GENETIC DIVERSITY STUDIES IN MAKOI (*Solanum nigrum* L.) GENOTYPES

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(T.Nandhini)

ABSTRACT

GENETIC DIVERSITY STUDIES IN MAKOI (*Solanum nigrum* L.) GENOTYPES

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An investigation was carried out with 45 makoi (*Solanum nigrum* L.) genotypes at the Horticultural College and Research Institute, Coimbatore during 2011 - 2013 to study the extent of variability, genetic divergence through morphological characters and molecular markers and association among yield and its component characters.

Morphological characterization of 45 genotypes based on 14 IPGRI descriptors was done and the cluster analysis grouped the genotypes into four major clusters. Ploidy analysis and DNA barcoding analysis revealed that TN *Sn* 10 was *S.nigrum* L. (hexaploid) and TN *Sn* 30, TN *Sn* 32, TN *Sn* 44 and TN *Sn* 47 were *S.villosum* Mill. (tetraploids) while genotypes TN *Sn* 08, TN *Sn* 12, TN *Sn* 23, TN *Sn* 38, TN *Sn* 52 and TN *Sn* 53 were *S.americanum* Mill.(diploids). The genotypes TN *Sn* 10 and TN *Sn* 30 recorded highest herbage and alkaloid yield and can be used in breeding programme to get high alkaloid yielding varieties. Though TN*Sn* 19 and TN *Sn* 22 recorded lesser yield than TN *Sn* 10 and TN *Sn* 30, they showed high nutritive value and palatable characters and hence they can be used for evolving varieties that can be used as green leafy vegetable.

In Mahalanobis D^2 analysis, the genotypes were grouped into eight clusters. Among the eight clusters, the cluster V was the largest with 15 genotypes followed by cluster I with 11 genotypes. The cluster IV and III has maximum inter cluster distance which showed maximum divergence between the two clusters. Fresh and dry herbage yield per plant contributed maximum towards divergence.

The genetic relationship and variation of 45 accessions were examined by SSR analysis using 10 primer pairs. A total of 22 fragments from ten SSR markers were formed of which seven primers were polymorphic. PIC was highest for the SSR primers STWIN 12

G (0.698). The Jaccard's similarity coefficient ranged from 0.36 to 1.00. Based on the banding pattern, the cluster analysis was done using UPGMA and the dendrogram was constructed which resulted in eight clusters.

In all the three species, the maximum phenotypic and genotypic coefficient of variations was observed for protein content, dry herbage yield per plant, fresh herbage yield per plant. Heritability estimates were higher for quality characters than the biometric characters. Ascorbic acid content, protein content, iron content and days to flower bud initiation recorded high estimates of heritability coupled with high genetic advance.

Correlation studies indicated that total alkaloid content expressed highly significant and positive correlation withdry herbage yield, crude fibre, number of leaves and number of primary branches. It also had significant negative association with protein content, number of berries per plantand days to fifty per cent flowering.

In a nutshell, wide diversity existed among the genotypes in morphological and biochemical characters and are beneficial for developing superior varieties through selection or hybridisation programme. Correlation revealed earlier flowering with lesser berry yield can be considered for higher herbage yield.

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ABBREVIATIONS

The following symbols and abbreviations are used in the text and tables

^{o}C	-	Degree Celsius
mg	-	Milligramme
g	-	Gram
kg	-	Kilogram
µg	-	Microgram
ng	-	Nanogram
g^{-1}	-	Per gram
%	-	Percentage
m	-	Meter
cm	-	Centimeter
mm	-	Millimeter
nm	-	Nanometer
kmph	-	Kilometer per hour
m^{-2}	-	Per square metre
ml	-	Millilitre
µl	-	Microlitre
M	-	Molar
mM	-	Millimolar
V	-	Volts
U	-	Unit
B	-	Beta
w/v	-	Weight by volume
v/v	-	Volume by volume
rpm	-	Revolution per minute
t	-	Tonnes
Fig.	-	Figure
ANOVA	-	Analysis of variance
CV	-	Coefficient of variation
CD	-	Critical difference
SE(d)	-	Standard error of mean deviation
GA	-	Genetic Advance
PCV	-	Phenotypic Coefficient of Variation

GCV	-	Genotypic Coefficient of Variation
RBD	-	Randomized Block Design
RF	-	Rain fall
RH	-	Relative Humidity
Viz	-	Namely
FW	-	Fresh weight
DW	-	Dry weight
RAPD	-	Randomly Amplified Polymorphic DNA
SSR	-	Simple Sequence Repeats
ISSR	-	Inter Simple Sequence Repeat
SNP	-	Single Nucleotide Polymorphism
SCAR	-	Sequence Characterized Amplified Regions
ICMR	-	Indian Council of Medical Research
MSL	-	Mean sea level
DAP	-	Days after planting
UPGMA	-	Unweighted pair group method with arithmetic average
CTAB	-	Cetyltrimethylammonium bromide
EDTA	-	Ethylenediaminetetraacetic acid
dNTPs	-	Deoxynucleotide Triphosphates
Taq	-	*Thermus aquaticus*

Introduction

CHAPTER I

INTRODUCTION

India, one of the twelve mega diversity hot spots regions of the world, has 2.4 per cent world's area with 8 per cent global diversity. India has one of the richest plant medical ethnicity in the world. The medicinal properties of plant species have made an amazing contribution in origin and evolution of many traditional herbal therapies. World health Organization (WHO) estimates that 80 per cent of the world's population depends on traditional medicine for their health needs. According to, All India Ethno biological Survey (2010) carried out by Ministry of Environment and Forests, Govt. of India, over 8000 plants are being used in traditional systems of medicine in India. Around 25,000 effective plant based formulations are being extensively used in folk medicine in India (Ramakrishnappa, 2002). In many developed countries traditional herbal remedies are making a comeback as alternates to modern medicines.

Medicinal plants trade is growing in volume and exports. India ranks second in the world after China in export of medicinal plants. According to the Pharmaceutical Export Promotion Council, exports of herbal products from India increased from ₹ 306.3 crores in 2005-06 to ₹ 570.8 crores in 2009-10 at a compound annual growth of 16.8%. The export of herbal products and medicines has the potential to reach ₹120 billion by 2012 (Saini Navdeep *et al*, 2011).

Solanaceae, a cosmopolitan family composed of approximately 90 genera and between 2000 and 3000 species comprises of many vegetables and medicinal plants. The family is widely distributed throughout tropical and temperate regions of the world, with its Centres of diversity occurring in Central and South America (Edmonds 1978, D'Arcy, 1991). *Solanum* is one of the most important and largest genera of the family Solanaceae, The generic name *Solanum* is generally considered to be derived from Latin *Solamen*, referring to the quieting or sedative effects associated with many of the species.

Makoi or Black night shade *(Solanum nigrum* Linn) belonging to Solanaceae family, is an upcoming medicinal crop valued for its leaves and matured green berries which contain steroidal glycol alkaloids. The total alkaloid content of leaf and berry is 0.431 and 0.101 per cent, respectively. The principle aglycone is solasodine, the nitrogen analogue of diosgenin which has been reported as a valuable steroidal precursor for the supplementary source of the commercial synthesis of several steroidal drugs. The plant also

contains other glycol alkaloids like solamargine, solanigrine and solasonine and two steroidal saponins namely nigrumins – I and II (Rodriguez *et al*., 1979; Sree *et al.,* 1982 and Tsuyoshi *et al,* 2000).

The herb has antiseptic antidysenteric properties and used against abdominal upsets, mouth ulcers, scrofulous dyscarasias (Pereeze *et al*., 1998). The leaves are used to suppress carcinogenesis and to alleviate the pain in inflammation of the kidneys and bladder. It has also been used in early Ayurveda along with other ingredients in curing heart diseases. The juice of the leaf is effective in the treatment of cirrhosis of liver and haemoptesis and also as an antidote in opium poisoning (Ravi *et al*., 2011). It has a great demand from pharmaceutical industries and become popular with a wide range of herbal products including Actilivforte, Geriforte, Herbolax, Manol, Liv-52 and Eve care etc., (Smitha *et al*., 2010).

Besides its medicinal properties, Makoi also has some unique nutritive value. The per capita consumption of green leafy vegetables was less than 14 grammes (Anon; 2011) as against 50-100 grammes recommended by ICMR (India Council for Medical Research). This disquieting phenomenon can be overcome by identifying the alternate source to meet up the need for growing demand. Makoi can serve this purpose as it was reported to be a rich source of vitamins and minerals such as riboflavin, Vitamin C, niacin, iron and calcium. In India, especially in Tamil Nadu, Makoi is a prime candidate for cultivation by the small farmers with its leaves and berries used as a nutritious vegetable.

Considering the importance of the crop and the vast genetic diversity existing in this species, the genotypes from different ecological regions are to be evaluated on different parameters. The growing demand for this crop necessitates attempts to preserve and utilize new variability with high yield, high alkaloid content and high nutritive value.

Hence, a systematic study to understand the nature and extent of variability has to be made and are imperative for promoting superior types among them as direct cultivars or harnessing the variability through appropriate breeding programme for developing improved varieties. The characters of economic importance are generally quantitative in nature and exhibit considerable degree of interaction with the environment. Thus, it becomes necessary to compute variability present in the material and its partitioning into genotypic, phenotypic, and environmental effects (Shukla *et al.,* 2006).

Solanum nigrum L. is found in different forms (n=12, 24 and 36), constituting a complex assemblage. Each form differs from the other in minor morphological characters. This complex assemblage is now named as *Solanum nigrum* Complex because it is composed of a large number (about 30) of morphologically distinct taxa with basic chromosome x=12 (Schilling and Andersen, 1990). This complicated polyploidy complex has lead to much of the taxonomic confusion surrounding this species.

The situation has been further complicated by a number of authors, who have persistently treated different members of the section as belonging to one species, *S. nigrum* L. The some medicinally important taxa belonging to *S. nigrum* Complex are *S. americanum* Mill., *S. nigrum* L. and *S. villosum* Mill. *Solanum scarbum* Mill., *S. chenopodioides* Lam., *S. retroflexum* Dunal. which displays similar morphological features with few phenotypic variations. Natural hybridization is probably more widespread in this section and is supposed to be the reason for complexity.

Genetic diversity is normally assessed by common morphological traits. However, such traits are affected by effects of environment, development stage of the plant, also the type of plant material. Assessment of genetic diversity with molecular markers is devoid of environmental effects and provides a true representation of the entire genome.

Simple sequence repeat markers (SSR) are being extensively used in genome studies, marker-assisted selection, and cultivar identification and are well-known for their versatility in providing a quick assay and for their highly informative data (Song *et al*., 1999; Cregan *et al*., 1999).

Recently taxonomist has started using number of techniques to solve the taxonomic confusion which could not be resolved by morphological markers. One of these could be the use of DNA barcoding which is an effective, reliable and simple tool to resolve the confusion in morphological identification. It is a diagnostic technique for species identification, using a short, standardized DNA region (www.barcoding.si.edu). It is a technique in which species identification is performed by using DNA sequences from a small fragment of the genome, with the aim of contributing to a wide range of ecological and conservation studies in which traditional taxonomic identification is not practical.

Keeping in view of all these aspects, the present investigation was taken with the following objectives

1. Morphological characterization of genotypes using descriptors and species confirmation
2. To study the *per se* performance of 45 genotypes
3. To study the magnitude of genetic divergence through D^2 analysis
4. To study the genetic divergence using SSR markers
5. To determine the association of various components with yield and their interrelations by estimating the correlation co-efficients
6. To study the direct and indirect effects of various components on yield by path analysis

Review of Literature

CHAPTER II

REVIEW OF LITERATURE

Medicinal plants are the local heritage with global importance. World is endowed with a rich wealth of medicinal herbs. These plants have always been the principal form of medicine in India and presently they are becoming popular throughout the World. Various drug potentials of these medicinal plants were already documented in Ayurvedic, Siddha, Unani and Homeopathy systems of medicine. Only few plants are cultivated and the remaining plants are being collected from wild source. Due to increasing demand in the pharmaceutical industries, the scientific cultivation and crop improvement of medicinal plants is the need of the day.

Among various medicinal plant species, *Solanum nigrum* L (Manathakkali in Tamil, Kakamachi in Sanskrit, and Makoi in Hindi) has great demand in the Indian system of medicine. This herb is recently gaining importance in pharmaceutical industry due to its amazing drug potential. The whole plant is recommended for use as cardiac tonic, alternative, diuretic, sedative, expectorant, diaphoretic, cathartic and anodyne. Decoction of its leaves is used in dropsy, chronic enlargement of liver and in jaundice. The juice of the plant is also prescribed in chronic skin disease, blood spitting, piles, inflammation of kidney and urinary bladder and in gonorrhoea. Berries of the plants are alterative, diuretic and are useful for curing fevers, diarrhoea and heart diseases (Chopra *et al*., 1986).

The active principle in this herb is solasodine, a glucosidal alkaloid, used as a starting material for the synthesis of hydrocortisones. It has great demand in the pharmaceutical industry. It is also indicated that the compound GMD-1630 obtained from the whole plant of *S. nigrum* L. has anticancer properties and its preparation has been patented by China. This therapeutic value indicates the herb as one of the commercially viable and valuable plants in future for exploitation.The scientific literature pertaining to *Solanum nigrum* L., are reviewed under different headings.

2.1. Description of the plant

Solanum nigrum L. is an erect to spreading, 90 to 100 cm tall, branching, annual to short-lived perennial herb to sub shrub. Roots are mainly fibrous with a shallow taproot. Stems are round to angular and covered with curved multicellular hairs sometimes tipped with a glandular head. Simple leaves are 2 to 8 cm long by 1 to 5.5 cm wide, ovate or lanceolate or rhamboidal in shape with wavy-toothed margins.

Inflorescences are simple, with pedicels closely clustered and forming an extended cyme or raceme-like cluster of 2 to 10 flowers. Flowers are 4 to 18 mm in diameter and bell-shaped, with a star-shaped, white corolla with a translucent to yellowish basal star. The calyx consists of ovate sepal lobes that are 1.2 to 2.5 mm long and a star-shaped corolla of five white lobes that are 1.8 to 7.5 mm long. Fruits are broadly ovoid and have dull purple to black or yellowish-green berries that are 6 to 10 mm broad. Berries may remain on the plant or fall to the ground when ripe. Fruit are without sclerotic granules on the surface. Seeds are tan, obovate, flat in cross-section, 1.7 to 2.4 mm long (Defelice, 2003).

2.2. Distribution of the plant

Solanum nigrum L. is one of the largest and most variable species groups distributed from temperate to tropical regions and from sea level to an altitudes over 3500 metres (D'Arcy, 1991). It is widely distributed in various habitats throughout the world particularly in Africa and South East Asia. In India, makoi is found growing in dry parts upto an elevation of 2,100m. Its wide tolerance to habitat types and its prolific seed production contribute to the success as a widespread species (Henderson, 1974).

2.3. Phytochemicals

S. nigrum L. has been investigated by many researchers especially for its steroidal alkaloids and flavonoids. Leaves and tender shoots are widely used as vegetable throughout the world and have provided a food source since early times, with *S. nigrum L.* being recorded as a ancient famine plant of Chinese (Henderson, 1974). Following is the summary of the compounds identified and isolated from *Solanum nigrum* L.

2.3. 1. List of phytochemicals reported in *Solanum nigrum* L.

Class of compound	Compound name	References
Steroidal Glycoalkaloid	Solasodine	Schreiber, 1958; Eltayeb *et al.,* 1997 and Ata *et al.,*2011
	Solasonine	Aslanov, 1971, Hu *et al.*1999 and Atanu *et al.,*2011
	α-Solamargine	Schreiber, 1958; Aslanov, 1971; Aslanov and Novruzov, 1978.
	Solanigridine	Schreiber, 1958.
	Solanine (α,β)	Abbas *et al.,* 1998; El-Ashaal *et al.*, 1999.
Steroidal saponins	Diosgenin	Khanna and Rathore, 1977.
	Nigrumnin I and II	Tsuyoshi *et al.*, 2000.
Sterols	Campesterol	Bhatt and Bhatt, 1984.
Flavonoids	Quercetin-3-galactoside	Nawwar *et al.,* 1989, Atanu *et al.,* 2011
	Isoquercitrin	Nawwar *et al.*, 1989.
Nutrients	Proteins, Calcium, Ir Vitamin C and Crude fiber	Edmonds and Chweya (1997) Veeraragavathatham *et al.* (1998) Akubugwo *et al*. (2007)

2.4.1. TAXONOMIC COMPLICATION IN *Solanum nigrum* L.

S. nigrum L. was first delimited in four taxa with polynomials by Dillenius. Linnaeus subsequently modified Dillenius's work, describing these in six varieties under the binomial *S. nigrum* (Edmonds and Chweya, 1997). The boundaries between many of the species are still ill-defined, with many of the new taxa proving to be no more than slight morphological variants of those already described. The situation is further complicated by the researchers who either treated different members of the section as varieties of *S. nigrum* or considered them as different species on the basis of morphological differences (Edmonds and Chweya, 1997; Schilling and Andersen, 1990). Hawkes & Edmonds (1972) and Baytop (1978) gave the rank of sub species to *S.villosum*. Nasir (1985) considered *Solanum nigrum L.*as species and villosum as as the variety while Jennifer and James (1997) gave the rank of species to all. The taxa of *S. nigrum* Complex are difficult to distinguish as they are morphologically similar and are all highly phenotypically plastic.

Three taxa belonging to *S. nigrum* Complex *viz. S. americanum* Mill., *S. nigrum* L. and *S. villosum* Mill. had been reported in India (Ganapathi and Rao,1986). Zubaida Yousaf *et al.* (2006) reported few morphological markers for distinguishing the species. *S. nigrum* L is different from *S. villosum* Mill. in the respect that the former has black matured berries with peduncles longer than pedicels while latter has orange or red matured berries and peduncles shorter than or equal to the pedicels. S.americanum Mill. can be distinguished from *S.nigrum* L. by its smaller seeds, umbellate inflorescence rather than the raciform, small anther and shiny fruits in *S.nigrum L.* Natural hybridization which is widespread in this section is supposed to be the reason for complexity. It is now named as *Solanum nigrum* Complex because it is composed of a large number (about 30) of morphologically distinct taxa (Schilling and Andersen, 1990).

2.4.1. Comparision of morphological characters of *Solanum nigrum* L.complex

Characters	*Solanum american* Mill.	*Solanum nigrum*	*Solanum villosum* Mill.	Reference
Plant height	Semi erect w spreading branches	-	Erect and branched	Beg *et al* (1989)
	Erect and spars branched	Erect and branch	Tall erect and branched	Anil kumar and Pushpangadan (2005)
Stem	Cylindrical, w smooth ridges	-	Dark green with purplish tints	Beg *et al* (1989)
	Thick green cylindr and sparsely pubesc with ridges	Cylindrical and gr with pubescence	Cylindrical and dul green with purplish tinge and prominen ridges	Anil kumar and Pushpangadan (2005)
Leaf shape	Lanceolate	Thick and ovate	Thick, dark green a ovate	Anil kumar Pushpangadan (2005)
	Ovate to lanceolate	Ovate to lanceolat	Rhombic to ovate	Mohy-ud-din (2008)
Leaf margin	Wavy	Toothed	Dentate	Anil kumar and Pushpangadan (2005)
	Entire to sinuate	Sinuate or dentate	Entire to sinuate	Mohy-ud-din (2008)
Leaf length(cm	4.40 - 7.60	-	3.20 - 5.20	Beg *et al* (1989)
	4.30 - 8.70	4.40 - 11.10	3.20 - 5.20	Anil kumar and Pushpangadan(2005)
	2.50 - 3.20	2.51 – 7.00	2.00 - 7.00	Mohy-ud-din (2008)
Leaf breadth (cm)	2.40 - 5.02	-	2.40 - 4.50	Beg *et al* (1989)
	3.20 - 5.20	2.80 - 5.90	3.50 - 5.10	Anil kumar and Pushpangadan(2005)
	1.10-4.00	2.00-4.50	1.50-4.00	Mohy-ud-din (2008)
Inflorescence type	Umbellate cymes	Extended cymes	Umbellate to solita cymes	Mohy-ud din (2008)
Flowers per inflorescence	4 - 9	-	3 - 9	Beg *et al* (1989)
	2 - 6	3 - 5	3 - 9	Anil kumar and Pushpangadan (2005)

Calyx length(mm)	1.1 - 2.0	1.2 - 2.5	1.2 - 2.2	Mohy-ud-din (2008)
Corolla color	White, occasionally purple	White	White	
Style length (mm)	1.2 - 3.5(4.5)	2.8 - 3.5 (4.5)	2.9 - 5.0 (6.0)	
Berry diamete (mm)	5 - 8	-	6 - 8	Beg *et al* (1989)
	6 - 8	4 - 7	4.50 - 9.00	Anil kumar and Pushpangadan (2005)
	4 – 7 (8)	6 -10	6 - 10	Mohy-ud-din (2008)
Berry shape	Globose	Broadly ovoid	Occasionally globo	Mohy-ud-din (2008)
Berry color	Bluish black	-	Orange red	Beg *et al* (1989)
	Shiny black	Purplish black	Orange red	Anil kumar and Pushpangadan(2005)
	Black, rarely dark green	Dull purple	Red, orange	Mohy-ud-din (2008)
Seeds per berr	30 - 50	-	24 - 40	Beg *et al* (1989)
	35 -50	40 - 50	30 - 45	Anil kumar and Pushpangadan (2005)

2.4.1.2.Polyploidy in *Solanum nigrum* L.

Polyploidy, the condition of having more than two genomes in a cell, is widespread in higher plants and represents a major evolutionary force. More than 75% of flowering plants experienced at least one polyploid event in their lineage, either by doubling of a single genome (autopolyploidy) or more commonly by combining two or more different genomes (allopolyploidy) (Masterson, 1994). World's most important crop plants are polyploids such as wheat, cotton, potato, banana, apple, peach, plum, strawberry, coffee and sugarcane, which indicates that genome doubling, has some advantages for the existence and diversification of crop plants. These advantages are known to include enhanced

vigour, increased heterozygosity with novel variation. (Birchler *et al.*, 2010 and Matsushita *et al.,* 2012).

The genus *Solanum* L. is one of the most abundantly represented among angiosperms in terms of species diversity. *Solanum* contains several plants of economic importance with basic chromosome number is x = 12. Among them, *Solanum nigrum* L. shows a polyploid series with *n* = 12, 24 and 36. There are a lot of controversies about the nature of polyploidy and the course of evolution in *Solanum nigrum L*. (Tandon and Rao, 1964)

Anil kumar and Pushpangadan (2005) studied molecular systematic study of variants of *Solanum nigrum L*. in India and found it to be a widely distributed polyploidy species with naturally occurring diploid, tetraploid and hexaploid forms.

Morphological and cytological studies conducted by Ganapathi and Rao (1986) concluded that the shiny bluish black, orange red and purplish black berried types were diploids (2*n*=2*x*=24), tetraploids (2*n*=4*x*=48) and hexaploids (2*n*=6*x*=72) in nature respectively.

Species belonging to the *Solanum nigrum* comlpex are predominantly self-pollinating, but out and crossbreeding can occur resulting in natural inter and infraspecific hybridizations which leads to the polyploidy series (Edmonds and Chweya, 1997).

2.4.1.1.2. Cytogenetical characters

In many of plant species, there are correlation between ploidy level and cytogenetic characteristics such as chloroplast number in guard cells, size of stomata cells, stomatal density and pollen grain diameter, which appears to be less influenced by environmental factors.

Relaiability of ploidy determination using stomatal measurements and pollen diameter were reported in banana by Elain Apsara (2000) and Ganga *et al.* (2002). In pepper, stomatal density and the number of chloroplast in guard cells seemed to be reliable for the estimation of ploidy level (Abak *et al.*, 1998). Stomatal size and changes in plant morphology were found to be useful indicators in the primary screening for new ploidy level in M1-generation of *Viola* × *Wittrockiana* Gams (Ajalin *et al*., 2002).

In *Coffea* sp., stomatal and epidermal cell frequency per unit leaf area decreased while stomatal guard cell length increased with an increase in ploidy. The reduction in

stomatal frequency at higher ploidy levels was mainly a result of larger epidermal cells. (Mishra, 1997).

Jaskani *et al.* (2002) studied the growth, morphology and fruit behavior of diploid and tetraploid trees of Kinnow mandarin *(Citrus retieulata* Blanco) and reported that tetraploids had thicker and broader leaves (5.29 cm). The diploids had high number of stomata/unit leaf area (7.42) but the stomata were larger in tetraploid leaves (28.18 µm). Fruit weight (153.3 g), fuit diameter (18.4 cm), rind thickness (0.70cm) were higher for tetraploids whereas diploids yielded higher TSS (9.5%), juice percentage (46.1 %) and number of developed seeds (19.0).

Murti *et al*. (2012) studied the morpho-anatomical, physiological and pollen characters in ploypoids of strawberry and reported that increasing ploidy level increased petiole, stem diameter, leaf thickness, stomata size, plastid number per cell, chlorophyll content and pollen size but decreased stomata density per unit area, pollen fertility and no fruit set as a result of seedlessness.

Vanessa *et al.* (2004) reported that higher the ploidy, greater was the size of the pollen and the stomata and the lower their number per unit area in *Stevia rebaudiana*. The triploid strain of stevia produced the shortest plants and the lowest number of inflorescences, whereas the tetraploid strain had the largest leaves.

Jaskani *et al.* (2005) reported in tetraploid watermelon that higher leaf area and larger flower size, larger pollens were observed. Tetraploid seed showed larger size and thicker seed coat and partially filled seed cavity due to less developed cotyledons as compared to diploids.

Cultivated alfalfa constitutes a complex taxonomic group called the *Medicago sativa-falcata* complex. which includes a number of diploid (2n = 2x = 16) and tetraploid (2n = 4x = 32) taxa. Sakiroglu and Brummer (2011) identified 67 diploid and 82 tetraploid among 149 accessions using flow cytometry.

Jaskani *et al.* (2005) used flow cytometry for ploidy clarification since chromosome counts were tedious and needed more time to identify tetraploids in watermelon. Autotetraploids in *Ocimum basilicum* was identified using flow cytometry (Omidbaigi *et al.*, 2010).

2.4.2. Species confirmation using DNA Barcoding

DNA barcoding is a molecular tool to identify the plant species using the unique short DNA sequences, located in the genome of nucleus or mitochondria or plastid. Plant DNA barcoding is gaining its importance in various fields which includes resolving the taxonomic disputes, biodiversity inventorization of new or cryptic species and conservation of biotic resources by studying the evolutionary relationships among the related taxa and ther by deriving phylogenetic linkage between the species. The important potential barcode candidates(short DNA sequences) proven to identify plant species includes *rpoc*1, *atp* B, *psb* A, *trn* H and Internal Transcribed Spacers (*ITS*).

Even though morphological keys are handy in identifying the right species, the assessment of the genetic variability are required. In plant DNA barcoding studies, nuclear DNA are proved best compared to chloroplast DNA because of their effective means of identification of poyploid species (Soltis and Kuzoff, 1995). The nuclear DNA contains both coding and non coding regions in which some of the regions like, Internal Transcribed Spacers (*ITS*) are involved more rapidly and these can be useful for phylogenetic analysis and to solve toxonomic disputes (Mindell and Honeycutt, 1990).

The amplication efficiency of the *ITS* region is high, when compared to the other barcode canditates and hence it can be further subjected to restriction digestion, which generates distinct bands at species level (Manhart and Mc.court, 1992 & Avise, 1994).

In recent years, efforts have been made to accurately identify medicinal plants used in raw drug trade to ensure the purity, quality and safety of drugs (Sucher and Carles, 2008). For this purpose several DNA barcoding methods have been developed for the identification of medicinal plants (Jayasinghe *et al.,* 2009). Asahina *et al*. (2010) investigated five medicinal *Dendrobium* species *viz., Dendrobium fimbriatum, D. moniliforme, D. nobile, D. pulchellum, and D. tosaense*. The phylogenetic trees constructed from matK barcode candidate successfully distinguished each species from each other.

Misra *et al.* (2006) developed an AFLP based detection of adulterants in crude drug preparations of the Safed musli (*Chlorophytum)* complex. Jain *et al.* (2008) developed

SCAR markers to identify three species of *Phyllanthus* used in dry leaf bulk herb trade. Srirama *et al*. (2010) used DNA barcoding technique to distinguish *Phyllanthus amarus* (Keezhanelli) from its species admixtures like *Phyllanthus fraternus* and *Phyllanthus maderaspatensis*.

Tribulus terrestris L. (Zygophyllaceae) is one of the highly traded raw drugs and also used as a stimulative food additive in Europe and USA. The nuclear ribosomal RNA genes and *ITS* (internal transcribed spacer) sequence were used to distinduish it from its adultrants *T. lanuginosus and T. subramanyamii* (Balasubramani *et al.,* 2010)

ITS region was used as barcode candidate in discrimination of coscinium fenestratrum species from its substituents arcangelisia flava in the Thai folk medicne (Wattanachaiying charoen *et al,* 2010).

ITS 2 can be used as an efficient and powerful marker and a potential barcode to distinguish Twenty four species in *Fabaceae* which were found in the Chinese Pharmacopoeia (Gao *et al*.,2010). Barcoding is used to identify *A. graveolens* and *F. vulgare* which are difficult to physically distinguish leaf material once it has been dried and packaged (Schori and Showalter, 2011).

Boerhavia diffusa whose roots are used in the treatment of jaundice and liver disorders are adultured with other species like *B.erecta, B.reanda, B.coccnea, B.diffusa.* Nuclear ribosomal internal transcibed spacer (*ITS)* was used as DNA barcode candidate to distinguish the species (Dhivya *et al.,* 2012)

The DNA barcode canditates, *matK* and *trnH-psbA* spacer are used to differentiate ethnomedicinal plants (*Catharanthus roseus* L., *Alstonia scholaris* L., *Thevetia Peruviana* (Pers.) Merrill, *Allamanda cathartica* L., *Tabernaemontana divaricata* L. *Calotropis gigantea* L. belonging to the family Apocynaceae (Mahadani *et al.,* 2013).

2.5. Morphological characterization

Morphological characters provided fundamental basis for the classification of plants. Specialized features of stem, leaf, flower, fruit and seed mostly characterize the family

Solanaceae. Plant habits, stem structure and colour, petiole status, petiole pubescence, leaf shape, leaf margin, leaf apex, leaf base, flower shape, flower colour, fruit shape, fruit type and seed colour are greatly helpful in the identification of the species (Symon, 1981; Nasir, 1985 and Pojarkova, 1997). In the identification of *Solanum* species qualitative characters play a key role as compared to quantitative characters. Therefore, they have significant contribution in the taxonomy of the genus *Solanum*. Within the species variation among the quantitative characters was least, therefore it is difficult to differentiate on the basis of quantitative characters (Symon, 1981; Hawkes and Edmond, 1972).

2.8. Genetic variabilty

Genetic variability in the base population plays a very important role in any crop-breeding programme. The extent of genetic variability is more important since greater genetic diversity leads to wide scope for crop selection. The characters of economic importance are generally quantitative in nature and exhibit considerable degree of interaction with the environment. Thus, it becomes imperative to compute variability present in the breeding material. The extent of variability of a character is measured by different statistics *viz.*, phenotypic variance, genotypic variance, phenotypic coefficient of variation and genotypic coefficient of variation (Allard, 1960). The work carried out by different authors on variability in medicinal and aromatic crops and important Solanaceous vegetables are reviewed here under.

2.8.1. Variability in morphological parameters

Pandita and Bhan (1999) reported that the phenotypic and genotypic coefficient of variations for plant height were low (10.35% and 9.04%) in *Asparagus* genotypes. Significant differences for plant height with high heritability estimate (96.10 %) and genetic gain (51.57%) were observed by Ibrahim and Hussein (2006) in *Hibiscus sabdariffa*.

Singh *et al*. (2000) observed phenotypic and genotypic coefficient of variation of 8.70 and 10.33 per cent respectively for plant height in *Mentha arvensis*. The same trait also had a positive and significant association with herb yield and oil yield.

Ahmad and Khaliq (2002) recorded PCV and GCV of 11.68 and 10.79 per cent respectively for plant height in four genotypes of *Ocimum sanctum*. Krishnamoorthy and Madalageri (2002) studied genetic diversity in fifteen ajowan genotypes. They observed that the heritability was high for number of seeds per umbel (0.977), number of leaves per plant

(0.976), essential oil yield (0.964). Singh *et al*. (2003) observed low variability and high heritability estimate of 87.0 per cent with a genetic advance of 13.6 per cent for plant height in *Opium poppy*.

Genetic diversity among thirty nine genotypes of coriander was estimated by Megeji and Korla (2002). The genotypic coefficient of variation was found to be higher for leaf yield per plot (63.94%) followed by seed vigour (51.95%)

Sudhir Shukla *et al*. (2003) reported significant differences for plant height ranging from 103.6 to 155.6 cm in fennel genotypes. The genotypic and phenotypic coefficient of variations was 8.35 per cent and 8.50 per cent respectively. High heritability (96.54 %) with genetic advance of 21.66 per cent was also obtained.

Among 20 genotypes of coriander significant differences for plant height with high heritability estimate (86.20%) and genetic gain (19.03%) were observed by Shah *et al.* (2003). Banerjee and Kope (2004) reported that phenotypic and genotypic coefficient of variation in fenugreek were high for stem weight, branches per plant, days to flowering and low for pod length and plant height.

Khurana *et al*, (2003) reported in chillies that among the characters studied, highest heritability value were observed for plant height and number of branches per plant.

Kavitha (2005) observed PCV and GCV values of 18.09 and 17.14 per cent respectively for stem girth in *Coleus forskohlii*. High heritability (90.00%) with GA of 33.47 per cent was also obtained.

Investigation by Hazra *et al.* (2000) recorded that number of leaves per plant at 180 days of planting exhibited high broad sense heritability and high genetic gain in turmeric. Shalini *et al.* (2001) reported higher genetic diversity in five *Bacopa monnieri* genotypes and the high divergence was contributed by small leaves and number of leaves.

Krishnamoorthy and Madalageri (2002) studied genetic diversity in fifteen Ajowan (*Trachyspermum ammi*) genotypes. They observed that the heritability was higher for number of leaves per plant and number of tertiary branches.

A wide range of variability was observed among fifteen accessions of *Solanum viarum* Dunal for days to flower, number of berries and yield per plant. The highest genotypic variance was observed for yield per plant and lowest for solasodine content.

Genetic advance was high for yield per plant followed by number of berries per plant (Singh *et al.,* 1985*)*.

Twenty six genotypes of ashwagandha were evaluated for fourteen quantitative traits. PCV, GCV and heritability were higher for important traits like number of berries per plant and fresh weight of berries per plant (Sangwan *et al*., 2013).

Misra and Yadav (1991) observed high heritability coupled with genetic advances for number of branches per plant in Safflower. Ibrahim and Hussein (2006) obtained wide variability at phenotypic and genotypic levels for number of branches per plant in *Hibiscus sabdariffa*. They also observed high heritability estimates of 39.00% and 53.00% in first and second season crop respectively.

Metha *et al.* (1992) reported in fenugreek that from a set of forty nine genotypes, the highest variability existed for number of branches per plant.

In fenugreek, significant differences among the genotypes for greens were observed by Hariharan (1996) for plant height, number of leaves per plant, total dry matter production, greens yield per plot, ascorbic acid content and leaf protein.

Tripathi *et al*. (2000) reported the estimates of high heritability and low genetic advance as per cent mean for plant height and low heritability with low genetic advance for primary branches per plant, whereas high heritability coupled with moderate genetic advance was observed for number of secondary branches per plant in coriander.

High heritability coupled with fairly high genetic advance were observed for plant height, yield of greens per plant, weight of leaves, weight of stem and leaf stem ratio, indicating the perponderance of additive gene action, for the traits could be very well utilised for selection of suitable genotypes in coriander (Ann Riya, 2001)

Mahalakhsmi (2006) observed the PCV and GCV values for number of branches were 19.87 and 19.01 respectively in *Phyllanthus amarus* genotypes. Heritability estimates of 91.54 per cent was observed on 90^{th} day after transplanting and the genetic advance as per cent of mean was 37.48 per cent.

In *Abrus precatorius,* all the accessions showed considerable variability for the majority of traits, the highest was in number of branches per plant (38.02%) observed by Jotshi *et al.* (2008).

Gaurav *et al. (*2008) in ten genotypes of *Stevia rebaudiana* reported higher estimation of PCV and GCV for leaf length, leaf width, plant height and stevioside content. The highest estimate of genetic advance was observed for the plant height (20.86) followed by number of leaves per plant (17.85).

Sivaneson and Ranwah (2009) reported that higher magnitude of GCV, PCV and heritability was for total phenolic compounds in leaves followed by husk yield per plant in 33 isabgol genotypes (*Plantago ovata*).

Variability studies conducted among 25 genotypes of *Ocimum spp* revealed highest estimates of heritability for plant height (99.70 %) followed by length of inflorescence (98.80 %) and fresh herb yield per plant (98.60%), The highest value of genetic advance was obtained for fresh herbage yield per plant (1018.02) followed by dry herb yield per plant (394.31) (Shehbaz Khan *et al.*2012).

2.8.2. Flower characters

Verma *et al*. (1998) studied genetic diversity in *Mentha* species where days to 50% flowering showed very high heritability. Ahmad and Khaliq (2002) found that high variability in days to 50 per cent flowering with high GCV (7.50%), PCV (8.30%), heritability (79.93%) and genetic advance (9.27) in four land races of *Ocimum sanctum*.

Genetic diversity in thirty genotypes of fennel was studied by Sudhir Shukla *et al*. (2003). The heritability was maximum for number of umbels per plant (96.54%) followed by umbellets per umbel. Ananya Banerjee and Kope (2004) reported that phenotypic and genotypic coefficient of variation in fenugreek was high for days to flowering and duration of flowering.

Singh *et al*. (2005) studied genetic diversity in thirty five genotypes of coriander and reported a high degree of both phenotypic and genotypic coefficient of variation for number of umbels per plant. In cumin, Panesar and Jadeja (2008) observed the umbels per plant showed significant positive correlation with grain yield at phenotypic level.

2.8.3. Yield characters

Hegde (1992) observed high heritability estimate of 78.47 per cent with 149.1 per cent genetic advance for dry weight of foliage in *C.forskohlii* at 190 days after planting.

In opium poppy, genetic diversity study carried out by Singh *et al*. (2003) in twenty two genotypes indicated that the capsule weight (23.38%) exhibited maximum diversity towards genetic divergence followed by capsule size (20.78%).

Data *et al*. (2005) studied genetic diversity in fenugreek and opined that the number of pods per plant had the highest direct effect on seed yield. High heritability (broad sense) and genetic gain was observed for pod weight (98.00% and 57.38% respectively) in *Pongamia pinnata* (Naresh Kaushik *et al.,* 2007).

Variability and correlation were investigated by Jotshi *et al.* (2008) in 7 accessions of *Abrus precatorius* for 19 phenological characters. Days to harvest had a positive significant correlation with pod width, number of seeds per pod and pod index (r= 0.258, 0.244, 0.336) respectively.

In *Withania somnifera* the highest GCV and PCV were recorded for dry root yield followed by plant canopy and the lowest for plant height. The genetic advance as percentage of mean was the highest for plant canopy followed by dry root yield. (Misra *et al.,* 2009)

2.8.4. Variability in physiological and biochemical parameters

Leaf chlorophyll content is one of the important physiological traits, which influences photosynthesis and thus it is indirectly associated with growth and yield of plants (Ekanayake *et al.,* 1996). Decrease in photosynthetic activity is often paralleled by a reduction in leaf chlorophyll content (Ekanayake *et al.,* 1998).

Ganjwala *et al. (*2000) studied genetic diversity in *Bacopa monnieri* and found a wide variation in bacoside content (0.45 -0.71 per cent). Zeinali *et al*. (2004) reported that genotypic and phenotypic coefficients of variation were high for the essential oil content in *Mentha* genotypes (38.5 per cent and 34.1 per cent respectively).

Sharma *et al.* (2009) studied variability among 15 *Andrographis paniculata* genotypes and reported that the andrographolide content ranged from 0.69 to 1.85 per cent and the genotype KI-2 had the highest estimated content. Chitra and Rajamani (2009) reported wide variation (0.02 to 1.33 per cent) in the colchicine content among 18 genotypes of glorylily and higher values were recorded in the genotype GS 15.

Sukanya *et al.* (2010) studied chemical diversity among one hundred and eight selected ashwagandha accessions and reported that the total withalonide content ranged from 0.066 to 0.588 per cent on dry root basis indicating considerable diversity in the collection for phyto-chemicals.

Ravi *et al*. (2011) evaluated 17 makoi genotypes and reported that total alkoloid content ranged from 0.08 to 0.22 per cent among the genotypes. Palanikumar *et al.* (2012 a) studied variability among 75 coriander genotypes and reported that the essential oil content ranged from 0.12 to 0.48 per cent and the genotype UD 685 had the highest estimated essential oil content.

2.9. Genetic divergence by D^2 statistic

The concept of D^2 statistic developed by Mahalanobis (1936) is useful in quantifying the degree of divergence between the biological populations at genotypic level and to assess the relative contribution of different components to the total divergence at both intra and inter cluster levels. Genetic divergence is more important for selecting parents for hybridization than eco-geographical isolation and is analysed using D^2 statistic. The studies on genetic divergence carried out by different workers are reviewed below.

Genetic diversity of twenty genotypes in *Ocimum viride* was studied by Pal *et al.* (1987) through D^2 for ten characters. They found that highest divergence was contributed by number of branches followed by oil yield, herb yield and oil content.

Dharmatti *et al.* (2001) assessed 402 tomato lines using multivariate analysis and grouped them into 4 cluster based on similarities of D^2 values. Cluster-I was the biggest having 217 genotypes, cluster-II consisting of 51 genotypes/ hybrids with potato leaf type and pink fruit and cluster-III and IV had 99 and 35 genotypes respectively.

Lal *et al*. (2001) evaluated thirty diverse citronella genotypes and grouped them into seven clusters. They reported that there existed negative correlation between leaf length

(71.41%) and citronellal per cent (10.85%). Shalini *et al*. (2001) reported genetic diversity in five *Baccopa monnieri* genotypes and that the high divergence was contributed by number of leaves.

In *Opium poppy*, genetic diversity study carried out by Singh *et al.* (2003) in twenty two genotypes indicated that the capsule weight (23.38 %) contributed maximum towards genetic divergence followed by plant height (17.32 %) and opium yield (14.29 %). Genetic diversity of thirty chilli genotypes was studied by Choudhary and Samadia (2004) through D^2 analysis for 17 characters. The plant height, number of fruits per plant, fruit yield per plant had maximum contribution towards divergence.

Seventy four genotypes of amaranthus were evaluated for diversity for green yield and related characters. D^2 analysis grouped the genotypes into twelve clusters. The study revealed that the clustering pattern of the genotypes showed a wide spectrum of variability and the genotypes did not resolve according to their geographical distribution. (Selvaraj, 2004).

A cluster analysis was carried out for twelve landraces of Iranian mint and was divided into 3 clusters. The number of nodes per main stem, number of lateral branches, spike width of main stem, leaf width, days to 50 % flowering, plant height and nodes per lateral branches were the major sources of diversity among the mint clones. Strong association was observed between leaf length and essential oil content ($r = 0.73$). (Zeinali *et al*., 2004)

Genetic diversity in thirty four genotypes of brinjal was studied by Kushwah and Bandhyopadhya (2005) through D^2 for thirteen characters and the number of fruits per plant, fruit diameter and number of pickings were found to be important characters contributing to divergence.

Deepti Rai and Mishra (2005) studied twenty characters in bael and grouped them into three clusters. Ascorbic acid content, fruit weight, fruit length, number of seeds per fruit, contribute maximum for genetic divergence. Mahesh *et al*. (2006) grouped 30 tomato genotypes into nine clusters based on D^2 analysis. The cluster mean indicated that days to 50% flowering, plant height, number of branches per plant, number of clusters per plant, number of fruits per cluster and fruit yield per plant were reported as main contributors towards divergence.

Studies of Kanthaswamy (2006) in seventy four genotypes of amaranthus revealed the grouping of genotypes into twelve clusters, where cluster I was the largest with fifty two genotypes. Based on the intra cluster values, cluster XI and XII were identified as genetically more divergent.

Based on D^2 analysis, Kalpande *et al.* (2007) conducted the genetic divergence of sixty one genotypes in grain amaranthus (*A. hypochondriacus* L.), which were grouped into ten clusters. The major characters contributing to genetic divergence were plant height, stem girth and grain yield.

Genetic divergence among 31 genotypes was determined using nine characters of *C. borivilianum* of indigenous origin using Mahalanobis D^2 statistic. The genotypes were grouped into eight clusters. Intra-cluster distance was largest for cluster VIII (nine genotypes), followed by cluster I (six genotypes) (Birendra Kumar *et al.*,2008).

Studies of Pandey and Singh (2010) in ninety eight genotypes of grain amaranthus (*A. hypochondriacus* L.) revealed the grouping of genotypes into eighteen clusters. The characters such as leaf size, grain yield and plant height contributed more towards genetic divergence. Genetic divergence among 75 coriander genotypes was determined using eleven characters using Mahalanobis D^2 statistic. The genotypes were grouped into eight clusters. Intra-cluster distance was largest for cluster I with 64 genotypes. Fresh weight of leaves contributed maximum to the genetic divergence (Palanikumar *et al* ., 2012d).

2.10. Genetic diversity analysis by molecular markers

The rapid advancement in molecular marker technology has provided new classes of genetic markers at the DNA level. The advent of DNA based markers has opened up new avenues and uncommon opportunities for biological science in the area of evolutionary studies (Gupta *et al.*,1992; Kapila *et al.*,1996), plant systematic and more recently in the tagging of genes coding for traits of agronomic importance. DNA markers are considered the best tools for determining genetic relationships or diversity, as they are unlimited in number, show high polymorphism and are independent of environment interaction i.e., highly heritable.

Though the morphological characters are important for initial genetic evaluation studies, it is often beset with many problems. They are subjected to environmental conditions to show differential expression which depends on ontogeny, not distributed

throughout the genome, and it necessitates the growth of plants to maturity and recording can be rather subjective.

2.10.1. SSR Markers

SSRs, called microsatellites, are randomly interpersed in eukaryotic genomes. They are highly variable in the number of repeats they contain and are co-dominantly inherited. Their polymorphisms have shown high efficiency for many studies. The existence of a set of single locus, codominant and highly polymorphic markers that are distributed along the whole genome, quickly and easily detectable would be a powerful tool for variability analysis and fingerprinting. SSRs are the only molecular markers currently available that fit all these requirements. The use of such a set by different research groups would allow direct comparison of results, which is hardly feasible with the most widely used DNA markers of the recent past, like RAPDs or AFLPs.

Markers commonly used in medicinal and aromatic plants mainly include random genomic markers, e.g., RAPD, SSR, SCAR, and SNP and ribosomal-DNA (Joshi *et al.* 2004 and Canter *et al.*, 2005). These markers have been used in *Cymbopogon* and *Mentha* (Khanuja *et al*., 2000 and 2005; Shasany *et al.* 2005) and have helped in characterizing elite varieties of these medicinal and aromatic plants. Efforts have also been made for the development of PCR-based high throughput markers (e.g., SSR, SNP, SCAR) in some medicinal plants (Blum *et al.*, 2003; McCallum *et al*., 2007), but they need to be developed in all medicinal plants. Different molecular markers have been found to be useful in differentiating accessions of medicinal plants are furnished below:

2.10.2. Different types of markers used in diversity studies of medicinal plants

S.No	Crops	Reference
RAPD		
1.	*Taxus wallichiana*	Ajit K. Shasany *et al.*, 2000
2.	*Podophyllum hexandrum*	Singh *et al.*, 2000
3.	*Cymbopogon* sps.	Shasany *et al.*, 2000
4.	*Digitalis*	Nebauer *et al.*, 2000
5.	*Mentha* sps.	Patra *et al.*, 2001
6.	*Bacopa monnieri*	Mahendra P. Darokar *et al.*, 2001
7.	*Echinaceae*	Kapteyn and Simon, 2002
8.	*Solanum* sps.	Brita Stedje *et al.*, 2003
9.	*Phyllanthus amarus*	Neeraj Jain *et al.*, 2003
10.	*Ocimum*	Ajay Pratap Singh *et al.*, 2004
11.	*Mentha arvensis and M. spicata*	Shasany *et al*., 2005
12.	*Coleus forskohlii*	Kavitha, 2005
13.	*Lycoris longituba*	Chuan-Liang Deng *et al.*, 2006
14.	*Salvia officinalis*	Echeverrigaray and Agostini, 2006
15.	*Chimonanthus praecox*	Kai-Ge Zhao *et al.*, 2007
16.	*Maytenus ilicifolia*	Mossi *et al.*, 2007
17.	*Curcuma longa*	Manoj Kumar Panda *et al.*, 2007
18.	*Oroxylum indicum*	Jayaram and Prasad, 2008
19.	*Gloriosa superba*	Seemanti Ghosh *et al.*, 2008
ISSR		
1.	*Phyllanthus amarus*	Meenakshi sundaram, 2002
2.	*Andrographis paniculata*	Panimalar, 2005
3.	*Curcuma longa*	Jagadeesan, 2006
4.	*Gaultheria fragrantissima*	Apte et al., 2006

5.	*Rehmannia glutinosa*	Yanqing Zhou *et al.*, 2007
6.	*Cannabis sativa*	Erdogan E. Hakki *et al*., 2007
7.	*Guizotia abyssinica*	Yohannes Petros *et al.*, 2007
8.	*Sagittaria natans*	Jin-Ming Chen *et al*., 2007
9.	*Swertia chirayita*	Joshi and Dhawan, 2007
10.	*Gycyrrhiza uralensis*	Yao *et al*., 2008
11.	*Artemisia herba-alba*	Haouari Mohsen and Ferchichi Ali, 2008
12.	*Podophyllum hexandrum*	Afroz Alam *et al*., 2008
SSR		
1.	*Medicago spp.*	Mahalakshmi *et al* .,2002
2.	*Cymbopogon* sp	Kumar *et al.*,2009
3.	*Lyceum granatum*	Zhao *et al.*,2010
4.	*Rhodiola rosea*	Soni *et al*.,2010
5.	*Curcuma longa*	Sigrist *et al.*,2011
6.	Medicinal *Dendrobium*	Pritam Chattopadhyay *et al* .,2012
7.	*Punica granatum*	Hasnaoui *et al.*,2012
8.	*Liriopa*	Li *et al.*,2011

2.11. Correlation

While formulating a selection programme for yield improvement in any crop, inter relationship of yield with other traits is considered as the most valuable one. Correlation studies pave way to know the association between highly heritable characters with the most economic character. Many authors worked in this aspect to bring out the relationship of different characters with yield and also within yield contributing characters. Reviews on association of yield with other characters and inter correlations among yield components are presented here.

2.11.1. Association of component characters with yield

Yield Character	Other characters	Type of associati	Reference
Makoi (*Solanum nigrum L.*)			
Plant height	Number of leaves per plant, fr and dry herbage yield per pl and total alkoloid content	Positive and significant	Shivanna *et al*.(2007
Total alkoloid conte	Plant spread	Negative	
Days to maturity	Plant height, plant spre number of leaves per plant, l area, fresh and dry herbage yi and total alkoloid	Negative	
Glory lily (*Gloriosa superba L.*)			
Plant height	Fresh seed weight per pod, fre seed yield per plant and dry s yield per plant	Positive	Chitra and Rajam (2010)
	Days to flowering, days to 50% flowering and dry seed recove	Negative	
Number of branche per plant	Fresh and dry seed yield per plant	Positive	
Days to 5(flowering	Fresh seed yield per plant and dry seed yield per plant	Negative	
Dry seed yield	Fresh seed yield per plant and number of pods per plant	Positive and significant	
Periwinkle (*Catharanthus roseus*)			
Number of primary branches	Total fresh leaf yield	Positive and significant	Samresh Dwivedi *et*
Leaf area	Total herbage yield	Positive	

Stem width	Number of primary branches, total herbage yield and catharanthine percentage	Negative and significant	*al.*(1999)
Opium poppy (*Papaver somniferum)*			
Opium yield	Capsule number and seed wei	Positive and significant	Khanna (1987)
	Days to flowering	Negative	
Safed musuli *(Chlorophytum borivilianum)*			
Root yield	Leaf number, leaf length and finger number	Positive	Birendra Kumar *et al.* (2008)
Aswagandha *(Withania somnifera*)			
Dry root yield	Root diameter and root length	Positive and significant	Misra *et al.(* 2009)
Plant canopy	Root length and dry root yield	Positive and significant	
Fresh root yield plant	Number of berries per plant, fresh weight of berries per pla seed yield per plant and root diameter	Positive and significant	Sangwan *et al.* (2013)
Phyllanthus amarus			
Herbage yield	Dry matter production, leaf ar plant height and number of leaves per plant	Positive and significant	Vasumathi (2001)
Stevia rebaudiana			
Number of leaves plant	Plant height	Positive and significant	Gaurav *et al. (*2008)
Leaf yield	Leaf length	Negative	
Vetiver (*Vetiveria zyzonoides*)			
Dry root yield and yield	Plant height	Positive and significant	Lal *et al.* (1999)

Ajowan (*Trachyspermum ammi)*			
Single plant yield	Plant height and number branches	Positive and significant	Dalkani *et al.* (2011)
Single plant yield	Ripening period	Negative	
Coriander (*Coriandrum sativum*)			
Biomass yield	Plant height, number of branches, number of leaves, weight of stem and leaves	Positive	Palanikumar *et al.*(201 b)
Amaranthus spp.			
Plant height	Number of leaves per plant, stem diameter, leaf size, ascorbic acid and protein	Positive and significant	Shukla *et al.* (2010)
Number of leaves plant	Number of branches per plant	Positive and significant	Shukla *et al.* (2010)
Protein	Plant height, leaf length and le width	Negative and significant	Rana *et al.* (2005)
Iron	Leaf weight	Negative	Mathai *et al.* (1981)

2.12. Path coefficient analysis

Path coefficient analysis is used to divide the observed correlation coefficient into direct and indirect effects of yield component that provide a more obvious picture of the character association for formulating efficient selection strategy. A path coefficient is a simple and standardized partial regression coefficient and as such, it measures the direct influence of one variable upon another (Dewey and Lu, 1959). From this, it could be possible to estimate the actual contribution of an attribute and its influence through other characters. Path analysis differs from simple correlation in that, it points out the causes and their relative importance, whereas, the latter measures simply the mutual association ignoring the causation (Jaiswal and Gupta, 1967). The literatures on path analysis are reviewed hereunder.

In fenugreek, Kholi *et al.* (1986) reported that number of shoots per plant gave maximum direct contribution towards yield followed by number of leaves per plant.

Sohoo and Bharadwaj (1986), in fenugreek, reported that plant height had maximum direct positive effect followed by number of shoots and number of leaves per plant suggesting that plant height and number of leaves per plant are major components of green yield.

The path coefficient analysis of 26 genotypes of periwinkle revealed that total herbage yield had the highest positive effect on total leaf alkaloid. The study indicated that short stature, thin-stem and high leaf stem ratio are important selection parameters for developing high alkaloid yielding periwinkle cultivar. (Samresh Dwivedi *et al.*1999)

Bhandari *et al*. (1997) reported direct negative effects of plant height, capsule number and seed yield on opium yield. Hence, a plant type with few capsules, dwarf stature, large leaf was suggested for increased latex yield.

Radjamany (1995) noticed a very low negative direct effect of number of leaves on the yield and the indirect effect of leaves through dry matter production was high followed by number of primary branches in coriander. Hariharan (1996) observed that plant height influenced directly on yield of greens per plot followed by total dry matter production and number of leaves per plant in path coefficient analysis of coriander.

Mahalakshmi (2006) reported direct positive effect of shoot weight (0.78), leaf breadth (0.42), plant height (0.36) and root weight (0.19) on yield in *Phyllanthus amarus*. Root length, number of branches and plant spread recorded direct negative effect on yield.

Ann Riya (2001) observed that yield of greens was directly and greatly influenced by weight of stem, plant height, number of branches per plant and weight of leaves. It also had a negative direct influence on yield via leaf stem ratio for initiation of selection programme in coriander.

Variability studies conducted in 30 *Ocimum* genotypes (20 exotic and 10 indigenous) revealed that fresh herbage yield per plant had highest positive direct effect with essential oil yield per plant and maximum direct negative effect for essential oil content.
(Panwar *et al.,* 2009).

Dalkani *et al.* (2011) studied path analysis on 10 genotypes of ajowan and reported that plant height and number of umbels have maximum direct effect on seed yield and can be used as selection criteria for improving seed yield in ajowan breeding programs.

Path analysis was carried out by Kumar *et al.* (2012) in 20 lines belonging to four different *Ocimum* species *i.e. O. basilicum, O. tenuiflorum, O. gratissimum* and *O. kilimandscharicum*. The yield traits (herbage yield, oil content and oil yield) revealed strong genetic association among them exhibiting positive direct path of herb yield (0.729) and oil content (0.532) towards oil yield.

Plant height, number of branches, number of leaves, fresh weight of leaves, weight of stem and weight of leaves exerted maximum positive direct effect on yield of biomass in coriander (Palanikumar *et al*., 2012c.).

Sangwan *et al.* (2013) reported that path analysis among 26 genotypes of aswagandha revealed that total alkaloid content showed the highest positive direct effect on fresh root yield per plant followed by biomass yield at maturity, suggesting that selection for these traits would be quite effective to improve fresh root yield in ashwagandha.

Materials and Methods

CHAPTER III

MATERIALS AND METHODS

The present investigation on **"Genetic diversity in Makoi (*Solanum nigrum* L.) Genotypes"** was carried out during 2011-2013 at the Department of Medicinal and Aromatic Crops and Micro Analytical Laboratory, Horticultural College & Research Institute, Tamil Nadu Agricultural University, Coimbatore. A brief account of the experimental materials and methodologies adopted for the study are given in this chapter.

3.1. METEOROLOGICAL DATA

3.1.1. Location

The field experiment was conducted at the Experimental field at Department of Medicinal and Aromatic Crops, Tamil Nadu Agricultural University, Coimbatore. Geographically, it is located at 11°02' North latitude, 77°03' East longitude and an altitude of 426.76 m above MSL.

3.1.2. Weather

The data on rainfall, maximum and minimum temperatures and relative humidity recorded during the cropping seasons are presented in Annexure I.

3.2. MATERIALS

Forty five accessions from different ecogeographical regions of India were collected and used in the study (Table 1.) The experiment was conducted for two seasons viz., Kharif (July – Nov 2011) and winter (Dec to April 2012) (Plate 1a and 1b).

3.3. METHOD

3.4. CULTIVATION DETAILS

3.4.1. Quality Seedling Production

Seedlings of forty five accessions were raised in portray nursery to get quality seedlings. The protrays of 98 cells (54 x 27 cm) of 3.5 cm diameter and 0.8 mm thickness was used for raising seedlings. Cocopeat, a soil - less media was used as a growing medium. The sterilized cocopeat at the rate of 300 kg / ha was mixed with *Azospirillum* and Phosphobacteria each at the rate of 1 Kg and 1.2 Kg of the cocopeat medium was used to

fill each protray. Seed sowing was done at the rate of 1 seed per cell. The seedlings of 30 days old with four leaves were transplanted in the main field.

3.4.2. Transplanting

The land was brought to a fine tilth by repeated ploughing and harrowing. Thirty days old healthy seedlings with intact media were used for planting and one seedling per hole was planted adopting a spacing of 60 x 45 cm in ridges and furrows.

3.4.3. Fertilizer application

Nitrogen, phosphorus and potassium at the rate of 100:50:50 Kg ha^{-1} (Farooqi and Sreeramu, 2004) was applied. The whole of P and K and half of N was applied as basal and the remaining half dose of nitrogen was applied as top dressing after 30 days after transplanting.

3.4.4. Irrigation

During first two weeks after transplanting, the crop was irrigated once in three days and thereafter at weekly interval.

3.4.5. Gap filling

Gap filling was done one week after transplanting with the reserved seedlings of the same age.

3.4.6. Cultural practices

Hand weeding was done at 25 - 30 days and again at 45-50 days after transplanting. Appropriate prophylactic plant protection measures were taken to keep the crop free from the insect pest and diseases.

3.4.7. Harvest

The crop was harvested three months after planting at matured green berry stage. The whole plant was harvested by cutting the plant at 15 cm above the ground level. (Farooqi and Sreeramu, 2004).

Table.1. List of forty five Germplasm collections

S.no	Accession number	Place of collection	2n	S.No.	Accession number	Place of collection	2n
1.	TN *Sn* 1	Coimbatore, Tamil Nadu	24	24.	TN *Sn* 24	Kolli hills, Tamil Nadu	24
2.	TN *Sn* 2	Namakkal, Tamil Nadu	24	25.	TN *Sn* 25	N.S.puram, Tamil Nadu	24
3.	TN *Sn* 3	Sirugamani, Tamil Nadu	24	26.	TN *Sn* 26	Rahuri, Maharastra	24
4.	TN *Sn* 4	Thagarapudur, Tamil Nadu	24	27.	TN *Sn* 27	Ottampatty -1, Tamil Nadu	24
5.	TN *Sn* 5	Ottampatty, Tamil Nadu	24	28.	TN *Sn* 28	P.Krishnapuram, TamilNadu	24
6.	TN *Sn* 6	Sobanapuram, Tamil Nadu	24	29.	TN *Sn* 29	E.patty-2, Tamil Nadu	24
7.	TN *Sn* 7	Paiyur, Tamil Nadu	24	30.	TN *Sn* 30	Coimbatore-2, Tamil Nadu	42
8.	TN *Sn* 8	Chinnakalvehalli,TamilNadu	24	31.	TN *Sn* 31	Pelukurichi, Tamil Nadu	42
9.	TN *Sn* 9	Theni, Tamil Nadu	24	32.	TN *Sn* 32	P.Krishnapuram, TamilNadu	42
10.	TN *Sn* 10	Ooty, Tamil Nadu	72	33.	TN *Sn* 33	Ottampatty, Tamil Nadu	24
11.	TN *Sn* 11	E.Patty, Tamil Nadu	24	34.	TN *Sn* 35	Pachamalai, Tamil Nadu	24
12.	TN *Sn* 12	Kolli hills, Tamil Nadu	24	35	TN *Sn* 36	Navaladipatti, Tamil Nadu	24
13.	TN *Sn* 13	Anthiyur, Tamil Nadu	24	36	TN *Sn* 37	Thuraiyur, Tamil Nadu	24
14.	TN *Sn* 14	S. mangalam, Tamil Nadu	24	37	TN *Sn* 38	Solan, H.P	24
15.	TN *Sn* 15	Salem, Tamil Nadu	24	38	TN *Sn* 40	KAU,Thrissur, Kerala	24
16.	TN *Sn* 16	Ottampatty, Tamil Nadu	24	39	TN *Sn* 41	Pattikkad Kerala	24
17.	TN *Sn* 17	Valasaiyur, Tamil Nadu	24	40	TN *Sn* 42	Kottakkal, Kerala	24
18.	TN *Sn* 18	Perur, Tamil Nadu	24	41	TN *Sn* 44	Nalhendra, Solan	42
19.	TN *Sn* 19	Kallipalayam, Tamil Nadu	24	42	TN *Sn* 47	Ottampatty-3, Tamil Nadu	42
20.	TN *Sn* 20	Trichy, Tamil Nadu	24	43	TN *Sn* 51	Odakali-2, Kerala	24
21.	TN *Sn* 21	Bhavanisagar, Tamil Nadu	24	44	TN *Sn* 52	Guddalore, Tamil Nadu	24
22.	TN *Sn* 22	Pelukurichi, Tamil Nadu	24	45	TN *Sn* 53	Kodaikanal, Tamil Nadu	24
23.	TN *Sn* -23	Namakkal, Tamil Nadu	24				

3.5. EXPERIMENT DETAILS

Present investigation consists of four experiments as follows

Experiment - I - Morphological characterization of genotypes using descriptors

Experiment - II - Species confirmation

Experiment - III - Study on *per se* performance of 45 genotypes

Experiment - IV - Molecular characterization of genotypes

3.5.1. Experiment - I: Morphological characterization of genotypes using descriptors

Observations on morphological characters were recorded in the form of multiscale scores by refering the IPGRI discriptors. Observations were taken on five randomly selected plants from each genotype. The list of morphological traits observed are given in Table 2.

3.5.2. Experiment – II: Species confirmation

Among forty five genotypes, thirteen genotypes were morphologically distinct from others (Table.3). They show wide variations in growth habit, stem, leaf, flower and fruit characters. The complicated polyploidy complex and taxonomic confusion surrounding this species necessitates species confirmation. So the distinct twelve genotypes were selected and the following analysis was conducted.

3.5.2.1. Ploidy analysis

3.5.2.1.1. Stomatal density

The sample for stomatal study was taken from the center portion of the physiological leaf. The sample leaves were cut into one centimetre square bits and boiled for two minutes in water and then transferred to 70 % ethanol, where it was kept for 24 hours to remove chlorophyll. The sample was washed with water and kept over a clean slide containing glycerin with the upper surface of the lamina bit in contact with the slide and sealed with a cover slip and examined under microscope of 45 X magnification. The number of stomata per microscopic field (0.152 mm^2) was

Table.2. List of morphological descriptors observed

S.No	Character	Description
1.	Plant growth habit	Prostrate
		Intermediate (compact)
		Erect
2.	Stem branching	Sparse
		Intermediate
		Dense
3.	Stem pubescence	Absent
		Sparse
		Intermediate
		Dense
4.	Stem shape	Cylindrical
		Angled
		Flattened
5.	Stem color	Green
		Light purple
		Purple
		Dark purple
6.	Leaf shape	Deltoid
		Ovate
		Lanceolate
7.	Leaf pubescence	Absent
		Sparse
		Intermediate
		Dense
8.	Leaf margin	Entire
		Undulate
		Sinuate
9.	Leaf color	Light green
		Green
		Dark green
10.	Flower size	Small
		Medium
		Big
11.	Corolla color	White
		Yellow
		White with purple stripes
12.	Fruit color	Purplish black
		Red
		Orange
13.	Seed color	Straw (deep yellow)
		Brown
		Black
14.	Ploidy level	Diploid
		Tetraploid
		Hexaploid

Table.3. List of morphologically distinct genotype

S.No	Accession number	2n	Place of collection
1.	TN *Sn* 8	24	Chinnakalvehalli, Tamil Nadu
2.	TN *Sn* 10	72	Ooty, Tamil Nadu
3.	TN *Sn* 12	24	Kolli hills, Tamil Nadu
4.	TN *Sn* 19	24	Kallipalayam, Tamil Nadu
5.	TN *Sn* 23	24	N-T – Namakkal, Tamil Nadu
6.	TN *Sn* 30	42	Coimbatore – 2, Tamil Nadu
7.	TN *Sn* 32	42	P.Krishnapuram -2, Tamil Nadu
8.	TN *Sn* 38	24	Solan, Himachal Pradesh
9.	TN *Sn* 44	42	Nalhendra, Solan, Himachal Pradesh
10.	TN *Sn* 47	42	Ottampatty – 4, Tamil Nadu
11.	TN *Sn* 52	24	Guddalore, Tamil Nadu
12.	TN *Sn* 53	24	Kodaikanal, Tamil Nadu

counted at least at ten different fields. The mean was arrived at and expressed as stomatal density per mm^2.

3.5.2.1.2. Stomatal size

The length and breadth of the stomata were also measured by using ocular micrometer and it was expressed in microns. The size of the stomata was calculated by multiplying the length and breadth and was expressed in μm^2.

3.5.2.2. Geometry of pollen grains

Pollen grains were collected from freshly dehisced anthers by gently tapping the anthers on glass slides containing a drop of glycerol. Then cover slips were placed over the pollens and slides were observed under a microscope and pollens were measured with the aid of ocular micrometers. The observations were recorded from 50 pollen grains in each genotype and the data were analysed in Q 500 MC WIN software programme.

The following observations were recorded using the Q 500 MC WIN software.

3.5.2.2.1. Diameter of the pollen grains

It refers to the equivalent circle diameter *i.e.,* the diameter of the circle having the same area of the pollen grain and expressed in microns.

3.5.2.2.2. Pollen fertility

The pollen fertility was tested with acetocarmine glycerine stain. The number of normal and shrivelled pollen grains was recorded under different fields of microscope. Pollen grains which are stained well and looked plump and normal are considered to be viable and the shrivelled and unstained ones as non vialble and the mean value was expressed in per cent.

3.5.2.3. Number of flowers per infloresence

Number of flowers in each infloresence of ten tagged plants of all accessions was counted and mean was tabulated.

3.5.2.4. Diameter of flower and berry

Diameter of all the flowers and berries were measured in ten selected plants of all accessions and mean was tabulated.

3.5.2.5. Number of seeds per berry

Number of seeds present in all berries of ten selelcted plants of all accessions was counted and mean was tabulated.

3.5.2.6. Root tip mitosis

Young root tips obtained directly from the seeds soaked in petridishes were fixed in saturated paradichlorobenzene for two hours, washed with distilled water and transferred into 1: 3 acetic alcohol for five minutes at 60º C. Then the roots were stained in 2% acetocarmine and squashed well. The chromosomes are then viewed at 40X objective.

3.5.2.7. DNA Barcoding for species confirmation

i. DNA extraction, PCR amplification and DNA sequencing

DNA was isolated from the fresh leaf samples of all distinct accessions by using the modified CTAB method (Khanuja *et al.,* 1999). Isolated DNA was used as the template for Polymerase Chain Reaction. The total volume of 20 µL PCR mixture contained 1 µL of 50 ng of DNA template, 2 µL of 10X Taq Buffer (Fermentas®), 2 µL of 25 mM $MgCl_2$ (Fermentas®), 2 µL of 2 mM dNTPs (Fermentas®), 0.5 µL of each Forward and Reverse primers (10 pM) and 0.1 µL of 5 U Taq DNA Polymerase (Fermentas®). The reaction was carried out in a thermal cycler (Eppendorf, Germany).

The forward and reverse primer sequences of internal transcribed spacer (*ITS*) region used in the present study are F- 5' GGA AGG AGG AGT CGT AAC AAG G 3'; R- 5' TCC TCC GCT TAT TGA TAT GC 3' (White *et al*, 1990). The PCR product was separated in 1% agarose gel and documented (Alpha Digidoc, USA). Sequencing of PCR product was outsourced to Chromous Biotech, Banglore, India.

ii. Sequencing alignment and phylogenetic analysis

The chromatographic traces of the forward and reverse sequences of *ITS* region were assembled and edited using the DNASTAR offline software (http://www.dnastar.com/). The contiguous alignments were removed and the sequences were aligned using Clustal W algorithm as implemented in the BioEdit tool (Hall, 1999). The individual *ITS* region was subjected to pairwise alignment with the reference sequence EF108406.1 obtained from the GenBank, which resulted in the split up of *ITS* region into *ITS1*, 5.8S rRNA and *ITS2*. The DNA sequences were subjected to BLAST (http://www.ncbi.nlm.nih.gov/blast/blast.cgi) for identification of species at species level. The aligned sequences were imported into the

Molecular evolutionary genetic analysis (MEGA 5) tool (Tamura *et al*, 2011). The phylogenetic analysis was carried out using the Neighbour Joining method by applying the test of phylogeny as bootstrap with 1000 replicates.

3.5.3. Experiment–III: Study on *per se* performance of 45 Makoi genotypes

3.5.3.1. Observations recorded

3.5.3.1.1. Plant characters

3.5.3.1.1.1. Plant height

The height of the plant was recorded from the ground level to the growing point of the main stem from randomly selected plants and the mean was calculated and expressed in centimeters.

3.5.3.1.1.2. Plant spread

The maximum plant spread along North- South and East- West directions were measured in randomly selected plants and the mean was calculated and expressed in centimeters.

3.5.3.1.1.3. Stem girth

Girth of the stem was measured at 10 cm above the ground level from randomly selected plants and the mean was calculated and expressed in centimeters.

3.5.3.1.1.4. Number of primary branches per plant

The number of primary branches of the plant was recorded from randomly selected plants and the mean was calculated and expressed in numbers.

3.5.3.1.1.5. Number of leaves per plant

The total number of leaves was counted from randomly selected plants and the mean was calculated and expressed in numbers.

3.5.3.1.1.6. Leaf length

The length of the leaf from the petiole to the tip was taken from randomly selected plants and the mean was calculated and expressed in centimeters.

3.5.3.1.1.7. Leaf breadth

The breadth of the leaf was recorded from randomly selected plants and the mean was calculated and expressed in centimeters.

3.5.3.1.1.8. Days to Flower bud initiation

The number of days taken for flower bud initiation was recorded from randomly selected plants in each genotype.

3.5.3.1.1.9. Days to 50 per cent flowering

The number of days taken for 50 per cent flowering was recorded from randomly selected plants in each genotype.

3.5.3.1.1.10. Number of berries per plant

The number of berries produced by selected plants from each replication was counted periodically and the mean value was expressed as number of berries per plant.

3.5.3.1.2. Yield parameters

3.5.3.1.2.1. Fresh herbage yield per plant

The plants were harvested by cutting the whole plant at 10 cm height above the ground level and fresh weight of the total harvested herbage from randomly selected plants were recorded and the mean value was expressed in grams.

3.5.3.1.2.2. Dry herbage yield per plant

The harvested herbage of randomly selected plants were cut into pieces and dried in hot air oven at 80°C for 72 hours till the moisture level of 8 per cent was obtained and weight was taken and the mean value was expressed in grams.

3.5.3.1.2.3. Fresh herbage yield per plot

The total harvested herbage from a single plot of each replication was recorded and the mean value was expressed in kilograms.

3.5.3.1.2.4. Dry herbage yield per plot

The dry herbage yield from a single plot of each replication was recorded and the mean value was expressed in kilograms.

3.5.3.1.2.5. Fresh herbage yield per hectare

The fresh herbage yield per hectare was estimated based on the fresh herbage yield per plot and expressed in tonnes.

3.5.3.1.2.6. Dry herbage yield per hectare

The dry herbage yield per hectare was estimated based on the dry herbage yield per plot and expressed in tonnes.

3.5.3.1.3. Physiological parameters

3.5.3.1.3.1. Total leaf area per plant

The leaf area of the plants was estimated by destructive sampling method by feeding the leaves into the photosensitive, automatic portable leaf area meter (Model LI-300)

3.5.3.1.3.2. Total chlorophyll content

Fully matured fresh leaves (fourth leaf from the tip of the shoot) from each genotypes was collected and the total chlorophyll were estimated by adopting the procedure of Yoshidha *et al.* (1971) and the contents were expressed as mg $g^{-1}$of fresh weight.

$$\text{Total chlorophyll} = \frac{\text{O.D. at 652 nm}}{34.5 \times W} \times V$$

Where,

V = Final volume of chlorophyll extract (ml)

W = Fresh weight of tissue extracted (g)

3.5.3.1.3.3. Soluble protein content

Soluble protein content was estimated in leaf sample following the method suggested by Lowry *et al*. (1951) and expressed in mg g^{-1} of fresh weight.

3.5.3.1.3.4. Total Phenol

The total phenol content in leaves of genotypes was estimated by Folin-Ciocalteau reagent method suggested by Malick and Singh (1980) and expressed in mgg^{-1} of fresh weight.

3.5.3.1.4. Biochemical parameters

3.5.3.1.4.1. Estimation of total alkaloid content

The total alkaloid content was estimated by the method suggested by Bakshi and Hamied (1972) and later modified by Kaul and Zutshi (1982). Powdered sample of 20 g was taken in a thimble and defatted in a Soxhlet extract apparatus with hexane till colorless solvent flowed back (4 hours). The defatted material was next extracted with 95 per cent ethanol in the Soxhlet till again the colorless solvent flowed (12 hours). The alcohol extract was concentrated to 33 ml by keeping it in a water bath for 2-3 hours and 3 ml HCl was added and the mixture was hydrolyzed in a boiling water bath for 4 hours under reflux, cooled and the pH made to 11 by using 10 N NaOH. Again, it was refluxed in boiling water bath for 1 hour and cooled. The mixture was filtered and the residue was washed with water till free of alkali and dried in oven.

The dried material was dissolved in chloroform and filtered through a thick pad of cotton, which was later washed with chloroform. The combined filtrate was concentrated in boiling water bath to remove the solvent. The residue was dried in oven at 80°C for 4 hours, cooled and weighed. The heating and weighing was repeated till constant weight was obtained. The weight of the residue is equal to the weight of the total alkaloid content and expressed in per cent.

3.5.3.1.4.3. Protein content

Protein content in leaves was estimated following the method of Lowry *et al.* (1951) and expressed in mg $100g^{-1}$.

3.5.3.1.4.4. Crude fibre content

The crude fibre analysis of leaves was estimated by the method of Maynard (1970). Two grams of the leaf sample were extracted with petroleum ether to remove fat (initial boiling temperature 35-38°C and final temperature, 52°C) and was transferred in a 400ml beaker. Two grams of dried sample was boiled with 200ml of sulphuric acid (H_2SO_4) for 30 minutes and was filtered through muslin cloth and washed with boiling water until washings are free of acid. The residue was boiled with 200 ml of sodium hydroxide (NaOH) for 30 minutes. The residue was filtered again through a muslin cloth and washed with 25 ml of boiling H_2SO_4, three 50 ml portions of water and 25 ml of alcohol. The residue was removed and transferred to pre-weighed crucible and it was dried for two hours at 130 $+2^0$ C. The

dish was cooled in a dessicator and weighed and then was ignited for 30 minutes. Finally, the residue was cooled in a dessicator and reweighed. The crude fibre content was expressed in percentage.

$$\text{Crude fibre (\%)} = \frac{\text{Weight of the residue with crucible} - \text{Weight of ash with crucible}}{\text{Weight of fat free sample}} \times 100$$

3.5.3.1.4.5. Iron content

The leaf samples were estimated calorimetrically from aliquot of digested triple acid extract of the plant material (Ezeonu *et al.*, 2002).

One gram of dried leaf sample was taken from each treatment and digested in a triple acid mixture (Nitric: Sulphuric: Perchloric acid at 9:3:1). The samples were allowed overnight for cold digestion and another day for hot digestion in a block digester. After thorough digestion, it was made up to 100ml with distilled water. The concentration of iron was recorded using the Atomic Absorption Spectrophotometer (AAS) was calculated as under and expressed as mg/100g of edible portion.

$$\text{Iron content (mg/100)} = \frac{\text{Absorbance of sample}}{\text{Weight of fat free sample}} \times 100$$

3.5.3.1.4.2. Ascorbic acid

The ascorbic acid content of leaves was estimated by the method of Rosenberg (1975) and expressed in mg $g^{-1}$of fresh sample.

3.5.3.1.4.6. Organoleptic evaluation

The first ten high yielding genotypes were taken and organoleptic evaluation was carried out. 250 grams of freshly harvested leaves from each genotype were taken and boiled in adequate water and salt. Specific sensory characteristics of each genotype (flavor, bitterness and overall acceptability) were rated separately on a scale of 1 to 3. Scores were defined as follows:

1 - low/ bad; 2 - medium/fair; 3 - high/ good. Numerical averages were then calculated for a composite test score.

3.5.3.2. STATISTICAL ANALYSIS

3.5.3.2.1. Analysis of variance

The analysis of variance for the above characters was carried out to find the significance of the variance as per the procedure suggested by Panse and Sukhatme (1967).

3.5.3.2.2. Range

Range was recorded with the lowest and the highest values presented in observation.

3.5.3.2.3. Mean

Mean was calculated by dividing the sum of the values of two replications by the total number of replications in an observation.

3.5.3.2.4. Standard error

Standard error was calculated by dividing the standard deviation to the square root of the number of observations.

3.5.3.2.5. Genetic divergence studies - D^2 analysis

The genetic diversity among forty five accessions of makoi was assessed by using D^2 Mahalanobis statistics (Mahalanobis, 1936). The grouping of accessions was done using Tocher's method, as described by Rao (1952). The steps involved in the analysis are:

a) Test of significance of difference by Wilk's static for aggregate traits.

b) Transformation of correlated variables

In terms of variance and covariances, the D^2 value was obtained by,

$$D^2 = w^{ij} (x_i^1 - x_i^2) (x_j^1 - x_j^2)$$

w^{ij} is the estimate of variance and covariance matrix.

Transformation was done by pivotal condensation method. Transformation of correlated variables into uncorrelated variables was dome by substituting these values of x_1, x_2, x_3 and x_4 in the transformed equation and the corresponding transformed values y_1, y_2, y_3 and y_4 were obtained (original mean to transformed data).

c) Computation of D^2 values

For each combination of population, the mean of deviation for the characters was computed and the D^2 was calculated as the sum of the squares of these deviations.

i.e. $D^2 = \Sigma (y_i^1 - y_i^2)^2$

Where i = 1, 2, 3.....p characters.

d) Test of significance of D^2 values

The significance of D^2 values for a pair of population was tested against the table values of χ^2 for p degrees of freedom.

Where p = Total number of characters

e) Grouping into clusters

The method suggested by Tocher (Rao, 1952) was followed for cluster formation. The genotypes were arranged in the order of their relative distance from each other. The values were arranged in ascending order of magnitude in each column. Two genotypes having smallest distance from each other were considered first to which a genotype having smallest average D^2 value from the first two genotypes was added. If at any stage, the average D^2 of a group appeared to be high from those already included, it was considered that the group does not fit with the former cluster and hence taken outside the first cluster and second cluster was formed. This process was continued and clusters were formed.

(i) Estimation of average intra cluster distances

The average of the distances of all possible combinations of genotypes included in a cluster was calculated.

(ii) Estimation of average inter cluster distances

This was calculated by measuring the distances between various combinations of clusters divided by the product of the number of genotypes in the concerned cluster combinations. The clusters were taken one by one and their distance from each other was calculated.

i.e. Average intercluster distance between cluster i and j = $\frac{\sum Di^2}{n_i \, X \, n_j}$

n_i = Number of genotypes in cluster i

n_j = Number of genotypes in cluster j

f) Contribution of individual characters towards divergence

In all the combinations, each character was ranked on the basis of (y_i^1 - y_i^2). Rank one was given to the highest mean difference and the rank 'p' to the lowest mean difference where 'p' is the total number of characters. The number of times appearing first in ranking for each character was counted and the per cent contribution was calculated taking the total number of combinations as 100.

3.5.3.2.6. Phenotypic and genotypic variances

The phenotypic and genotypic variances were estimated according to Lush (1940).

a) Genotypic variance ($\sigma^2 g$) = $\frac{MS_1 - MS_2}{r}$

Where,

MS_1 = Mean sum of squares for genotypes.

MS_2 = Mean sum of squares for error or error variance,

r = Number of replications.

a) Phenotypic variance ($\sigma^2 p$) = $\sigma^2 g + \sigma^2 e$

Where,

$\sigma^2 g$ = Genotypic variance.

$\sigma^2 e$ = Error variance.

3.5.3.2.7. Phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV)

The Phenotypic and Genotypic Coefficient of Variation was worked out as per the methods suggested by Burton (1952).

Phenotypic coefficient of variation = $\frac{\sqrt{\sigma^2 p}}{General\ \ mean} \times 100$

Genotypic coefficient of variation = $\frac{\sqrt{\sigma^2 g}}{General\ \ mean} \times 100$

Environmental coefficient of variation $= \frac{\sqrt{\sigma^2 e}}{General\ \ mean} \times 100$

The PCV and GCV are classified as follows as suggested by Sivasubramanian and Madhavamenon (1973).

Low : Less than 15 %

Moderate : 15 – 30 %

High : More than 30 %

3.5.3.2.8. Heritability and genetic advance

Heritability in the broad sense (h^2) was derived based on the formula proposed by Lush (1940) and expressed in %.

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

Where,

$(\sigma^2 g)$ - Genotypic Variance

$(\sigma^2 p)$ - Phenotypic Variance

As suggested by Johnson *et.al*. (1955), heritability values are categorized as follows:

Low : Less than 30 %

Moderate : 30 – 60 %

High : More than 60 %

Genetic advance was estimated by the following formulae as per the method of Johnson *et.al*. (1955).

$$\text{Genetic advance} = \frac{\sigma^2 g}{\sqrt{\sigma^2 p}} \text{ x } 2.06$$

Where

$\sigma^2 g$ - Genotypic variance

$\sigma^2 p$ - Phenotypic Variance

k - Selection differential, the value of which is 2.06 at 5% selection intensity

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

The range of genetic advance as per cent of mean was classified as suggested by Johnson *et.al*. (1955).

Low	:	Less than 10 per cent
Moderate	:	10 – 20 per cent
High	:	More than 20 per cent

3.5.3.2.9. Association analysis

3.5.3.2.9.1. Correlation coefficient

The association between yield and component traits and among themselves was computed based on *per se* performance of the genotypes as genotypic correlation coefficient (Goulden, 1952). The variance and covariance components were utilized to calculate genotypic correlation coefficient as outlined by Al – Jibour *et al*. (1958).

Genotypic correlation coefficient

$$rg\ 1.2 = \frac{CoVg\ 1.2}{\sqrt{(\sigma^2 g_1 + \sigma^2 g_2)}}$$

Where,

rg 1.2 = Genotypic correlation coefficient between the traits 1 and 2

CoVg 1.2 = Genotypic covariance between characters 1 and 2

$\sigma^2 g_1$ = Genotypic variance for the character 1

$\sigma^2 g_2$ = Genotypic variance for the character 2

3.5.3. Experiment – IV: Molecular characterization of genotypes

3.5.3.1. Materials

The experiment was conducted in the Micro Analytical Laboratory, Department of Spices and Plantation Crops, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore. Molecular analysis was carried out for all forty five makoi accessions.

3.5.3.2. Methods

3.5.3.2.1. DNA isolation

The young fresh leaves of ten days old plants raised in protrays were collected and the DNA was extracted by CTAB method. The reagents used for extraction and procedure for extractions are given in Annexure II and III (Dixit, 1998). The extracted DNA was purified for RNA contamination by RNase treatment.

3.5.3.2.2. Quantification of genomic DNA

To check the quality and quantity of the extracted genomic DNA, gel electrophoresis was carried out on 0.8 per cent agarose gel. DNA concentration for PCR amplification was estimated by comparing the band intensity of a sample with the band intensities of known dilutions that gave good amplifications. Based on the band intensity, the DNA was further diluted to the required concentration (25-50 ng) using double distilled water.

3.5.3.2.3. SSR analysis

DNA from forty five Makoi genotypes was amplified using a set of 10 SSR primer pairs (Angeline *et al*., 2010). The list of SSR primers used in the study along with their annealing temperature is presented in Table 4. Amplification reaction was used in a volume of 25 µl containing 50ng of genomic DNA and amplification was performed in PTC thermal (AB PCR). PCR reaction mixture was followed in Annexure IV.

3.5.3.2.4. PCR Programme for SSR

Initial denaturation at 94^0 C for 3 minutes

Denaturation at 94^0 C for 1 minute

Annealing at 50-60^0 C for 1 minute } 34 cycles

Extension at 72^0C for 2 minute

Final extension at 72^0C for 10 minutes

Holding at 4^0C for 10 minutes

3.5.3.2.5. Gel electrophoresis

PCR amplified products were subjected to gel electrophoresis in a 1.5 per cent agarose gel in 1X TBE at 90 V for 2 hours using gel electrophoresis unit. The Ethidium

bromide stained gels were documented using gel documentation unit. The materials required and protocol for agarose gel electrophoresis is given in Annexure V.

3.5.3.3. Statistical analysis for construction of dendrogram using molecular data

3. 5.3.3.1. Polymorphism survey of SSR markers

Polymorphism survey of SSR markers was carried out by considering only the clear and unambiguous bands. Markers were scored for the presence and absence of the corresponding band among the different trees. The scores '1' and '0' were given for the presence and absence of bands, respectively.

3. 5.3.3.2. Cluster analysis

The data obtained by scoring the SSR profiles of different primers were subjected to cluster analysis. Similarity matrix was constructed using Jaccard's coefficient and the similarity values were used for cluster analysis and dendrogram was constructed by Unweighted Pair-Group Method using Arithmetic averages (UPGMA) with the Sequential Agglomerative Hierarchical and Nested (SHAN) function (Sneath and Sokal, 1973). Data analysis was done using NTSYS-pc version 2.02i.

3. 5.3.3.3.Genetic diversity estimation

After visualizing the gel, amplified fragments of each SSR marker were scored as ''1'' and ''0'', where ''1'' indicated the presence of a specific allele (band) and ''0'' indicated its absence. Polymorphism information content (PIC) of SSR markers was calculated using the below formula. A PIC value of each locus was calculated as:

$$PIC_j = 1 - \sum_{l=1 to L} P^2_{ij},$$

Where P_{ij} is the relative frequency of the i^{th} allele for the locus j and was summed across all the alleles (L) over all lines. PIC provided an estimate of the discriminatory power of a locus by taking into account, not only the number of alleles that are expressed, but also the relative frequencies of those alleles. PIC values ranges from 0 (monomorphic) to 1 (very highly discriminative), with many alleles in equal frequencies.

Genetic diversity estimate related analyses were done using NTSYSpc ver.2.02i (Rohlf, 2005). Genetic similarities (GS) between pairs of trees were measured by the DICE similarity coefficient based on the proportion of shared alleles with SIMQUAL module.

Genetic distances between pairs of lines were estimated as GD or D = 1 - GS. The clustering of trees was done based on a similarity matrix using an unweighted pair group method with arithmetic average (UPGMA) algorithm following SAHN module. The clustering result was used to construct a dendrogram following TREE module.

Table.4. List of SSR primer pairs used in the study

Sl. N	Primer Name	Primer Sequence 5' to 3'	Annealin Temp (ºC
1	STWIN 12 G Forward Sequence	TGT TGA TTG TGG TGA TAA	49
	STWIN 12 G Reverse Sequence	TGT TGG ACG TGA CTT GTA	
2	SB4-32 Forward Sequence	CTC GGC GGT TAG CAC AGT CAC	59
	SB4-32 Reverse Sequence	GCC CAT AGA CAG ACA GCA AAG CC	
3	SB6-36 Forward Sequence	GCA TAA TGA CGG CGT GCT	60
	SB6-36 Reverse Sequence	CTT CCA AGT GAA AGA AAC CAT CA	
4	SB6-57 Forward Sequence	ACA GGG CTT TAG GGA AAT CG	60
	SB6-57 Reverse Sequence	CCA TCA CCG TCG GCA TCT	
5	SB6-84 Forward Sequence	CGC TCT CGG GAT GAA TGA	58
	SB6-84 Reverse Sequence	TAA CGG ACC ACT AAC AAA TGA TT	
6	TMS29 Forward Sequence	AGC CAC CCA TCA CAA AGA TT	55
	TMS29 Reverse Sequence	GTC GCA CTA TCG GTC ACG TA	
7	TMS37 Forward Sequence	CCT TGC AGT TGA GGT GAA TT	55
	TMS37 Reverse Sequence	TCA AGC ACC TAC AAT CAA TCA	
8	TMS 39 Forward Sequence	CGG CGT ATT CAA ACT CTT GG	60
	TMS 39 Reverse Sequence	GCG GAC CTT TGT TTT GGT AA	
9	CM2 Forward Sequence	GTA CCT ATG GGA ATA AGC AAA	52
	CM2 Reverse Sequence	CCA ATT TGT CTG AAG TTG AGT	
10	CM6 Forward Sequence	AGT TAA CAA CTT TGG TGC TGT	54
	CM6Reverse Sequence	TAA TAT GGT AAG CAC ATT CCA	

Experimental Results

Chapter IV

EXPERIMENTAL RESULTS

The present investigation was carried out in the Department of Medicinal and Aromatic Crops, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore to understand the genetic diversity present in the makoi (*Solanum nigrum* L.) genotypes collected from various sources. The results were statistically analysed and the data are presented in appropriate tables with suitable figures.

4.1. Morphological characterization of genotypes using descriptors

Morphological characters were recorded for 45 makoi genotypes using the IPGRI descriptors. Observations for fourteen morphological traits were taken from ten randomly selected plants of genotypes (Table 5).

4.1.1.1. Growth habit and branching

The data recorded for plant growth habit and branching revealed that among the forty five genotypes, TN *Sn* 10 alone was erect with dense branching, TN *Sn* 44 was prostrate with intermediate branching, Fourteen genotypes were of spreading type with dense branching and the rest were of indeterminate type.

4.1.1.2. Stem pubescence

Stem pubescence was dense in five genotypes, sparse in seven genotypes and completely absent in remaining genotypes.

4.1.1.3. Stem shape

Stem shape was cylindrical in all the genotypes except in five genotypes which had angled stem

4.1.1.4. Stem color

Stem color was dark purple only in TN *Sn* 10 and purplish in TN *Sn* 30 and TN *Sn* 31 while it was dark green in TN *Sn* 44 and TN *Sn* 47. All the remaining genotypes had green stem.

4.1.1.5. Leaf shape

Leaf shape was deltoid in TN *Sn* 10, TN *Sn* 30 and TN *Sn* 31 while it was ovate in all the remaining genotypes.

4.1.1.6. Leaf pubescence

Leaf pubescence was dense in five genotypes, sparse in six genotypes and completely absent in remaining genotypes.

4.1.1.7. Leaf margin

Leaf margin was sinuate in ten genotypes and in TN *Sn* 10 it was undulate while the remaining genotypes had smooth entire leaf margin.

4.1.1.8. Leaf color

Leaf color was dark green in four genotypes and light green in six genotypes while others had normal green leaves.

4.1.1.9. Flower size

Five genotypes have big sized flowers and six genotypes have medium sized flowers while remaining all genotypes had small sized flowers.

4.1.1.10. Flower color

Flower color was white in all the genotypes except in TN *Sn* 47 which had purple stripes in its corolla.

4.1.1.11. Berry size

Four genotypes have big sized berries and seventeen genotypes have medium sized berries while remaining genotypes had smaller berries.

4.1.1.12. Berry color

Berry color was red in TN *Sn* 30 and TN *Sn* 31 and orange in TN *Sn* 44 and TN *Sn* 47. All the remaining genotypes had black colored berries.

4.1.1.13. Seed color

Straw colored seed was seen in TN *Sn* 30, TN *Sn* 31, TN *Sn* 44 and TN *Sn* 47. All the remaining genotypes had brown colored seeds.

4.1.2. Cluster analysis

Cluster analysis was carried out using data recorded on morphological characters with the help of multivariate analysis system (NTSYS 2.11) and the resulting dendrogram is presented in Figure 5. The dendrogram separated the genotypes into four major clusters. Cluster I was further divided into two sub clusters, Ia and Ib. Cluster Ia comprised of

22 genotypes, cluster Ib contained 11 genotypes. Cluster II consists of 6 genotypes and cluster III contains two distinct genotypes TN *Sn* 10 and TN *Sn* 44. Cluster IV was further divided into two sub clusters, IV a (TN *Sn* 30, TN *Sn* 31 and TN *Sn* 32) and IV b (TN *Sn* 47).

4.2. Ploidy analysis

Among the forty five genotypes, thirteen genotypes were morphologically distinct from others (Table.3). They showed wide variations in growth habit, stem, leaf, flower and berry characters. The complicated polyploidy complex and taxonomic confusion surrounding this species necessitates ploidy analysis. The determination of ploidy level is a prerequisite for using the germplasm in taxonomic, genetic or breeding experiments. So the distinct thirteen genotypes were selected and the following analysis were conducted and the data are tabulated in Table.6

4.2.1. Number of stomata per unit area

TN *Sn* 10 (9.11) recorded less number of stomata per unit area followed by TN *Sn* 44 (10.67), TN *Sn* 30 (11.22), TN *Sn* 32 (11.33) and TN *Sn* 47 (11.44). The genotypes TN *Sn* 51 (16.11) and TN *Sn* 19 (16.67) recorded more number of stomata per unit area.

4.2.2. Stomatal length

Stomatal length was maximum in TN *Sn* 10 (25.03 µ) followed by TN *Sn* 44 (24.18 µ) and TN *Sn* 47 (24.12 µ). Minimum stomatal length was recorded in TN *Sn* 19 (20.13 µ) and TN *Sn* 51 (20.16 µ).

4.2.3. Stomatal breadth

Stomatal breadth was maximum in TN *Sn* 10 (16.70 µ) followed by TN *Sn* 44 (15.31 µ) and TN *Sn* 47 (15.06 µ). Minimum stomatal breadth was recorded in TN *Sn* 51 (13.28 µ) and TN *Sn* 19 (13.54 µ).

4.2. 4.Pollen diameter

Pollen diameter was maximum in TN *Sn* 10 (27.06 µ) followed by TN *Sn* 44 (26.95 µ) and TN *Sn* 32 (26.91 µ). Minimum pollen diameter was recorded in TN *Sn* 51 (23.03 µ) and TN *Sn* 19 (23.05 µ).

4.2.5. Pollen fertility

Pollen fertility was maximum in TN *Sn* 10 (98.56 %) followed by TN *Sn* 30 (96.23 %) and TN *Sn* 47 (96.04%). Minimum pollen diameter was recorded in TN *Sn* 51 (92.45 %) and TN *Sn* 19 (92.47 %).

4.2.6. Number of flowers per inflorescence

TN *Sn* 10 had eight flowers per inflorescence. TN *Sn* 44, TN *Sn* 47, TN *Sn* 30 and TN *Sn* 32 had five flowers per inflorescence. Remaining genotypes have three flowers per inflorescence.

4.2.7. Flower diameter

Flower diameter was maximum in TN *Sn* 10 (0.96 cm) followed by TN *Sn* 44 (0.71 cm), TN *Sn* 32(0.70 cm) and TN *Sn* 47 (0.70 cm). Minimum flower diameter was recorded in TN *Sn* 51 (0.50 cm) and TN *Sn* 19 (0.49 cm).

4.2.8. Berry diameter

Berry diameter was maximum in TN *Sn* 10 (0.92 cm) followed by TN *Sn* 44 (0.78 cm) and TN *Sn* 32 (0.76 cm). Minimum berry diameter was recorded in TN *Sn* 51 (0.58 cm) and TN *Sn* 19 (0.58 cm).

4.2. 9.Number of seeds per berry

Number of seeds per berry was minimum in TN *Sn* 10 (31.33) followed by TN *Sn* 44 (33.17. Maximum number of seeds per berry was recorded in TN *Sn* 38 (38.50) and TN *Sn* 51 (38.17).

4.2.10. Confirmation of ploidy level using flow cytometry

The ploidy status of all distinct genotypes was analysed using flow cytometry. A diploid genotype TN *Sn* 19 (2n = 24) was used as an internal reference standard. The ploidy level was determined by measuring the size of the nuclear genome in the form of histograms (Plate.6a and 6b). With the help of histograms it is inferred that TN *Sn* 10 was hexaploid and TN *Sn* 30, TN *Sn* 31, TN *Sn* 32, TN *Sn* 44 and TN *Sn* 47 (red and orange fruited types) were tetraploid. Although the genotypes TN *Sn* 8, TN *Sn* 12, TN *Sn* 23, TN *Sn* 38 and TN *Sn* 53 were distinct from other genotypes, they were also diploids similar to other genotypes.

4.2.11. Root tip mitosis

The ploidy status was furthur confirmed using root tip mitosis. The results revealed that, the genotype TN *Sn* 10 was hexaploid with 2n = 72 and the genotypes TN *Sn* 30, TN *Sn* 32, TN *Sn* 44 and TN *Sn* 47 were tetraploid with 2n = 48 while the other distinct genotypes *viz.,* TN *Sn* 8, TN *Sn* 12, TN *Sn* 23, TN *Sn* 38 and TN *Sn* 53 were diploids with 2n = 24.

4.2.12. Species confirmation

DNA barcoding community has proposed several molecular markers for identification of plant species. Among the proposed barcode candidates, nuclear *ITS* region usually exhibit high levels of variation, including indel polymorphism (Graham *et al.* 2000) and serve as an efficient candidate for species identification, provides relative ease of sequencing and alignment (Kress et al. 2005, Hollingsworth *et al*. 2011, Taberlet 2007, Baldwin et al. 1995).

The multiple sequence alignment of the *Solanum* sp. displayed distinct nucleotide variation between the three important species *S. americanum* Miller, *S. villosum* Miller, *S. nigrum* L. The evolutionary analysis conducted in the MEGA5 tool involved 685 codon positions in the final data set. All the positions containing gaps and missing data were eliminated. The *ITS* region exhibited 97.5% overall constant sites and 2.5% variable sites; among which 2.3% constituted parsimony informative sites. The pairwise comparison of *S. americanum*, *S. nigrum* and *S. villosum* showed 98.5% identity. The overall average pairwise distance was estimated to be 0.01. Most of the parsimony informative characters were shared between *S. nigrum* and *S. americanum*. Blast analysis also confirmed the closeness between, *S. nigrum* and *S. americanum*. The best nucleotide substitution model predicted using the MEGA5 tool was attributed to be Tamura-3- parameter (T92) model.

The phylogenetic analysis performed using Neighbour joining statistical method produced three distinct clades, wherein the three species *S. americanum* Miller, *S. nigrum* L., *S. villosum* Miller were grouped under clade I, II and III respectively (Fig 2).

4.4. *Per se* performance of genotypes

4.4.1. Analysis of variance

The analysis of variance was carried to test the significance of difference among the genotypes of three *Solanum sp.* studied. Analysis of variance indicated that there is an existence of significant variability among the genotypes for all the characters studied. (Table 7-9).

4.4.2. Mean performance of genotype

The mean performance of the genotypes of three *Solanum* species for all the characters studied is furnished in Tables 10 – 36.

4.3.1. Plant height

***a. Solanum nigrum* L.**

In the first season, the mean of the plant height was 89.63 cm. Among the genotypes, TN *Sn* 10 recorded maximum plant height of 127.71 cm followed by TN *Sn* 19 (111.47 cm). The genotype TN *Sn* 27 recorded the lowest plant height of 70.56 cm.

In the second season, the mean of plant height was 92.93 cm. As in the first season, TN *Sn* 10 recorded maximum plant height of 130.22 cm and TN *Sn* 27 recorded minimum plant height of 74.10 cm. Similar trend was recorded in pooled analysis also (Table.10).

b. Solanum americanum Mill.

In the first season, the mean of the plant height was 109.67 cm. Among the genotypes, TN *Sn* 08 recorded maximum plant height of 116.10 cm followed by TN *Sn* 52 (114.42 cm). The genotype TN *Sn* 23 recorded the lowest plant height of 88.13 cm.

In the second season, the mean of plant height was 112.63 cm. As in the first season, TN *Sn* 08 recorded maximum plant height of 119.94 cm and TN *Sn* 23 recorded minimum plant height of 90.48 cm. Similar trend was recorded in pooled analysis also (Table.10).

***c. Solanum villosum* Mill.**

In the first season, the mean of the plant height was 109.45 cm. Among the genotypes, TN *Sn* 47 recorded maximum plant height of 123.28 cm followed by TN *Sn* 30 (121.61 cm). The genotype TN *Sn* 44 recorded the lowest plant height of 73.11 cm.

In the second season, the mean of plant height was 112.63 cm. As in the first season, TN *Sn* 08 recorded maximum plant height of 119.94 cm and TN *Sn* 23 recorded minimum plant height of 90.48 cm. Similar trend was recorded in pooled analysis also (Table.10).

4.3.2. Plant spread (N –S)

***a. Solanum nigrum* L.**

In both the seasons the plant spread was higher in genotype TN *Sn* 19 (50.84 cm and 56.32 cm respectively). Sixteen genotypes recorded plant spread exceeding the mean of

43.29 cm in both the seasons. In the pooled analysis, the mean performance of genotypes ranged from 51.88 cm to 30.86 cm (Table.11).

b. Solanum americanum Mill.

In both the seasons the plant spread was higher in genotype TN *Sn* 08 (54.11 cm and 57.12 cm respectively) (Table.11).

***c. Solanum villosum* Mill.**

In both the seasons the plant spread was higher in genotype TN *Sn* 30 (58.69 cm and 58.41 cm respectively) (Table.11).

4.3.2. Plant spread (E-W)

***a. Solanum nigrum* L.**

TN *Sn* 19 recorded the maximum plant spread of 48.32 cm in first season and the plant spread was minimum in the genotype TN *Sn* 03 (29.20 cm). Twenty genotypes recorded plant spread exceeding the general mean (41.04 cm).

I n the second season also TN *Sn* 19 recorded the maximum plant spread of 49.63 cm and twenty genotypes recorded plant spread exceeding the general mean (43.76 cm). Similar trend was recorded in pooled analysis also (Table.12).

b. Solanum americanum Mill.

In both the seasons, TN *Sn* 08 recorded the maximum plant spread of 50.19 cm 53.21 cm respectively (Table.12).

***c. Solanum villosum* Mill.**

TN *Sn* 30 recorded the maximum plant spread of 56.18 cm in first season and 59.23 cm in second season (Table.12).

4.3.3. Number of primary branches per plant

***a. Solanum nigrum* L.**

In the first season, the genotype TN *Sn* 10 (29.52) recorded maximum number of primary branches. The genotype TN *Sn* 20 (9.15) recorded lesser primary branches per plant. Fifteen genotypes exceeded the grand mean (13.79).

In the second season, TN *Sn* 10 (31.25) recorded maximum number of primary branches followed by TN *Sn 19* (24.12). Similar trend was recorded in pooled mean analysis (Table.13)

b. Solanum americanum Mill.

In the first season, the genotype TN *Sn* 53 (19.13) recorded maximum number of primary branches with the grand mean of 18.41. Similar trend was recorded in season II pooled mean analysis (Table.13)

***c. Solanum villosum* Mill.**

In the first season, the genotype TN *Sn* 10 (29.52) recorded maximum number of primary branches. The genotype TN *Sn* 20 (9.15) recorded lesser primary branches per plant. Fifteen genotypes exceeded the grand mean (13.79).

In the second season, TN *Sn* 31 (25.14) recorded maximum number of primary branches and similar trend was recorded in pooled mean analysis (Table.13)

4.3.4. Stem girth

***a. Solanum nigrum* L.**

During the first season, the highest mean value for stem girth was recorded in genotype TN *Sn* 17 (4.58 cm) and the lowest mean value in TN *Sn* 3 (2.04 cm) (Table 14).

In the second season also, the highest mean value for stem girth was recorded in genotype TN *Sn* 17 (4.96 cm) and the lowest mean value in TN *Sn* 15 (2.19 cm). In the pooled analysis the mean ranged from 4.41 cm to 2.15 cm with the grant mean of 3.55 cm.

b. Solanum americanum Mill.

During the first season, the highest mean value for stem girth was recorded in genotype TN *Sn* 53 (4.86 cm) and in the second season also, the highest mean value for stem girth was recorded in genotype TN *Sn* 53 (4.96 cm). In the pooled analysis the mean ranged from 4.93 cm to 3.08 cm with the grant mean of 4.47 cm.

***c. Solanum villosum* Mill.**

During the first season, the highest mean value for stem girth was recorded in genotype TN *Sn* 30 (5.28 cm) and in the second season also, the highest mean value for stem girth was recorded in genotype TN *Sn* 30 (5.46 cm).

4.3.5. Number of leaves per plant

***a. Solanum nigrum* L.**

Among the genotypes, TN *Sn* 10 (145.16) recorded the highest number of leaves per plant and TN *Sn* 20 (82.49) recorded the least number of leaves (Table 15) during the first season. Similar trend was also observed in the second season as well as in the pooled mean analysis.

b. Solanum americanum Mill.

Among the genotypes, TN *Sn* 12 (133.56) recorded the highest number of leaves per plant and TN *Sn* 52 (110.69) recorded the least number of leaves (Table 15) during the first season. Similar trend was also observed in the second season as well as in the pooled mean analysis.

***c. Solanum villosum* Mill.**

Among the genotypes, TN *Sn* 30 (142.96) recorded the highest number of leaves per plant and TN *Sn* 44 (120.42) recorded the least number of leaves (Table 15) during the first season. Similar trend was also observed in the second season as well as in the pooled mean analysis.

4.3.6. Leaf length

***a. Solanum nigrum* L.**

Among the genotypes, the highest leaf length was recorded in TN *Sn* 19 (7.52 cm) in the first season. The grand mean for this trait was 5.74 cm (Table 16). During the second season also TN *Sn* 19 (7.56 cm) recorded the highest leaf length. Similar trend was also observed in the pooled mean analysis.

b. Solanum americanum Mill.

Among the genotypes, the highest leaf length was recorded in TN *Sn* 12 (7.26 cm) in the first season. The grand mean for this trait was 6.59 cm (Table 16). During the second season also TN *Sn* 12 (7.36 cm) recorded the highest leaf length. Similar trend was also observed in the pooled mean analysis.

***c. Solanum villosum* Mill.**

Among the genotypes, the highest leaf length was recorded in TN *Sn* 32 (7.38 cm) in the first season. The grand mean for this trait was 6.03 cm (Table 16). During

the second season also TN *Sn* 32 (7.28 cm) recorded the highest leaf length. Similar trend was also observed in the pooled mean analysis.

4.3.7. Leaf breadth

***a. Solanum nigrum* L.**

During the first season, the highest leaf breadth was recorded in TN *Sn* 51 (3.93 cm) and TN *Sn* 29 (3.81 cm) and TN *Sn* 15 (Table 17). Similar trend was also observed in the second as well as in the pooled mean analysis.

b. Solanum americanum Mill.

During the first season, the highest leaf breadth was recorded in TN *Sn* 08 (3.53 cm) (Table 17). Similar trend was also observed in the second as well as in the pooled mean analysis.

***c. Solanum villosum* Mill.**

During the first season, the highest leaf breadth was recorded in TN *Sn* 30 (4.62 cm) (Table 17). Similar trend was also observed in the second as well as in the pooled mean analysis.

4.3.8. Days to flower bud initiation

***a. Solanum nigrum* L.**

TN *Sn* 04 took only 23.14 days for flower initiation in first season and 23.45 days in second season (Table 18). TN *Sn* 19 took more number of days for flower bud initiation both in season I (29.06 days) and season II (29.36 days). Similar trend was also observed in the pooled mean analysis.

b. Solanum americanum Mill.

TN *Sn* 08 took only 21.04 days for flower initiation in first season and 21.36 days in second season (Table 18). TN *Sn* 23 took more number of days for flower bud initiation both in season I (29.45 days) and season II (29.36 days). Similar trend was also observed in the pooled mean analysis.

***c. Solanum villosum* Mill.**

TN *Sn* 30 took only 19.05 days for flower initiation in first season and 20.69 days in second season (Table 18). TN *Sn* 32 took more number of days for flower bud initiation

both in season I (20.14 days) and season II (21.33 days). Similar trend was also observed in the pooled mean analysis.

4.3.9. Days to fifty per cent flowering

***a. Solanum nigrum* L.**

TN *Sn* 04 took only 55.15 days in first season and 55.24 days in second season for fifty per cent flowering. TN *Sn* 19 took more number of days for fifty per cent flowering both in season I (57.80 days) and season II (57.89 days). Similar trend was also observed in the pooled mean analysis (Table 19).

b. Solanum americanum Mill.

TN *Sn* 08 took only 53.16 days in first season and 53.45 days in second season for fifty per cent flowering. TN *Sn* 23 took more number of days for fifty per cent flowering both in season I (57.43 days) and season II (57.54 days). Similar trend was also observed in the pooled mean analysis (Table 19).

***c. Solanum villosum* Mill.**

TN *Sn* 30 took only 50.09 days in first season and 50.23 days in second season for fifty per cent flowering.

4.3.10. Number of berries per plant

***a. Solanum nigrum* L.**

In the first season TN *Sn* 7 recorded more number of berries per plant (285.52) followed by TN *Sn* 4 (280.12). TN *Sn* 19 recorded less number of berries per plant (245.78). Twenty four genotypes exceeded the grand mean (253.70). Similar trend was also observed in the season II (Table 20).

In pooled analysis, mean ranged from 288.37 to 198.26 with twenty four genotypes exceeding the grand mean (255.97).

b. Solanum americanum Mill.

In the both seasons TN *Sn* 52 recorded more number of berries per plant (249.42 and 251.23 respectively). TN *Sn* 23 recorded less number of berries per plant (212.29 and 210.23 respectively) (Table 20).

***c. Solanum villosum* Mill.**

In the both seasons TN *Sn* 30 recorded more number of berries per plant (240.14 and 245.36 respectively). TN *Sn* 47 recorded less number of berries per plant (210.19 and 215.28 respectively) (Table 20).

4.3.11. Fresh herbage yield per plant

***a. Solanum nigrum* L.**

In both first (496.98 g) and second (485.23 g) seasons TN *Sn* 10 recorded higher fresh herbage yield per plant. TN *Sn* 14 recorded lesser fresh herbage yield per plant both in first season (207.01 g) and second season (215.21 g). Sixteen genotypes exceeded the grand mean (300.89 g) in season I and seventeen genotypes exceeded the grand mean (310.78 g) in season II. Similar trend was also observed in the pooled mean analysis (Table 21).

b. Solanum americanum Mill.

In both first (426.74 g) and second (439.12 g) seasons TN *Sn* 38 recorded higher fresh herbage yield per plant. TN *Sn* 23 recorded lesser fresh herbage yield per plant both in first season (324.14 g) and second season (332.10 g). Similar trend was also observed in the pooled mean analysis (Table 21).

***c. Solanum villosum* Mill.**

In both first (468.53 g) and second (475.21 g) seasons TN *Sn* 30 recorded higher fresh herbage yield per plant. TN *Sn* 32 recorded lesser fresh herbage yield per plant both in first season (292.70 g) and second season (300.12 g). Similar trend was also observed in the pooled mean analysis (Table 21).

4.3.12. Dry herbage yield per plant

***a. Solanum nigrum* L.**

TN *Sn* 10 recorded highest dry herbage yield per plant both in first season (247.02 g) and second season (239.21 g). The lowest dry herbage yield per plant was noticed in TN *Sn* 14 both in season I (78.55 g) and season II (85.23 g) (Table 22). Seventeen genotypes exceeded the grand mean in both season I (132.78 g) and season II (137.97 g).

b. Solanum americanum Mill.

TN *Sn* 38 recorded highest dry herbage yield per plant both in first season (202.80 g) and second season (202.17 g). The lowest dry herbage yield per plant was noticed in TN *Sn* 23 both in season I (138.63 g) and season II (142.36 g) (Table 22).

***c. Solanum villosum* Mill.**

TN *Sn* 30 recorded highest dry herbage yield per plant both in first season (224.88 g) and second season (228.36 g). The lowest dry herbage yield per plant was noticed in TN *Sn* 32 both in season I (122.36 g) and season II (127.23 g) (Table 22).

4.3.13. Fresh herbage yield per plot

***a. Solanum nigrum* L.**

The highest and the lowest yield per plot was recorded in TN *Sn* 10 (15.30 kg) and TN *Sn* 14 (6.80 kg) respectively during the first season, where the respective grand mean was 9.62 kg (Table 23). Similar trend was also observed in season II.

In pooled mean analysis, yield per plot was recorded highest in accession TN *Sn* 10 (15.49 kg) and lowest in TN *Sn* 14 (6.79 kg). Sixteen genotypes exceeded the grand mean (9.78 kg).

b. Solanum americanum Mill.

The highest and the lowest yield per plot was recorded in TN *Sn* 38 (13.40 kg) and TN *Sn* 23 (10.34 kg) respectively during the first season, where the respective grand mean was 12.62 kg (Table 23). Similar trend was also observed in season II. In pooled mean analysis, yield per plot was recorded highest in accession TN *Sn* 38 (13.67 kg) and lowest in TN *Sn* 23 (10.53 kg).

***c. Solanum villosum* Mill.**

The highest and the lowest yield per plot was recorded in TN *Sn* 30 (14.33 kg) and TN *Sn* 32 (9.30 kg) respectively during the first season, where the respective grand mean was 12.06 kg (Table 23). Similar trend was also observed in season II.

In pooled mean analysis, yield per plot was recorded highest in accession TN *Sn* 30 (14.54 kg) and lowest in TN *Sn* 32 (9.45 kg).

4.3.14. Dry herbage yield per plot

***a. Solanum nigrum* L.**

Highest dry herbage yield of 7.18 kg (season I) and 7.25 kg (season II) per plot was recorded in TN *Sn* 10. The dry herbage yield was lower in TN *Sn*14 both in season I (2.49 kg) and season II (2.62 kg). Similar trend was also observed in pooled analysis (Table 24).

b. Solanum americanum Mill.

Highest dry herbage yield of 6.42 kg (season I) and 6.68 kg (season II) per plot was recorded in TN *Sn* 38. The dry herbage yield was lower in TN *Sn* 23 both in season I (4.42 kg) and season II (4.50 kg). Similar trend was also observed in pooled analysis (Table 24).

***c. Solanum villosum* Mill.**

Highest dry herbage yield of 7.02 kg (season I) and 7.12 kg (season II) per plot was recorded in TN *Sn* 30. The dry herbage yield was lower in TN *Sn* 32 both in season I (3.92 kg) and season II (4.05 kg). Similar trend was also observed in pooled analysis (Table 24).

4.3.15. Fresh herbage yield per hectare

***a. Solanum nigrum* L.**

The highest and the lowest fresh herbage yield per hectare (estimated) was recorded in genotype TN *Sn* 10 in both the seasons (16.89 and 16.90 t ha^{-1} respectively) and TN *Sn* 14 (7.38 and 7.82 t ha^{-1}) respectively. Sixteen genotypes exceeded the grand mean of 10.60 t ha^{-1}(season I) and 11.04 t ha^{-1}(season II) (Table 25).

b. Solanum americanum Mill.

The highest fresh herbage yield per hectare (estimated) was recorded in genotype TN *Sn* 38 in both the seasons (15.03 and 15.15 t ha^{-1} respectively). Similar results were got in season II (Table 25).

***c. Solanum villosum* Mill.**

The highest and the lowest fresh herbage yield per hectare (estimated) was recorded in genotype TN *Sn* 30 in both the seasons (15.82 and 15.90 t ha^{-1} respectively) and TN *Sn* 32 (9.51 and 9.99 t ha^{-1}) respectively. Similar results were got in season II (Table 25).

4.3.16. Dry herbage yield per hectare

***a. Solanum nigrum* L.**

TN *Sn* 10 recorded highest estimated dry herbage yield in season I (7.82 t ha^{-1}) and season II (8.13 t ha^{-1}). Sixteen genotypes exceeded the grand mean in both season I (4.67 t ha^{-1}) and season II (4.86 t ha^{-1}) (Table 26).

b. Solanum americanum Mill.

TN *Sn* 38 recorded highest estimated dry herbage yield in season I (7.08 t ha^{-1}) and season II (7.36 t ha^{-1}) (Table 26).

***c. Solanum villosum* Mill.**

TN *Sn* 30 recorded highest estimated dry herbage yield in season I (7.73t ha^{-1}) and season II (8.04 t ha^{-1}).

4.3.17. Total leaf area

***a. Solanum nigrum* L.**

TN *Sn* 22 recorded maximum total leaf area in season I (3149.21 cm^2) and season II (3500.12 cm^2). TN *Sn* 14 recoded the minimum total leaf area in season I (1144.21 cm^2) and season II (1204.23 cm^2). Similar trend was also observed in pooled analysis (Table 27).

b. Solanum americanum Mill.

TN *Sn* 38 recorded maximum total leaf area in season I (2927.50 cm^2) and season II (3000.47 cm^2). Similar trend was also observed in pooled analysis (Table 27).

***c. Solanum villosum* Mill.**

TN *Sn* 30 recorded maximum total leaf area in season I (3257.03 cm^2) and season II (3421.00 cm^2). Similar trend was also observed in pooled analysis.

4.3.18. Total chlorophyll

***a. Solanum nigrum* L.**

In season I total chlorophyll was highest in TN *Sn* 10 (2.13 mg g^{-1}) and lowest in TN *Sn* 14 (1.47 mg g^{-1}). Similar trend was also observed in season II and pooled analysis (Table 28).

b. Solanum americanum Mill.

In season I total chlorophyll was highest in TN *Sn* 08 (1.95 mg g^{-1}) and lowest in TN *Sn* 14 (1.80 mg g^{-1}). Similar trend was also observed in season II and pooled analysis (Table 28).

***c. Solanum villosum* Mill.**

In season I total chlorophyll was highest in TN *Sn* 30 (2.05 mg g^{-1}) and lowest in TN *Sn* 14 (1.95 mg g^{-1}). Similar trend was also observed in season II and pooled analysis.

4.3.19. Total soluble protein

***a. Solanum nigrum* L.**

The total soluble protein was highest in TN *Sn* 10 in both seasons (16.36 mg g^{-1} and15.55 mg g^{-1} respectively) and was least in TN *Sn*14 (12.64 mg g^{-1} and 12.01 mg g^{-1} respectively). Similar trend was also observed in pooled analysis (Table 29).

b. Solanum americanum Mill.

The total soluble protein was highest in TN *Sn* 38 in both seasons (16.41 mg g^{-1} and 15.59 mg g^{-1} respectively) and was least in TN *Sn* 23 (13.24 mg g^{-1} and 12.58 mg g^{-1} respectively). Similar trend was also observed in pooled analysis.

***c. Solanum villosum* Mill.**

The total soluble protein was highest in TN *Sn* 30 in both seasons (16.98 mg g^{-1} and 16.13mg g^{-1} respectively) and was least in TN *Sn* 32 (13.17 mg g^{-1} and 12.51 mg g^{-1} respectively). Similar trend was also observed in pooled analysis.

4.3.20. Total phenols

***a. Solanum nigrum* L.**

Total phenols were highest in TN *Sn* 10 (1.89 mg g^{-1} and1.98 mg g^{-1} respectively) and was least in TN *Sn*13 (1.47 mg g^{-1} and 1.53 mg g^{-1} respectively). Similar trend was also observed in pooled analysis (Table 30).

b. Solanum americanum Mill.

Total phenols were highest in TN *Sn* 08 (1.69 mg g^{-1} and1.76 mg g^{-1} respectively) and was least in TN *Sn* 23 (1.62 mg g^{-1} and 1.69 mg g^{-1} respectively). Similar trend was also observed in pooled analysis (Table 30).

***c. Solanum villosum* Mill.**

Total phenols were highest in TN *Sn* 30 (1.77 mg g^{-1} and1.84 mg g^{-1} respectively) and was least in TN *Sn* 32 (1.48 mg g^{-1} and 1.53 mg g^{-1} respectively). Similar trend was also observed in pooled analysis.

4.3.21. Total alkaloid content

***a. Solanum nigrum* L.**

TN *Sn* 10 registered highest total alkaloid content in both season I (0.475%) and season II (0.479%) (Table 31). Similar trend was also observed in pooled analysis.

b. Solanum americanum Mill

TN *Sn* 12 registered highest total alkaloid content in both season I (0.425%) and season II (0.435%) (Table 31). Similar trend was also observed in pooled analysis.

***c. Solanum villosum* Mill.**

TN *Sn* 30 registered highest total alkaloid content in both season I (0.438%) and season II (0.442%). Similar trend was also observed in pooled analysis.

4.3.22. Protein content

***a. Solanum nigrum* L.**

Among the genotypes, the highest protein content was recorded in TN *Sn* 19 in both the seasons (5.00 mg and 5.17 mg respectively). Next to it, TN *Sn* 22 recorded highest protein content (4.58 mg and 4.74 mg respectively) (Table.32). A similar trend was seen in pooled analysis.

b. Solanum americanum Mill.

Among the genotypes, the highest protein content was recorded in TN *Sn* 23 in both the seasons (1.83 mg and 1.93 mg respectively). A similar trend was seen in pooled analysis.

***c. Solanum villosum* Mill.**

Among the genotypes, the highest protein content was recorded in TN *Sn* 30 in both the seasons (1.39 mg and 1.57 mg respectively). A similar trend was seen in pooled analysis.

4.3.23. Crude fiber

***a. Solanum nigrum* L.**

In case of crude fiber, TN *Sn* 01 (0.80 per cent and 0.84 per cent respectively) recorded maximum percentage in both the seasons (Table.33). A similar trend was seen in pooled analysis.

b. Solanum americanum Mill.

In case of crude fiber, TN *Sn* 52 and TN *Sn* 53 (0.59 per cent and 0.63 per cent respectively) recorded maximum percentage in both the seasons (Table.33). A similar trend was seen in pooled analysis.

***c. Solanum villosum* Mill.**

In case of crude fiber, TN *Sn* 30 (0.81 per cent and 0.85 per cent respectively) recorded maximum percentage in both the seasons (Table.33). A similar trend was seen in pooled analysis.

4.3.24. Iron content

***a. Solanum nigrum* L.**

In both the seasons, iron content were higher in TN *Sn* 19 (6.07 mg and 6.13 mg respectively) and was least in TN *Sn* 29 (4.26 mg and 4.52 mg respectively). (Table 34). A similar trend was seen in pooled analysis.

b. Solanum americanum Mill.

In both the seasons, iron content were higher in TN *Sn* 23 (4.76 mg and 4.91 mg respectively) and was least in TN *Sn* 38 (2.27 mg and 2.38 mg respectively). A similar trend was seen in pooled analysis.

***c. Solanum villosum* Mill.**

In both the seasons, iron content were higher in TN *Sn* 30 (3.12 mg and 3.17 mg respectively) and was least in TN *Sn32* (2.31 mg and 2.38 mg respectively). A similar trend was seen in pooled analysis.

4.3.25. Ascorbic acid content

***a. Solanum nigrum* L.**

TN *Sn* 19 registered highest ascorbic acid content in both the seasons (21.36 mg and 21.96 mg respectively) followed by TN *Sn* 22 (20.85 mg and 21.66 mg respectively) (Table35).

b. Solanum americanum Mill.

TN *Sn* 23 registered highest ascorbic acid content in both the seasons (14.31 mg and 15.63 mg respectively).

***c. Solanum villosum* Mill.**

TN *Sn* 30 registered highest ascorbic acid content in both the seasons (13.40 mg and 14.76 mg respectively).

4.3.26. Organoleptic evaluation of genotypes

The organoleptic evaluation was carried out for the high yielding genotypes. Over all acceptability was high for TN *Sn* 19 (2.93) and TN *Sn* 22 (2.82) as they have less bitterness but the other high yielding genotypes like TN *Sn* 10 (1.25) and TN *Sn* 30 (1.19) are not acceptable due to biterness. (Table 36).

4.4. Genetic divergence

4.4.1. D^2 analysis

D^2 values were computed for all possible pairs of combinations from the means of forty five genotypes. D^2 analysis was carried out using eleven important morphological characters.

4.4.1.1. Group constellations

A method suggested by Toucher (Rao, 1952) was used to group the genotypes into different clusters based on D^2 values. Forty five germplasm were grouped into eight clusters. Among eight clusters, cluster V was the largest with 15 genotypes followed by cluster I with 11 genotypes and Cluster VIII with 6 genotypes. Cluster II, IV and VI had 2 genotypes each and cluster III, VII have 3 genotypes each. The clustering composition and the distribution of genotypes into different clusters are presented in Table.35.

4.4.1.2. Intra cluster D^2 values and distance (D)

Intra cluster D^2 values and distance (D) values are given in Table.36. Maximum difference among the genotypes within the same cluster (intra cluster) was shown by cluster I (6049.74) with the distance of 77.78, followed by cluster VIII (4978.65) with the distance of 70.56. Cluster II recorded the minimum difference (98.30) with a distance of 9.915.

4.4.1.3. Inter cluster D^2 values and distance (D)

Diversity among the cluster varied with inter cluster D^2 values of 1791.55 to 19274.14. The cluster IV and III showed maximum inter cluster D^2 value (19274.14) with a distance of 138.83 followed by VII and III with a inter cluster D^2 value (17007.17) and a distance of 130.14. The lowest inter cluster D^2 value (1791.55) with a distance of 42.32 was shown by V and II (Table. 36).

4.4.1.4. Per cent contribution of characters towards divergence

The per cent contribution of each character towards divergence is presented in Table.37. It was observed that fresh herbage yield per plant (29.49 %) followed by dry herbage yield per plant (21.31 %) and number of branches per plant (20.10 %) contributed maximum towards diversity. Number of berries per plant and number of leaves per plant contributed 11.72 % and 10.40 % respectively towards divergence. Minimum contribution toward divergence was seen in days to fifty per cent flowering (2.73%) plant height (1.92) and plant spread (1.24 and 1.20). However, stem girth and days to flower bud initiation did not contribute towards divergence.

4.4.1.5. Cluster mean values for characters

The mean values of 11 characters for eight clusters are presented in Table.38. Cluster VI recorded the highest mean value for plant height (119.05) followed by cluster III (117.84) and cluster I (97.05). Cluster IV recorded the lowest mean value for plant height (73.87). Cluster VI recorded the highest mean value for plant spread (56.70 and 54.76) Cluster IV recorded the lowest mean value for plant spread (32.12 and 29.65).

Cluster VI recorded the maximum stem girth of 5.16. Cluster IV recorded the minimum stem girth of 2.23. The highest mean values for number of branches was recorded by cluster VI (29.37), followed by cluster III (24.15) and the lowest was recorded by cluster VII (13.89). The highest mean for number of leaves per plant was recorded by cluster III (145.15) and the lowest by cluster IV (101.65). The highest number of berries per plant was

recorded by cluster IV (274.61) while, the lowest mean for number of berries per plant was recorded by cluster II (206.46). The minimum mean value for flower bud initiation was recorded by cluster VI (20.02) and cluster II recorded more number of days for flower bud initiation (29.36).

Cluster VI took less days for fifty per cent flowering (50.35) and cluster II more days to fifty per cent flowering (57.55). Cluster VI recorded the maximum value for fresh herbage yield per plant (463.11) followed by cluster III (460.74) while, cluster VII recorded the lowest mean value for fresh herbage yield per plant (262.39). Cluster VI recorded the highest mean value for dry herbage yield per plant (223.44) and cluster II recorded lowest mean value for dry herbage yield per plant (121.92).

4.4.2. Genetic Diversity studies based on molecular markers

4.4.2.1. SSR primer polymorphism

In present study, 45 makoi genotypes were evaluated for genetic diversity using simple sequence repeat (SSR) markers.

All the 10 primer pairs used in the study showed amplification. Out of the 10 primers used, 7 were found to be polymorphic and 3 primer pairs were found to be monomorphic. The 10 primer pairs generated a total of 22 alleles. The number of alleles produced by different primers ranged from two to four with an average of 2.30 markers per primer. Among the 10 primers used, primer STWIN 12 G produced maximum number of four amplified products. The SSR marker profiles of 45 accessions generated by the primers STWIN 12 G is given in Plate 8.

4.4.2.2. Polymorphism Information Content

The polymorphism information content (PIC) was calculated for SSR markers. PIC was highest for the SSR primers STWIN 12 G (0.698) lowest for the primer SB6-84 (0.340). The results are presented in Table 39. Higher the PIC, more informative is the SSR marker. Hence, primer STWIN 12 G was found to be highly informative.

4.4.2.3. Similarity index and hierarchical clustering

The banding pattern of the SSR markers scored in the form of binary data was used for computing Jaccard's similarity index. The similarity index values obtained for each pair wise comparison among the 45 genotypes are presented in the Table 40. The similarity coefficients based on 10 SSR markers ranged from 0.36 to 1.00. Among the 45 genotypes

studied, the highest similarity index (1.00) was recorded in all combinations of TN *Sn* 01, TN *Sn* 02, TN *Sn* 03, TN *Sn* 04, TN *Sn* 05, TN *Sn* 13, TN *Sn* 15, TN *Sn* 16, TN *Sn* 21, TN *Sn* 24, TN *Sn* 25, TN *Sn* 26, TN *Sn* 27, TN *Sn* 28, TN *Sn* 33, TN *Sn* 35, TN *Sn* 36 and TN *Sn* 37. The lowest similarity index (0.36) was recorded between the genotype TN *Sn* 32 and TN *Sn* 01, TN *Sn* 02, TN *Sn* 03, TN *Sn* 04, TN *Sn* 05, TN *Sn* 06, TN *Sn* 07, TN *Sn* 13, TN *Sn* 15, TN *Sn* 16, TN *Sn* 21, TN *Sn* 24, TN *Sn* 25, TN *Sn* 26, TN *Sn* 27, TN *Sn* 28, TN *Sn* 33, TN *Sn* 35, TN *Sn* 36 and TN *Sn* 37. The similarity values obtained for each pair wise comparison of SSR markers among the 45 genotypes were used to construct dendrogram based on hierarchical clustering and the results are presented in Fig.10. 45 genotypes were grouped into 8 clusters at nearly 36 % similarity level. The cluster size varied from 32 (cluster I) to 1 (Clusters II, V, VII and VIII). The list of all the 8 clusters is presented in Table 41.

The cluster I consisted of 32 genotypes and the cluster II consisted of TN *Sn* 19. The cluster III consisted of TN *Sn* 8, TN *Sn* 12, TN *Sn* 38 and TN *Sn* 23. The cluster IV consisted of TN *Sn* 52 and TN *Sn* 53. The cluster V consisted of TN *Sn* 10. The cluster VI consisted of TN *Sn* 30, TN *Sn* 31 and TN *Sn* 32. The cluster VII consisted of TN *Sn* 44 and the cluster VIII consisted of TN *Sn* 47.

4.5. Studies on variability and genetic parameters

A measure of variability and an understanding of genetic makeup of the crop are important constituents of any crop improvement programme. The estimates of variability on the basis of genotypic coefficient of variation, phenotypic coefficient of variation, heritability and genetic advance as per cent of mean are presented in Tables 43 - 45.

4.5.1. Phenotypic and genotypic coefficient of variability

The GCV per cent was found to be less than PCV per cent for all the traits studied in all the three species studied. In *Solanum nigrum* L., the maximum PCV and GCV were observed for protein content (20.42 and 20.34) and dry herbage yield per plant (19.45 and 18.81). The minimum values of PCV and GCV was recorded for total alkaloid content (3.81 and 3.01) and days to fifty per cent flowering (4.29 and 4.19).

In *Solanum americanum* Mill, the maximum PCV and GCV were observed for iron content (26.79 and 26.77) and dry herbage yield per plant (19.29 and 19.24). The minimum values of PCV and GCV was recorded for total alkaloid content (5.61 and 4.61) and days to fifty per cent flowering (2.89 and 2.79). In *Solanum villosum* Mill, the maximum PCV and

GCV were observed for dry herbage yield (26.70 and 26.69) and plant spread (21.55 and 21.45). The minimum values of PCV and GCV was recorded for total alkaloid content (3.32 and 3.31) and number of berries per plant (5.61 and 5.58)

4.5.2. Heritability and genetic advance

Estimates of heritability (h^2) and GA as percentage of mean for 15 traits of pooled data of all three species are furnished in Table 43 -45.

In *Solanum nigrum L*., heritability (h^2) value was recorded as maximum for quality characters than the biometric characters. Total alkaloid (92.69), dry herbage yield per plant (91.59) and iron content (90.72) recorded higher estimates of heritability (h^2). A moderate estimate of heritability was recorded for crude fibre (89.34) and ascorbic acid (89.67). The heritability was low for plant height (73.10), stem girth (72.66) and number of berries per plant (69.79).

In *Solanum americanum* Mill., also heritability (h^2) value was recorded as maximum for quality characters than the biometric characters. Total alkaloid (91.49), dry herbage yield per plant (90.18) and iron content (94.89) recorded higher estimates of heritability (h^2).

In *Solanum villosum* Mill., heritability (h^2) value was recorded as maximum for total alkaloid (90.28), dry herbage yield per plant (91.86), iron content (95.49) and ascorbic acid (93.48) recorded higher estimates of heritability (h^2).

The GA expressed as percentage of mean was maximum for fresh and dry herbage yield (37.50 per cent and 36.59 per cent respectively) followed by protein content (30.00 per cent) among the *Solanum nigrum* L. genotypes. In case of *Solanum americanum* Mill. The GA was high for iron content (35.13 per cent) and number of leaves (34.08 per cent) followed by number of leaves (34.08) and crude fiber (28.76 per cent).

In *Solanum villosum* Mill. The characters like protein content (30.09 per cent) and number of branches (35.94 per cent) followed by crude fiber (28.71 per cent).

4.6. Character association through correlation and path analysis

Yield is a complex character and depends upon number of yield contributing traits. In order to understand the inter-character associations among different yield contributing characters, it is necessary to interpret correlation in crop plants. The selection practiced for one character may simultaneously bring change in the other related character. Thus, the

information of the magnitude and direction of association between the component characters is essential for the improvement in the desirable direction. Therefore, it is worthwhile to work out the association of the component characters with yield as well as with the other characters in order to suggest a suitable selection index that can be used for selection of the germplasm for the improvement in the yield. Simple correlation coefficient was worked out for all three species separately (Table.46 -48)

4.6. Correlation studies

4.6.1.1. Correlation between alkaloid yield and yield components in *S.nigrum* L.

In case of *S.nigrum* L. genotypes, total alkaloid yield expressed highly significant and positive correlation with dry herbage yield per plant (0.465), crude fiber (0.433), fresh herbage yield per plant (0.368) and number of primary branches (0.342). It also has significant negative association with days to 50 per cent flowering (-0.360) and protein content (-0.433) (Table.46).

4.6.1.2. Inter correlation among yield components in *S.nigrum* L.

Plant height exhibited positive correlation with plant spread (0.819 and 0.809), fresh herbage yield per plant (0.683), dry herbage yield per plant (0.425), number of leaves per plant (0.466) and protein content (0.415). Plant spread expressed significant positive correlation with number of primary branches (0.655) and number of leaves per plant (0.452).

Number of primary branches had highly significant positive correlation with number of leaves per plant (0.506), fresh herbage yield per plant (0.473), protein content (0.443) and significant negative correlation fifty per cent flowering (-0.561).

Number of leaves per plant registered significant positive correlation with protein content (0.551), crude fiber (0.553) and iron content (0.470) and negative association with fifty per cent flowering (-0.492).

Number of berries per plant had significant positive association with days to flower bud initiation (0.616). Days to fifty per cent flowering had significant negative association with fresh herbage yield per plant (-0.390), protein content (-0.563), crude fiber (-0.445) and iron content (-0.409).

Fresh herbage yield expressed highly significant and positive correlation with dry herbage yield per plant (0.825), protein content (0.322), crude fiber (0.365) and iron content

(0.330). Dry herbage yield expressed highly significant and positive correlation with protein content (0.545) and crude fiber (0.608).

The protein content expressed highly significant and positive correlation with crude fiber (0.638) iron content (0.709) and ascorbic acid (0.564). The crude fiber expressed highly significant and positive correlation with iron content (0.599) and ascorbic acid (0.309). the iron content expressed high positive correlation with ascorbic acid (0.495).

4.6.2.1. Correlation between alkaloid yield and yield components in *S.americanum* Mill.

In case of *S.americanum Mill*. genotypes, total alkaloid yield expressed highly significant and positive correlation with dry herbage yield per plant (0.590), crude fiber (0.392), fresh herbage yield per plant (0.344) and number of primary branches (0.481). It also has significant negative association with days to 50 per cent flowering (-0.360) and protein content (-0.433) (Table.47).

4.6.2.2. Inter correlation among yield components in *S.americanum* Mill.

Plant height exhibited positive correlation with plant spread (0.869 and 0.878), fresh herbage yield per plant (0.843), dry herbage yield per plant (0.755) and ascorbic acid content (0.417). Plant spread expressed significant positive correlation with number of primary branches (0.433) and fresh herbage yield per plant (0.564).

Number of primary branches had highly significant positive correlation with number of leaves per plant (0.500), fresh herbage yield per plant (0.527), dry herbage yield per plant(0.528) and significant negative correlation with ascorbic acid (-0.837).

Number of leaves per plant registered significant positive correlation with fresh herbage yield per plant (0.592), protein content (0.551), crude fiber (0.532) and ascorbic acid (0.511) and negative association with fifty per cent flowering (-0.657).

Days to flower bud initiation expressed highly negative correlation with fresh herbage yield per plant (-0.670), dry herbage yield per plant (-0.493). Fresh herbage yield expressed highly significant and positive correlation with dry herbage yield per plant (0.797) and protein content (0.452). Dry herbage yield expressed highly significant and positive correlation with protein content (0.445). No significant correlation was noticed among protein content, crude fiber, iron content and ascorbic acid.

4.6.2.1. Correlation between alkaloid yield and yield components in *S.villosum* Mill.

In case of *S.villosum Mill*. genotypes, total alkaloid yield expressed highly significant and positive correlation with primary branches (0.617), ascorbic acid (0.507) and plant spread (0.463 and 0.522). It also has significant negative association with iron content (-0.452) (Table.49).

4.6.2.2. Inter correlation among yield components in *S.villosum* Mill.

Plant height exhibited positive correlation with plant spread (0.739 and 0.756), fresh herbage yield per plant (0.504), dry herbage yield per plant (0.424) and number of leaves per plant (0.893). Plant spread expressed significant positive correlation with number of primary branches (0.447) and number of leaves per plant (0.493).

Number of primary branches had highly significant positive correlation with number of leaves per plant (0.677), fresh herbage yield per plant (0.558) and dry herbage yield per plant (0.525). Number of leaves per plant registered significant positive correlation with fresh herbage yield per plant (0.826) and dry herbage yield per plant (0.538).

Number of berries per plant has significant positive correlation with days to flower bud initiation (0.567). Days to flower bud initiation expressed highly positive correlation with days to fifty per cent flowering (0.567) and negative correlation with fresh herbage yield per plant (-0.534). Fresh herbage yield expressed highly significant and positive correlation with dry herbage yield per plant (0.897) and iron content (0.453). Dry herbage yield expressed highly significant and positive correlation with protein content (0.372). The protein content expressed significant positive correlation with iron content (0.351) and the iron content has positive correlation with ascorbic acid (0.304).

Table 5. Morphological characterization of genotypes

Characters	Growth habit	Branching	Stem Pubescence	Stem Shape	Stem Color	Leaf Shape	Leaf Pubescence	Leaf Margin	Leaf Color	Flower Size	Flower Color	Berry Size	Berry Color	Seed color	Ploidy
TN Sn 1	Intermediate	Intermediate	Absent	Cylinderical	Green	Ovate	Absent	Entire	Green	Small	White	Small	Black	Brown	2x
TN Sn 2	Intermediate	Intermediate	Absent	Cylinderical	Green	Ovate	Absent	Entire	Green	Small	White	Small	Black	Brown	2x
TN Sn 3	Intermediate	Intermediate	Absent	Cylinderical	Green	Ovate	Absent	Entire	Green	Small	White	Small	Black	Brown	2x
TN Sn 4	Intermediate	Intermediate	Absent	Cylinderical	Green	Ovate	Absent	Entire	Green	Small	White	Small	Black	Brown	2x
TN Sn 5	Spreading	Dense	Absent	Cylinderical	Green	Ovate	Absent	Entire	Green	Small	White	Medium	Black	Brown	2x
TN Sn 6	Spreading	Dense	Absent	Cylinderical	Green	Ovate	Absent	Entire	Green	Small	White	Medium	Black	Brown	2x
TN Sn 7	Intermediate	Intermediate	Absent	Cylinderical	Green	Ovate	Absent	Entire	Green	Small	White	Small	Black	Brown	2x
TN Sn 8	Intermediate	Intermediate	Sparse	Cylinderical	Green	Ovate	Sparse	Sinuate	Light green	Medium	White	Medium	Black	Brown	2x
TN Sn 9	Intermediate	Intermediate	Absent	Cylinderical	Green	Ovate	Absent	Entire	Green	Small	White	Small	Black	Brown	2x
TN Sn 10	Erect	Dense	Dense	Angled	Dark purple	Deltoid	Dense	Undulate	Dark green	Big	White	Big	Black	Brown	4x
TN Sn 11	Spreading	Dense	Absent	Cylinderical	Green	Ovate	Absent	Entire	Green	Small	White	Medium	Black	Brown	2x
TN Sn 12	Intermediate	Intermediate	Sparse	Cylinderical	Green	Ovate	Sparse	Sinuate	Green	Medium	White	Medium	Black	Brown	2x
TN Sn 13	Intermediate	Intermediate	Absent	Cylinderical	Green	Ovate	Absent	Entire	Green	Small	White	Small	Black	Brown	2x
TN Sn 14	Spreading	Dense	Absent	Cylinderical	Green	Ovate	Absent	Entire	Green	Small	White	Small	Black	Brown	2x
TN Sn 15	Intermediate	Intermediate	Absent	Cylinderical	Green	Ovate	Absent	Entire	Green	Small	White	Small	Black	Brown	2x

Morphological characterization of genotypes

Characters	Growth habit	Branching	Stem Pubescence	Stem Shape	Stem Color	Leaf Shape	Leaf Pubescence	Leaf Margin	Leaf Color	Flower Size	Flower Color	Berry Size	Berry Color	Seed color	Ploidy
TN Sn 16	Intermediate	Intermediate	Absent	Cylinderical	Green	Ovate	Absent	Entire	Green	Small	White	Small	Black	Brown	2x
TN Sn 17	Intermediate	Intermediate	Absent	Cylinderical	Green	Ovate	Absent	Entire	Green	Small	White	Small	Black	Brown	2x
TN Sn 18	Intermediate	Intermediate	Absent	Cylinderical	Green	Ovate	Absent	Entire	Green	Small	White	Small	Black	Brown	2x
TN Sn 19	Spreading	Dense	Absent	Cylinderical	Green	Ovate	Absent	Entire	Green	Small	White	Big	Black	Brown	2x
TN Sn 20	Spreading	Dense	Absent	Cylinderical	Green	Ovate	Absent	Entire	Green	Small	White	Medium	Black	Brown	2x
TN Sn 21	Intermediate	Intermediate	Absent	Cylinderical	Green	Ovate	Absent	Entire	Green	Small	White	Small	Black	Brown	2x
TN Sn 22	Spreading	Dense	Absent	Cylinderical	Green	Ovate	Absent	Entire	Green	Small	White	Medium	Black	Brown	2x
TN Sn 23	Intermediate	Intermediate	Sparse	Cylinderical	Green	Ovate	Sparse	Sinuate	Light green	Medium	White	Medium	Black	Brown	2x
TN Sn 24	Intermediate	Intermediate	Absent	Cylinderical	Green	Ovate	Absent	Entire	Green	Small	White	Small	Black	Brown	2x
TN Sn 25	Spreading	Dense	Absent	Cylinderical	Green	Ovate	Absent	Entire	Green	Small	White	Medium	Black	Brown	4x
TN Sn 26	Intermediate	Intermediate	Absent	Cylinderical	Green	Ovate	Absent	Entire	Green	Small	White	Small	Black	Brown	2x
TN Sn 27	Intermediate	Intermediate	Absent	Cylinderical	Green	Ovate	Absent	Sinuate	Green	Small	White	Small	Black	Brown	2x
TN Sn 28	Intermediate	Intermediate	Absent	Cylinderical	Green	Ovate	Absent	Entire	Green	Small	White	Small	Black	Brown	2x
TN Sn 29	Intermediate	Intermediate	Absent	Cylinderical	Green	Ovate	Absent	Entire	Green	Small	White	Small	Black	Brown	2x
TN Sn 30	Spreading	Dense	Dense	Angled	Purple	Deltoid	Dense	Sinuate	Dark green	Big	White	Medium	Red	Straw	4x

Morphological characterization of genotypes

Characters	Growth habit	Branching	Stem Pubescence	Stem Shape	Stem Color	Leaf Shape	Leaf Pubescence	Leaf Margin	Leaf Color	Flower Size	Flower Color	Berry Size	Berry Color	Seed color	Ploidy
TN Sn 31	Spreading	Dense	Dense	Angled	Purple	Deltoid	Dense	Sinuate	Dark green	Big	White	Medium	Red	Straw	4x
TN Sn 32	Intermediate	Intermediate	Absent	Cylinderical	Green	Ovate	Absent	Entire	Green	Small	White	Small	Red	Brown	4x
TN Sn 33	Intermediate	Intermediate	Absent	Cylinderical	Green	Ovate	Absent	Entire	Green	Small	White	Small	Black	Brown	2x
TN Sn 35	Intermediate	Intermediate	Absent	Cylinderical	Green	Ovate	Absent	Entire	Green	Small	White	Small	Black	Brown	2x
TN Sn 36	Intermediate	Intermediate	Absent	Cylinderical	Green	Ovate	Absent	Entire	Green	Small	White	Small	Black	Brown	2x
TN Sn 37	Intermediate	Intermediate	Absent	Cylinderical	Green	Ovate	Absent	Entire	Green	Small	White	Small	Black	Brown	2x
TN Sn 38	Intermediate	Intermediate	Sparse	Cylinderical	Green	Ovate	Sparse	Sinuate	Light green	Medium	White	Medium	Black	Brown	2x
TN Sn 40	Spreading	Dense	Sparse	Cylinderical	Green	Ovate	Absent	Entire	Light green	Small	White	Medium	Black	Brown	2x
TN Sn 41	Spreading	Dense	Absent	Cylinderical	Green	Ovate	Absent	Entire	Green	Small	White	Medium	Black	Brown	2x
TN Sn 42	Spreading	Dense	Absent	Cylinderical	Green	Ovate	Absent	Entire	Green	Small	White	Medium	Black	Brown	4x
TN Sn 44	Prostrate	Intermediate	Dense	Angled	Dark green	Ovate	Dense	Sinuate	Green	Big	White	Big	Orange	Straw	4x
TN Sn 47	Spreading	Dense	Dense	Angled	Dark green	Ovate	Dense	Sinuate	Dark green	Big	White with purple stripes	Big	Orange	Straw	2x
TN Sn 51	Intermediate	Intermediate	Absent	Cylinderical	Green	Ovate	Absent	Entire	Green	Small	White	Small	Black	Brown	2x
TN Sn 52	Intermediate	Intermediate	Sprase	Cylinderical	Green	Ovate	Sparse	Sinuate	Light green	Medium	White	Medium	Black	Brown	2x
TN Sn 53	Intermediate	Intermediate	Sprase	Cylinderical	Green	Ovate	Sparse	Sinuate	Light green	Medium	White	Medium	Black	Brown	4x

Table.6.a. Variations in Stomatal, pollen, flower and berry characters in distinct genotypes

S.No	Genotypes	No. of stomata per unit area	Stomatal length (µ)	Stomatal breath (µ)	Pollen diameter (µ)	Pollen fertility (%)	No.of flowers per inflorescence	Flower diameter (cm)	Berry diameter (cm)	No.of seeds per berry	Root tip mitosis results
1.	TN *Sn* 08	15.22	20.97	13.82	23.49	92.14	3	0.53	0.62	37.83	Diploid (2n = 24)
2.	TN *Sn* 10	9.11	25.03	16.70	27.06	98.56	8	0.96	0.92	31.33	Hexaploid (2n = 72)
3.	TN *Sn* 12	15.56	20.84	13.90	23.46	93.04	3	0.55	0.65	37.83	Diploid (2n = 24)
4.	TN *Sn* 19	16.67	20.13	13.54	23.05	92.47	3	0.49	0.58	37.83	Diploid (2n = 24)
5.	TN *Sn* 23	15.11	20.89	13.92	23.47	93.25	3	0.56	0.64	37.67	Diploid (2n = 24)
6.	TN *Sn* 30	11.22	23.91	14.98	26.90	96.23	5	0.69	0.72	34.83	Tetraploid (2n = 48)
7.	TN *Sn* 32	11.33	23.99	15.08	26.91	95.14	5	0.70	0.76	34.83	Tetraploid (2n = 48)
8.	TN *Sn* 38	14.67	21.03	13.94	23.56	92.89	3	0.55	0.63	38.50	Diploid (2n = 24)
9.	TN *Sn* 44	10.67	24.18	15.31	26.95	95.47	5	0.71	0.78	33.17	Tetraploid (2n = 48)
10.	TN *Sn* 47	11.44	24.12	15.06	26.87	96.04	5	0.70	0.71	33.17	Tetraploid (2n = 48)
12.	TN *Sn* 52	15.56	20.93	13.93	23.50	93.74	3	0.55	0.65	37.00	Diploid (2n = 24)
13.	TN *Sn* 53	15.76	21.01	13.90	23.52	93.56	3	0.54	0.62	37.67	Diploid (2n = 24)

Table 6.b. List of genotypes subjected to DNA barcode analysis

S.No	Accession number	Ploidy	Identified as	GenBank Accession number
1	TN*Sn* 08	Diploid	*Solanum americanum* Miller.	KC540784
2	TN*Sn* 10	Hexaploid	*Solanum nigrum* L	KC540785
3	TN*Sn* 12	Diploid	*Solanum americanum* Miller.	KC540786
4	TN*Sn* 19	Diploid	*Solanum nigrum* L	KC540796
5	TN*Sn* 23	Diploid	*Solanum americanum* Miller.	KC540787
6	TN*Sn* 30	Tetraploid	*Solanum villosum* Miller.	KC540788
7	TN*Sn* 32	Tetraploid	*Solanum villosum* Miller.	KC540789
8	TN*Sn* 38	Diploid	*Solanum americanum* Miller.	KC540790
9	TN*Sn* 44	Tetraploid	*Solanum villosum* Miller.	KC540795
10	TN*Sn* 47	Tetraploid	*Solanum villosum* Miller.	KC540791
12	TN*Sn* 52	Diploid	*Solanum americanum* Miller.	KC540793
13	TN*Sn* 53	Diploid	*Solanum americanum* Miller.	KC540794

Table 7. Analysis of variance for different characters of *Solanum nigrum* L. genotypes

Sl. No.	Characters	Mean sum of squares		
		Season I		
		Replication	Treatment	Error
1.	Plant height (cm)	20.05	181.72**	28.23
2.	Plant spread (N-S)	7.71	73.22**	5.64
3.	Plant spread (E-W)	19.29	93.98**	3.70
4.	Stem girth (cm)	0.85	1.13**	0.04
5.	Leaf length (cm)	0.07	2.05**	0.06
6.	Leaf breadth (cm)	0.25	0.67**	0.02
7.	Number of branches per plant	1.56	21.82**	0.94
8.	Number of leaves per plant	0.51	657.06**	103.22
9.	Days to flower bud initiation	2.93	6.93**	0.10
10.	Days to 50 per cent flowering	0.59	1.00**	0.07
11.	Number of berries per plant	10.72	981.82**	2.06
12.	Fresh herbage yield per plant (g)	22.19	6851.01**	226.12
13.	Dry herbage yield per plant (g)	31.35	2059.93**	4.23
14.	Total alkaloid content (%)	0.001	0.0003*	0.0001
15.	Protein content (mg $100g^{-1}$)	0.17	0.83**	0.002
16.	Crude fibre (%)	0.02	0.003*	0.0001
17.	Iron content (mg $100g^{-1}$)	0.34	0.26**	0.003
18.	Ascorbic acid (mg g^{-1})	1.04	5.31**	0.09

* Significance at 5% level, ** Significance at 1% level

Table 8. Analysis of variance for different characters of *Solanum americanum* Mill. genotypes

Sl. No.	Characters	Mean sum of squares		
		Season I		
		Replication	Treatment	Error
1.	Plant height (cm)	30.69	194.30**	0.81
2.	Plant spread (N-S)	0.36	45.46**	3.13
3.	Plant spread (E-W)	17.78	62.19**	1.55
4.	Stem girth (cm)	0.002	1.01**	0.01
5.	Leaf length (cm)	0.022	0.80**	0.01
6.	Leaf breadth (cm)	0.02	0.18**	0.02
7.	Number of branches per plant	9.24	1.06**	0.13
8.	Number of leaves per plant	25.90	159.63**	0.83
9.	Days to flower bud initiation	1.21	17.95**	0.40
10.	Days to 50 per cent flowering	0.004	4.93**	0.04
11.	Number of berries per plant	12.34	389.45**	2.61
12.	Fresh herbage yield per plant (g)	101.72	2793.99**	2.72
13.	Dry herbage yield per plant (g)	40.66	1045.77**	4.30
14.	Total alkaloid content (%)	0.0007	0.0009*	0.0002
15.	Protein content (mg $100g^{-1}$)	0.013	0.078**	0.0003
16.	Crude fibre (%)	0.003	0.013*	0.02
17.	Iron content (mg $100g^{-1}$)	0.06	1.56**	0.01
18.	Ascorbic acid (mg g^{-1})	5.07	1.83**	0.02

* Significance at 5% level, ** Significance at 1% level

Table 9. Analysis of variance for different characters of *Solanum villosum* Mill. genotypes

Sl. No.	Characters	Mean sum of squares		
		Season I		
		Replication	Treatment	Error
1.	Plant height (cm)	10.22	850.38**	0.44
2.	Plant spread (N-S)	0.55	196.74**	1.96
3.	Plant spread (E-W)	7.77	215.88**	1.04
4.	Stem girth (cm)	0.072	1.66**	0.003
5.	Leaf length (cm)	0.05	3.56**	0.02
6.	Leaf breadth (cm)	0.13	0.68**	0.003
7.	Number of branches per plant	1.90	76.39**	1.05
8.	Number of leaves per plant	20.24	170.11**	0.83
9.	Days to flower bud initiation	2.45	0.39**	0.12
10.	Days to 50 per cent flowering	0.005	0.49**	0.08
11.	Number of berries per plant	21.53	324.60**	1.45
12.	Fresh herbage yield per plant (g)	137.04	12757.71**	1.12
13.	Dry herbage yield per plant (g)	19.00	4600.84**	3.16
14.	Total alkaloid content (%)	0.0001	0.0004*	0.0001
15.	Protein content (mg $100g^{-1}$)	0.038	0.026**	0.008
16.	Crude fibre (%)	0.001	0.003*	0.0004
17.	Iron content (mg $100g^{-1}$)	0.070	0.29**	0.0067
18.	Ascorbic acid (mg g^{-1})	2.77	1.51**	0.05

* Significance at 5% level, ** Significance at 1% level

Table. 10. Mean performance of *Solanum* genotypes for plant height (cm)

***Solanum nigrum* L.**							
Genotypes	Season I	Season II	Pooled	Genotypes	Season I	Season II	Pooled
TN *Sn* 1	97.09	99.17	98.13	TN *Sn* 21	85.43	85.66	85.54
TN *Sn* 2	80.66	81.76	81.21	TN *Sn* 22	106.61	110.72	108.67
TN *Sn* 3	73.93	77.04	75.49	TN *Sn* 24	83.47	85.53	84.50
TN *Sn* 4	90.96	93.70	92.33	TN *Sn* 25	91.80	93.65	92.73
TN *Sn* 5	84.60	85.42	85.01	TN *Sn* 26	93.31	96.47	94.89
TN *Sn* 6	96.65	97.03	96.84	TN *Sn* 27	70.56	74.10	72.33
TN *Sn* 7	87.24	87.07	87.15	TN *Sn* 28	80.44	81.15	80.80
TN *Sn* 9	93.12	95.14	94.13	TN *Sn* 29	80.49	125.24	102.87
TN *Sn* 11	99.00	101.13	100.07	TN Sn 33	92.10	95.93	94.02
TN *Sn* 13	91.74	93.97	92.86	TN Sn 35	88.93	90.64	89.79
TN *Sn* 14	94.08	97.58	95.83	TN Sn 36	88.81	90.79	89.81
TN *Sn* 15	74.01	75.32	74.67	TN Sn 37	79.15	80.58	79.87
TN *Sn* 16	78.78	80.38	79.58	TN Sn 40	93.97	96.46	95.21
TN *Sn* 17	96.87	98.28	97.58	TN Sn 41	90.38	92.73	91.55
TN *Sn* 18	94.45	96.53	95.49	TN Sn 42	86.50	88.10	87.30
TN *Sn* 19	111.47	112.49	111.98	TN Sn 51	102.50	105.78	104.14
TN *Sn* 20	98.85	101.12	99.98				
		Season I		Season II		Pooled	
MEAN		**89.63**		**92.93**		**91.01**	
SED		5.31		5.29		5.34	
CV		5.82		6.03		6.04	
CD (P= 0.05)		10.67		10.45		10.67	

Solanum americanum Mill.				***Solanum villosum Mill.***			
Genotypes	Season I	Season II	Pooled	Genotypes	Season I	Season II	Pooled
TN *Sn* 8	116.1	119.94	118.03	TN *Sn* 30	121.61	124.59	123.10
TN *Sn* 12	113.94	114.47	114.21	TN Sn 31	111.43	113.61	112.53
TN *Sn* 23	88.13	90.48	89.31	TN Sn 32	117.81	118.49	118.15
TN Sn 38	112.11	116.65	114.39	TN Sn 44	73.11	75.88	74.50
TN Sn 52	114.42	117.90	116.16	TN Sn 47	123.28	124.78	124.03
TN Sn 53	113.33	116.36	114.85				
MEAN	**109.67**	**112.63**	**111.16**	**MEAN**	**109.45**	**111.47**	**110.46**
SED	0.90	0.89	0.86	**SED**	0.66	0.76	0.70
CV	0.81	0.87	0.79	**CV**	0.60	0.67	0.83
CD (P=0.05)	1.81	1.76	1.80	**CD(P=0.05)**	1.34	1.45	1.40

Table 11. Mean performance of Solanum genotypes for plant spread (N-S) (cm)

***Solanum nigrum* L.**							
Genotypes	Season I	Season II	Pooled	Genotypes	Season I	Season II	Pooled
TN *Sn* 1	50.74	50.24	50.49	TN *Sn* 21	39.62	37.76	38.69
TN *Sn* 2	38.72	37.23	37.98	TN *Sn* 22	50.81	48.65	49.73
TN *Sn* 3	38.73	38.21	35.47	TN *Sn* 24	42.81	43.14	42.98
TN *Sn* 4	42.22	42.23	42.73	TN *Sn* 25	47.95	46.34	47.15
TN *Sn* 5	43.66	42.25	42.96	TN *Sn* 26	49.08	47.56	48.32
TN *Sn* 6	50.58	53.21	51.90	TN *Sn* 27	31.34	34.64	32.99
TN *Sn* 7	41.23	45.32	43.28	TN *Sn* 28	33.67	39.56	36.62
TN *Sn* 9	51.88	52.10	38.63	TN *Sn* 29	48.39	42.39	45.39
TN *Sn* 11	48.21	51.03	49.62	TN Sn 33	48.56	52.21	50.39
TN *Sn* 13	36.95	39.31	38.63	TN Sn 35	39.82	33.21	36.52
TN *Sn* 14	30.86	42.15	45.38	TN Sn 36	48.13	49.67	48.90
TN *Sn* 15	32.90	33.99	33.45	TN Sn 37	38.34	39.56	38.95
TN *Sn* 16	36.29	34.23	35.26	TN Sn 40	48.85	51.23	50.04
TN *Sn* 17	42.71	41.40	42.06	TN Sn 41	44.79	47.34	46.07
TN *Sn* 18	42.62	42.36	42.39	TN Sn 42	39.25	39.23	39.24
TN *Sn* 19	50.84	56.32	53.58	TN Sn 51	49.88	50.33	50.11
TN *Sn* 20	48.29	46.89	47.59				
		Season I		Season II		Pooled	
MEAN		**43.29**		**43.98**		**43.44**	
SED		2.37		2.45		2.40	
CV		5.44		5.30		5.35	
CD (P= 0.05)		4.77		4.82		4.80	

Solanum americanum Mill.				***Solanum villosum Mill.***			
Genotypes	Season I	Season II	Pooled	Genotypes	Season I	Season II	Pooled
TN *Sn* 8	54.11	57.12	55.62	TN *Sn* 30	58.69	58.41	58.55
TN *Sn* 12	53.02	50.13	51.58	TN Sn 31	54.71	55.32	55.02
TN *Sn* 23	41.36	42.89	42.13	TN Sn 32	51.09	47.25	49.17
TN Sn 38	52.80	50.45	51.63	TN Sn 44	32.75	34.01	33.38
TN Sn 52	53.69	51.29	52.49	TN Sn 47	54.34	54.23	54.29
TN Sn 53	53.84	54.85	54.35				
MEAN	**51.47**	**51.12**	**51.30**	**MEAN**	**50.32**	**49.84**	**50.08**
SED	1.77	1.75	1.78	**SED**	1.40	1.39	1.42
CV	3.45	3.44	3.52	**CV**	2.79	2.80	3.01
CD (P=0.05)	3.55	3.50	3.54	**CD(P=0.05)**	2.81	2.79	2.85

Table 12. Mean performance of Solanum genotypes for plant spread (E-W) (cm)

***Solanum nigrum* L.**							
Genotypes	Season I	Season II	Pooled	Genotypes	Season I	Season II	Pooled
TN *Sn* 1	47.21	49.23	48.22	TN *Sn* 21	35.34	38.00	36.67
TN *Sn* 2	35.23	36.21	35.72	TN *Sn* 22	47.21	49.63	48.42
TN *Sn* 3	29.2	30.25	29.73	TN *Sn* 24	32.21	35.21	33.71
TN *Sn* 4	42.27	44.34	43.31	TN *Sn* 25	42.12	45.23	43.68
TN *Sn* 5	39.04	56.23	47.64	TN *Sn* 26	46.34	48.2	47.27
TN *Sn* 6	47.02	49.63	48.33	TN *Sn* 27	27.87	30.14	29.01
TN *Sn* 7	38.32	40.23	39.28	TN *Sn* 28	30.14	33.78	31.96
TN *Sn* 9	48.99	49.33	49.16	TN *Sn* 29	45.59	47.66	46.63
TN *Sn* 11	45.20	48.23	46.72	TN Sn 33	45.48	47.23	46.36
TN *Sn* 13	43.13	45.23	44.18	TN Sn 35	37.41	39.12	38.27
TN *Sn* 14	45.46	47.23	46.35	TN Sn 36	45.02	49.36	47.19
TN *Sn* 15	29.32	31.25	30.29	TN Sn 37	34.38	36.23	35.31
TN *Sn* 16	32.05	34.23	33.14	TN Sn 40	47.39	51.23	49.31
TN *Sn* 17	49.05	52.13	50.59	TN Sn 41	42.43	45.23	43.83
TN *Sn* 18	46.03	48.23	47.13	TN Sn 42	35.20	37.14	36.17
TN *Sn* 19	48.32	49.63	48.98	TN Sn 51	47.41	50.21	48.81
TN *Sn* 20	46.99	49.00	48.00				
		Season I		Season II		Pooled	
MEAN		**41.04**		**43.76**		**42.41**	
SED		1.92		2.01		1.90	
CV		4.54		4.82		4.50	
CD (P= 0.05)		3.87		4.02		3.85	
Solanum americanum Mill.				***Solanum villosum Mill.***			
Genotypes	Season I	Season II	Pooled	Genotypes	Season I	Season II	Pooled
TN *Sn* 8	49.62	53.2	51.41	TN *Sn* 30	56.18	59.23	57.71
TN *Sn* 12	50.19	53.21	51.70	TN Sn 31	51.21	53.23	52.22
TN *Sn* 23	36.43	39.23	37.83	TN Sn 32	47.87	49.14	48.51
TN Sn 38	50.39	53.21	51.80	TN Sn 44	29.20	32.15	30.68
TN Sn 52	50.29	53.78	52.04	TN Sn 47	52.69	52.22	52.46
TN Sn 53	50.33	49.23	49.78				
MEAN	**47.88**	**50.31**	**49.09**	**MEAN**	**47.43**	**49.19**	**48.32**
SED	1.24	1.30	1.25	**SED**	1.02	1.23	1.12
CV	2.53	3.20	3.15	**CV**	2.11	2.15	2.10
CD (P=0.05)	2.50	2.60	2.51	**CD (P=0.05)**	2.05	2.45	2.24

Table 13. Mean performance of Solanum genotypes for number of primary branches

***Solanum nigrum* L.**							
Genotypes	Season I	Season II	Pooled	Genotypes	Season I	Season II	Pooled
TN *Sn* 1	16.42	15.42	15.92	TN *Sn* 21	9.62	12.36	10.99
TN *Sn* 2	12.98	13.56	13.27	TN *Sn* 22	22.15	24.23	23.19
TN *Sn* 3	10.41	12.36	11.39	TN *Sn* 24	12.49	14.23	13.36
TN *Sn* 4	9.56	11.25	10.41	TN *Sn* 25	14.92	16.36	15.64
TN *Sn* 5	15.01	19.23	17.12	TN *Sn* 26	15.86	17.56	16.71
TN *Sn* 6	9.92	12.01	10.97	TN *Sn* 27	16.73	19.20	17.97
TN *Sn* 7	16.12	16.56	16.34	TN *Sn* 28	10.49	11.52	11.01
TN *Sn* 9	10.42	14.20	12.31	TN *Sn* 29	13.49	15.20	14.35
TN *Sn* 11	15.12	18.23	16.68	TN Sn 33	12.01	14.89	13.45
TN *Sn* 13	15.20	18.23	16.72	TN Sn 35	11.92	16.32	14.12
TN *Sn* 14	14.98	18.23	16.61	TN Sn 36	9.49	11.22	10.36
TN *Sn* 15	15.12	16.35	15.74	TN Sn 37	12.34	15.23	13.79
TN *Sn* 16	16.00	17.23	16.62	TN Sn 40	17.94	19.63	18.79
TN *Sn* 17	15.92	15.23	15.78	TN Sn 41	12.96	14.23	13.60
TN *Sn* 18	13.12	17.56	15.34	TN Sn 42	13.02	15.63	14.33
TN *Sn* 19	24.12	23.01	23.57	TN Sn 51	10.13	12.36	11.25
TN *Sn* 20	9.15	10.56	9.36				
		Season I		Season II		Pooled	
MEAN		**13.79**		**15.74**		**14.76**	
SED		0.97		1.02		1.04	
CV		6.58		6.54		6.23	
CD (P= 0.05)		1.95		2.04		2.08	

Solanum americanum Mill.				***Solanum villosum Mill.***			
Genotypes	Season I	Season II	Pooled	Genotypes	Season I	Season II	Pooled
TN *Sn* 8	18.12	19.23	18.68	TN *Sn* 30	24.49	24.23	24.36
TN *Sn* 12	18.26	19.36	18.81	TN Sn 31	25.14	26.23	25.69
TN *Sn* 23	17.42	19.63	18.53	TN Sn 32	18.59	19.63	19.11
TN Sn 38	19.02	21.03	20.03	TN Sn 44	12.92	12.33	12.63
TN Sn 52	18.49	20.36	19.43	TN Sn 47	11.23	14.32	12.78
TN Sn 53	19.13	21.36	20.25				
MEAN	**18.41**	**20.16**	**19.29**	**MEAN**	**18.47**	**19.35**	**18.91**
SED	0.36	0.35	0.38	**SED**	1.02	1.04	1.03
CV	1.90	1.95	1.89	**CV**	5.42	5.50	5.45
CD (P=0.05)	0.74	0.70	0.74	**CD(P=0.05)**	2.06	2.08	2.06

Table 14. Mean performance of Solanum genotypes for stem girth (cm)

***Solanum nigrum* L.**							
Genotypes	Season I	Season II	Pooled	Genotypes	Season I	Season II	Pooled
TN *Sn* 1	4.02	4.01	4.02	TN *Sn* 21	2.94	3.00	2.97
TN *Sn* 2	2.98	2.52	2.75	TN *Sn* 22	4.05	4.13	4.09
TN *Sn* 3	2.04	2.36	2.20	TN *Sn* 24	2.13	2.56	2.35
TN *Sn* 4	3.65	3.56	3.61	TN *Sn* 25	3.48	3.89	3.69
TN *Sn* 5	3.41	4.78	3.60	TN *Sn* 26	3.99	4.12	4.06
TN *Sn* 6	4.03	4.23	4.13	TN *Sn* 27	2.08	2.56	2.32
TN *Sn* 7	3.56	4.01	3.79	TN *Sn* 28	2.24	2.89	2.57
TN *Sn* 9	4.50	4.59	4.55	TN *Sn* 29	3.81	3.96	3.89
TN *Sn* 11	3.86	3.96	3.91	TN Sn 33	3.87	3.78	3.83
TN *Sn* 13	3.78	3.89	3.84	TN Sn 35	3.24	3.56	3.40
TN *Sn* 14	3.56	3.87	3.72	TN Sn 36	3.87	4.01	3.94
TN *Sn* 15	2.10	2.19	2.15	TN Sn 37	2.75	2.89	2.82
TN *Sn* 16	2.16	2.34	2.25	TN Sn 40	4.18	4.26	4.22
TN *Sn* 17	4.58	4.96	4.77	TN Sn 41	3.50	3.87	3.69
TN *Sn* 18	4.01	4.23	4.12	TN Sn 42	2.89	3.25	3.07
TN *Sn* 19	4.36	4.45	4.41	TN Sn 51	4.16	4.59	4.38
TN *Sn* 20	3.98	4.01	4.00				
		Season I		Season II		Pooled	
MEAN		**3.45**		**3.68**		**3.55**	
SED		0.20		0.28		0.25	
CV		5.83		5.92		5.78	
CD (P= 0.05)		0.41		0.50		0.50	

Solanum americanum Mill.				***Solanum villosum Mill.***			
Genotypes	Season I	Season II	Pooled	Genotypes	Season I	Season II	Pooled
TN *Sn* 8	4.52	4.21	4.37	TN *Sn* 30	5.28	5.46	5.37
TN *Sn* 12	4.69	4.59	4.64	TN Sn 31	4.87	5.01	4.94
TN *Sn* 23	3.01	3.15	3.08	TN Sn 32	4.25	4.56	4.41
TN Sn 38	4.80	4.96	4.88	TN Sn 44	3.06	3.16	3.11
TN Sn 52	4.83	5.02	4.93	TN Sn 47	5.14	5.26	5.20
TN Sn 53	4.86	4.96	4.91				
MEAN	**4.45**	**4.48**	**4.47**	**MEAN**	**4.52**	**4.69**	**4.61**
SED	0.13	0.15	0.16	**SED**	0.05	0.08	0.07
CV	3.10	3.17	3.40	**CV**	1.28	1.25	1.23
CD (P=0.05)	0.27	0.30	0.30	**CD(P=0.05)**	0.11	0.16	0.14

Table15. Mean performance of Solanum genotypes for number of leaves per plant

***Solanum nigrum* L.**							
Genotypes	Season I	Season II	Pooled	Genotypes	Season I	Season II	Pooled
TN *Sn* 1	119.57	121.02	120.30	TN *Sn* 21	86.72	89.36	88.04
TN *Sn* 2	100.42	102.35	101.39	TN *Sn* 22	140.12	146.25	143.19
TN *Sn* 3	92.59	95.23	93.91	TN *Sn* 24	116.82	118.56	117.69
TN *Sn* 4	89.42	92.35	90.89	TN *Sn* 25	97.62	96.32	96.97
TN *Sn* 5	114.92	115.21	115.07	TN *Sn* 26	99.59	104.25	101.92
TN *Sn* 6	115.89	120.3	118.10	TN *Sn* 27	103.42	108.23	105.83
TN *Sn* 7	82.49	85.63	84.06	TN *Sn* 28	114.92	117.25	116.09
TN *Sn* 9	85.92	7.25	46.59	TN *Sn* 29	89.42	92.35	90.89
TN *Sn* 11	112.19	115.24	113.72	TN Sn 33	100.41	105.21	102.81
TN *Sn* 13	110.42	112.32	111.37	TN Sn 35	112.96	114.25	113.61
TN *Sn* 14	100.36	105.36	102.86	TN Sn 36	108.52	112.35	110.44
TN *Sn* 15	96.40	98.56	97.48	TN Sn 37	102.96	108.25	105.61
TN *Sn* 16	105.42	107.23	106.33	TN Sn 40	125.49	127.89	126.69
TN *Sn* 17	116.86	118.56	117.71	TN Sn 41	119.4	121.23	120.32
TN *Sn* 18	102.28	110.78	106.53	TN Sn 42	122.93	125.2	124.07
TN *Sn* 19	141.92	149.23	145.58	TN Sn 51	101.48	104.25	102.87
TN *Sn* 20	82.49	85.26	83.88				
		Season I		Season II		Pooled	
MEAN		**106.43**		**107.06**		**106.75**	
SED		10.15		9.87		10.04	
CV		9.51		8.52		9.25	
CD (P= 0.05)		20.42		19.01		20.08	
Solanum americanum Mill.				***Solanum villosum Mill.***			
Genotypes	Season I	Season II	Pooled	Genotypes	Season I	Season II	Pooled
TN *Sn* 8	130.49	133.02	131.76	TN *Sn* 30	142.96	146.21	144.59
TN *Sn* 12	133.56	135.63	134.60	TN Sn 31	140.42	145.21	142.82
TN *Sn* 23	131.86	135.25	133.56	TN Sn 32	133.42	135.24	134.33
TN Sn 38	132.52	135.21	133.87	TN Sn 44	120.42	123.25	121.84
TN Sn 52	110.69	112.35	111.52	TN Sn 47	139.69	141.23	140.46
TN Sn 53	129.92	135.21	132.57				
MEAN	**128.17**	**131.11**	**129.65**	**MEAN**	**135.38**	**138.23**	**136.81**
SED	0.91	1.02	1.04	**SED**	0.91	0.89	0.90
CV	0.70	0.84	0.80	**CV**	0.66	0.68	0.72
CD (P=0.05)	1.83	2.04	2.07	**CD(P=0.05)**	1.83	1.98	1.85

Table 16. Mean performance of Solanum genotypes for leaf length (cm)

***Solanum nigrum* L.**							
Genotypes	Season I	Season II	Pooled	Genotypes	Season I	Season II	Pooled
TN *Sn* 1	7.37	6.23	6.80	TN *Sn* 21	5.57	6.00	5.79
TN *Sn* 2	4.66	4.23	4.45	TN *Sn* 22	6.65	6.58	6.62
TN *Sn* 3	6.31	5.33	5.82	TN *Sn* 24	4.02	4.23	4.13
TN *Sn* 4	4.44	5.00	4.72	TN *Sn* 25	4.63	4.89	4.76
TN *Sn* 5	6.05	6.32	6.19	TN *Sn* 26	4.48	4.56	4.52
TN *Sn* 6	6.22	6.24	6.23	TN *Sn* 27	4.48	4.57	4.53
TN *Sn* 7	6.07	6.21	6.14	TN *Sn* 28	4.69	4.87	4.78
TN *Sn* 9	5.91	6.01	5.96	TN *Sn* 29	6.57	6.87	6.72
TN *Sn* 11	7.26	7.56	7.41	TN Sn 33	6.88	6.98	6.93
TN *Sn* 13	6.76	6.98	6.87	TN Sn 35	6.21	6.54	6.38
TN *Sn* 14	6.26	6.35	6.31	TN Sn 36	6.58	6.87	6.73
TN *Sn* 15	4.29	4.23	4.26	TN Sn 37	4.98	4.79	4.89
TN *Sn* 16	4.57	4.87	4.72	TN Sn 40	6.91	7.01	6.96
TN *Sn* 17	6.63	6.69	6.66	TN Sn 41	5.08	5.26	5.17
TN *Sn* 18	4.57	5.23	4.90	TN Sn 42	5.91	5.87	5.89
TN *Sn* 19	7.52	7.56	7.54	TN Sn 51	6.29	6.23	6.26
TN *Sn* 20	4.73	4.56	4.65				
		Season I		Season II		Pooled	
MEAN		**5.74**		**5.81**		**5.78**	
SED		0.25		0.28		0.27	
CV		4.41		4.45		4.50	
CD (P= 0.05)		0.51		0.52		0.52	
Solanum americanum Mill.				***Solanum villosum Mill.***			
Genotypes	Season I	Season II	Pooled	Genotypes	Season I	Season II	Pooled
TN *Sn* 8	6.76	6.56	6.66	TN *Sn* 30	5.40	5.56	5.48
TN *Sn* 12	7.26	7.36	7.30	TN Sn 31	5.35	5.87	5.61
TN *Sn* 23	5.52	5.56	5.54	TN Sn 32	7.38	7.28	7.30
TN Sn 38	7.15	7.23	7.19	TN Sn 44	4.24	4.35	4.30
TN Sn 52	6.23	6.56	6.40	TN Sn 47	7.40	7.46	7.48
TN Sn 53	6.61	6.78	6.70				
MEAN	**6.59**	**6.68**	**6.63**	**MEAN**	**5.95**	**6.10**	**6.03**
SED	0.12	0.19	0.12	**SED**	0.16	0.18	0.15
CV	1.85	1.97	1.85	**CV**	2.68	2.70	2.70
CD (P=0.05)	0.24	0.25	0.25	**CD(P=0.05)**	0.32	0.38	0.31

Table 17. Mean performance of Solanum genotypes for leaf breadth (cm)

***Solanum nigrum* L.**							
Genotypes	Season I	Season II	Pooled	Genotypes	Season I	Season II	Pooled
TN *Sn* 1	4.29	4.01	4.15	TN *Sn* 21	2.66	2.88	2.77
TN *Sn* 2	2.53	3.00	2.77	TN *Sn* 22	3.57	3.87	3.72
TN *Sn* 3	3.49	3.86	3.68	TN *Sn* 24	3.36	3.38	3.37
TN *Sn* 4	3.10	3.24	3.17	TN *Sn* 25	2.74	2.87	2.81
TN *Sn* 5	3.02	3.21	3.12	TN *Sn* 26	2.78	2.98	2.88
TN *Sn* 6	2.40	2.56	2.48	TN *Sn* 27	2.36	2.58	2.47
TN *Sn* 7	3.36	3.45	3.41	TN *Sn* 28	2.81	3.21	3.01
TN *Sn* 9	2.95	3.00	2.98	TN *Sn* 29	3.81	3.89	3.85
TN *Sn* 11	2.53	2.98	2.76	TN Sn 33	3.08	3.45	3.27
TN *Sn* 13	2.74	3.22	2.98	TN Sn 35	3.34	3.21	3.28
TN *Sn* 14	2.53	2.33	2.43	TN Sn 36	3.78	3.41	3.60
TN *Sn* 15	1.49	1.41	1.45	TN Sn 37	3.76	3.84	3.80
TN *Sn* 16	2.18	2.11	2.14	TN Sn 40	3.78	3.74	3.76
TN *Sn* 17	2.81	2.98	2.90	TN Sn 41	3.57	3.41	3.49
TN *Sn* 18	2.64	2.87	2.76	TN Sn 42	3.29	3.32	3.31
TN *Sn* 19	3.74	3.89	3.82	TN Sn 51	3.93	3.98	3.96
TN *Sn* 20	2.76	3.12	2.94				
		Season I		Season II		Pooled	
MEAN		**3.07**		**3.19**		**3.13**	
SED		0.15		0.16		0.15	
CV		4.86		4.85		4.98	
CD (P= 0.05)		0.30		0.32		0.35	
Solanum americanum Mill.				***Solanum villosum Mill.***			
Genotypes	Season I	Season II	Pooled	Genotypes	Season I	Season II	Pooled
TN *Sn* 8	3.53	3.56	3.55	TN *Sn* 30	4.62	4.99	4.81
TN *Sn* 12	2.53	2.87	2.70	TN Sn 31	4.34	4.57	4.46
TN *Sn* 23	2.91	3.24	3.08	TN Sn 32	3.74	3.89	3.82
TN Sn 38	3.36	3.12	3.24	TN Sn 44	3.34	3.58	3.46
TN Sn 52	3.36	3.52	3.44	TN Sn 47	3.47	3.65	3.56
TN Sn 53	3.08	3.00	3.02				
MEAN	**3.13**	**3.22**	**3.17**	**MEAN**	**3.90**	**4.14**	**4.02**
SED	0.15	0.14	0.15	**SED**	0.05	0.07	0.06
CV	2.86	2.89	2.51	**CV**	1.48	1.54	1.50
CD (P=0.05)	0.30	0.34	0.30	**CD (P=0.05)**	0.12	0.15	0.13

Table 18. Mean performance of Solanum genotypes on days to flower bud initiation

***Solanum nigrum* L.**							
Genotypes	Season I	Season II	Pooled	Genotypes	Season I	Season II	Pooled
TN *Sn* 1	28.12	28.21	28.18	TN *Sn* 21	29.13	29.36	29.25
TN *Sn* 2	28.17	28.36	28.27	TN *Sn* 22	28.14	28.69	28.42
TN *Sn* 3	25.00	25.23	25.12	TN *Sn* 24	28.78	28.69	28.74
TN *Sn* 4	23.14	23.45	23.30	TN *Sn* 25	24.13	25.32	24.73
TN *Sn* 5	28.14	28.56	28.35	TN *Sn* 26	29.87	30.21	30.04
TN *Sn* 6	24.36	24.53	24.45	TN *Sn* 27	29.01	30.25	29.63
TN *Sn* 7	25.14	25.36	25.25	TN *Sn* 28	29.35	30.25	29.80
TN *Sn* 9	29.45	29.36	29.41	TN *Sn* 29	29.14	30.56	29.85
TN *Sn* 11	25.69	25.36	25.53	TN Sn 33	28.01	29.36	28.69
TN *Sn* 13	25.41	25.36	25.39	TN Sn 35	27.26	28.36	27.81
TN *Sn* 14	27.26	27.96	27.61	TN Sn 36	28.01	28.36	28.19
TN *Sn* 15	28.09	28.35	28.22	TN Sn 37	27.13	27.56	27.35
TN *Sn* 16	28.15	28.36	28.26	TN Sn 40	28.36	29.00	28.68
TN *Sn* 17	29.00	29.36	29.18	TN Sn 41	28.14	28.36	28.25
TN *Sn* 18	24.01	24.23	24.12	TN Sn 42	28.56	28.69	28.63
TN *Sn* 19	29.06	29.36	29.21	TN Sn 51	28.45	29.36	28.91
TN *Sn* 20	28.56	28.36	28.46				
		Season I		Season II		Pooled	
MEAN		**27.52**		**27.94**		**27.74**	
SED		0.31		0.32		0.30	
CV		1.14		1.15		1.20	
CD (P= 0.05)		0.64		0.64		0.61	

Solanum americanum Mill.				***Solanum villosum Mill.***			
Genotypes	Season I	Season II	Pooled	Genotypes	Season I	Season II	Pooled
TN *Sn* 8	21.04	21.36	21.20	TN *Sn* 30	19.05	20.69	19.87
TN *Sn* 12	22.01	22.36	22.19	TN Sn 31	19.99	20.33	20.16
TN *Sn* 23	29.45	29.36	29.41	TN Sn 32	20.14	21.33	20.74
TN Sn 38	26.47	26.35	26.41	TN Sn 44	19.47	20.14	19.81
TN Sn 52	23.14	25.36	24.25	TN Sn 47	20.14	21.25	20.70
TN Sn 53	23.00	24.14	23.57				
MEAN	**24.19**	**24.82**	**24.51**	**MEAN**	**19.76**	**20.75**	**20.26**
SED	0.63	0.62	0.64	**SED**	0.35	0.36	0.35
CV	2.59	2.54	2.57	**CV**	1.74	1.75	1.82
CD (P=0.05)	1.27	1.34	1.32	**CD (P=0.05)**	0.71	0.72	0.72

Table 19. Mean performance of Solanum genotypes for days to fifty per cent flowering

***Solanum nigrum* L.**							
Genotypes	Season I	Season II	Pooled	Genotypes	Season I	Season II	Pooled
TN *Sn* 1	57.38	57.36	57.38	TN *Sn* 21	57.13	57.28	57.13
TN *Sn* 2	56.28	56.36	56.28	TN *Sn* 22	56.78	56.89	56.78
TN *Sn* 3	55.15	55.24	55.15	TN *Sn* 24	56.62	56.89	56.76
TN *Sn* 4	56.29	56.36	56.29	TN *Sn* 25	55.48	55.48	55.48
TN *Sn* 5	56.27	56.98	56.27	TN *Sn* 26	57.24	57.89	57.57
TN *Sn* 6	55.33	55.28	55.33	TN *Sn* 27	56.97	56.36	56.67
TN *Sn* 7	56.31	56.98	56.31	TN *Sn* 28	57.39	57.88	57.64
TN *Sn* 9	57.31	57.89	57.31	TN *Sn* 29	57.03	57.85	57.44
TN *Sn* 11	56.93	56.28	56.93	TN Sn 33	57.17	57.89	57.53
TN *Sn* 13	56.6	56.98	56.6	TN Sn 35	57.15	57.23	57.19
TN *Sn* 14	57.05	57.56	57.05	TN Sn 36	57.02	57.56	57.29
TN *Sn* 15	57.39	57.48	57.39	TN Sn 37	56.60	56.23	56.42
TN *Sn* 16	56.98	56.39	56.98	TN Sn 40	57.17	57.69	57.43
TN *Sn* 17	57.38	57.89	57.38	TN Sn 41	56.64	56.36	56.50
TN *Sn* 18	55.71	55.69	55.71	TN Sn 42	57.14	57.45	57.30
TN *Sn* 19	57.80	57.89	57.82	TN Sn 51	56.01	56.32	56.17
TN *Sn* 20	56.26	56.36	56.26				
		Season I		Season II		Pooled	
MEAN		**56.73**		**56.92**		**56.78**	
SED		0.27		0.25		0.25	
CV		1.48		1.56		1.62	
CD (P= 0.05)		0.55		0.51		0.50	

Solanum americanum Mill.				***Solanum villosum Mill.***			
Genotypes	Season I	Season II	Pooled	Genotypes	Season I	Season II	Pooled
TN *Sn* 8	53.16	53.45	53.16	TN *Sn* 30	50.09	50.23	50.16
TN *Sn* 12	53.72	53.87	53.72	TN Sn 31	50.30	50.14	50.22
TN *Sn* 23	57.43	57.54	57.43	TN Sn 32	51.00	51.22	51.11
TN Sn 38	54.69	54.23	54.46	TN Sn 44	50.83	50.12	50.48
TN Sn 52	53.34	53.22	53.28	TN Sn 47	51.09	51.36	51.23
TN Sn 53	54.18	54.45	54.32				
MEAN	**54.42**	**54.46**	**54.40**	**MEAN**	**50.66**	**50.61**	**50.64**
SED	0.20	0.22	0.19	**SED**	0.28	0.30	0.31
CV	0.37	0.35	0.34	**CV**	0.56	0.57	0.54
CD (P=0.05)	0.40	0.44	0.40	**CD(P=0.05)**	0.57	0.61	0.62

Table 20. Mean performance of Solanum genotypes for number of berries per plant

***Solanum nigrum* L.**							
Genotypes	Season I	Season II	Pooled	Genotypes	Season I	Season II	Pooled
TN *Sn* 1	250.01	252.35	251.18	TN *Sn* 21	273.42	279.36	276.39
TN *Sn* 2	274.92	280.14	277.53	TN *Sn* 22	200.36	210.23	205.29
TN *Sn* 3	265.49	270.14	267.81	TN *Sn* 24	236.62	238.52	237.57
TN *Sn* 4	280.12	285.23	282.67	TN *Sn* 25	269.82	271.25	270.53
TN *Sn* 5	245.47	249.36	247.41	TN *Sn* 26	271.96	275.63	273.79
TN *Sn* 6	247.49	251.23	249.36	TN *Sn* 27	267.42	275.12	271.27
TN *Sn* 7	285.52	291.23	288.37	TN *Sn* 28	242.89	249.36	246.10
TN *Sn* 9	264.19	270.14	267.16	TN *Sn* 29	270.62	275.36	272.99
TN *Sn* 11	220.94	224.12	222.53	TN Sn 33	265.12	270.14	267.63
TN *Sn* 13	250.91	253.21	252.06	TN Sn 35	253.17	262.13	257.65
TN *Sn* 14	256.41	258.36	257.38	TN Sn 36	259.49	263.12	261.30
TN *Sn* 15	279.64	281.25	280.44	TN Sn 37	246.12	250.14	248.13
TN *Sn* 16	270.49	274.23	272.36	TN Sn 40	225.95	230.21	228.08
TN *Sn* 17	240.62	245.12	242.87	TN Sn 41	230.14	233.14	231.64
TN *Sn* 18	253.69	256.35	255.02	TN Sn 42	229.26	235.21	232.23
TN *Sn* 19	196.42	200.10	198.26	TN Sn 51	268.12	275.23	271.67
TN *Sn* 20	279.26	285.10	282.18				
		Season I		Season II		Pooled	
MEAN		**253.70**		**258.24**		**255.97**	
SED		1.43		1.58		1.50	
CV		7.56		8.21		7.55	
CD (P= 0.05)		2.88		2.98		2.75	
Solanum americanum Mill.				***Solanum villosum Mill.***			
Genotypes	Season I	Season II	Pooled	Genotypes	Season I	Season II	Pooled
TN *Sn* 8	212.96	214.23	213.59	TN *Sn* 30	240.14	245.36	242.75
TN *Sn* 12	224.12	228.21	226.16	TN Sn 31	230.15	235.12	232.63
TN *Sn* 23	212.29	210.23	211.26	TN Sn 32	215.19	216.35	215.77
TN Sn 38	220.85	224.23	222.54	TN Sn 44	231.42	235.36	233.39
TN Sn 52	249.42	251.23	250.32	TN Sn 47	210.19	215.28	212.73
TN Sn 53	225.68	229.36	227.52				
MEAN	**224.22**	**226.25**	**225.23**	**MEAN**	**225.42**	**229.49**	**227.45**
SED	1.61	1.65	1.60	**SED**	1.20	1.23	1.25
CV	2.71	2.87	2.56	**CV**	2.53	2.54	2.58
CD (P=0.05)	3.24	3.30	3.24	**CD(P=0.05)**	2.42	2.87	2.50

Table 21. Mean performance of Solanum genotypes for fresh herbage yield per plant (g)

***Solanum nigrum* L.**							
Genotypes	Season I	Season II	Pooled	Genotypes	Season I	Season II	Pooled
TN *Sn* 1	392.46	340.12	366.29	TN *Sn* 21	290.62	297.23	293.93
TN *Sn* 2	292.87	300.12	296.50	TN *Sn* 22	444.83	454.23	449.53
TN *Sn* 3	363.04	370.12	366.58	TN *Sn* 24	306.86	314.20	310.53
TN *Sn* 4	248.32	256.21	252.27	TN *Sn* 25	377.91	381.21	379.56
TN *Sn* 5	377.90	385.21	381.56	TN *Sn* 26	287.16	398.21	342.69
TN *Sn* 6	367.31	374.20	370.76	TN *Sn* 27	316.30	327.21	321.76
TN *Sn* 7	258.26	265.23	261.75	TN *Sn* 28	282.64	298.21	290.43
TN *Sn* 9	277.35	289.32	283.34	TN *Sn* 29	315.30	329.21	322.26
TN *Sn* 11	229.09	238.21	233.65	TN Sn 33	272.44	285.14	278.79
TN *Sn* 13	236.97	245.21	241.09	TN Sn 35	240.79	251.21	246.00
TN *Sn* 14	207.01	215.21	211.11	TN Sn 36	272.15	284.21	278.18
TN *Sn* 15	321.00	334.21	327.61	TN Sn 37	227.80	230.12	228.96
TN *Sn* 16	247.05	256.32	251.69	TN Sn 40	322.11	332.01	327.06
TN *Sn* 17	303.44	312.04	307.74	TN Sn 41	299.99	301.24	300.62
TN *Sn* 18	252.61	259.32	255.97	TN Sn 42	280.58	288.52	284.55
TN *Sn* 19	446.96	456.21	451.59	TN Sn 51	303.55	308.21	305.88
TN *Sn* 20	266.68	278.12	272.40				
		Season I		Season II		Pooled	
MEAN		**300.89**		**310.78**		**305.84**	
SED		15.03		15.10		14.98	
CV		4.91		5.01		4.98	
CD (P= 0.05)		30.22		30.21		30.20	

Solanum americanum Mill.				***Solanum villosum Mill.***			
Genotypes	Season I	Season II	Pooled	Genotypes	Season I	Season II	Pooled
TN *Sn* 8	408.69	416.20	412.45	TN *Sn* 30	468.53	475.21	471.87
TN *Sn* 12	416.30	425.21	420.76	TN Sn 31	449.37	459.32	454.35
TN *Sn* 23	324.14	332.10	328.12	TN Sn 32	292.70	300.12	296.41
TN Sn 38	426.74	439.12	432.93	TN Sn 44	310.09	316.21	313.15
TN Sn 52	406.49	412.05	409.27	TN Sn 47	394.40	401.25	397.83
TN Sn 53	406.08	412.96	409.52				
MEAN	**398.07**	**406.27**	**402.18**	**MEAN**	**383.02**	**390.42**	**386.72**
SED	1.64	1.68	1.58	**SED**	1.05	1.14	1.17
CV	3.41	3.50	3.49	**CV**	4.27	4.21	4.25
CD (P=0.05)	3.31	3.28	3.27	**CD(P=0.05)**	2.12	2.28	2.24

Table 22. Mean performance of Solanum genotypes for dry herbage yield per plant (g)

Genotypes	Season I	Season II	Pooled	Genotypes	Season I	Season II	Pooled
TN *Sn* 1	181.50	182.56	182.03	TN *Sn* 21	121.52	129.36	125.44
TN *Sn* 2	180.12	184.23	182.18	TN *Sn* 22	202.88	210.23	206.56
TN *Sn* 3	159.21	163.25	161.23	TN *Sn* 24	127.81	134.23	131.02
TN *Sn* 4	100.99	102.23	101.61	TN *Sn* 25	176.41	183.23	179.82
TN *Sn* 5	177.55	180.21	178.88	TN *Sn* 26	120.55	124.23	122.39
TN *Sn* 6	174.77	176.32	175.55	TN *Sn* 27	135.16	138.36	136.76
TN *Sn* 7	115.79	119.32	117.56	TN *Sn* 28	120.17	124.36	122.27
TN *Sn* 9	121.88	129.36	125.62	TN *Sn* 29	136.00	139.23	137.62
TN *Sn* 11	90.96	95.23	93.10	TN Sn 33	118.69	124.22	121.46
TN *Sn* 13	96.91	101.23	99.07	TN Sn 35	99.27	104.25	101.76
TN *Sn* 14	78.55	85.23	81.89	TN Sn 36	120.29	127.25	123.77
TN *Sn* 15	137.38	141.23	139.31	TN Sn 37	92.54	95.36	93.95
TN *Sn* 16	113.56	118.23	115.90	TN Sn 40	140.89	143.23	142.06
TN *Sn* 17	126.12	135.21	130.67	TN Sn 41	127.72	132.25	129.99
TN *Sn* 18	116.04	129.32	122.68	TN Sn 42	121.92	124.25	123.09
TN *Sn* 19	201.21	214.23	207.72	TN Sn 51	128.92	135.21	132.07
TN *Sn* 20	118.53	126.23	122.38				
		Season I		Season II		Pooled	
MEAN		**132.78**		**137.97**		**135.38**	
SED		2.05		2.10		2.12	
CV		3.51		4.09		4.23	
CD (P= 0.05)		4.13		4.21		4.20	

Solanum americanum Mill.				***Solanum villosum Mill.***			
Genotypes	Season I	Season II	Pooled	Genotypes	Season I	Season II	Pooled
TN *Sn* 8	187.94	196.23	192.09	TN *Sn* 30	224.88	228.36	226.62
TN *Sn* 12	196.21	200.14	198.18	TN Sn 31	219.38	223.12	221.25
TN *Sn* 23	138.63	142.36	140.50	TN Sn 32	122.36	127.23	124.80
TN Sn 38	202.80	202.17	202.49	TN Sn 44	132.22	135.23	133.73
TN Sn 52	190.76	195.36	193.06	TN Sn 47	187.17	196.36	191.77
TN Sn 53	191.07	193.24	192.16				
MEAN	**184.57**	**188.25**	**186.41**	**MEAN**	**177.20**	**182.06**	**179.63**
SED	2.10	2.07	2.04	**SED**	1.77	1.78	1.84
CV	4.56	5.11	5.14	**CV**	4.99	4.58	4.81
CD (P=0.05)	4.20	4.16	4.01	**CD(P=0.05)**	3.57	3.45	3.59

Table 23. Mean performance of Solanum genotypes for fresh herbage yield per plot (Kg)

***Solanum nigrum* L.**							
Genotypes	Season I	Season II	Pooled	Genotypes	Season I	Season II	Pooled
TN *Sn* 1	12.52	12.60	12.56	TN *Sn* 21	9.31	9.71	9.51
TN *Sn* 2	9.23	9.44	9.34	TN *Sn* 22	14.23	14.72	14.48
TN *Sn* 3	11.73	11.81	11.77	TN *Sn* 24	9.70	9.76	9.73
TN *Sn* 4	7.57	7.70	7.64	TN *Sn* 25	12.18	12.77	12.48
TN *Sn* 5	12.20	12.61	12.41	TN *Sn* 26	9.18	9.70	9.44
TN *Sn* 6	11.81	11.73	11.77	TN *Sn* 27	10.09	10.72	10.41
TN *Sn* 7	8.51	8.78	8.65	TN *Sn* 28	8.75	9.76	9.26
TN *Sn* 9	9.14	9.84	9.49	TN *Sn* 29	10.14	10.70	10.42
TN *Sn* 11	7.32	7.68	7.51	TN Sn 33	8.72	8.85	8.78
TN *Sn* 13	7.63	7.72	7.68	TN Sn 35	7.69	7.73	7.71
TN *Sn* 14	6.80	6.78	6.79	TN Sn 36	8.63	8.97	8.80
TN *Sn* 15	10.34	10.77	10.55	TN Sn 37	7.39	7.69	7.54
TN *Sn* 16	7.83	7.92	7.87	TN Sn 40	10.36	10.78	10.57
TN *Sn* 17	9.55	9.79	9.67	TN Sn 41	9.69	10.07	9.88
TN *Sn* 18	8.23	8.30	8.26	TN Sn 42	8.59	8.94	8.77
TN *Sn* 19	14.41	14.73	14.57	TN Sn 51	9.43	9.81	9.62
TN *Sn* 20	8.51	8.87	8.69				
		Season I		Season II		Pooled	
MEAN		**9.62**		**9.93**		**9.78**	
SED		0.16		0.15		0.17	
CV		2.69		2.75		2.87	
CD (P= 0.05)		0.33		0.30		0.34	

Solanum americanum Mill.				***Solanum villosum Mill.***			
Genotypes	Season I	Season II	Pooled	Genotypes	Season I	Season II	Pooled
TN *Sn* 8	13.13	12.00	12.07	TN *Sn* 30	14.33	14.75	14.54
TN *Sn* 12	13.34	13.63	13.49	TN Sn 31	14.21	14.65	14.43
TN *Sn* 23	10.34	10.72	10.53	TN Sn 32	9.30	9.59	9.45
TN Sn 38	13.40	13.94	13.67	TN Sn 44	9.82	10.21	10.01
TN Sn 52	12.72	13.22	12.97	TN Sn 47	12.66	13.17	12.92
TN Sn 53	12.78	13.28	13.03				
MEAN	**12.62**	**12.80**	**12.63**	**MEAN**	**12.06**	**12.47**	**12.27**
SED	0.45	0.38	0.46	**SED**	0.05	0.07	0.08
CV	3.60	3.58	4.01	**CV**	2.46	2.52	2.56
CD (P=0.05)	0.92	0.70	0.98	**CD (P=0.05)**	0.11	0.14	0.16

Table 24. Mean performance of Solanum genotypes for dry herbage yield per plot (Kg)

***Solanum nigrum* L.**							
Genotypes	Season I	Season II	Pooled	Genotypes	Season I	Season II	Pooled
TN *Sn* 1	5.66	6.16	5.91	TN *Sn* 21	3.81	3.83	3.82
TN *Sn* 2	5.63	5.78	5.70	TN *Sn* 22	6.41	6.69	6.55
TN *Sn* 3	4.90	5.12	5.01	TN *Sn* 24	4.11	4.19	4.15
TN *Sn* 4	3.14	3.35	3.24	TN *Sn* 25	5.67	5.74	5.71
TN *Sn* 5	5.58	5.82	5.70	TN *Sn* 26	3.87	3.75	3.81
TN *Sn* 6	5.48	5.91	5.70	TN *Sn* 27	4.38	4.64	4.51
TN *Sn* 7	3.68	3.70	3.70	TN *Sn* 28	3.80	3.85	3.82
TN *Sn* 9	3.76	4.00	3.88	TN *Sn* 29	4.26	4.36	4.31
TN *Sn* 11	2.80	3.13	2.97	TN Sn 33	3.82	3.99	3.90
TN *Sn* 13	3.06	3.22	3.14	TN Sn 35	3.13	3.29	3.22
TN *Sn* 14	2.49	2.62	2.55	TN Sn 36	3.66	3.81	3.74
TN *Sn* 15	4.30	4.38	4.34	TN Sn 37	2.81	2.92	2.87
TN *Sn* 16	3.52	3.77	3.65	TN Sn 40	4.47	4.64	4.56
TN *Sn* 17	3.93	3.62	3.77	TN Sn 41	3.92	4.08	4.00
TN *Sn* 18	3.63	3.76	3.70	TN Sn 42	3.86	4.02	3.94
TN *Sn* 19	6.48	6.58	6.54	TN Sn 51	4.03	4.19	4.11
TN *Sn* 20	3.75	3.91	3.84				
		Season I		Season II		Pooled	
MEAN		**4.18**		**4.33**		**4.25**	
SED		0.10		0.14		0.15	
CV		2.37		2.56		3.01	
CD (P= 0.05)		0.20		0.28		0.30	

Solanum americanum Mill.				***Solanum villosum Mill.***			
Genotypes	Season I	Season II	Pooled	Genotypes	Season I	Season II	Pooled
TN *Sn* 8	5.95	5.94	5.95	TN *Sn* 30	7.02	7.12	7.17
TN *Sn* 12	6.12	6.52	6.32	TN Sn 31	6.87	6.89	6.88
TN *Sn* 23	4.42	4.50	4.46	TN Sn 32	3.92	4.05	3.99
TN Sn 38	6.42	6.68	6.55	TN Sn 44	4.16	4.33	4.24
TN Sn 52	6.00	6.23	6.12	TN Sn 47	5.69	5.92	5.81
TN Sn 53	5.82	6.05	5.94				
MEAN	**5.79**	**5.99**	**5.89**	**MEAN**	**5.53**	**5.66**	**5.62**
SED	0.10	0.12	0.11	**SED**	0.05	0.06	0.07
CV	1.73	1.97	1.95	**CV**	0.99	1.07	1.20
CD (P=0.05)	0.20	0.24	0.22	**CD(P=0.05)**	0.11	0.12	0.14

Table 25. Mean performance of Solanum genotypes for fresh herbage yield per hectare (t) (estimated)

***Solanum nigrum* L.**							
Genotypes	Season I	Season II	Pooled	Genotypes	Season I	Season II	Pooled
TN *Sn* 1	13.89	13.92	13.91	TN *Sn* 21	10.35	10.86	10.61
TN *Sn* 2	10.33	11.05	10.69	TN *Sn* 22	14.57	15.36	14.97
TN *Sn* 3	12.78	13.22	13.00	TN *Sn* 24	10.85	11.39	11.12
TN *Sn* 4	8.83	8.72	8.77	TN *Sn* 25	13.36	14.03	13.70
TN *Sn* 5	13.49	13.95	13.72	TN *Sn* 26	10.16	10.67	10.42
TN *Sn* 6	13.17	13.41	13.29	TN *Sn* 27	11.20	11.76	11.49
TN *Sn* 7	9.18	10.33	9.76	TN *Sn* 28	9.92	10.42	10.17
TN *Sn* 9	10.14	10.41	10.27	TN *Sn* 29	11.19	11.75	11.47
TN *Sn* 11	8.07	8.11	8.10	TN Sn 33	10.59	11.11	10.85
TN *Sn* 13	8.54	9.13	8.83	TN Sn 35	8.08	8.48	8.28
TN *Sn* 14	7.38	7.82	7.60	TN Sn 36	9.61	10.09	9.85
TN *Sn* 15	11.39	11.75	11.57	TN Sn 37	8.09	8.49	8.29
TN *Sn* 16	8.87	8.87	8.87	TN Sn 40	11.46	12.04	11.75
TN *Sn* 17	8.98	9.08	9.03	TN Sn 41	10.55	11.07	10.81
TN *Sn* 18	9.68	9.63	9.66	TN Sn 42	9.91	10.4	10.16
TN *Sn* 19	15.19	15.95	15.58	TN Sn 51	10.66	11.2	10.93
TN *Sn* 20	9.34	9.81	9.57				
		Season I		Season II		Pooled	
MEAN		**10.60**		**11.04**		**10.82**	
SED		0.18		0.20		0.19	
CV		1.72		2.10		2.00	
CD (P= 0.05)		0.37		0.40		0.38	
Solanum americanum Mill.				***Solanum villosum Mill.***			
Genotypes	Season I	Season II	Pooled	Genotypes	Season I	Season II	Pooled
TN *Sn* 8	14.62	14.91	14.76	TN *Sn* 30	15.82	15.90	15.86
TN *Sn* 12	14.69	14.91	14.80	TN Sn 31	10.36	10.89	10.63
TN *Sn* 23	11.36	11.92	11.64	TN Sn 32	9.51	9.99	9.75
TN Sn 38	15.03	15.15	15.09	TN Sn 44	10.77	11.32	11.05
TN Sn 52	14.57	15.29	14.93	TN Sn 47	14.75	15.44	15.10
TN Sn 53	14.33	15.05	14.69				
MEAN	**14.10**	**14.54**	**14.32**	**MEAN**	**12.24**	**12.71**	**12.48**
SED	0.18	0.20	0.20	**SED**	0.16	0.14	0.15
CV	1.29	2.04	2.05	**CV**	1.30	1.28	1.32
CD (P=0.05)	0.37	0.40	0.40	**CD (P=0.05)**	0.32	0.28	0.30

Table 26. Mean performance of Solanum genotypes for dry herbage yield per hactare (t) (estimated)

***Solanum nigrum* L.**							
Genotypes	Season I	Season II	Pooled	Genotypes	Season I	Season II	Pooled
TN *Sn* 1	6.40	6.66	6.53	TN *Sn* 21	4.26	4.42	4.34
TN *Sn* 2	6.22	6.47	6.35	TN *Sn* 22	7.13	7.41	7.27
TN *Sn* 3	5.49	5.71	5.60	TN *Sn* 24	6.22	6.47	6.35
TN *Sn* 4	3.75	3.89	3.82	TN *Sn* 25	4.22	4.39	4.31
TN *Sn* 5	6.13	6.38	6.25	TN *Sn* 26	4.72	4.91	4.82
TN *Sn* 6	6.04	6.28	6.16	TN *Sn* 27	4.25	4.42	4.34
TN *Sn* 7	4.05	4.21	4.13	TN *Sn* 28	4.63	4.82	4.72
TN *Sn* 9	4.20	4.37	4.29	TN *Sn* 29	4.19	4.36	4.27
TN *Sn* 11	3.14	3.27	3.21	TN Sn 33	4.44	4.63	4.54
TN *Sn* 13	2.70	2.80	2.75	TN Sn 35	3.49	3.63	3.56
TN *Sn* 14	4.73	4.92	4.83	TN Sn 36	4.19	4.36	4.28
TN *Sn* 15	3.93	4.09	4.01	TN Sn 37	3.25	3.38	3.31
TN *Sn* 16	4.35	4.52	4.43	TN Sn 40	4.88	5.07	4.97
TN *Sn* 17	4.05	4.20	4.13	TN Sn 41	4.43	4.62	4.53
TN *Sn* 18	4.58	4.77	4.68	TN Sn 42	4.22	4.39	4.31
TN *Sn* 19	7.16	7.45	7.31	TN Sn 51	4.48	4.66	4.57
TN *Sn* 20	4.13	4.30	4.22				
		Season I		Season II		Pooled	
MEAN		**4.67**		**4.86**		**4.76**	
SED		0.03		0.04		0.04	
CV		1.66		1.58		1.45	
CD (P= 0.05)		0.06		0.08		0.08	

Solanum americanum Mill.				***Solanum villosum Mill.***			
Genotypes	Season I	Season II	Pooled	Genotypes	Season I	Season II	Pooled
TN *Sn* 8	6.60	6.86	6.73	TN *Sn* 30	7.73	8.04	7.89
TN *Sn* 12	6.71	6.98	6.85	TN Sn 31	4.23	4.40	4.32
TN *Sn* 23	4.88	5.07	4.97	TN Sn 32	4.16	4.34	4.25
TN Sn 38	7.08	7.36	7.22	TN Sn 44	4.59	4.77	4.68
TN Sn 52	6.76	7.03	6.90	TN Sn 47	6.29	6.54	6.41
TN Sn 53	6.38	6.64	6.51				
MEAN	**6.40**	**6.66**	**6.53**	**MEAN**	**5.40**	**5.62**	**5.51**
SED	0.02	0.03	0.02	**SED**	0.04	0.04	0.04
CV	0.35	0.42	0.40	**CV**	0.77	0.74	0.78
CD (P=0.05)	0.04	0.06	0.04	**CD (P=0.05)**	0.08	0.08	0.08

Table 27. Mean performance of Solanum genotypes for total leaf area (cm^2) per plant

***Solanum nigrum* L.**							
Genotypes	Season I	Season II	Pooled	Genotypes	Season I	Season II	Pooled
TN *Sn* 1	2011.21	2088.21	2049.50	TN *Sn* 21	1287.24	1300.41	1293.50
TN *Sn* 2	1792.58	1800.21	1796.50	TN *Sn* 22	3149.21	3500.12	3324.50
TN *Sn* 3	1547.24	1620.12	1583.50	TN *Sn* 24	2093.24	2147.23	2120.00
TN *Sn* 4	1298.13	1321.04	1309.50	TN *Sn* 25	1411.56	1522.01	1467.00
TN *Sn* 5	2255.14	2250.12	2252.50	TN *Sn* 26	1610.42	1700.11	1655.00
TN *Sn* 6	2280.12	2314.02	2297.00	TN *Sn* 27	1971.96	1952.00	1962.00
TN *Sn* 7	1863.54	1958.23	1911.00	TN *Sn* 28	2114.24	2235.14	2174.50
TN *Sn* 9	1257.14	1820.11	1538.50	TN *Sn* 29	1292.04	1358.01	1325.00
TN *Sn* 11	2299.24	2300.14	2299.50	TN Sn 33	1989.01	2000.78	1995.00
TN *Sn* 13	1958.01	2001.78	1980.00	TN Sn 35	2052.87	2123.01	2088.00
TN *Sn* 14	1144.21	1204.23	1174.00	TN Sn 36	2006.25	2178.23	2092.00
TN *Sn* 15	1409.14	1536.24	1472.50	TN Sn 37	1997.23	2014.10	2005.50
TN *Sn* 16	1987.24	2006.23	1996.50	TN Sn 40	2907.46	3452.01	3179.50
TN *Sn* 17	2186.47	2300.12	2243.00	TN Sn 41	2289.21	2500.14	2394.50
TN *Sn* 18	1957.54	1987.20	1972.50	TN Sn 42	2967.66	3014.78	2991.50
TN *Sn* 19	2929.01	3000.00	2964.50	TN Sn 51	1995.21	2010.47	2002.50
TN *Sn* 20	1272.14	1350.14	1311.00				
		Season I		Season II		Pooled	
MEAN		**1957.06**		**2056.57**		**2006.71**	
SED		97.09		96.52		90.05	
CV		4.83		5.23		5.14	
CD (P= 0.05)		195.15		185.10		180.24	
Solanum americanum Mill.				***Solanum villosum Mill.***			
Genotypes	Season I	Season II	Pooled	Genotypes	Season I	Season II	Pooled
TN *Sn* 8	2946.25	2304.21	2625.00	TN *Sn* 30	3257.03	3421.00	3339.00
TN *Sn* 12	2940.14	3145.10	3042.50	TN Sn 31	3154.32	3258.14	3206.00
TN *Sn* 23	2901.35	3004.21	2952.50	TN Sn 32	2957.01	3004.12	2980.50
TN Sn 38	2927.50	3000.47	2964.00	TN Sn 44	2867.29	2921.41	2894.00
TN Sn 52	2040.14	2078.24	2059.00	TN Sn 47	3170.45	3214.01	3192.00
TN Sn 53	2096.10	2114.78	2105.50				
MEAN	**2641.91**	**2607.84**	**2624.75**	**MEAN**	**3081.22**	**3163.74**	**3122.30**
SED	215.62	214.01	220.12	**SED**	36.51	37.01	36.87
CV	8.21	9.23	8.99	**CV**	7.16	7.58	7.45
CD(P=0.05)	433.41	430.14	445.12	**CD (P=0.05)**	73.39	74.53	78.14

Table 28. Mean performance of Solanum genotypes for total chlorophyll (mg g^{-1})

***Solanum nigrum* L.**							
Genotypes	Season I	Season II	Pooled	Genotypes	Season I	Season II	Pooled
TN *Sn* 1	1.94	1.84	1.89	TN *Sn* 21	1.85	1.76	1.81
TN *Sn* 2	1.71	1.63	1.67	TN *Sn* 22	1.98	1.88	1.93
TN *Sn* 3	1.84	1.75	1.80	TN *Sn* 24	1.66	1.58	1.62
TN *Sn* 4	1.63	1.55	1.59	TN *Sn* 25	1.76	1.68	1.72
TN *Sn* 5	1.88	1.79	1.84	TN *Sn* 26	1.87	1.78	1.83
TN *Sn* 6	1.81	1.72	1.76	TN *Sn* 27	1.85	1.75	1.80
TN *Sn* 7	1.64	1.56	1.60	TN *Sn* 28	1.84	1.75	1.80
TN *Sn* 9	1.71	1.63	1.67	TN *Sn* 29	1.83	1.75	1.79
TN *Sn* 11	1.73	1.64	1.69	TN Sn 33	1.55	1.48	1.52
TN *Sn* 13	1.65	1.57	1.61	TN Sn 35	1.57	1.49	1.53
TN *Sn* 14	1.47	1.49	1.48	TN Sn 36	1.78	1.70	1.74
TN *Sn* 15	1.87	1.78	1.83	TN Sn 37	1.57	1.49	1.53
TN *Sn* 16	1.82	1.73	1.78	TN Sn 40	1.85	1.75	1.80
TN *Sn* 17	1.65	1.57	1.61	TN Sn 41	1.71	1.62	1.66
TN *Sn* 18	1.67	1.59	1.63	TN Sn 42	1.66	1.57	1.62
TN *Sn* 19	2.06	1.96	2.01	TN Sn 51	1.64	1.56	1.61
TN *Sn* 20	1.71	1.63	1.67				
		Season I		Season II		Pooled	
MEAN		**1.75**		**1.67**		**1.71**	
SED		0.01		0.01		0.01	
CV		0.83		0.85		0.91	
CD (P= 0.05)		0.02		0.02		0.02	

Solanum americanum Mill.				***Solanum villosum Mill.***			
Genotypes	Season I	Season II	Pooled	Genotypes	Season I	Season II	Pooled
TN *Sn* 8	1.95	1.85	1.90	TN *Sn* 30	2.05	1.95	2.00
TN *Sn* 12	1.88	1.79	1.84	TN Sn 31	2.10	2.00	2.05
TN *Sn* 23	1.88	1.79	1.84	TN Sn 32	1.68	1.59	1.63
TN Sn 38	1.80	1.71	1.76	TN Sn 44	1.62	1.54	1.58
TN Sn 52	1.92	1.82	1.87	TN Sn 47	1.90	1.81	1.86
TN Sn 53	1.90	1.81	1.86				
MEAN	**1.89**	**1.80**	**1.85**	**MEAN**	**1.87**	**1.78**	**1.82**
SED	0.003	0.003	0.004	**SED**	0.005	0.005	0.005
CV	0.19	0.20	0.21	**CV**	0.32	0.41	0.40
CD (P=0.05)	0.007	0.006	0.008	**CD(P=0.05)**	0.01	0.01	0.01

Table 29. Mean performance of Solanum genotypes for total soluble proteins (mg g^{-1})

***Solanum nigrum* L.**							
Genotypes	Season I	Season II	Pooled	Genotypes	Season I	Season II	Pooled
TN *Sn* 1	15.34	14.58	14.96	TN *Sn* 21	13.41	12.74	13.08
TN *Sn* 2	13.48	12.80	13.14	TN *Sn* 22	16.78	15.94	16.36
TN *Sn* 3	13.12	12.46	12.79	TN *Sn* 24	15.30	14.54	14.92
TN *Sn* 4	13.30	12.63	12.96	TN *Sn* 25	13.22	12.56	12.89
TN *Sn* 5	15.23	14.47	14.86	TN *Sn* 26	13.24	12.58	12.91
TN *Sn* 6	14.34	13.63	13.99	TN *Sn* 27	13.20	12.53	12.87
TN *Sn* 7	13.30	12.64	12.97	TN *Sn* 28	13.34	12.66	13.00
TN *Sn* 9	13.26	12.61	12.94	TN *Sn* 29	13.57	12.89	13.23
TN *Sn* 11	12.72	12.09	12.40	TN Sn 33	13.18	12.53	12.86
TN *Sn* 13	13.17	12.51	12.85	TN Sn 35	12.92	12.28	12.60
TN *Sn* 14	12.64	12.01	12.33	TN Sn 36	13.07	12.43	12.75
TN *Sn* 15	13.43	12.76	13.10	TN Sn 37	13.20	12.55	12.88
TN *Sn* 16	13.23	12.57	12.90	TN Sn 40	12.67	12.04	12.36
TN *Sn* 17	13.20	12.54	12.87	TN Sn 41	13.35	12.68	13.02
TN *Sn* 18	13.13	12.47	12.80	TN Sn 42	13.30	12.64	12.97
TN *Sn* 19	14.86	14.11	14.49	TN Sn 51	13.40	12.73	13.07
TN *Sn* 20	13.24	12.58	12.91				
		Season I		Season II		Pooled	
MEAN		**13.58**		**12.90**		**13.24**	
SED		0.03		0.03		0.03	
CV		1.24		1.54		1.42	
CD (P= 0.05)		0.06		0.06		0.06	

Solanum americanum Mill.				***Solanum villosum Mill.***			
Genotypes	Season I	Season II	Pooled	Genotypes	Season I	Season II	Pooled
TN *Sn* 8	16.32	15.50	15.91	TN *Sn* 30	16.98	16.13	16.56
TN *Sn* 12	16.23	15.41	15.82	TN Sn 31	16.93	16.09	16.51
TN *Sn* 23	13.24	12.58	12.91	TN Sn 32	13.17	12.51	12.85
TN Sn 38	16.41	15.59	16.00	TN Sn 44	13.46	12.80	13.13
TN Sn 52	15.56	14.78	15.17	TN Sn 47	16.86	16.02	16.44
TN Sn 53	15.51	14.73	15.12				
MEAN	**15.55**	**14.77**	**15.16**	**MEAN**	**15.48**	**14.71**	**15.10**
SED	0.04	0.04	0.04	**SED**	0.07	0.08	0.08
CV	0.28	0.42	0.52	**CV**	0.47	0.52	0.50
CD (P=0.05)	0.08	0.08	0.08	**CD(P=0.05)**	0.14	0.16	0.16

Table 30. Mean performance of Solanum genotypes for total phenols (mg g^{-1})

***Solanum nigrum* L.**							
Genotypes	Season I	Season II	Pooled	Genotypes	Season I	Season II	Pooled
TN *Sn* 1	1.74	1.81	1.77	TN *Sn* 21	1.60	1.65	1.63
TN *Sn* 2	1.7	1.76	1.73	TN *Sn* 22	1.77	1.84	1.81
TN *Sn* 3	1.72	1.79	1.76	TN *Sn* 24	1.5	1.56	1.53
TN *Sn* 4	1.68	1.75	1.71	TN *Sn* 25	1.54	1.60	1.57
TN *Sn* 5	1.75	1.82	1.79	TN *Sn* 26	1.57	1.63	1.60
TN *Sn* 6	1.74	1.81	1.78	TN *Sn* 27	1.65	1.73	1.69
TN *Sn* 7	1.72	1.78	1.75	TN *Sn* 28	1.59	1.64	1.62
TN *Sn* 9	1.61	1.66	1.64	TN *Sn* 29	1.62	1.68	1.65
TN *Sn* 11	1.62	1.68	1.65	TN Sn 33	1.44	1.50	1.47
TN *Sn* 13	1.47	1.53	1.50	TN Sn 35	1.54	1.60	1.57
TN *Sn* 14	1.48	1.54	1.51	TN Sn 36	1.66	1.73	1.70
TN *Sn* 15	1.64	1.71	1.68	TN Sn 37	1.60	1.67	1.63
TN *Sn* 16	1.63	1.69	1.66	TN Sn 40	1.61	1.66	1.64
TN *Sn* 17	1.6	1.66	1.63	TN Sn 41	1.59	1.65	1.62
TN *Sn* 18	1.58	1.64	1.61	TN Sn 42	1.63	1.69	1.66
TN *Sn* 19	1.82	1.89	1.86	TN Sn 51	1.52	1.58	1.55
TN *Sn* 20	1.61	1.68	1.64				
		Season I		Season II		Pooled	
MEAN		**1.62**		**1.69**		**1.65**	
SED		0.005		0.005		0.005	
CV		0.30		0.40		0.45	
CD (P= 0.05)		0.01		0.01		0.01	

Solanum americanum Mill.				***Solanum villosum Mill.***			
Genotypes	Season I	Season II	Pooled	Genotypes	Season I	Season II	Pooled
TN *Sn* 8	1.69	1.76	1.73	TN *Sn* 30	1.77	1.84	1.81
TN *Sn* 12	1.66	1.74	1.70	TN Sn 31	1.76	1.83	1.80
TN *Sn* 23	1.62	1.69	1.65	TN Sn 32	1.48	1.53	1.51
TN Sn 38	1.66	1.74	1.70	TN Sn 44	1.89	1.97	1.93
TN Sn 52	1.69	1.75	1.72	TN Sn 47	1.77	1.85	1.81
TN Sn 53	1.66	1.74	1.70				
MEAN	**1.66**	**1.74**	**1.70**	**MEAN**	**1.73**	**1.80**	**1.77**
SED	0.005	0.005	0.005	**SED**	0.008	0.006	0.007
CV	0.33	0.40	0.45	**CV**	0.48	0.58	0.47
CD (P=0.05)	0.01	0.01	0.01	**CD(P=0.05)**	0.01	0.01	0.01

Table 31. Mean performance of Solanum genotypes for total alkaloid content (%)

***Solanum nigrum* L.**							
Genotypes	Season I	Season II	Pooled	Genotypes	Season I	Season II	Pooled
TN *Sn* 1	0.355	0.356	0.356	TN *Sn* 21	0.380	0.391	0.386
TN *Sn* 2	0.350	0.358	0.354	TN *Sn* 22	0.345	0.354	0.350
TN *Sn* 3	0.370	0.379	0.375	TN *Sn* 24	0.365	0.358	0.362
TN *Sn* 4	0.355	0.358	0.357	TN *Sn* 25	0.365	0.375	0.370
TN *Sn* 5	0.365	0.368	0.367	TN *Sn* 26	0.370	0.389	0.380
TN *Sn* 6	0.370	0.375	0.373	TN *Sn* 27	0.370	0.384	0.377
TN *Sn* 7	0.355	0.365	0.360	TN *Sn* 28	0.373	0.374	0.374
TN *Sn* 9	0.365	0.371	0.368	TN *Sn* 29	0.378	0.391	0.385
TN *Sn* 11	0.375	0.378	0.377	TN Sn 33	0.395	0.421	0.408
TN *Sn* 13	0.370	0.387	0.379	TN Sn 35	0.363	0.401	0.382
TN *Sn* 14	0.370	0.356	0.363	TN Sn 36	0.360	0.356	0.358
TN *Sn* 15	0.345	0.398	0.372	TN Sn 37	0.373	0.387	0.380
TN *Sn* 16	0.370	0.387	0.379	TN Sn 40	0.365	0.367	0.366
TN *Sn* 17	0.370	0.378	0.374	TN Sn 41	0.355	0.362	0.359
TN *Sn* 18	0.355	0.369	0.362	TN Sn 42	0.375	0.378	0.377
TN *Sn* 19	0.335	0.345	0.340	TN Sn 51	0.375	0.378	0.377
TN *Sn* 20	0.365	0.369	0.367				
		Season I		Season II		Pooled	
MEAN		**0.37**		**0.37**		**0.37**	
SED		0.008		0.007		0.007	
CV		2.32		2.14		2.10	
CD (P= 0.05)		0.01		0.01		0.01	

Solanum americanum Mill.				***Solanum villosum Mill.***			
Genotypes	Season I	Season II	Pooled	Genotypes	Season I	Season II	Pooled
TN *Sn* 8	0.415	0.421	0.418	TN *Sn* 30	0.438	0.442	0.440
TN *Sn* 12	0.425	0.435	0.430	TN Sn 31	0.413	0.419	0.416
TN *Sn* 23	0.385	0.396	0.391	TN Sn 32	0.430	0.435	0.433
TN Sn 38	0.378	0.384	0.381	TN Sn 44	0.408	0.412	0.410
TN Sn 52	0.400	0.452	0.426	TN Sn 47	0.405	0.413	0.409
TN Sn 53	0.390	0.394	0.392				
MEAN	**0.40**	**0.41**	**0.41**	**MEAN**	**0.42**	**0.42**	**0.42**
SED	0.01	0.01	0.01	**SED**	0.01	0.01	0.01
CV	3.20	2.14	2.10	**CV**	0.28	0.25	0.30
CD (P=0.05)	0.02	0.02	0.02	**CD(P=0.05)**	0.02	0.02	0.02

Table 32. Mean performance of Solanum genotypes for protein content (mg $100g^{-1}$)

***Solanum nigrum* L.**							
Genotypes	Season I	Season II	Pooled	Genotypes	Season I	Season II	Pooled
TN *Sn* 1	3.48	3.45	3.41	TN *Sn* 21	2.36	2.62	2.47
TN *Sn* 2	2.85	2.84	2.84	TN *Sn* 22	4.58	4.74	4.62
TN *Sn* 3	1.60	1.64	1.62	TN *Sn* 24	2.61	2.77	2.74
TN *Sn* 4	2.25	2.31	2.27	TN *Sn* 25	2.40	2.51	2.47
TN *Sn* 5	2.16	2.18	2.15	TN *Sn* 26	2.50	2.62	2.55
TN *Sn* 6	2.19	2.29	2.24	TN *Sn* 27	2.91	3.00	2.94
TN *Sn* 7	2.30	2.37	2.34	TN *Sn* 28	2.60	2.82	2.74
TN *Sn* 9	2.34	2.43	2.35	TN *Sn* 29	2.46	2.55	2.51
TN *Sn* 11	2.15	2.18	2.13	TN Sn 33	2.61	2.85	2.74
TN *Sn* 13	2.29	2.32	2.31	TN Sn 35	2.40	2.56	2.48
TN *Sn* 14	2.51	2.55	2.52	TN Sn 36	2.51	2.74	2.69
TN *Sn* 15	2.41	2.55	2.47	TN Sn 37	2.64	2.69	2.65
TN *Sn* 16	2.30	2.36	2.34	TN Sn 40	2.42	2.51	2.48
TN *Sn* 17	2.55	2.62	2.52	TN Sn 41	2.41	2.46	2.43
TN *Sn* 18	2.64	2.71	2.66	TN Sn 42	2.61	2.74	2.67
TN *Sn* 19	5.00	5.17	5.12	TN Sn 51	2.23	2.28	2.25
TN *Sn* 20	2.71	2.91	2.85				
		Season I		Season II		Pooled	
MEAN		**2.61**		**2.71**		**2.65**	
SED		0.05		0.06		0.06	
CV		1.94		2.01		2.04	
CD (P= 0.05)		0.10		0.12		0.12	

Solanum americanum Mill.				***Solanum villosum Mill.***			
Genotypes	Season I	Season II	Pooled	Genotypes	Season I	Season II	Pooled
TN *Sn* 8	1.24	1.31	1.28	TN *Sn* 30	1.39	1.57	1.47
TN *Sn* 12	1.69	1.74	1.68	TN Sn 31	1.34	1.31	1.32
TN *Sn* 23	1.83	1.93	1.78	TN Sn 32	1.28	1.31	1.29
TN Sn 38	1.57	1.66	1.62	TN Sn 44	1.34	1.49	1.37
TN Sn 52	1.61	1.66	1.64	TN Sn 47	1.43	1.72	1.65
TN Sn 53	1.58	1.62	1.59				
MEAN	**1.59**	**1.65**	**1.60**	**MEAN**	**1.36**	**1.48**	**1.42**
SED	0.01	0.02	0.02	**SED**	0.08	0.09	0.08
CV	1.05	2.01	1.23	**CV**	2.30	2.10	2.14
CD (P=0.05)	0.03	0.04	0.04	**CD (P=0.05)**	0.17	0.18	0.16

Table 33. Mean performance of Solanum genotypes for crude fibre (%)

***Solanum nigrum* L.**							
Genotypes	Season I	Season II	Pooled	Genotypes	Season I	Season II	Pooled
TN *Sn* 1	0.80	0.84	0.82	TN *Sn* 21	0.64	0.67	0.66
TN *Sn* 2	0.65	0.72	0.69	TN *Sn* 22	0.77	0.80	0.79
TN *Sn* 3	0.69	0.74	0.72	TN *Sn* 24	0.66	0.67	0.67
TN *Sn* 4	0.70	0.73	0.72	TN *Sn* 25	0.72	0.74	0.73
TN *Sn* 5	0.72	0.75	0.74	TN *Sn* 26	0.71	0.72	0.72
TN *Sn* 6	0.70	0.73	0.72	TN *Sn* 27	0.67	0.73	0.70
TN *Sn* 7	0.66	0.69	0.68	TN *Sn* 28	0.67	0.70	0.69
TN *Sn* 9	0.65	0.71	0.68	TN *Sn* 29	0.67	0.71	0.69
TN *Sn* 11	0.64	0.67	0.66	TN Sn 33	0.67	0.70	0.69
TN *Sn* 13	0.64	0.67	0.66	TN Sn 35	0.70	0.74	0.72
TN *Sn* 14	0.68	0.71	0.70	TN Sn 36	0.72	0.76	0.74
TN *Sn* 15	0.67	0.72	0.70	TN Sn 37	0.71	0.74	0.73
TN *Sn* 16	0.70	0.74	0.72	TN Sn 40	0.68	0.71	0.70
TN *Sn* 17	0.71	0.74	0.73	TN Sn 41	0.70	0.74	0.72
TN *Sn* 18	0.67	0.72	0.70	TN Sn 42	0.73	0.76	0.75
TN *Sn* 19	0.80	0.82	0.81	TN Sn 51	0.69	0.73	0.71
TN *Sn* 20	0.69	0.71	0.70				
		Season I		Season II		Pooled	
MEAN		**0.69**		**0.73**		**0.71**	
SED		0.009		0.01		0.01	
CV		1.31		2.10		2.14	
CD (P= 0.05)		0.01		0.03		0.02	

Solanum americanum Mill.				***Solanum villosum Mill.***			
Genotypes	Season I	Season II	Pooled	Genotypes	Season I	Season II	Pooled
TN *Sn* 8	0.42	0.44	0.43	TN *Sn* 30	0.81	0.85	0.83
TN *Sn* 12	0.57	0.60	0.59	TN Sn 31	0.80	0.85	0.83
TN *Sn* 23	0.66	0.69	0.68	TN Sn 32	0.71	0.75	0.73
TN Sn 38	0.58	0.61	0.60	TN Sn 44	0.79	0.78	0.79
TN Sn 52	0.59	0.63	0.61	TN Sn 47	0.78	0.77	0.78
TN Sn 53	0.59	0.63	0.61				
MEAN	**0.57**	**0.60**	**0.59**	**MEAN**	**0.78**	**0.80**	**0.79**
SED	0.005	0.005	0.006	**SED**	0.02	0.04	0.04
CV	0.91	0.87	1.10	**CV**	2.64	2.58	2.54
CD (P=0.05)	0.01	0.01	0.01	**CD(P=0.05)**	0.04	0.08	0.09

Table 34. Mean performance of Solanum genotypes for iron content (mg $100g^{-1}$)

***Solanum nigrum* L.**							
Genotypes	Season I	Season II	Pooled	Genotypes	Season I	Season II	Pooled
TN *Sn* 1	5.18	5.38	5.28	TN *Sn* 21	4.84	4.94	4.89
TN *Sn* 2	4.12	4.18	4.15	TN *Sn* 22	5.04	5.13	5.09
TN *Sn* 3	4.29	4.36	4.33	TN *Sn* 24	4.28	4.58	4.43
TN *Sn* 4	4.79	4.81	4.80	TN *Sn* 25	4.37	4.57	4.47
TN *Sn* 5	4.32	4.46	4.39	TN *Sn* 26	4.44	4.64	4.54
TN *Sn* 6	4.60	4.70	4.65	TN *Sn* 27	4.53	4.56	4.55
TN *Sn* 7	4.70	4.82	4.76	TN *Sn* 28	4.82	4.95	4.89
TN *Sn* 9	4.22	4.32	4.27	TN *Sn* 29	4.26	4.52	4.39
TN *Sn* 11	4.41	4.57	4.49	TN Sn 33	4.88	5.00	4.94
TN *Sn* 13	4.41	4.52	4.47	TN Sn 35	4.37	4.73	4.55
TN *Sn* 14	4.33	4.44	4.39	TN Sn 36	4.33	4.57	4.45
TN *Sn* 15	4.77	4.87	4.82	TN Sn 37	4.84	4.92	4.88
TN *Sn* 16	4.83	4.98	4.91	TN Sn 40	4.64	4.88	4.76
TN *Sn* 17	4.74	4.93	4.84	TN Sn 41	4.64	4.73	4.69
TN *Sn* 18	4.54	4.78	4.66	TN Sn 42	4.86	5.01	4.94
TN *Sn* 19	6.07	6.13	6.10	TN Sn 51	4.88	5.00	4.94
TN *Sn* 20	5.00	5.13	5.07				
		Season I		Season II		Pooled	
MEAN		**4.65**		**4.79**		**4.72**	
SED		0.05		0.05		0.05	
CV		1.16		1.23		1.20	
CD (P= 0.05)		0.11		0.10		0.10	

Solanum americanum Mill.				***Solanum villosum Mill.***			
Genotypes	Season I	Season II	Pooled	Genotypes	Season I	Season II	Pooled
TN *Sn* 8	3.51	3.65	3.58	TN *Sn* 30	3.12	3.21	3.17
TN *Sn* 12	3.28	3.42	3.35	TN Sn 31	3.13	3.27	3.20
TN *Sn* 23	4.76	4.91	4.84	TN Sn 32	2.31	2.38	2.35
TN Sn 38	2.27	2.38	2.33	TN Sn 44	3.10	3.46	3.28
TN Sn 52	2.51	2.73	2.62	TN Sn 47	2.99	3.17	3.08
TN Sn 53	3.02	3.13	3.08				
MEAN	**3.23**	**3.37**	**3.30**	**MEAN**	**2.93**	**3.10**	**3.02**
SED	0.02	0.01	0.01	**SED**	0.08	0.09	0.08
CV	0.86	1.02	1.24	**CV**	2.71	2.87	2.99
CD (P=0.05)	0.05	0.02	0.03	**CD (P=0.05)**	0.16	0.18	0.18

Table 35. Mean performance of Solanum genotypes for ascorbic acid (mg g^{-1})

Genotypes	Season I	Season II	Pooled	Genotypes	Season I	Season II	Pooled
***Solanum nigrum* L.**							
TN *Sn* 1	19.39	20.32	19.86	TN *Sn* 21	18.41	19.44	18.93
TN *Sn* 2	17.24	17.76	17.50	TN *Sn* 22	20.85	21.66	21.26
TN *Sn* 3	19.22	20.33	19.78	TN *Sn* 24	17.29	18.53	17.91
TN *Sn* 4	17.34	19.24	18.29	TN *Sn* 25	15.31	16.67	15.99
TN *Sn* 5	15.36	16.28	15.82	TN *Sn* 26	17.89	18.36	18.13
TN *Sn* 6	15.03	15.63	15.33	TN *Sn* 27	18.14	19.36	18.75
TN *Sn* 7	15.93	16.16	16.05	TN *Sn* 28	17.38	18.73	18.06
TN *Sn* 9	17.12	17.76	17.44	TN *Sn* 29	16.76	17.88	17.32
TN *Sn* 11	18.23	19.49	18.86	TN Sn 33	15.83	16.67	16.25
TN *Sn* 13	17.13	17.71	17.42	TN Sn 35	16.35	16.84	16.60
TN *Sn* 14	16.15	17.16	16.66	TN Sn 36	15.83	16.02	15.93
TN *Sn* 15	15.64	16.74	16.19	TN Sn 37	18.84	19.51	19.18
TN *Sn* 16	16.30	17.40	16.85	TN Sn 40	18.37	19.41	18.89
TN *Sn* 17	17.64	17.97	17.81	TN Sn 41	17.40	18.31	17.86
TN *Sn* 18	18.74	19.18	18.96	TN Sn 42	15.13	14.83	14.98
TN *Sn* 19	21.36	21.96	21.66	TN Sn 51	17.90	18.44	18.17
TN *Sn* 20	19.60	20.35	19.98				
		Season I		Season II		Pooled	
MEAN		**17.43**		**18.25**		**17.84**	
SED		0.29		0.24		0.36	
CV		1.68		2.01		2.00	
CD (P= 0.05)		0.60		0.48		0.52	
Solanum americanum Mill.				***Solanum villosum Mill.***			
TN *Sn* 8	14.22	15.63	14.93	TN *Sn* 30	13.40	14.76	14.08
TN *Sn* 12	14.26	15.33	14.80	TN Sn 31	12.51	13.73	13.12
TN *Sn* 23	14.31	15.63	14.97	TN Sn 32	11.31	12.57	11.94
TN Sn 38	13.25	14.88	14.07	TN Sn 44	12.00	12.63	12.32
TN Sn 52	12.60	13.69	13.15	TN Sn 47	11.82	12.62	12.22
TN Sn 53	12.14	13.42	12.78				
MEAN	**13.46**	**14.76**	**14.12**	**MEAN**	**12.21**	**13.26**	**12.74**
SED	0.14	0.15	0.16	**SED**	0.22	0.24	0.25
CV	1.04	2.04	2.07	**CV**	1.77	2.14	2.04
CD (P=0.05)	0.29	0.30	0.30	**CD(P=0.05)**	0.45	0.48	0.50

Table 36. Organoleptic evaluation of high yielding Solanum genotypes

Genotypes	Color	Flavor	Bitterness	Over all acceptability
TN *Sn* 8	2.25	2.35	2.50	2.38
TN *Sn* 10	2.20	1.20	3.00	1.25
TN *Sn* 12	2.30	2.38	2.42	2.25
TN *Sn* 19	2.82	2.89	1.32	2.93
TN *Sn* 22	2.75	2.85	1.56	2.82
TN *Sn* 30	2.19	1.10	2.92	1.19
TN Sn 38	2.27	2.40	2.42	2.29
TN Sn 47	2.22	1.12	3.00	1.14
TN Sn 52	2.15	2.36	2.39	2.32
TN Sn 53	2.14	2.28	2.38	2.28

Scores	1	2	3
Color	Bad	Fair	Good
Flavor	Bad	Fair	Good
Bitterness	Low	Medium	High
Over all acceptability	Not acceptable	Acceptable	Highly acceptable

Table 37. Distribution of genotypes into different clusters based on D^2 analysis

Cluster No.	No. of types	Accession No.	Source
I	11	TN *Sn* 1	Coimbatore, Tamil Nadu
		TN *Sn* 2	Namakkal, Tamil Nadu
		TN *Sn* 3	Sirugamani, Tamil Nadu
		TN *Sn* 4	Thagarapudur, Tamil Nadu
		TN *Sn* 5	Ottampatty, Tamil Nadu
		TN *Sn* 6	Sobanapuram, Tamil Nadu
		TN *Sn* 7	Paiyur, Tamil Nadu
		TN *Sn* 8	Chinnakalvehalli,Tamil Nadu
		TN *Sn* 9	Theni, Tamil Nadu
		TN *Sn* 12	Kolli hills, Tamil Nadu
		TN *Sn* 53	Kodaikanal, Tamil Nadu
II	2	TN *Sn* 26	Rahuri, Maharastra
		TN *Sn* 33	Ottampatty, Tamil Nadu
III	3	TN *Sn* 10	Ooty, Tamil Nadu
		TN *Sn* 19	Kallipalayam, Tamil Nadu
		TN *Sn* 22	Pelukurichi, Tamil Nadu
IV	2	TN *Sn* 15	Salem, Tamil Nadu
		TN *Sn* 27	Ottampatty -1, Tamil Nadu
V	15	TN *Sn* 11	E.Patty, Tamil Nadu
		TN *Sn* 13	Anthiyur, Tamil Nadu
		TN *Sn* 14	S. mangalam, Tamil Nadu
		TN *Sn* 16	Ottampatty, Tamil Nadu
		TN *Sn* 17	Valasaiyur, Tamil Nadu
		TN *Sn* 18	Perur, Tamil Nadu
		TN *Sn* 20	Trichy, Tamil Nadu
		TN *Sn* 21	Bhavanisagar, Tamil Nadu
		TN *Sn* 23	Namakkal, Tamil Nadu
		TN *Sn* 24	Kolli hills, Tamil Nadu
		TN *Sn* 25	N.S.puram, Tamil Nadu
		TN *Sn* 28	P.Krishnapuram, Tamil Nadu
		TN *Sn* 29	E.patty-2, Tamil Nadu
		TN *Sn* 41	Pattikkad ,Kerala
		TN *Sn* 42	Kottakkal, Kerala
VI	2	TN *Sn 30*	Coimbatore-2, Tamil Nadu
		TN *Sn 31*	Pelukurichi, Tamil Nadu
VII	3	TN *Sn 32*	P.Krishnapuram, Tamil Nadu
		TN *Sn 35*	Pachamalai, Tamil Nadu
		TN *Sn 36*	Navaladipatti, Tamil Nadu
		TN *Sn 37*	Thuraiyur, Tamil Nadu
VIII	6	TN *Sn* 38	Solan, H.P
		TN *Sn* 40	KAU,Thrissur, Kerala
		TN *Sn* 44	Nalhendra, Solan
		TN *Sn* 47	Ottampatty-3, Tamil Nadu
		TN *Sn* 51	Odakali-2, Kerala
		TN *Sn* 52	Guddalore, Tamil Nadu

Table 38. Inter and intra cluster D^2 and D (with in parentheses) values

Cluster No.	I	II	III	IV	V	VI	VII	VIII
I	6049.74 (77.78)	4601.21 (67.83)	11502.52 (107.25)	5694.26 (75.46)	5696.36 (75.47)	9115.32 (95.47)	7151.90 (84.56)	5781.58 (76.03)
II		98.30 (9.915)	16521.88 (128.53)	2859.50 (53.47)	1791.55 (42.32)	13747.20 (117.24)	2435.01 (49.34)	6024.13 (77.61)
III			1132.35 (33.65)	19274.14 (138.83)	15584.05 (124.83)	1836.27 (42.85)	17007.17 (130.41)	6930.89 (83.25)
IV				190.35 (13.79)	4661.30 (68.27)	15674.90 (125.19)	6934.19 (83.27)	8923.75 (94.46)
V					3039.77 (55.13)	13834.50 (117.62)	3219.74 (56.74)	6375.39 (79.84)
VI						289.72 (17.02)	15611.90 (124.94)	5682.94 (75.38)
VII							3726.43 (61.04)	7205.62 (84.88)
VIII								4978.65 (70.56)

Intra cluster divergence : Diagonal values

Inter cluster divergence : Off- diagonal value

Table 39. Relative contribution of eleven agronomic traits towards divergence

S.No.	Characters	Number of first rank	Percentage of contribution towards divergence
1.	Plant height	19	1.92
2.	Plant spread (N-S)	14	1.24
3.	Plant spread (E-W)	10	1.20
4.	Stem girth	0	0.00
5.	Number of branches per plant	0	20.10
6.	Number of leaves per plant	4	10.40
7.	Number of berries per plant	215	11.72
8.	Days to flower bud initiation	0	0.00
9.	Days to 50% flowering	27	2.73
10.	Fresh herbage yield per plant	292	29.49
11.	Dry herbage yield per plant	409	21.31

Table 40. Cluster Mean values for eleven characters of *Solanum* genotypes

S.No	Cluster No.	I	II	III	IV	V	VI	VII	VIII
1.	Plant height	97.05	95.05	117.84	73.87	92.48	119.05	95.46	105.88
2.	Plant spread (N-S)	46.88	48.82	52.75	32.12	44.16	56.70	44.34	49.72
3.	Plant spread (E-W)	44.25	45.66	50.21	29.65	40.22	54.96	43.47	46.81
4.	Stem girth	3.86	3.94	4.54	2.23	3.45	5.16	3.64	4.27
5.	Number of branches per plant	14.83	15.08	24.15	16.85	14.64	29.37	13.89	15.81
6.	Number of leaves per plant	109.93	102.37	145.15	101.65	108.67	143.70	115.99	122.87
7.	Number of berries per plant	254.25	270.71	206.46	274.61	250.88	237.69	245.09	236.46
8.	Days to flower bud initiation	25.89	29.36	25.98	28.93	27.81	20.02	26.02	24.79
9.	Days to 50% flowering	55.71	57.55	55.20	57.05	56.86	50.35	55.50	53.87
10.	Fresh herbage yield per plant	349.25	285.22	460.74	324.68	285.58	463.11	262.39	364.35
11.	Dry herbage yield per plant	164.28	121.92	215.80	138.03	123.49	223.94	111.07	165.86

Table 41. SSR primers showing total and polymorphic amplicons generated pattern for 45 genotypes of Solanum genotypes

S. No.	Primers	Number of alleles	Number of polymorphic alleles	% Polymorphism (b/a x 100)	PIC Value
1.	STWIN 12 G	4	4	100	0.698
2.	SB4-32	3	3	100	0.502
3.	SB6-36	3	3	100	0.604
4.	SB6-57	1	0	0	0.000
5.	SB6-84	2	2	100	0.340
6.	TMS29	3	2	66	0.432
7.	TMS37	1	0	0	0.000
8.	TMS 39	2	2	100	0.480
9.	CM2	1	0	0	0.000
10.	CM6	3	3	100	0.544

Table 43. Clustering of 45 genotypes based on SSR markers

Cluster number	Number of genotypes	List of genotypes included
I	32	TN *Sn* 01, TN *Sn* 02, TN *Sn* 03, TN *Sn* 04, TN *Sn* 05, TN *Sn* 06, TN *Sn* 07, TN *Sn* 09, TN *Sn* 11, TN *Sn* 13, TN *Sn* 14, TN *Sn* 15, TN *Sn* 16, TN *Sn* 17, TN *Sn* 18, TN *Sn* 20, TN *Sn* 21, TN *Sn* 22, TN *Sn* 24, TN *Sn* 25, TN *Sn* 26, TN *Sn* 27, TN *Sn* 28, TN *Sn* 29, TN *Sn* 33, TN *Sn* 35, TN *Sn* 36 TN *Sn* 37, TN *Sn* 40, TN *Sn* 41, TN *Sn* 42 and TN *Sn* 51
II	1	TN *Sn* 19
III	4	TN *Sn* 08, TN *Sn* 12, TN *Sn* 23 and TN *Sn* 38
IV	2	TN *Sn* 52 and TN *Sn* 53
V	1	TN *Sn* 10
VI	3	TN *Sn*30, TN *Sn* 31 and TN *Sn* 32
VII	1	TN *Sn* 44
VIII	1	TN *Sn* 47

Table 44. Estimates of variability and genetic parameters of *Solanum nigrum* L. genotypes

S.No.	Characters	Mean	Range	PCV %	GCV %	h^2	GA (% mean)
1.	Plant height (cm)	91.01	125.24-74.1	11.22	10.59	73.10	16.90
2.	Plant spread (N-S)	43.44	53.58-32.99	14.39	13.32	85.69	25.40
3.	Plant spread (E-W)	42.41	50.59-29.01	13.48	12.84	82.40	24.37
4.	Stem girth (cm)	3.55	4.77-2.15	13.52	12.71	72.66	14.08
5.	Number of branches per plant	14.76	23.57-9.36	18.85	19.88	88.69	37.16
6.	Number of leaves per plant	106.75	149.23-17.25	16.26	15.58	72.84	27.40
7.	Leaf length (cm)	5.78	7.54-4.13	10.81	9.26	66.87	24.45
8.	Leaf breadth (cm)	3.13	4.15-1.45	11.85	10.21	68.34	26.24
9.	Days to flower bud initiation	27.74	30.04-23.3	16.76	16.66	88.11	33.53
10.	Days to 50% flowering	56.78	57.82-55.15	4.29	4.19	85.83	22.28
11.	Number of berries per plant	255.97	288.37-198.26	8.66	8.64	69.57	17.77
12.	Fresh herbage yield per plant (g)	305.84	451.59-211.11	19.45	18.81	89.60	37.50
13.	Dry herbage yield per plant (g)	135.38	207.72-81.89	20.73	20.68	91.59	36.59
14.	Total alkaloid content (%)	0.37	0.408-0.340	3.81	3.01	92.69	4.92
15.	Protein content (mg $100g^{-1}$)	2.65	5.17-1.64	20.42	20.34	79.36	30.00
16.	Crude fibre (%)	0.71	0.84-0.67	5.54	5.38	89.34	15.77
17.	Iron content (mg $100g^{-1}$)	4.72	6.10-4.15	7.73	7.64	90.72	15.57
18.	Ascorbic acid (mg g^{-1})	17.84	21.66-14.98	9.21	9.06	89.67	18.35

Table 45. Estimates of variability and genetic parameters of *Solanum americanum* Mill. genotypes

S.No.	Characters	Mean	Range	PCV %	GCV %	h^2	GA (% mean)
1.	Plant height (cm)	111.16	118.03-89.31	18.88	18.84	79.16	18.15
2.	Plant spread (N-S)	51.30	55.62-42.13	9.60	8.96	77.10	17.24
3.	Plant spread (E-W)	49.09	52.04-37.83	11.49	11.21	75.12	22.53
4.	Stem girth (cm)	4.47	4.93-3.08	16.10	15.80	76.29	21.95
5.	Number of branches per plant	19.29	20.25-18.53	14.01	13.53	77.42	26.40
6.	Number of leaves per plant	129.65	134.60-111.52	10.90	10.87	88.95	34.08
7.	Leaf length (cm)	6.63	7.30-5.54	9.68	9.50	76.34	19.21
8.	Leaf breadth (cm)	3.17	3.55-2.70	10.27	8.89	74.95	15.86
9.	Days to flower bud initiation	24.51	29.41-21.2	12.36	12.08	75.59	24.34
10.	Days to 50% flowering	54.40	57.43-53.16	2.89	2.87	88.36	15.87
11.	Number of berries per plant	225.23	250.32-211.26	6.21	6.17	78.66	12.63
12.	Fresh herbage yield per plant (g)	402.18	432.93-328.12	12.29	12.28	79.80	19.11
13.	Dry herbage yield per plant (g)	186.41	202.49-140.50	19.29	19.24	90.18	25.11
14.	Total alkaloid content (%)	0.41	0.43-0.38	5.61	4.61	91.49	7.81
15.	Protein content (mg $100g^{-1}$)	1.60	1.78-1.28	12.25	12.20	79.25	25.05
16.	Crude fibre (%)	0.59	0.68-0.43	14.02	13.99	89.57	28.76
17.	Iron content (mg $100g^{-1}$)	3.30	4.84-2.33	26.79	26.77	94.89	35.13
18.	Ascorbic acid (mg g^{-1})	14.12	14.97-12.78	6.81	6.73	87.63	13.71

Table 46. Estimates of variability and genetic parameters of *Solanum villosum* Mill. genotypes

S.No.	Characters	Mean	Range	PCV %	GCV %	h^2	GA (% mean)
1.	Plant height (cm)	110.46	124.03-74.49	18.67	18.66	79.89	32.44
2.	Plant spread (N-S)	50.08	58.55-33.38	20.90	20.70	78.02	20.19
3.	Plant spread (E-W)	48.32	57.71-30.68	21.55	21.45	75.03	23.97
4.	Stem girth (cm)	4.61	5.37-3.11	19.82	19.78	71.58	20.66
5.	Number of branches per plant	18.91	25.69-12.63	12.90	12.45	87.28	35.94
6.	Number of leaves per plant	136.81	144.59-121.84	6.75	6.72	79.02	13.78
7.	Leaf length (cm)	6.03	7.48-4.30	12.23	12.07	68.54	15.14
8.	Leaf breadth (cm)	4.02	4.99-3.58	14.61	14.53	68.96	19.79
9.	Days to flower bud initiation	20.26	20.74-19.81	12.51	11.80	71.56	22.66
10.	Days to 50% flowering	50.64	51.23-50.16	11.06	10.90	71.63	21.57
11.	Number of berries per plant	227.45	242.75-212.73	5.61	5.58	79.10	11.46
12.	Fresh herbage yield per plant (g)	386.75	471.87-296.41	16.65	16.65	81.98	22.53
13.	Dry herbage yield per plant (g)	179.63	226.62-124.80	26.70	26.69	91.86	24.94
14.	Total alkaloid content (%)	0.42	0.44-0.40	3.32	3.31	90.28	26.79
15.	Protein content (mg $100g^{-1}$)	1.42	1.65-1.29	19.21	18.71	93.17	30.09
16.	Crude fibre (%)	0.79	0.83-0.73	15.50	14.82	86.92	28.71
17.	Iron content (mg $100g^{-1}$)	3.02	3.28-2.35	12.77	12.48	95.49	25.13
18.	Ascorbic acid (mg g^{-1})	12.74	14.08-11.94	16.94	16.71	93.48	13.38

Table 47. Estimates of simple correlations among 15 biometric traits in *Solanum nigrum* L. genotypes

Characters	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}	X_{12}	X_{13}	X_{14}	X_{15}
X_1	1.000	0.819**	0.809**	0.677**	0.466**	0.016	0.170	-0.433**	0.683**	0.425**	0.475**	0.337*	0.415**	0.335*	0.263
X_2		1.000	0.778**	0.655**	0.452**	-0.041	0.054	-0.391*	0.360*	0.311*	0.270	0.268	0.263	0.197	0.215
X_3			1.000	0.202	0.444**	-0.015	0.078	-0.298	0.216	0.187	0.176	0.271	0.137	0.063	0.203
X_4				1.000	0.506**	0.107	0.245	-0.561**	0.473**	0.295	0.443**	0.333*	0.262	0.275	0.342*
X_5					1.000	0.095	0.179	-0.492**	0.423**	0.379**	0.551**	0.553**	0.470**	0.245	0.296
X_6						1.000	0.616**	-0.128	-0.187	0.101	0.128	0.102	0.191	0.127	0.144
X_7							1.000	0.246	-0.043	-0.162	-0.378**	0.111	0.338*	0.061	-0.106
X_8								1.000	-0.390**	-0.317	-0.563**	-0.445**	-0.409**	-0.377*	-0.360*
X_9									1.000	0.825**	0.250	0.322*	0.365*	0.330*	0.368*
X_{10}										1.000	0.545**	0.608**	0.300	0.273	0.465**
X_{11}											1.000	0.638**	0.709**	0.564**	-0.316*
X_{12}												1.000	0.599**	0.309*	0.433**
X_{13}													1.000	0.495**	-0.248
X_{14}														1.000	-0.165
X_{15}															1.000

* Significant at 5% level; ** Significant at 1% level; G – Genotypic correlation.

X_1 – Plant height, X_2 – Plant spread (N-S), X_3 – Plant spread (E-W), X_4 – Number of primary branches, X_5 – Number of leaves per plant, X_6 – Number of berries per plant, X_7 – Days to flower bud initiation, X_8 – Days to 50% flowering, X_9 - Fresh herbage yield per plant, X_{10} – Dry herbage yield per plant, X_{11} – Protein content, X_{12} – Crude fibre, X_{13} – Iron content, X_{14} – Ascorbic acid, X_{15} –Total alkaloid content

Table 48. Estimates of simple correlations among 15 biometric traits in *Solanum americanum Mill*. genotypes

Characters	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}	X_{12}	X_{13}	X_{14}	X_{15}
X_1	1.000	0.869**	0.878**	0.467**	0.364**	0.214	0.261	0.264	0.843**	0.755**	0.236	0.244	0.229	0.417**	0.364*
X_2		1.000	0.907**	0.433**	0.172	-0.274	-0.928**	0.343*	0.564**	0.480**	0.240	0.121	0.198	-0.410**	0.341*
X_3			1.000	0.440**	0.266	-0.180	-0.954**	0.217	0.574**	0.485**	0.207	0.154	0.166	-0.369**	0.415**
X_4				1.000	0.500**	0.011	-0.245	0.412**	0.527**	0.528**	0.050	0.193	0.140	-0.837**	0.481**
X_5					1.000	0.080	-0.172	-0.657**	0.592**	0.242	0.532**	0.167	0.266	0.511**	0.456**
X_6						1.000	0.289	-0.219	-0.197	-0.223	0.207	0.075	0.181	0.092	0.368*
X_7							1.000	-0.518**	-0.670**	-0.493**	-0.133	-0.183	-0.118	0.305*	0.206
X_8								1.000	-0.201	-0.253	-0.187	-0.194	-0.244	-0.218	-0.433**
X_9									1.000	0.797**	0.452**	0.197	0.187	0.137	0.344*
X_{10}										1.000	0.445**	0.188	0.190	0.178	0.590**
X_{11}											1.000	0.269	0.197	0.197	-0.237
X_{12}												1.000	0.180	-0.151	0.392**
X_{13}													1.000	0.108	-0.052
X_{14}														1.000	0.1396
X_{15}															1.000

* Significant at 5% level; ** Significant at 1% level; G – Genotypic correlation.

X_1 – Plant height, X_2 – Plant spread (N-S), X_3 – Plant spread (E-W), X_4 – Number of primary branches, X_5 – Number of leaves per plant, X_6 – Number of berries per plant, X_7 – Days to flower bud initiation, X_8 – Days to 50% flowering, X_9 - Fresh herbage yield per plant, X_{10} – Dry herbage yield per plant, X_{11} – Protein content, X_{12} – Crude fibre, X_{13} –Iron content, X_{14} – Ascorbic acid, X_{15} –Total alkaloid content.

Table 49. Estimates of simple correlations among 15 biometric traits in *Solanum villosum Mill*. genotypes

Characters	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}	X_{12}	X_{13}	X_{14}	X_{15}
X_1	1.000	0.739**	0.756**	0.446**	0.893**	0.266	0.253	0.285	0.504**	0.424**	0.262	0.203	0.165	0.273	0.378**
X_2		1.000	0.996	0.447**	0.493**	0.291	0.071	0.009	0.261	0.273	0.320*	0.334*	0.288	0.244	0.463**
X_3			1.000	0.335*	0.479**	0.214	0.038	0.008	0.220	0.229	0.312*	0.278	0.250	0.230	0.522**
X_4				1.000	0.677**	0.135	0.245	0.237	0.558**	0.525**	0.124	0.125	0.110	0.123	0.617**
X_5					1.000	-0.215	-0.156	0.078	0.826**	0.538**	-0.216	0.234	0.117	0.295	0.402**
X_6						1.000	0.585**	0.339*	-0.320*	0.268	0.100	0.216	0.126	0.237	-0.059
X_7							1.000	0.567**	-0.284	-0.534**	0.145	0.245	0.118	0.115	-0.238
X_8								1.000	-0.118	-0.460**	-0.258	-0.189	-0.106	-0.134	0.329*
X_9									1.000	0.897**	0.333*	0.262	0.453**	0.253	0.194
X_{10}										1.000	0.372**	0.344*	0.351*	0.213	0.143
X_{11}											1.000	0.145	0.351*	0.168	-0.283
X_{12}												1.000	0.240	0.146	-0.020
X_{13}													1.000	0.304*	-0.452**
X_{14}														1.000	0.507**
X_{15}															1.000

* Significant at 5% level; ** Significant at 1% level; G – Genotypic correlation.

X_1 – Plant height, X_2 – Plant spread (N-S), X_3 – Plant spread (E-W), X_4 – Number of primary branches, X_5 – Number of leaves per plant, X_6 – Number of berries per plant, X_7 – Days to flower bud initiation, X_8 – Days to 50% flowering, X_9 - Fresh herbage yield per plant, X_{10} – Dry herbage yield per plant, X_{11} – Protein content, X_{12} – Crude fibre, X_{13} –Iron content, X_{14} – Ascorbic acid, X_{15} –Total alkaloid content.

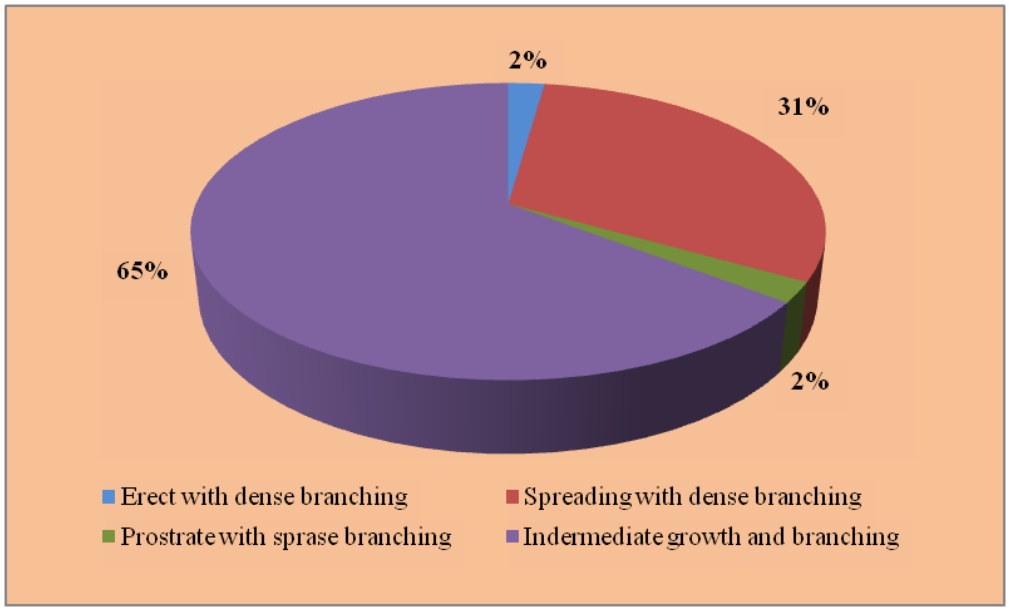

Fig.1. Diversity in growth habit and branching

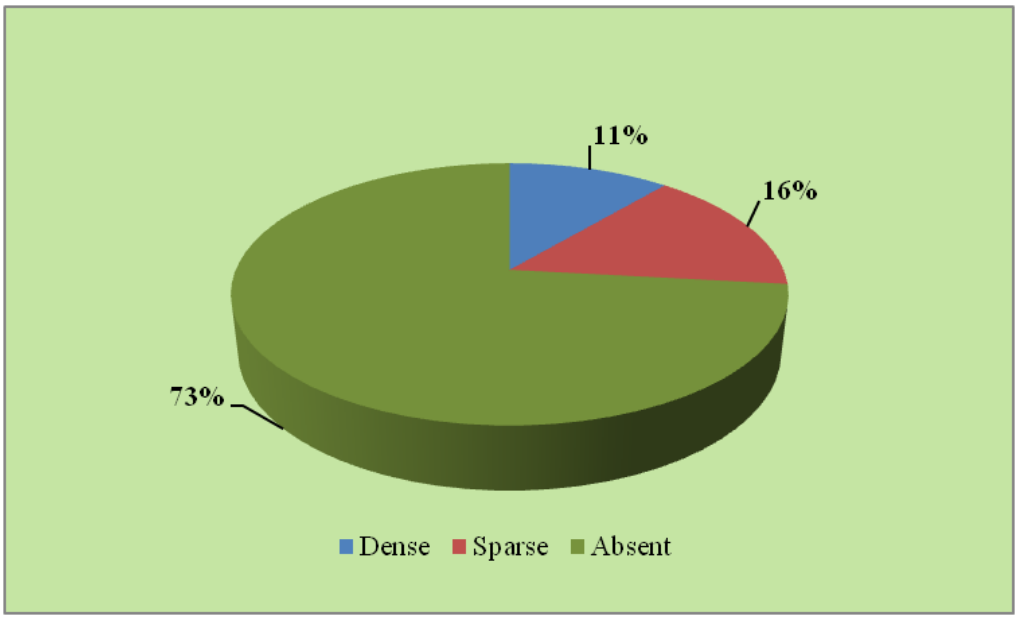

Fig.2. Diversity in stem and leaf pubescence

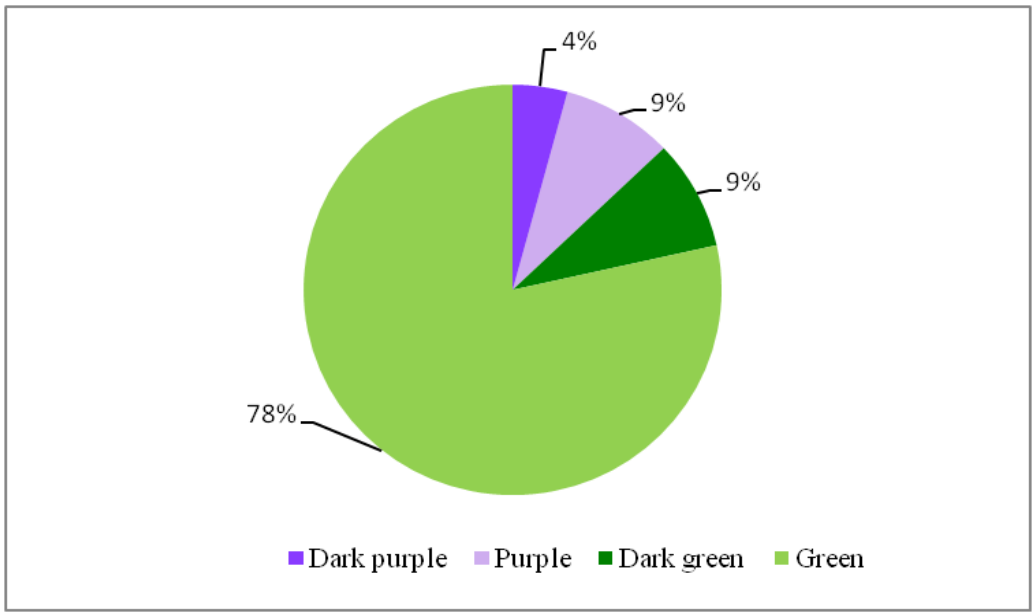

Fig.3. Diversity in stem color

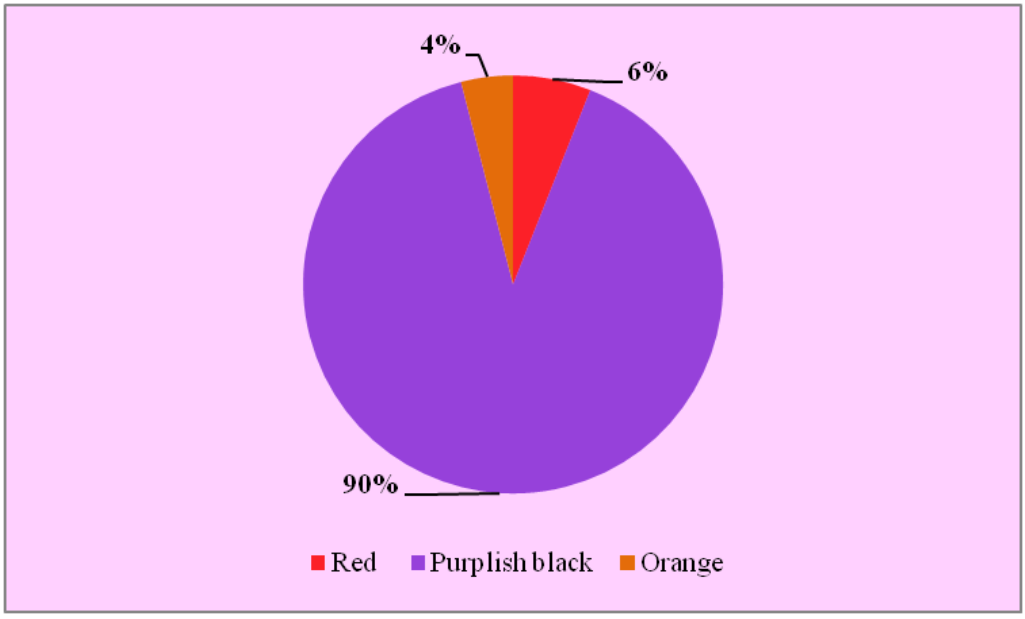

Fig.4. Diversity in berry color

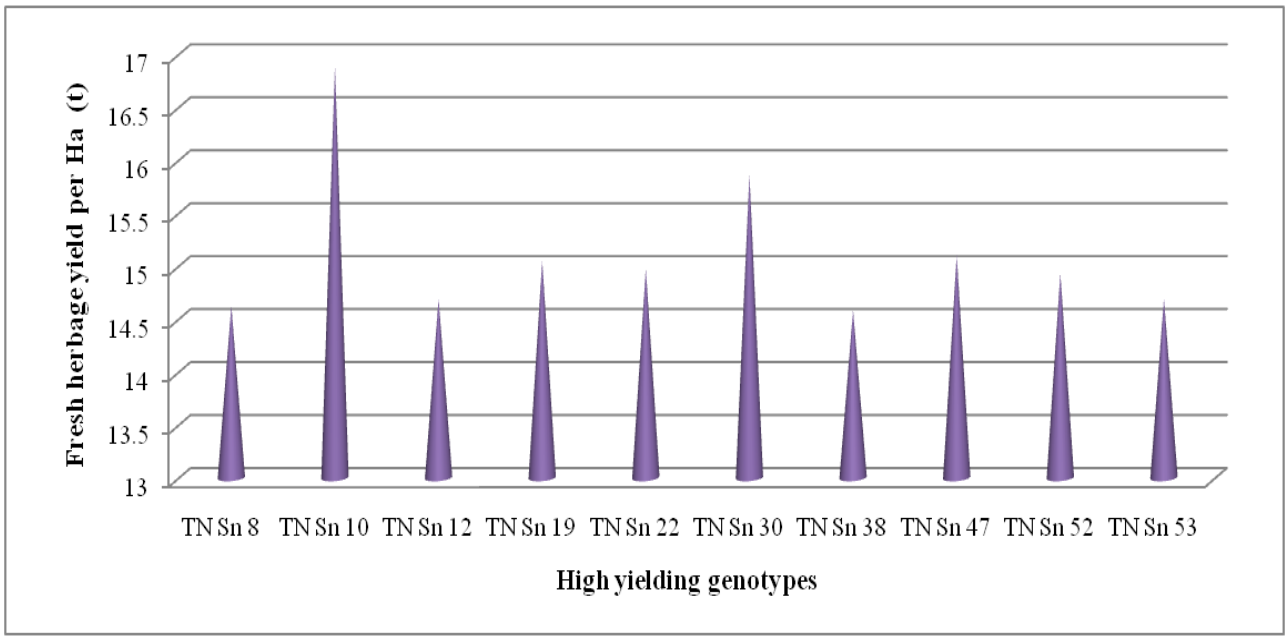

Fig.6. Fresh herbage yield per hectare of high yielding Makoi genotypes

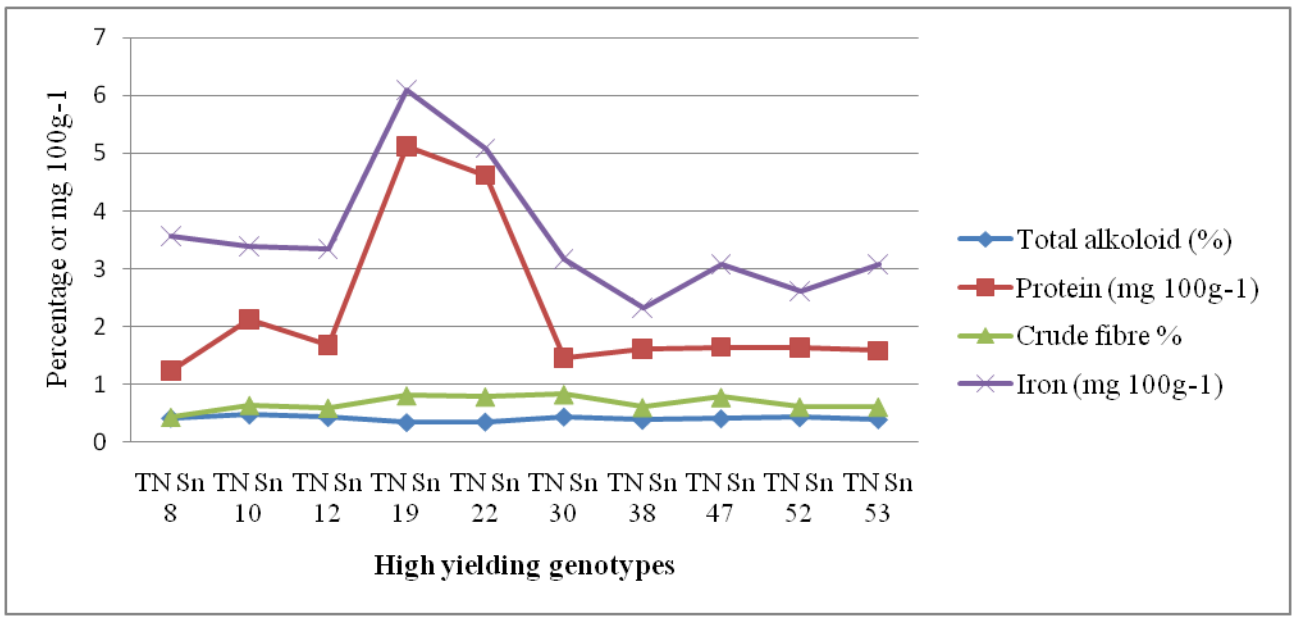

Fig.7. Biochemical contents in high yielding Makoi genotypes

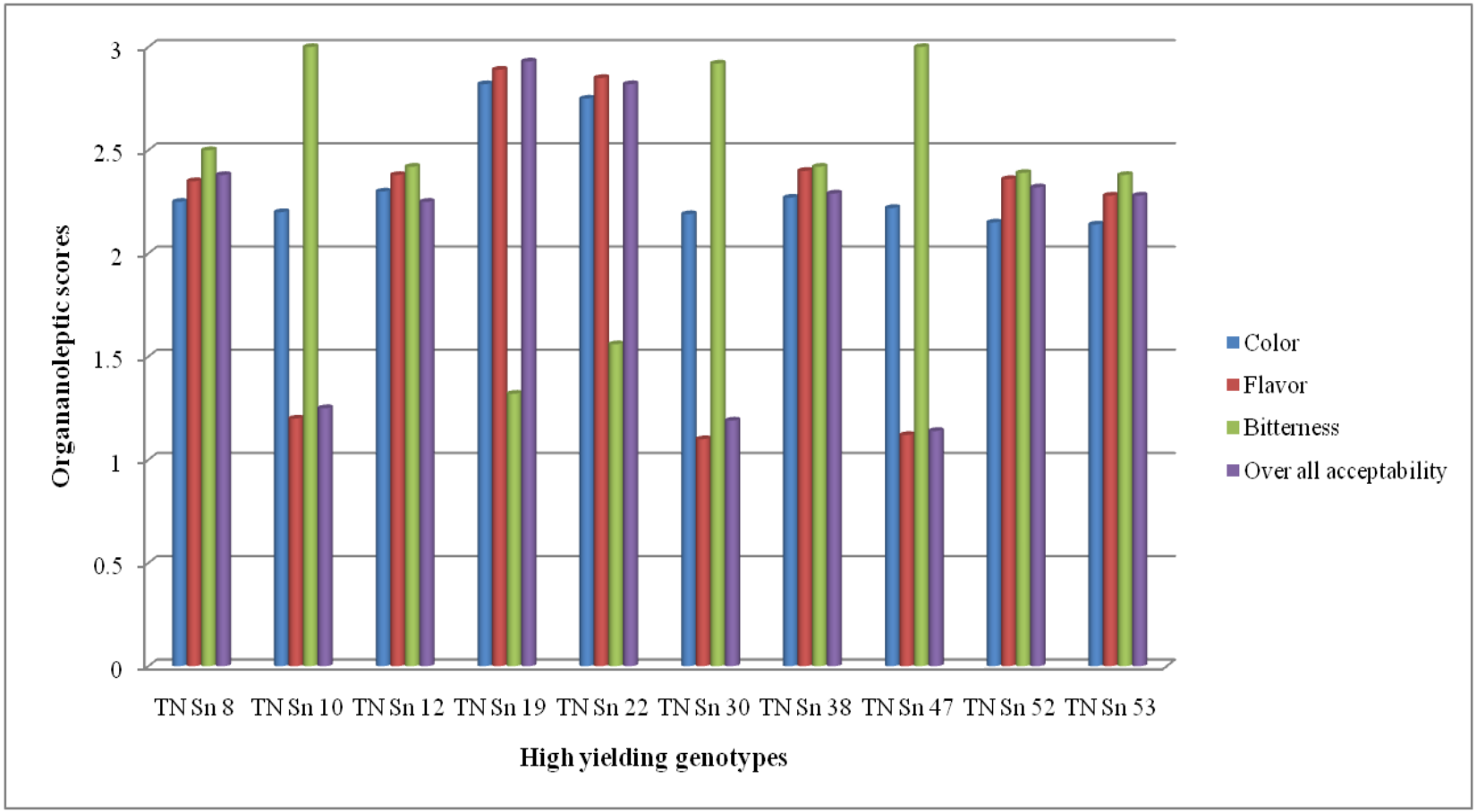

Fig.8. Organoleptic evaluation of high yielding genotypes

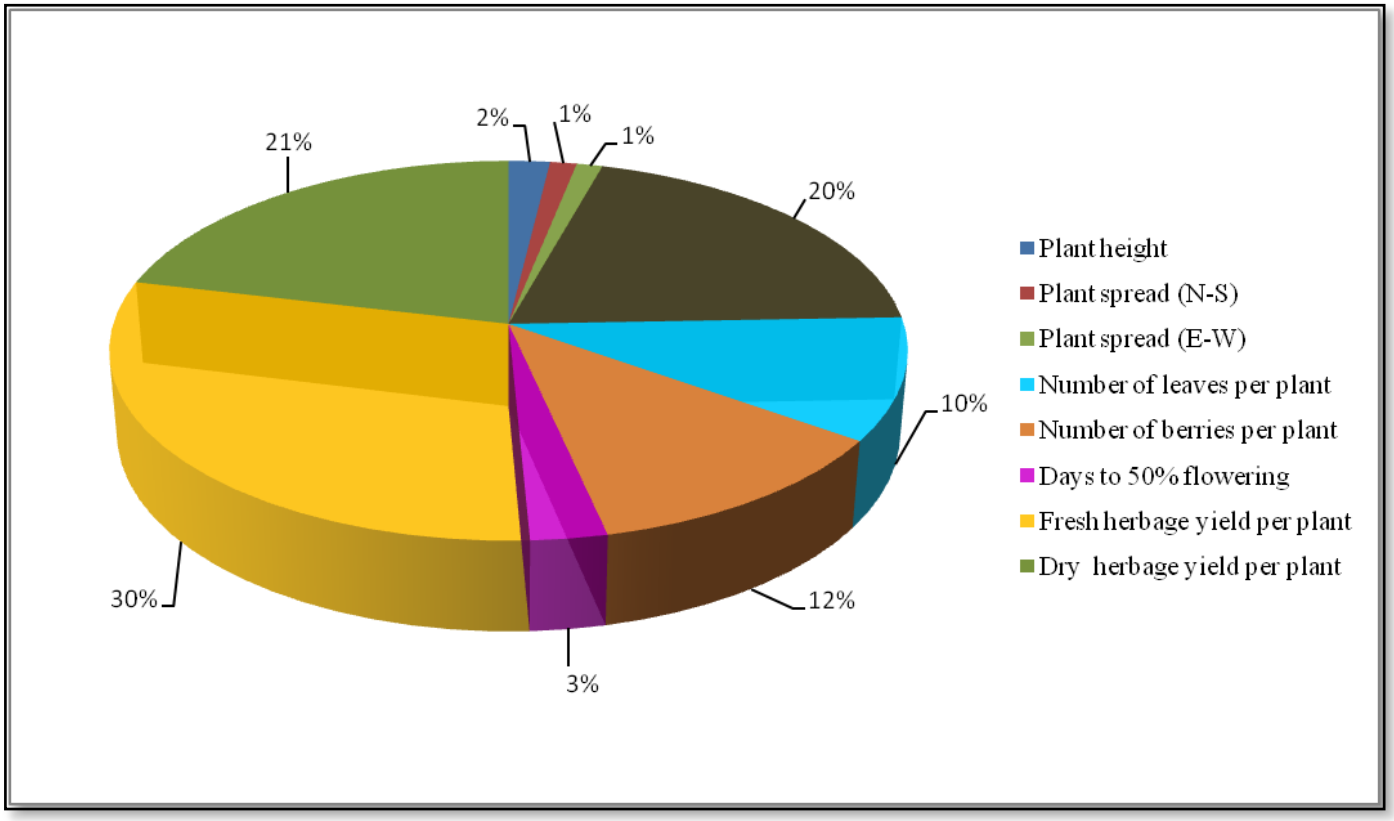

Fig.9. Relative contribution of characters towards divergence in Makoi genotypes

Discussion

CHAPTER V

DISCUSSION

Medicinal plants are still an accessible, affordable and culturally appropriate source of primary health care for majority of the Indian population. A tropical country like India is the cradle of many medicinal plant species. Studies on the genetic resources, protection of wild races and imparting the genes in crop improvement forms the foremost task.

Solanum nigrum L. is a potential medicinal plant, gaining importance in the global herbal industry. The principle aglycone is solasodine, the nitrogen analogue of diosgenin which has been reported as a valuable steroidal precursor for the supplementary source of the commercial synthesis of several steroidal drugs. Knowledge on genetic variability encompassed within species will greatly aid direct exploitation of variability as cultivars and indirectly as base materials for different breeding programmes.

The presence of adequate genetic variability is an essential prerequisite for breeding programme, and therefore a thorough knowledge of existing variability within the population is of prime importance. The study of variability for different plant characters in the genetic stocks and an estimation of the part played by the genes and environment in the expression of the traits will greatly facilitate to understand the basis for diversity / variability existing among the genotypes within a reasonable period.

In the present study, forty five makoi genotypes collected from different eco geographical regions of India were evaluated with the following objectives

i. To study the variability in morphological characters based on descriptors and species identification using ploidy studies and barcoding.
ii. To identify superior types for alkaloid yield and nutritive content of all three *Solanum sp* based on their *per se* performance.
iii. To identify yield components which exhibits high heritability and genetic advance
iv. To study the association of characters with yield and to characterize genotypes based on molecular markers.

5.1. Variability assessment through morphological markers

Morphological characterization is considered to be an important first step in description and classification of crop germplasm because any breeding programme in crop mainly depends

upon the magnitude of genetic variability (Smith and Smith, 1989). The extent of genetic diversity assessed in makoi based on 14 qualitative traits with the minimum set of descriptors (IPGRI) revealed certain satisfactory measures of diversity (Plate.2). These easily observable morphological traits are useful tool for preliminary evaluation, because they offer a fast and useful approach for assessing the extent of diversity. Characterization of makoi using morphological characters had been used early by several researchers *viz.,* Beg *et al.* (1989), Edmonds and Chweya (1997), Schilling and Anderson (1990), Anil Kumar and Pushpangadan (2005) and Ayesha Mohy –ud-din (2008).

In the present study, considerable variations were observed in plant growth habit *viz.,* spreading, erect and prostrate (TN *Sn* 44). Among the forty five genotypes, TN *Sn* 10 alone was erect with dense branching while fourteen genotypes were of spreading type with dense branching and the rest were of indeterminate type (Fig.1). Since the leaves are the main economic part, the dense branching genotypes with erect growth are highly preferred. When there is more number of branches, number of leaves will also increase, followed by increased leaf yield. Erect growing genotypes are ideal than spreading and prostrate types since it allows maximum and uniform exposure of sunlight and would result in an increase in dry matter production and subsequently increase in yield. Moreover, there is less chance of leaves trenching the ground (or) soil.

Two types of leaf shape were noticed in germplasm collection. Leaf shape was deltoid in TN *Sn* 10, TN *Sn* 30 and TN *Sn* 31 while it was ovate in all the remaining genotypes. Leaf and stem pubescence was dense in five genotypes, sparse in seven genotypes and completely absent in remaining genotypes (Fig. 2). Genotypes having pubescence may have some resistance to pests and disease compared with rest of the genotypes.

Stem color varied from green, dark green to purple and dark purple (Fig. 3). Variations were also noticed in flower size and berry size (Big, medium and small). Regarding the flower color, one distinct genotype (TN *Sn* 47) had purple stripes in its corolla and all others had white colored corolla. Berry color varied from black, red to orange (Fig. 4). In case of seed color, red and orange berried genotypes had straw color while others had brown colored seeds. The variations may be due to natural hybridization occurring in this species and ploidy variations reported in this species (Schilling and Andersen, 1990).

Cluster analysis was carried out using observations recorded in different morphological characters with the help of multivariate analysis system (NTSYS 2.11). The dendrogram separated the genotypes into four major clusters (Fig.5). Cluster I was further divided into two sub clusters, Ia and Ib. Cluster Ia comprised of 22 genotypes, cluster Ib contained 11 genotypes. Cluster II consisted of 6 genotypes and cluster III contains two distinct genotypes TN *Sn* 10 and TN *Sn* 44. Cluster IV was further divided into two sub clusters, IV a (three genotypes) and IV b (TN *Sn* 47).

Morphological description could provide unique identification of genotypes, however their ability to provide reliably discriminating identification is cumbersome. Morphological descriptions reflect not only the genetic constitution of the cultivar, but also the interaction of the genotypes with the environment (G x E) within which it is expressed. G x E interaction effects have been found to cause aberrant means for traits such that morphological markers collected in field plots can provide, at best, only an initial screening of genotype identity or distinctiveness. Morphological variations have been eliminated with the consequence that most of the genotypes outwardly appear similar due to their unknown genetic control.

The present study indicated that morphological markers could be employed for genetic diversity analysis, provided clear cut procedures with identification of gaps and the construction of a comprehensive collection of phenotypic types within each ecological germplasm pool are made available as indicated by Crossa *et al*. (1994). The availability of few number of morphological markers, their poor or unknown genetic control and environmental influence on the phenotypic expression, stage specific identification and practical difficulties are known impediments in using these as genetic markers in genetic diversity analysis.

5.2. Species identification

5.2.1. Ploidy variations

Ploidy is an important aspect of plant genomes and a majority of plant species are proposed to have undergone polyploidisation in their history (Grant, 1971). About 80% of all angiosperms are polyploids (Masterson, 1994), including the majority of economically important crops such as banana, wheat, maize, sugarcane, potato, coffee, alfalfa and cotton (Leitch and Bennett, 1997). Determination of its ploidy level is a prerequisite for using germplasm in taxonomic, genetic or breeding experiments. Polyploidy is important for certain crop species because desirable agronomic and horticultural traits such as size and vigour are expressed in polyploids more often than in their diploid counterparts (Elliot, 1958) as morphological measurements were also correlated to ploidy level.

In the present study, an attempt was made to study the ploidy variation in makoi genotypes by measuring the variations in stomatal and pollen as they are correlated with the ploidy level (Plate.5). Further, the number of flowers per inflorescence, flower diameter, berry diameter increases with increase in ploidy level (Plate 3a and 3b). The number of seeds per berry alone decreases with increase in ploidy level. The ploidy status was also confirmed using flow cytometry. Among the genotypes, TN *Sn* 10 was found to be hexaploid and TN *Sn* 30, TN *Sn* 31, TN *Sn* 32, TN *Sn* 44 and TN *Sn* 47 (red and orange fruited types) are found to be tetraploid. Although the genotypes TN *Sn* 8, TN *Sn* 12, TN *Sn* 23, TN *Sn* 38 and TN *Sn* 53 were distinct from other genotypes, they were also diploids similar to other genotypes. The genotypes TN *Sn* 10 and TN *Sn* 30 excelled in all growth characters and alkaloid content and this may be due to the increased vigor caused by its ploidy status. Ploidy difference has been studied in makoi by several researchers *viz.*, Bhiravamurty and Rethy (1983), Beg *et al.* (1989); Edmonds and Chweya (1997), Schilling and Anderson (1990), Anil Kumar and Pushpangadan (2005) and Ayesha Mohy –ud-din (2008).

5.2.2. DNA Barcoding

DNA barcoding community has proposed several molecular markers for identification of plant species. Among the proposed barcode candidates, nuclear *ITS* region usually exhibit high levels of variation, including indel polymorphism (Graham *et al.* 2000) and serve as an efficient candidate for species identification, provides relative ease of

sequencing and alignment (Kress et al. 2005, Hollingsworth *et al*. 2011, Taberlet 2007, Baldwin et al. 1995). In the present study, the interrelationship between the thirteen *Solanum nigrum* L. complex collected from different geographical location was analyzed using the barcode candidate *ITS*. The multiple sequence alignment of the *Solanum* sp. displayed distinct nucleotide variation between the three important species *S. americanum* Miller, *S. villosum* Miller, *S. nigrum* L. The evolutionary analysis conducted in the MEGA5 tool involved 685 codon positions in the final data set. The *ITS* region exhibited 97.5% overall constant sites and 2.5% variable sites; among which 2.3% constituted parsimony informative sites. The pairwise comparison of *S. americanum*, *S. nigrum* and *S. villosum* showed 98.5% identity.

Most of the parsimony informative characters were shared between *S. nigrum* and *S. americanum*. Blast analysis also confirmed the closeness between, *S. nigrum* and *S. americanum*. The best nucleotide substitution model predicted using the MEGA5 tool was attributed to be Tamura-3- parameter (T92) model. The phylogenetic analysis performed using Neighbour joining statistical method produced three distinct clades, wherein the three species *S. americanum* Miller, *S. nigrum* L., *S. villosum* Miller were grouped under clade I, II and III respectively (Fig 2).

5.3. *Per se* performance of genotypes

In any selection programme, the *per se* performance serves as an important criterion for discarding the undesirable types. This offers a better scope for the germplasm to be used as the potential source and also for selecting the high yielding accessions. Hence, the genotypes chosen for the present study were assessed for their *per se* performance.

The *per se* performance of forty five genotypes revealed significant difference among the genotypes for the traits studied. The genotypes TN*Sn* 10 (*S.nigrum* L., 2n = 72), TN*Sn* 30 (*S.villosum* Mill), TN*Sn* 19 (*S.nigrum* L.) and TN*Sn* 22 (*S.nigrum* L.) registered the highest mean values for plant height, number of branches per plant, number of leaves per plant, stem girth, leaf length, leaf breadth, fresh and dry herbage yield per plant (Fig.6). The expression of maximum plant characters by these genotypes may be due to the higher ploidy level (TN*Sn* 10 and TN*Sn* 30) and the differential ability of genotypes for the synthesis of phytohormones as reported by Anshebo (2002), Vanessa *et al.* (2004) in Stevia, Jaskani *et al.* (2002) in Kinnow mandarin and Murti *et al*. (2012) in Strawberry.

Earliness is one of the most important criteria in crop improvement programme. The red and orange fruited genotypes like TN*Sn* 30 (*S.villosum* Mill) and TN*Sn* 44 (*S.villosum* Mill) took lesser days to flower initiation and 50 per cent flowering. So these genotypes can be used to evolve earlier varieties.

Total leaf area, total chlorophyll, total soluble protein and total phenols were highest in TN *Sn* 10(*S.nigrum* L., 2n = 72) and TN *Sn* 30 (*S.villosum* Mill). Higher total leaf area and total chlorophyll are the important traits which influence higher solar interception and increased photosynthetic efficiency that improves the synthesis of phyto assimilate production resulting in better portioning of reserved food.

In the present study, variation in total alkaloid content had been recorded among the genotypes. The genotype TN *Sn* 10 (*S.nigrum* L., 2n = 72) registered highest total alkaloid content (0.475%) followed by TN *Sn* 30 (*S.villosum* Mill) (0.438 %). The genotypes TN *Sn* 10, TN *Sn* 30 (*S.villosum* Mill), TN *Sn* 19 and TN *Sn* 22 showed superior performance for morpho-economic traits which might serve as ideal parents for developing hybrids with high herbage yield (Plate. 7).

The variability in the chemical constituents among the accessions may be due to the differences in ploidy status. TN *Sn* 19 (*S.nigrum* L.) and TN *Sn* 22 (*S.nigrum* L.) recorded highest protein, iron content and ascorbic acid content in both seasons. The organoleptic evaluation was carried out for the high yielding genotypes. Over all accepetability was good for TN *Sn* 19 (*S.nigrum* L.) and TN *Sn* 22 (*S.nigrum* L.) as they have less bitterness (due to low alkaloid content) whereas, the other high yielding genotypes like TN *Sn* 10 (*S.nigrum* L., 2n =72) and TN *Sn* 30 (*S.villosum* Mill) had more biterness (high alkoloid content) and over all acceptability was not fair (Fig. 8). Hence, the high yielding genotypes TN *Sn* 10(*S.nigrum* L., 2n =72) and TN *Sn* 30 (*S.villosum* Mill) can be used in breeding programme for gettting high alkaloid yielding vareities as they recorded high alkaloid content in both the seasons.

Though TN *Sn* 19 (*S.nigrum* L.) and TN *Sn* 22 (*S.nigrum* L.) recoded low yield than TN *Sn* 10(*S.nigrum* L., 2n=72) and TN *Sn* 30 (*S.villosum* Mill), they showed high nutritive value and hence they can be used for evolving varirties that can be used as green leafy vegetable (Fig.7).

5.4. Genetic diversity studies

5.4.1. Assessment of genetic diversity using D^2 statistics

Collection and conservation of genetic resources of crop are only preliminary stage in crop improvement. Cataloguing and indexing of germplasm are important for practical utilisation. The study of genetic variability for diverse morpho-economic traits in the available germplasm is a prelude for potential crop improvement. Genetic divergence is mainly studied to identify highly divergent clusters with promising genotypes for higher biomass yield.

Mahalanobis (1936) generalized distance matrix D^2 to measure the genetic divergence which was used in the present study to measure the genetic divergence available in the makoi genotypes. The analysis of variance revealed significant differences among the genotypes for all the eleven characters observed, indicating the existence of considerable genetic variability among the genotypes. The presence of high variability among the genotypes for different characters has confirmed the pattern of distribution of forty five genotypes into eight clusters, such that the genotypes within a cluster have lesser D^2 values among themselves than those belonging to other clusters.

In the present investigation, cluster V had the maximum number of genotypes (fifteen genotypes) followed by cluster I (11 genotypes). Cluster I contains genotypes collected from Tamil Nadu (Coimbatore, Trichy, Namakkal, Salem and Theni districts). On the contrary, cluster V incorporated the genotypes from Tamil Nadu and Kerala. The Cluster VIII composed of genotypes from Tamil Nadu, Kerala and Himachal Pradesh. The distribution pattern of genotypes of diverse origin in a single cluster indicates that genetic diversity observed within makoi was not related to geographic origin. Noted differences in plant characters probably occurred over time with the free movement of plant material from location to location and spontaneous or natural hybridization over the time. Thus, the makoi genotypes to be used in crop improvement programme should be selected on the basis of quantified degree of divergence as opposed to geographic origin of genotypes.

Thus, it is evident that the geographic diversity, though important, may not be the only factor quantifying degree of divergence. The present study has shown that the factors other than geographic diversity might have been responsible for the clustering of genotypes. A similar finding was reported by Lal *et al.* (1999) in vetiver, Choudhary *et al.* (2002) in

celery, Lal *et al.* (2001) in Java citronella, Lal *et al.* (2002) in Shankhapushpi and Kavitha (2005) in *Coleus forskohlii*.

High intra cluster divergence was recorded for the cluster I and cluster VIII and it showed that genotypes within the cluster were highly variable for the characters studied. Cluster II recorded the least intra cluster distance suggesting the closeness between the two genotypes present in that cluster. The cluster IV and III showed maximum inter cluster D^2 value followed by VII and III, which indicated that the genotypes included in these two clusters are highly divergent and hybridization would produce heterotic hybrids and a greater spectrum of variability in the segregating generations. Lal *et al.* (1999) stated that clustering pattern could be utilized in selecting prospective parents for breeding programme to develop the highest possible variability for various economic characters.

Regarding the cluster means for 11 characters, cluster VI and cluster III recorded the maximum cluster mean values for most of the characters. The cluster VI had two genotypes viz., TN*Sn* 30 and TN*Sn* 31 and cluster III had three genotypes TN*Sn* 10, TN*Sn* 19 and TN*Sn* 22. Based on the mean values of clusters for different characters and mean performance of the genotypes they are grouped in the respective clusters, the genotypes can be selected for a viable hybridization programme for improving a particular character.

The relative contribution of various characters towards genetic divergence is also exhibited by D^2 analysis. Anshebo (2002) suggested that in the D^2 analysis, the relative importance of the different characters in relation to their contribution to total genetic divergence is given by their respective rank total. Higher the rank total of characters, higher is its contribution to divergence and *vice versa*. In the present study, the highest contribution towards genetic divergence was recorded by fresh and dry herbage yield per plant followed by number of branches per plant (Fig.9).

5.4.2. Genetic diversity studies using SSR markers

Genetic diversity is desirable for long term crop improvement and reduction of vulnerability to undesirable characters. Molecular characterization of germplasm aids plant breeders in selecting appropriate materials for further genetic improvement of cultivars. Since the morphometric markers are highly influenced by the environment, it led to search of new alternate marker systems and resulted in the DNA based molecular markers. The major advantage of molecular markers is their genotypic nature. They reflect change at the

DNA level and hence reflect the actual genetic distance between genotypes and their common ancestry more than phenotypic markers.

In the present study, apart from using the morphological traits, SSR markers were also used to estimate the extent of molecular diversity among the 45 genotypes. Ten SSR primers were used to generate SSR marker profiles. The number of alleles detected by different primers ranged from 0 to 4 and the level of polymorphism was 0 to 100%. To characterize the capacity of each primer to detect polymorphic loci, the PIC was calculated for each primer. The PIC value ranged from 0.000 to 0.698. The higher the PIC value, the more informative is the SSR marker. Hence, primer STWIN 12 G was highly informative which indicated that these primers are useful to determine the genetic differences among the makoi genotypes and to study the phylogenetic relationship.

Among 10 primers used, seven primers were found to be highly informative in the present study. This indicated that these primers are useful to determine the genetic differences among the cocoa trees and to study the phylogenetic relationship. The similarity matrix was computed using SSR markers based on Jaccard's coefficient following the UPGMA method using SHAN programme of NTSYS-pc version 2.02i. This matrix was used for cluster analysis. The Jaccard's similarity coefficient for the SSR data set varied from 0.36 to 1.00. The SSR marker profiles resulted in eight clusters (Fig. 10).

In the cluster analyses, the groups involved genotypes from different origins, with no clear evidence of regionalization of the genetic variability. This situation indicated no parallelism between genetic diversity and geographical distribution. They suggest that genetic drift and selection in different environments could cause greater diversity than the geographic distance. The frequent movement of seed material and subsequent adaptation to agro climatic condition may also be responsible for such variation. Therefore, the selection of genotypes for hybridization should be based on genetic diversity rather than geographical diversity.

5.4.3. Comparison of clustering based on D^2 statistics and SSR based clustering

When we compare the clusters formed by the SSR markers and the clusters formed by the D^2 analysis using morphometric traits, the genotypes of cluster I in SSR marker clusters were present in cluster I, II, IV, V, VII of D^2 analysis. Similarly, genotypes of cluster II (TN *Sn* 19) and V (TN *Sn* 10) were grouped in cluster III of D^2 analysis.

Genotypes in cluster IV of SSR clustering (TN *Sn* 52 and TN *Sn* 53) were present in different clusters in D^2 analysis. The genotypes in cluster VI (TN *Sn* 30 and TN *Sn* 32) were present in the same cluster VI in D^2 analysis. The genotypes in cluster VII (TN *Sn* 44) and VIII (TN *Sn* 47) of SSR clustering were present in same cluster in D^2 analysis. This shows the potentiality of SSR markers for the characterization of genotypes, though some sort of deviation was observed between the clusters of molecular and morphological data. In the present study, both the morphological and SSR marker data were able to reveal the existence of a wide genetic diversity among the makoi genotypes. The genetic diversity analysis will facilitate the breeder towards the development of well-defined heterotic groups for this crop.

5.5. Variability, Heritability (h^2) and genetic advance (GA)

The variability of the genetic stocks could be increased by increasing the collection from diversified origin and geographical distribution. Mode of origin and subsequent adaptations to varied environments are the primary causes for the heritable variations found among the genotypes (Anshebo, 2002). The phenotypic and genotypic variances, estimated from the total variance were used to assess the variability among the genotypes. The genotypic coefficient of variance helps in measurement of range of diversity in a character and provides means to compare the genetic variability in quantitative characters. The genotypic coefficient of variance along with phenotypic coefficient of variance was used to ascertain the value of diversity among the genotypes.

In the present investigation, the genotypes exhibited considerable amount of variability for all the 18 traits studied for all three *Solanum sp*. Among the various characters observed for this study, higher variability range was observed for protein content, dry herbage yield per plant and fresh herbage yield per plant while moderate variability was recorded for iron content, ascorbic acid, number of branches, number of leaves per plant, plant height, plant spread, and days to flower bud initiation in all the three species. Thus, there existed immense scope for selection based on these characters. Similar results were reported by Shalini Mathur *et al.* (2003) in *Centella asiatica*, Sethi (1991) in palmrosa, Sharma *et al*.(1990) in fenugreek, Varalakshmi and Reddy (1997) in leafy amaranthus and Rana *et al.* (2005) in grain amaranthus (*A. hypochondriacus*) accessions. Low estimates of variability were observed for the rest of the traits.

The difference between the genotypic and phenotypic variances indicated the contribution of environmental variance (Ram and Singh, 1993). Smaller the values of difference between phenotypic and genotypic variance, the lesser will be the environment effect on the character. Similarly, higher the values of difference between phenotypic and genotypic variance, the greater will be the environmental effect. These results were similar to earlier findings in isabgol by Lal *et al.* (1999), *Ocimum sanctum* by Ahmad and Khaliq (2002), chilli by Prabhakaran *et al*. (2004) and brinjal by Sunita Kushwah and Bandhyopadhya (2005). The presence of comparatively little difference between PCV and GCV estimate for all the characters indicated that variability was primarily due to genotypic difference and scope for selection based on these components would be much greater in. genotypes.

Simple measures of variability like range, phenotypic and genotypic coefficient of variation indicated the extent of variability of different characters. As yield and its attributes are highly influenced by the environment, it is difficult to conclude whether the observed variability is heritable or not. Therefore it becomes essential to partition the observed variability into heritable and nonheritable components. The genotypic coefficient alone is not sufficient for the determination of the amount of heritable variation.

According to Singh *et al.* (1972), the genes cannot cause a character to develop unless they have the proper environment. The variability observed in some characters is caused primarily by the differences in the genes which were carried by different individuals and the variability in other character is due to primary differences in the environment to which individuals have been exposed. For assessing the heritable variation, the estimation of heritability gains importance.

Heritability and genetic advance are the two important parameters and a relative comparison of heritability estimates and expected genetic advance as percentage of mean will give an idea about the nature of gene action governing a particular character (Johnson *et al*., 1955).

According to Burton (1952), heritability estimates also have a bearing on the population response to selection. Heritability denotes the additive genetic variance in per cent of the total variance. High estimates of heritability for certain traits suggest that they are under genetic control. Higher the value of genetic advance, better and surer the progress will be on the mean in the succeeding generation under directional selection.

In the present investigation, many characters like ascorbic acid content, protein content, iron content and days to flower bud initiation registered high heritability indicating that these traits could be governed by additive genes and therefore these characters could be readily fixed by selection. These results are in confirmation with the findings of Krishnamoorthy and Madalageri (2002) in ajowan, Sudhir Shukla *et al*. (2003) in fennel, Ibrahim and Hussein (2006) in *Hibiscus sabdariffa*, Mahalakhsmi (2006) in *Phyllanthus amarus* and Naresh Kaushik *et al.,* (2007) in *Pongamia pinnata*.

In all the three species, low heritability estimates were recorded for plant height, plant spread, leaf length and breadth, stem girth and days to flower bud initiation. For these traits, the environment and non additive gene effect constituted a major portion of the total phenotypic variance, and thus improvement for these characters cannot be made by selection. The above finding confirms the earlier report of Ananya Banerjee and Kope (2004) in coriander and Kousar Makeen *et al*. (2007) in mungbean.

Even though, heritability estimates represent the heritable portion of variation, they do not indicate the effectiveness with which selection of a phenotype could be made based on the phenotypic performance (Johnson *et al.*, 1955), and thus high heritability could not be considered as an indication of greater genetic gain. The results of present study have clearly brought out that high heritability linked with high genetic advance of the traits like protein content, iron content, number of branches per plant, ascorbic acid content and number of leaves per plant indicates that the expressions of these characters are governed by additive genes and improvement could be possible through selection. High heritability value along with high genetic advance as per cent of mean is more useful in predicting genetic progress that would result from selecting the best individuals. A similar finding was reported by Lal *et al* (1999) in *Vetiver*, Singh *et al*.(2005) in coriander, Megeji and Korla (2002) and Singh *et al*. (2003) in opium poppy.

5.6. Correlation of yield and yield attributing characters

The studies on correlation values indicate the intensity and direction of association of a character with yield. The interrelationship of component characters of yield provides the information about the likely consequences of selection for simultaneous improvement of desirable characters under selection.

Grafius (1959) suggested that there might not be many genes for yield *per se*, but for its components. Hence, the knowledge on interrelationship of plant characters with herbage yield and among themselves is of paramount importance to the breeder for making improvement in complex characters, for which direct selection is not much effective. Hence, the association analysis was undertaken to determine the direction of selection and the characters to be considered in improving it.

The simple correlation coefficients were worked out based on the pooled data for all solanum species. The correlation analysis made in this study revealed positive and highly significant association of alkaloid yield with dry and fresh herbage yield, number of primary branches, number of leaves per plant, plant height and plant spread. Hence, it may be concluded that these traits may be considered as the most important yield contributing attributes. These results coincide with the findings of Shivanna *et al*. (2007) in Makoi, Samresh Dwivedi *et al*. (1999) in Catharanthus, Vasumathi (2001) in Phyllanthus, Gaurav *et al. (*2008) in Stevia, Dalkani *et al.* (2011) in ajowan, Palanikumar *et al.* (2012 b) in Coriander, Data *et al*. (2005) in fenugreek, Shukla *et al.,* (2010) in vegetable amaranth.

There was negative and significant association of number of berries per plant, days to flower bud initiation and days to fifty per cent flowering with herbage yield per plant which indicated that late flowering favoured more foliage development and increased berry yield and decreases the alkaloid yield. These results coincide with the findings of Shivanna *et al*. (2007) in Makoi, Chitra and Rajamani (2010) in Glory lily, Khanna (1987) in opium poppy,

Therefore, while exercising selection, emphasis must be laid on the yield components, which had significant correlation with herbage yield. However, the information about the association with herbage yield and yield attributes alone is not sufficient. The interrelationship between these component characters themselves may affect the overall influence of the characters on yield. Hence, the selection based on the yield components would be effective in improving yield, provided the components are highly heritable and genotypic correlations among them are not negative (Doku, 1970)

The characters *viz*., plant height, plant spread, stem girth, number of leaves per plant number of branches per plant and herbage yield per plant had highly significant positive correlation with most of the characters except days to flowering, days to fifty per cent flowering and number of berries per plant. The inter correlation between various yield traits were studied by several authors *viz*., Singh *et al*. (2000) in *Mentha arvensis*, Panesar and

Jadeja (2008) in cumin and Jotshi *et al.* (2008) in *Abrus precatorius*. Their conclusions generally are in agreement with the results of the present study. Highly negative correlation was seen in total alkaloid content with protein, iron content and ascorbic acid. This indicated that high nutritive genotypes have less alkaloid content.

The studies on *perse* performance of genotypes revealed that, the high yielding genotypes TN *Sn* 10 (*S.nigrum* L.2n =72)) and TN *Sn* 30 (*S.villosum* Mill.) can be used in breeding programme for gettting high alkaloid yielding vareities as they had recorded high herbage yiled with high alkaloid content in both the seasons. Though TN*Sn* 19 (*S.nigrum* L) and TN *Sn* 22 (*S.nigrum* L) recoded lesser yield than TN *Sn* 10 and TN *Sn* 30, they showed high nutritive value and palatable characters and hence they can be used for evolving varirties that can be used as green leafy vegetable. The studies on association of characters through correlation revealed that, earlier flowering with lesser berry yield should be considerd for the selecting genotypes for more herbage yield.

Summary

CHAPTER VI

SUMMARY

The study entitled "Genetic diversity studies in Makoi (*Solanum nigrum* L.) genotypes" has been carried out at the Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore. The genetic diversity analysis of 45 genotypes was carried out using morphological markers, D^2 statistic and molecular markers. In addition, variability for growth, physiological and biochemical parameters were also analysed for the same set of genotypes. The results obtained from the experiments are summarized below,

- Morphological characterization of 45 genotypes based on 14 IPGRI descriptors was done and the cluster analysis grouped the genotypes into four major clusters.
- Ploidy analysis carried out in morphologically distinct genotypes revealed that, TN *Sn* 10 was an hexaploid (2n = 72) and TN *Sn* 30, TN *Sn* 32, TN *Sn* 44 and TN *Sn* 47 were tetraploids (2n = 48) while remaining distinct genotypes (TN *Sn* 8, TN *Sn* 12, TN *Sn* 23, TN *Sn* 38, TN *Sn* 52 and TN *Sn* 53) were diploid (2n=24) like the other genotypes.
- DNA Barcoding carried out in morphologically distinct genotypes revealed that the diploids were *S.nigrum* L.(TN *Sn* 19) and *S.americanum* Mill. (TN *Sn* 8, TN *Sn* 12, TN *Sn* 23, TN *Sn* 38, TN *Sn* 52 and TN *Sn* 53) and tetraploids were *S.villosum* Mill. (TN *Sn* 30, TN *Sn* 32, TN *Sn* 44 and TN *Sn* 47). The TN *Sn* 10 though it was hexaploid it was grouped under *S.nigrum L.*
- Based on mean performance for different characters, the genotype TN *Sn* 10 *(S.nigrum L)*followed by TN *Sn* 30 *(S.villosum* Mill) was the best in both seasons with regard to herbage yield, its component traits and alkaloid content. The protein content, iron content and ascorbic acid content were higher in TN *Sn* 19*(S.nigrum L.)* and TN *Sn* 22 *(S.nigrum L.*)

- Based on Mahalanobis D^2 analysis, the genotypes were grouped into eight clusters. Among the eight clusters, the cluster V was the largest with 15 genotypes followed by cluster I with 11 genotypes and Cluster VIII with 6 genotypes. Cluster II, IV and VI had 2 genotypes each and cluster III, VII have 3 genotypes each. The Cluster IV and III (138.83) have maximum inter cluster distance which showed maximum divergence between these two clusters. Fresh and dry herbage yield per plant contributed maximum towards divergence followed by number of branches per plant.
- In this study, SSR markers were used to estimate the extent of molecular diversity among genotypes. SSR (10) primers were used to generate marker profiles. The number of alleles detected by different primers ranged from 0 to 4 and the level of polymorphism was 0 – 100 %. The PIC value ranged from 0.000 to 0.698. Primer STWIN 12 G was found to be highly informative as it has the highest PIC value (0.698).
- The Jaccard's similarity coefficient for the SSR data set varied from 0.36 to 1.00. Based on the banding pattern, the cluster analysis was done using UPGMA and the dendrogram was constructed which resulted in eight clusters.
- For all the three species, the maximum phenotypic and genotypic coefficient of variations were observed for protein content, dry herbage yield per plant and fresh herbage yield per plant followed by iron content, ascorbic acid, plant height, plant spread, number of branches, number of leaves per plant and days to flower bud initiation.
- For all the three species, heritability estimates were higher for biochemical characters like ascorbic acid content, protein content, iron content follwed by biometrical characters like days to flower bud initiation followed by plant spread, number of branches, number of leaves, days to 50 per cent flowering, fresh herbage yield per plant, total alkaloid content and crude fiber.
- For all the three species, the GA expressed as percentage of mean was high for protein content, iron content, number of branches per plant, ascorbic acid

content and number of leaves per plant followed by plant height, plant spread, leaf breadth, days to flower bud initiation and crude fiber.

- In correlation studies, total alkaloid yield expressed highly significant and positive correlation with dry herbage yield, number of leaves per plant, plant height and plant spread and crude fibre. It also has significant negative association with number of berries per plant, days to flower bud initiation and days to fifty per cent flowering.

CONCLUSION

- The high yielding genotypes TN *Sn* 10 (*S.nigrum* L. 2n =72) and TN *Sn* 30 (*S.villosum* Mill.) can be used in breeding programme for gettting high alkaloid yielding varieties. TN*Sn* 19 (*S.nigrum* L) and TN *Sn* 22 (*S.nigrum* L) showed high nutritive value and palatable characters and hence they can be used for evolving varieties that can be used as green leafy vegetable.
- Morphological and boichemical characters showed considerable diversity and is confirmed by SSR markers. Such diversity is beneficial to develop new superior varieties through selection or through hybridization programme.
- The correlation studies revealed that, early flowering with lesser berry yield should be considered for the selecting genotypes for more herbage yield.

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* Originals not seen

Plate.1a. Field view of experimental plot

(Season I)

Plate.1b. Field view of experimental plot

(Season II)

Plate 2. Morphological variations in Makoi genotypes

Variations in Leaf shape

Leaf pubescence

Flower size

Stem colour

Purple stripes in corolla

Stem shape

Stem pubescence

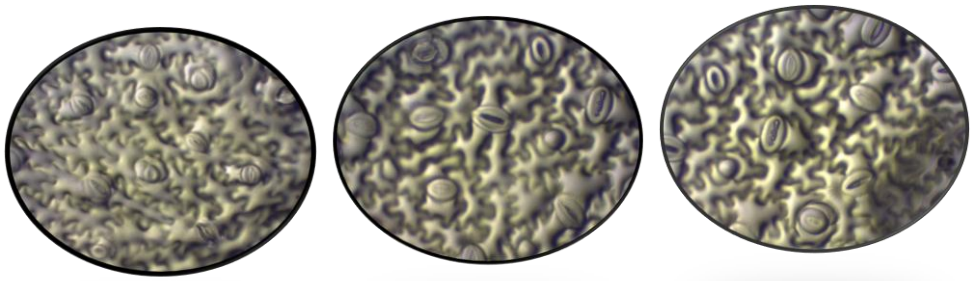
Plate 3a.Variation in number of flowers & berries per infloresence

Plate.3b.Variation in berry colour and size

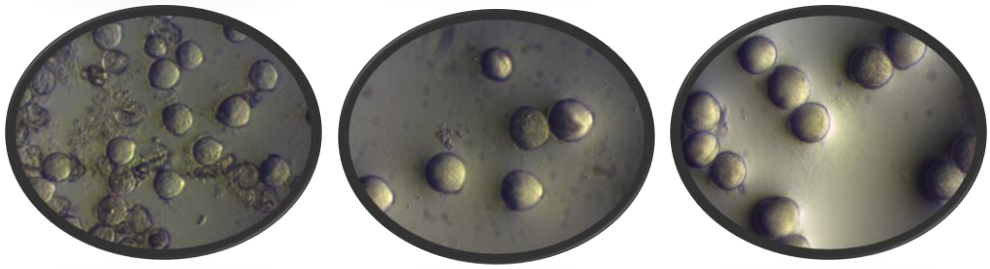
Plate 5. Ploidy analysis

Diploid **Tetraploid** **Hexaploid**

Variations in stomatal size



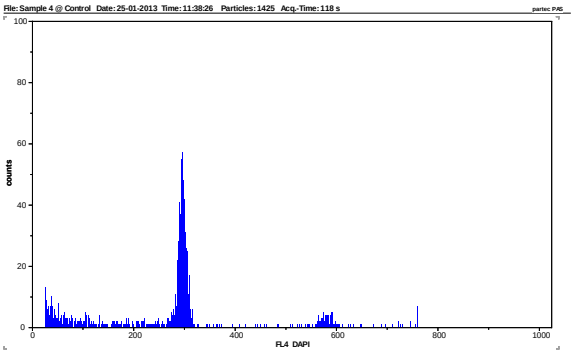
Variations in pollen size



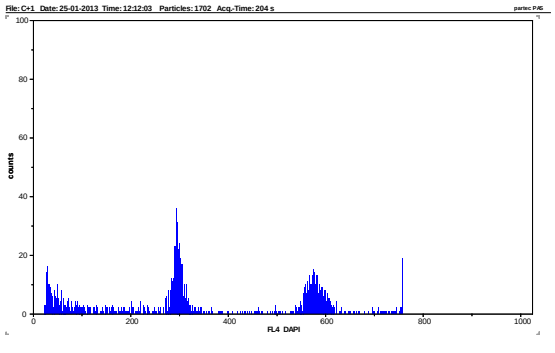
Root tip Mitosis

2n= 24 **2n= 48** **2n= 72**

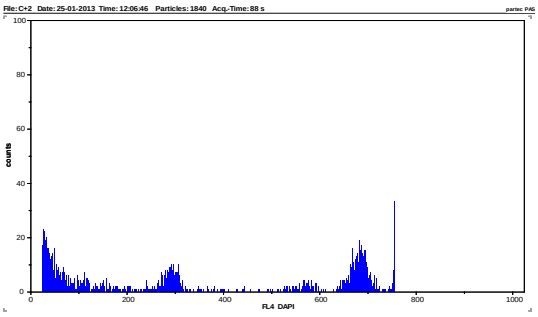
PLATE 6a. Confirmation of ploidy level of genotypes using Flow cytometric histograms



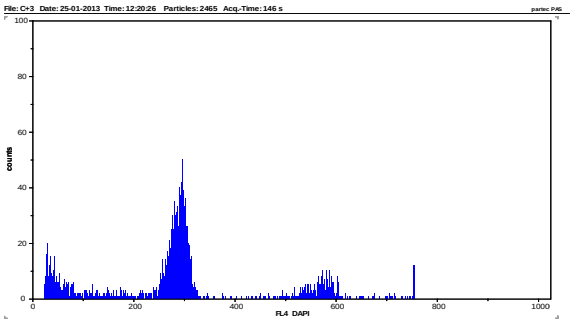
TN *Sn* 19 (Standard) Diploid



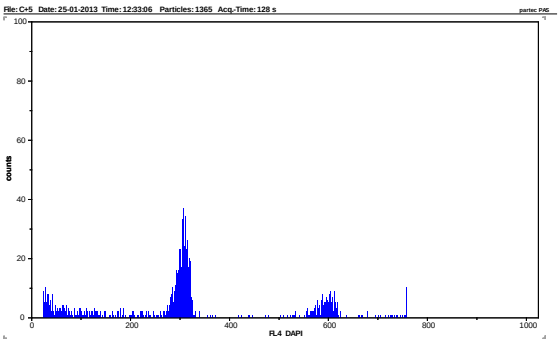
TN *Sn* 08 – Diploid



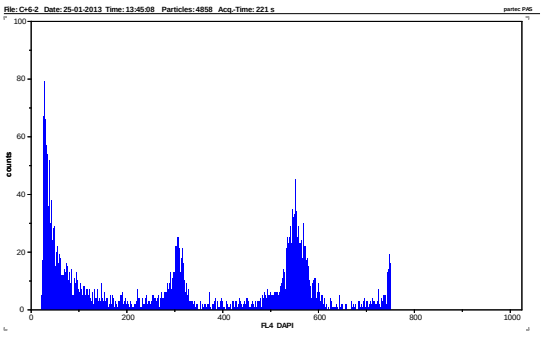
TN *Sn* 10 – Hexaploid



TN *Sn* 12 - Diploid

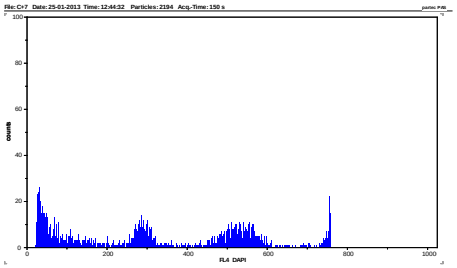


TN *Sn* 23 - Diploid

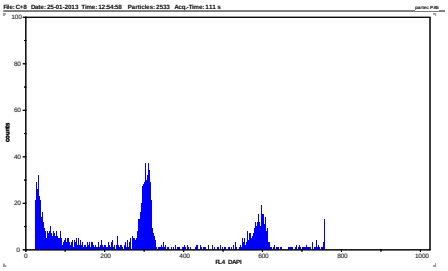


TN *Sn* 30 - Tetraploid

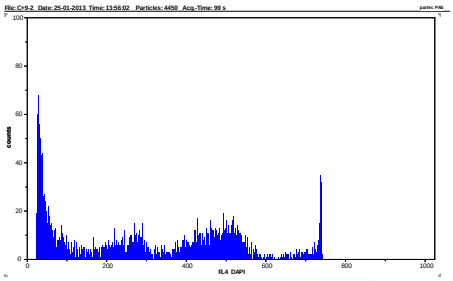
PLATE 6b. PLOIDY CONFIRMATION OF GENOTYPES USING FLOW CYTOMETRIC HISTOGRAMS



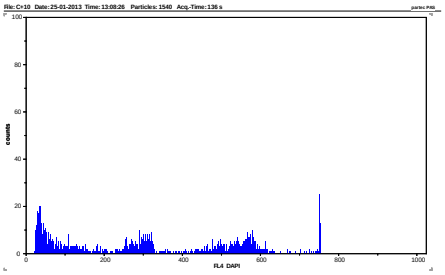
TN *Sn* 32 - Tetraploid



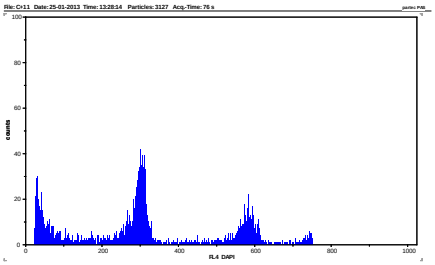
TN *Sn* 38 - Diploid



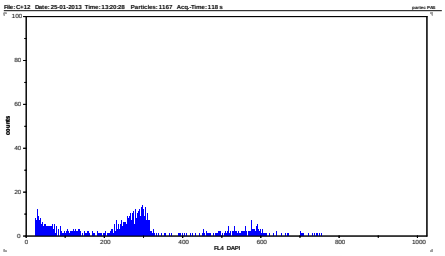
TN *Sn* 44 – Tetraploid



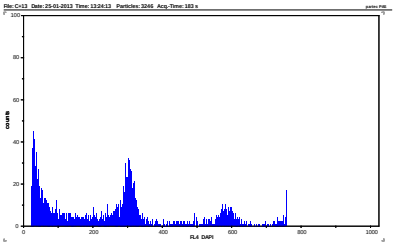
TN *Sn* 47 – Tetraploid



TN *Sn* 51 – Diploid



TN *Sn* 52– Diploid



TN *Sn* 53 – Diploid

Plate 7. High yielding genotypes

TN *Sn* 10

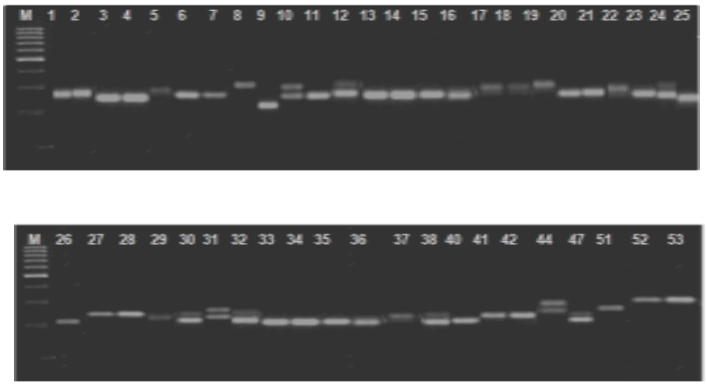
TN *Sn* 30

TN *Sn* 19

TN *Sn* 22

PLATE 7. SSR MARKER PROFILES OF 45 MAKOI GENOTYPES

SSR PROFILES GENERATED BY THE PRIMER - STWIN 12 G



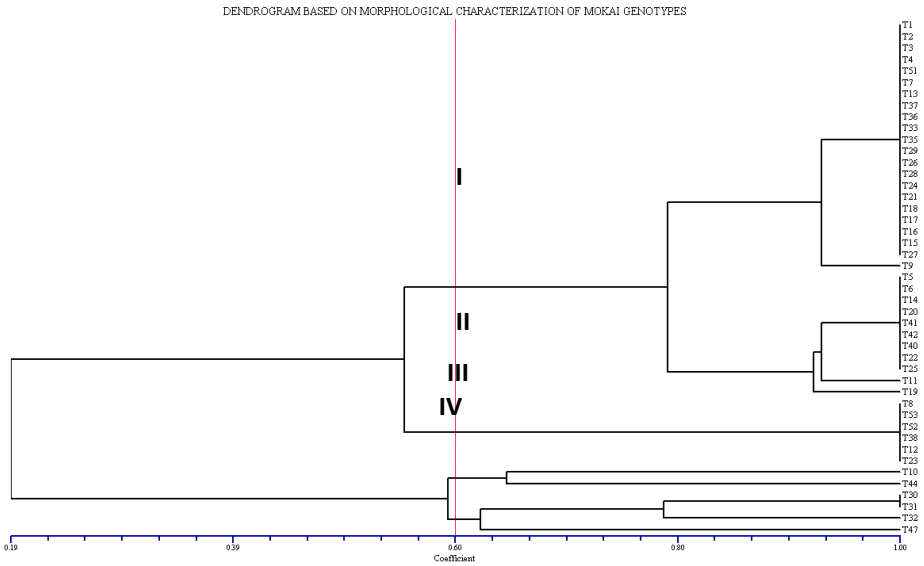


Fig.5. Dendrogram based descriptors of forty five genotypes

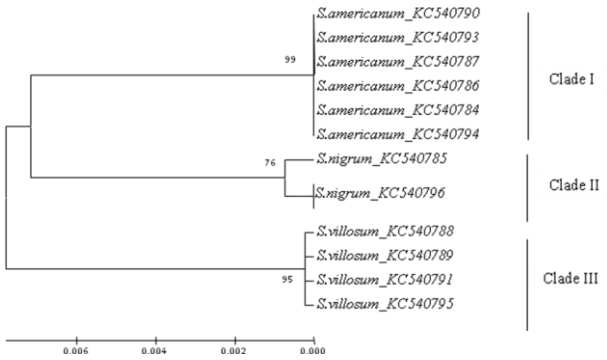


Fig 6. Phylogenetic analysis of the *ITS* sequences of the *Solanum species* using MEGA5 software

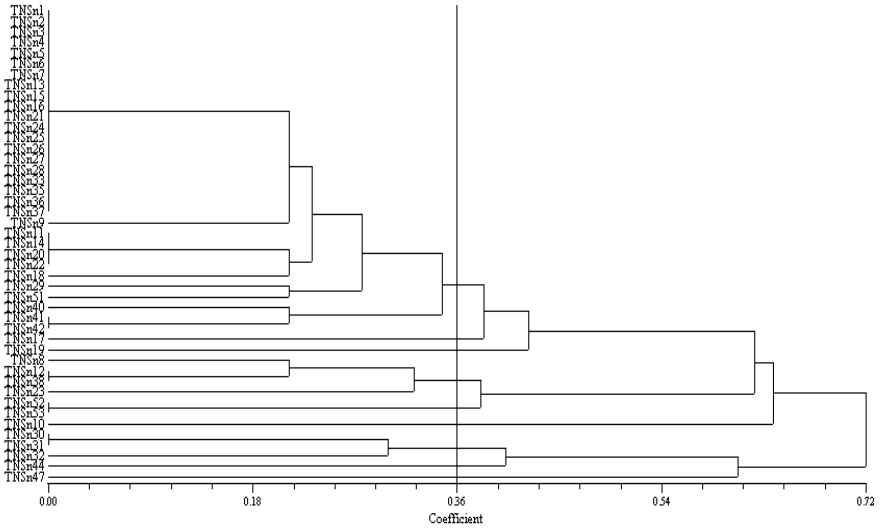


Fig.10. Dendrogram based on SSR markers of forty five genotypes