54	0.26	1									
ARKA LALIMA	0.15	0.24	0.47	0.53	0.24	0.38	0.31	0.23	0.32	0.31	0.58	1								
ARKA KIRTIMAN	0.33	0.50	0.375	0.30	0.29	0.47	0.20	0.44	0.19	0.00	0.35	0.36	1							
DOGR-HY-1	0.33	0.50	0.16	0.18	0.50	0.38	0.20	0.19	0.48	0.06	0.42	0.36	0.23	1						
DOGR-HY-2	0.25	0.10	0.59	0.55	0.21	0.33	0.44	0.13	0.48	0.44	0.52	0.50	0.26	0.17	1					
JRDO-07-13	0.53	0.41	0.22	0.39	0.33	0.39	0.26	0.19	0.46	0.26	0.44	0.25	0.22	0.38	0.17	1				
LUCIFER	0.40	0.30	0.43	0.48	0.30	0.23	0.24	0.05	0.70	0.53	0.46	0.35	0.14	0.33	0.38	0.43	1			
BSS-827	0.43	0.27	0.52	0.71	0.27	0.32	0.40	0.10	0.64	0.47	0.61	0.38	0.13	0.24	0.48	0.52	0.56	1		
PUNE RED	0.22	0.18	0.36	0.70	0.30	0.23	0.37	0.26	0.62	0.63	0.52	0.42	0.20	0.20	0.45	0.36	0.60	0.49	1	
ORIENT	0.50	0.33	0.52	0.71	0.22	0.26	0.40	0.15	0.57	0.47	0.61	0.38	0.24	0.19	0.409	0.52	0.55	0.80	0.65	1



RAPD Dendrogram



SSR Dendrogram

Plate 9: RAPD and SSR Dendrogram showing clusters of 20 onion genotypes

The entries in this study were grouped into four clusters based on Jaccard's similarity matrix. Cluster I consisted of five genotypes whereas Cluster II included twelve and Cluster III had two genotypes and Cluster IV includes one genotype (Table 4.17).

Table 4.18: Cluster of different genotypes using RAPD primers

Cluster	Genotypes
I	IIHR-C-606, DOGR-571-LR, BHIMA KIRAN, ARKA KIRTIMAN, JRDO-07-13
II	DOGR-546-DR, JWO-08-09, BHIMA SWETA, RO-628, RO-636, BHIMA SHAKTI, ARKA LALIMA, DOGR-HY-2, LUCIFER, BSS-827,PUNE RED, ORIENT
III	LINE-355, DOGR-HY-1
IV	PKV WHITE

4.8.3. Molecular typing using codominant markers

Codominant markers are allowing the analysis of only a single locus per experiment, so they are more informative because the allelic variation for that locus can be distinguished. SSRs were used as codominant markers in this study.

4.8.3.a. Characterization using SSRs

Analysis of polymorphism and marker efficiency parameters

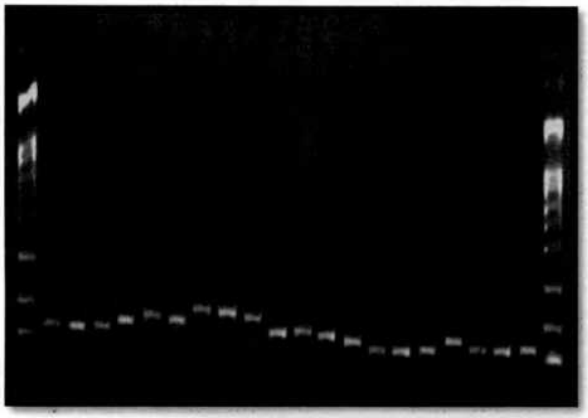
In this study nine SSR primers were used which had been developed by Mc Callum *et al.* (2008). They showed differential ability to identify unique multiband phenotypes among twenty genotypes. Primer ACM-004, ACM-068, ACM-187, ACM-326, ACM-046 ACM-240 and ACM-300 produced more bands and primer ACM-318 amplified the fewest. The percentage of polymorphic sites ranged from (76.5 % to 100 %) and mean was (94.72 %) (Table 4.18). Thus, these primers at particular loci can also be used as specific primers for the identification of cultivars. Fischer and Bachmann (2000) referred microsatellite markers as an efficient and relatively inexpensive successful method in onion. Jakse *et al.* (2001) also reported microsatellite markers as powerful tool for cultivar identification and genetic diversity studies. Similarly, Oh *et al.* (2004) used microsatellite markers for characterization of an apomictic species *Allium senescens.*

The markers with higher PIC values are highly informative for genetic studies and are very useful in distinguishing the polymorphic rate of a marker at a specific

locus (Hildebrand *et al.* 1992). PIC value gives information on the relative ability of the marker to detect the genetic variability between accessions, which was differed between SSR primers, highest for ACM-326 (0.56) and lowest for ACM-018 and ACM-318 (0.24) with mean value was (0.38) (Table 4.18). This means that all the primers selected were good indicators of the genetic diversity of the genotypes. Compared with the PIC values reported by other researchers, Khar *et al.* (2011) obtained the maximum PIC value of 0.89 in 34 onion accessions while Mallor *et al.* (2014) obtained the maximum PIC value of 0.77 in 85 Spanish onion genotypes. The result are also in agreement with previous studies carried out by Hanci and Gocke (2016) who evaluated variability at 46 microsatellite loci and identified 308 alleles with these markers, out of which 303 were polymorphic.

The nine SSR primers revealed the usefulness of a marker in distinguishing genotypes with DI values ranging from (0.19) for ACM-300 to (0.94) for ACM-240 with an average of 0.67 (Table 4.18). Except for two SSR primers that had DI values lower than (0.50), seven markers had high DI value (DI>0.50). The high DI values observed in this study indicated that the seven SSR markers used were informative. The maximum MI was observed for the primer ACM-004 (1.12) followed by the primer ACM -046 (0.98), ACM-326 (0.91), ACM-240 (0.87), ACM-318 (0.74), ACM-018 (0.54) and ACM-068 (0.52). Similar results were highlighted by Mohapatra (2017) who found SSR primers; ACM-004, ACM-068, ACM-187, ACM-326, ACM-046 AND ACM-240 produced more bands and primer ACM-318, ACM-018 and ACM-300 amplified the fewest. Other authors reported different allele sizes. For example, ACM004 gave bands between 201 bp and 212 bp in Czech onion varieties (Mitrová *et al.* 2015); 220 bp and 230 bp in Spanish onion accessions (Mallor *et al.* 2014); and 201 bp and 210 bp in Indian onion accessions (Khar *et al.* 2011).

The remaining SSRs primers had the MI value less than 0.50. The minimum MI was observed for the primer ACM-300 (0.12). The estimates of RP were found to be the highest for the primer ACM-018 (0.85) followed by the primer ACM-318 (0.61). The ACM-187 primer had the lowest RP values 0.22. Similar results were reported by González *et al.* (2015) who identified eleven polymorphic markers 32 alleles in the whole collection, with an average of 2.9 alleles per locus. Reddy *et al.* (2013) reviewed the use of DNA markers in Alliums for cultivar identification, diversity studies, SSRs development, colour improvement, total soluble solids (TSS), cytoplasmic male sterility



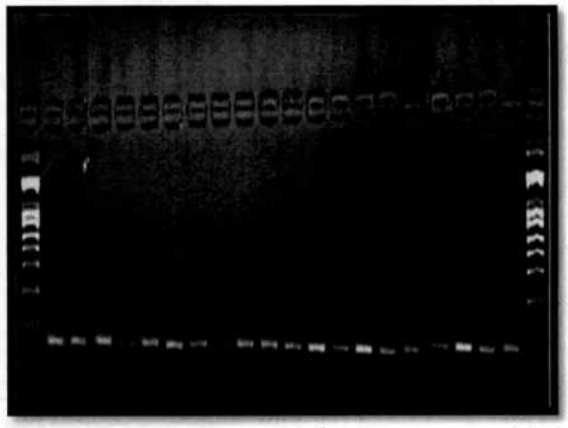
ACM-068

ACM-004

ACM-240

ACM-318

ACM-326



ACM-046

Plate 10: SSRs Profile of 20 Onion genotypes using primers ACM-068, ACM-004, ACM-240, ACM-318, ACM-326, ACM-046

(CMS) and efforts of DNA sequencing. In onion breeding, the traits such as size, shape, colour, pungency, soluble solids and disease resistance were targeted. McCallum *et al.* (2008) also worked on open pollinated (OP) bulb onion populations of wide geographical adaptation and surveyed genetic variation in a cultivated onion germplasm using simple sequence repeats (SSRs) markers.

SSRs primer similarity was noticed to be lowest between DOGR-HY-1 with RO-628 (0.05) followed by RO-636 with PKV White and DOGR-HY-1 with RO-636 (0.06) whereas, highest between Pune Red with Lucifer (0.81) followed by Pune Red with RO-636 (0.79). Mahajan *et al.* (2009) observed Similar results *i.e.* Nashik Red and Poona Red cultivars showed 100% similarity and were indistinguishable, whereas, cultivars N-53 and Bombay Red showed 95% similar fragments. Thus, to study the similarity among the cultivars the picture will be clear using more microsatellite primer pairs. These studies will be helpful in selecting the diverse parents (inbreds) for the development of suitable hybrids of onion besides the DNA fingerprinting of the cultivars, promising germplasm and parents for protection under Plant Cultivar Protection Act. It will also save the time for grow out tests for testing the purity of seeds.

Table 4.19: Various parameters related to efficiency of nine primers for SSRs analysis

Sl. no.	Marker name	Sequence (5′ -3′)	TSB	NTB	NPB	PPB Value (%)	PIC	MI	DI	RP
1	**ACM004**	F-TTTCGTATAGTACGCGATAAG	13	2	4	100	0.46	1.12	0.84	0.54
		R-GCGTGCAGATGTAGTCACA								
2	**ACM018**	F-GATCAAGGTCGGAGTATATGA	6	1	2	100	0.24	0.54	0.92	0.85
		R-TAGAGACGCTAGACGAGTG								
3	**ACM046**	F-CGATCGACATCAGCACAG	15	2	2	86.5	0.37	0.98	0.81	0.54
		R-CAGATAGAGAGCAGTGCAG								
4	**ACM068**	F-AGTCGTGTAGCGTGTACTT	16	3	2	100	0.39	0.52	0.72	0.45
		R-CATAGGGCAGCTATAGGCTA								
5	**ACM187**	F-TATACTCGAGCACTGTATGTA	17	1	2	76.5	0.28	0.21	0.24	0.22
		R-GCAGTGCTCATAATCCTACG								
6	**ACM240**	F-GTACTACCCTAATGGTAGCG	10	4	3	100	0.47	0.87	0.94	0.34
		R-AGATTGACGGCTATGACTTG								
7	**ACM300**	F-AGTTGCTGATTCGTGCTATG	5	2	2	89.5	0.38	0.12	0.19	0.51
		R-GCACTTGATAGTCAAGGTC								
8	**ACM318**	F-TCCTAGTCCACAACTACGAC	12	2	1	100	0.24	0.74	0.83	0.61
		R-ATCGAGATACTGCTGCGAC								
9	**ACM326**	F-AGACTAGAATCAAGCATTA	16	2	2	100	0.56	0.91	0.9	0.42
		R-AGGTTAGGCGAGTAGACATA								
		Mean	12.22	2.11	2.22	94.72	0.38	0.67	0.71	0.50

[**TSB:** Total scorable bands; **NTB**: Number of Total Bands; **NPB**: Number of Polymorphic Band; **PPB**: Percentage of Polymorphic bands; **PIC**: Polymorphic Information Content; **MI**: Marker Index; **DI**: Diversity Index; **RP**: Resolving Power]

Table 4.20: SSRs Similarity Matrix

	IIHR-C-606	DOGR-571-LR	DOGR-546-DR	JWO-08-09	LINE-355	Bhima Kiran	Bhima Sweta	PKV White	RO-628	RO-636	Bhima Shakti	Arka Lalima	Arka Kirtiman	DOGR-HY-1	DOGR-HY-2	JRDO-07-13	Lucifer	BSS-827	Pune Red	Orient
IIHR-C-606	1																			
DOGR-571-LR	0.62	1																		
DOGR-546-DR	0.25	0.26	1																	
JWO-08-09	0.35	0.19	0.67	1																
LINE-355	0.27	0.38	0.10	0.14	1															
Bhima Kiran	0.38	0.40	0.39	0.35	0.23	1														
Bhima Sweta	0.40	0.42	0.48	0.37	0.39	0.40	1													
PKV White	0.07	0.15	0.12	0.11	0.30	0.23	0.22	1												
RO-628	0.32	0.33	0.33	0.48	0.30	0.26	0.52	0.15	1											
RO-636	0.15	0.10	0.37	0.56	0.11	0.21	0.32	0.06	0.68	1										
Bhima Shakti	0.50	0.44	0.50	0.46	0.33	0.35	0.74	0.24	0.48	0.33	1									
Arka Lalima	0.20	0.15	0.42	0.45	0.24	0.333	0.50	0.39	0.41	0.39	0.53	1								
Arka Kirtiman	0.27	0.38	0.29	0.20	0.23	0.46	0.39	0.44	0.24	0.18	0.41	0.31	1							
DOGR-HY-1	0.14	0.25	0.27	0.17	0.30	0.33	0.23	0.250	0.05	0.06	0.17	0.29	0.12	1						
DOGR-HY-2	0.26	0.21	0.59	0.45	0.24	0.33	0.58	0.13	0.35	0.32	0.53	0.63	0.24	0.29	1					
JRDO-07-13	0.35	0.22	0.30	0.40	0.33	0.35	0.32	0.13	0.36	0.26	0.22	0.25	0.11	0.21	0.32	1				
Lucifer	0.23	0.24	0.50	0.53	0.20	0.23	0.50	0.11	0.70	0.65	0.39	0.38	0.20	0.17	0.45	0.33	1			
BSS-827	0.45	0.27	0.46	0.70	0.24	0.32	0.52	0.10	0.66	0.50	0.56	0.41	0.13	0.10	0.41	0.50	0.56	1		
Pune Red	0.14	0.10	0.50	0.61	0.11	0.20	0.36	0.13	0.55	0.79	0.32	0.44	0.17	0.20	0.37	0.32	0.81	0.48	1	
Orient	0.35	0.19	0.50	0.68	0.09	0.29	0.44	0.17	0.56	0.47	0.46	0.53	0.14	0.10	0.38	0.47	0.46	0.79	0.53	1

The entries in this study were grouped into four clusters based on Jaccard's similarity matrix. Cluster I consisted of four genotypes whereas Cluster II included thirteen and Cluster III had two genotypes and Cluster IV had one genotype (Table 4.20).

Table 4.21: Cluster of different genotypes using SSRs

Cluster	Genotypes
I	IIHR-C-606, DOGR-571-LR, BHIMA KIRAN, ARKA KIRTIMAN
II	DOGR-546-DR, JWO-08-09, BHIMA SWETA, RO-628, RO-636, BHIMA SHAKTI, ARKA LALIMA DOGR-HY-2, JRDO-07-13, LUCIFER, BSS-827, PUNE RED, ORIENT
III	LINE-355,
IV	DOGR-HY-1

Molecular markers provide powerful means to test the hybrid status of plants resulting from crosses and to monitor the introgression of foreign germplasm into *A. cepa.* The analysis of hybrid plants is another important application of molecular markers. Markers allow verification of the hybrid's cytoplasm, the composition of the nuclear genome, and investigation of introgression in backcrossing experiments.

However, more number of primers should be tested for effective screening of diverse germplams that will be helpful in designing any future breeding programs. The clear banding pattern and the easy detection of the presence of this gene shows the potentiality of using this technique for screening of wider germplasm preferably using more number of related primers that can be very helpful in selecting the parents for any breeding programs or biotechnological manipulations for improving the resistance for this disease in onion in future.

CHAPTER 5

Summary and Conclusion

SUMMARY AND CONCLUSION

The present investigation entitled "Characterization of onion genotypes using molecular markers and its field performance in the plains of West Bengal" was carried out at the research field of All India Network Research Project on Onion and Garlic (ICAR), C- Block Farm, Bidhan Chandra Krishi Viswavidyalaya, Kalyani, West Bengal during *Rabi* seasons of 2015-16 and 2016-17. Twenty genotypes were laid out in Randomized Block Design with three replications. The genotypes were planted in flat beds of 2x1.5 m size at a spacing of 15x10 cm with its scheduled cultural practices. Genotypes were evaluated based on their different morphological, biochemical and yield parameters grown under field condition. The varieties exhibited wide range of variation on different growth parameters and qualitative characters, yield components and yield.

The performance over two years of pooled data revealed the superiority of different genotypes for different characters. So, the best solution for selecting superior genotypes is to select according to the concerned parameter in demand. Among twenty genotypes of onion, Bhima Kiran was the best in respect of highest yield (264.35 q/ha) in the year 2015-16 and (270.58 q/ha) yield in the year of 2016-17. In all respect of trait, the results indicated that estimation of PCV were greater than the corresponding GCV for all the traits in the both the years as well as over pooled analysis. Difference between GCV and PCV were found minimum for most of the characters except double bulb (%) which exhibited high difference between GCV and PCV. The narrow difference between GCV and PCV indicated that these characters were least influenced by the environment. On the other hand, the wide difference between GCV and PCV designated that environment had major role for phenotypic expression of these traits. Low to moderate heritability accompanied by low genetic advance was estimated in Grade A bulb number in both the years along with pooled analysis and therefore it can be concluded that this character was high influence of the environmental factors. As a result, selection of this character was not to be effective. The characters like Double bulb (%), Neck thickness (mm), Days to maturity, Grade B bulb, Grade C bulb, Marketable yield (kg/plot), Total yield (kg/plot), Total soluble solids (^{0}Brix) and Physiological weight loss percentage after one month showed low to moderate heritability with low genetic advance, whereas Marketable yield (q/ha) and Total yield (q/ha) showed moderate heritability with high genetic advance in the year 2015-16. On the contrary, high heritability concomitant with

low genetic advance had exhibited by most of the characters except Marketable (bulb/plot), Marketable yield (q/ha), Total yield (q/ha), Percent disease index, which showed high heritability with moderate to high genetic advance.

Plant height is positively correlated with Polar diameter and Equatorial diameter, at phenotypic levels both the years and over pooled data analysis. Similarly, Days to maturity is significantly negatively correlated with Polar diameter (mm), Equatorial diameter (mm) at phenotypic level for the both the years and over pooled data analysis. Individual bulb weight (g) consistently significantly positive associations with a number of characters like Marketable yield (kg/plot), Marketable yield (q/ha), Total yield (kg/plot) and Total yield (q/ha) at phenotypic level, therefore, this trait may be considered important and undeviating relationship with yield. When interrelationship among these characters considered, then selection of any other traits had capacity to generate a strong correlated response selecting individually as well as among them.

Based on multivariate analysis, the varieties were grouped into 5 clusters. The maximum number of genotypes (7) was included in cluster IV followed by cluster II (5) and (5) in cluster I and cluster III whereas, minimum number of genotypes was recorded in cluster V (2). The genotypes of cluster I recorded the superior performance for total yield, marketable yield, days to maturity, individual bulb weight, minimum double bulb percentage, Physiological weight loss percentage after one month, whereas the genotypes of cluster – II had higher days to maturity, total yield, marketable yield, desirable minimum values for PWL after one month and double bulb percentage. Similarly, the genotypes of clusters-III, clusters-IV cluster-V showed maximum values for days to maturity. Principal component analysis simplifies the complex data by transforming the number of correlated variables into a smaller number of variables called principal components. According to PCA results, Eigen value was maximum in PC-I followed by PC-II and PC-III. Total variance percentage was maximum in PC-I followed by PC-II and PC-III. The first six PCs with Eigen values >1 contributed (81.96 %) of the variability amongst genotypes.

In terms of disease incidence, several genotypes were impressively of low percentage of disease incidence (PDI). Lowest PDI observed in RO-636 followed by PKV White, Bhima Sweta and Arka Kirtiman, whereas, highest PDI observed in DOGR-546-DR followed by DOGR-571-LR, Line-355 and DOGR-HY-1. In the controlled condition after artificial inoculation of purple blotch, almost every plant got infected but

disease severity was varies with the varieties; DOGR-546-DR, DOGR-571-LR and Line-355 got severe infection but genotype like Arka Kirtiman and Arka Sweta, PKV White and RO-636 showed less severity. So, based on field and controlled conditions, we can suggest some of the varieties which are less susceptible to purple blotch disease.

The molecular marker based studies with RAPD and SSRs efficiently characterized the onion genotype diversity for the studied genotypes. The polymorphic bands produced were efficient in assessing the genetic diversity among the cultivars. Out of 10 Primers six primers showed 100% polymorphism (Oligo-02, Oligo-04, Oligo-05, OPC-04, OPQ-06 and OPG-13). Oligo-01 had (94.9%) moderate polymorphism and OPA-09 had the lowest polymorphism (78.4 %). The polymorphic bands proved to be useful in differentiating between the genotypes and group them in to categories. Among SSR primers, ACM-004, ACM-068, ACM-187, ACM-326, ACM-046 ACM-240 and ACM-300 produced more bands and primer ACM-318 amplified the fewest. The SSR based grouping was more or less similar with phenotype and RAPD based grouping. Though these different studies could not differentiate the genotypes accurately for average genotypes, but the best genotypes for different type of parameters were distinctly grouped from each other.

Conclusion:

From the above results, it is concluded on the basis of phenotypic and genotypic coefficients of variability, heritability, genetic advance as percent of mean, phenotypic and genotypic correlation coefficients and genetic diversity analysis that there is a great possibility of improvement in attributes of this valuable vegetable crop. Most of the characters had high heritability and moderate to high heritability indicating additive gene effect for their inheritance and the variation observed was mainly under genetic control and less influenced by environment, offering ample opportunities for selection. The higher values of genotypic than those of phenotypic correlation suggested that genotypic effect were more important than the environmental factors. The highest distance between clusters indicates that genotypes might have originated from different breeding programmes bearing maximum heterogeneity within the population. Therefore, crossing between two genotype selected from each cluster will produce more heterotic response and produce wide variability with desirable segregants due to recombination diverse gene complex. The genotype from different origin was accommodated in the same cluster indicating their close affinity due to similar gene complexes. The results

suggested that the genotypes with in a cluster might have some degree of ancestral relationship. As a result the genotype within the same cluster originated from nearest geographical region with more or less similar kind of gene complexes. Further, the molecular marker based studies (RAPD and SSR) proved to be very efficient in differentiating between the onion genotypes. Moreover, because of their efficient polymorphism, reproducibility, and transferability, these markers will provide new opportunities for germplasm identification, genetic diversity analysis, phylogenetic relationships studies, chromosome mapping, and even map-based cloning in the onion and other important related *Allium* species.

On the basis of all the parameters studied, different genotypes proved to be best for one or more than one character. So, it is very difficult to conclude a single genotype with all of the best studied parameters suitable for cultivation. However, the major concern of the farmers are to produce high yielding quality onions in minimum crop duration with lesser disease incidence. Throughout the study, it was observed that all these major parameters do not introgress together in their best form in any genotype. So, it is advised to practice the cultivation of the genotypes based on their particular character which is of higher market value such as bulb weight or yield or pungency *etc.* Otherwise, the farmers may also choose such genotype for cultivation which ensures better than average performance in terms of all the major characters like short duration, less disease susceptibility, high yield and other yield attributing characters.

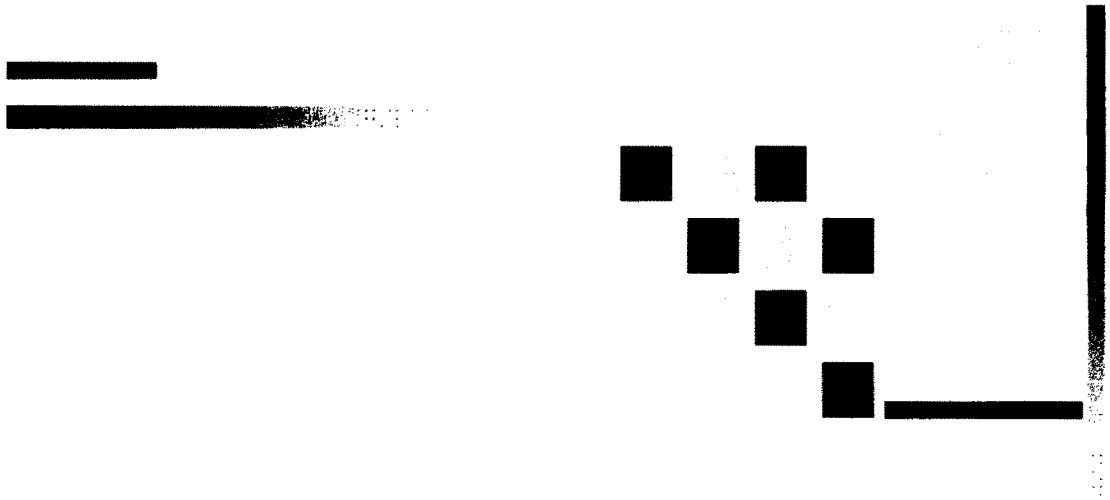
CHAPTER 6

Future Scope of Research

FUTURE SCOPE OF RESEARCH

Onion is an important vegetable which is being cultivated for a wide range of applications. From medicinal values to huge culinary purposes, longer shelf life to high foreign exchange value, Onions play very pivotal role in national economy is undisputedly brilliant. But, the limitations of this vegetable production in India are mainly cultivation under particular season (Rabi), lack of suitable high yielding varieties, proper screening techniques (both phenotypic and genotypic) and few other factors. In this background of issues and the current research outcome, an enormous scope for further research on onion is possible to take out.

- More number of genotypes can be screened by means of observing selected morphological, biochemical and different yield attributing parameters.
- The selected onion genotypes can be further screened through multi-location trials to check for yield stability and genotype performance in farmer's field condition.
- The selected high yielding genotypes as well as highly purple blotch tolerant genotypes can be further incorporated in advance generation production in breeding programmes.
- The selected highly polymorphic RAPD and SSRs can be further utilized for genotype screening and character association.
- More number of primers should be tested for effective screening of diverse germplams that will be helpful in designing any future breeding programs.
- The resistant gene specific markers can be utilized for higher number of genotype screening and diversity analysis.
- Lastly, the targeted genes can be studied for expression analysis and genetic engineering programmes in varietal development pipeline.



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