

**ASSESSMENT OF GENETIC DIVERSITY IN CHRYSANTHEMUM
(*Dendranthema grandiflora* Tzvelev) CULTIVARS BASED ON
PHENOTYPIC AND MOLECULAR MARKERS**

By

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AUGUST, 2012

CERTIFICATE

Smt. P.Lalitha Kameswari has satisfactorily prosecuted the course of research and that the thesis entitled **“ASSESSMENT OF GENETIC DIVERSITY IN CHRYSANTHEMUM (*Dendranthema grandiflora* Tzvelev) CULTIVARS BASED ON PHENOTYPIC AND MOLECULAR MARKERS”** submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that the thesis or part thereof has not been previously submitted by her for a degree of any University.

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This is to certify that the thesis entitled, “**ASSESSMENT OF GENETIC DIVERSITY IN CHRYSANTHEMUM (*Dendranthema grandiflora* Tzvelev) CULTIVARS BASED ON PHENOTYPIC AND MOLECULAR MARKERS**” submitted in partial fulfillment for the degree of **DOCTOR OF PHILOSOPHY IN HORTICULTURE** of the Dr.Y.S.R Horticultural University, Venkataramannagudem, is a record of the bonafide research work carried out by **Smt. P.Lalitha Kameswari** under our guidance and supervision. The subject of the thesis has been approved by the Student’s Advisory Committee.

No part of the thesis has been submitted by the student for any other degree or diploma or has been published. All the assistance and help received during the course of the investigations have been duly acknowledged by the author of the thesis.

(Dr. M. PRATAP)

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DECLARATION

I, **Smt. P.LALITHA KAMESWARI** hereby declare that the thesis entitled **“ASSESSMENT OF GENETIC DIVERSITY IN CHRYSANTHEMUM (*Dendranthema grandiflora* Tzvelev) CULTIVARS BASED ON PHENOTYPIC AND MOLECULAR MARKERS”** submitted to Dr. Y. S. R. Horticultural University, Venkataramannagudem for the degree of **DOCTOR OF PHILOSOPHY IN HORTICULTURE** is the result of original research work done by me. I also declare that the material contained in this thesis has not been published earlier.

Date :
Place: HYDERABAD

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(P.Lalitha Kameswari)

LIST OF ABBREVIATIONS AND SYMBOLS

@	:	at the rate of
%	:	Per cent
Σ	:	Sum of
$^{\circ}\text{C}$	:	Degree Celsius
$\sigma^2\text{g}$	:	Genotypic variance
$\sigma^2\text{p}$	:	Phenotypic variance
<	:	Less than
$\geq$	:	More than or equal
μl	:	Microlitre
$^{\circ}\text{brix}$	:	Degrees brix
AFLP	:	Amplified Fragment Length Polymorphic DNAs
ANOVA	:	Analysis of variance
bp	:	Base pair
CD	:	Critical Difference
cm	:	Centimetre
CTAB	:	Cetyl Trimethyl Ammonium Bromide
Cv	:	Coefficient of variation
df	:	Degrees of freedom
DAT	:	Days after transplanting
DNA	:	Deoxy ribo nucleic acid
dNTP's	:	De-oxy nucleotide triphosphate
EDTA	:	Ethylene Diamine Tetra Acetic acid
<i>et. al.</i>	:	Et alia
etc	:	Etcetera
Fig.	:	Figure
g	:	Gram
GA	:	Genetic advance
GAM	:	Genetic Advance as per cent mean
GCV	:	Genotypic coefficient of variance
GV	:	Genetic variability

Gy	:	Gray
H	:	Heritability in broad sense
i.e.	:	That is
ICBN	:	International Code of Botanical Nomenclature
IPGRI	:	International Plant Genetic Resources Institute
ISSR	:	Inter-Simple Sequence Repeat
kb	:	kilo base pairs
kg	:	kilogram
mg	:	milligram
ml	:	millilitre
mm	:	millimetre
mM	:	millimolar
ng	:	Nanogram
nM	:	Nanometre
no.	:	Number
NIAB	:	National Institute of Agricultural Botany
NS	:	Not significant
NTSYS	:	Numerical Taxonomy and Multivariate Analysis system
PCA	:	Principal Component Analysis
PCR	:	Polymerase Chain Reaction
PCV	:	Phenotypic Coefficient of Variance
pH	:	Power of hydrogen
pmols	:	picomoles
RAPD	:	Random Amplified Polymorphic DNA
RFLP	:	Restriction Fragment Length Polymorphism
RF	:	Rainfall
r_g	:	Genotypic correlation coefficient
r_p	:	Phenotypic correlation coefficient
RH	:	Relative humidity
rpm	:	Revolutions per minute
SCAR	:	Sequence Characterized Amplified Regions
SEm	:	Standard Error of mean

SPSS	:	Statistical Package for the Social Sciences
SSR	:	Simple Sequence Repeats
STS	:	Sequence-Tagged Sites
Taq	:	Thermus aquaticus
TBE	:	Tris Borate EDTA buffer
TE buffer	:	Tris buffer
t/ha	:	Tones per hectare
U	:	Units
UPGMA	:	Unweighted Pair Group Method using Arithmetic means
<i>Viz.,</i>	:	Namely
$\overline{X}$	:	Grand mean

Title of the thesis : **Assessment of genetic diversity in Chrysanthemum (*Dendranthema grandiflora* Tzvelec) cultivars based on phenotypic and molecular markers.**

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ABSTRACT

The present investigation was carried out to evaluate genetic diversity in chrysanthemum genotypes maintained at Floricultural Research Station, ARI, Rajendranagar, Hyderabad using morphological and molecular (RAPD and ISSRs) markers. The 30 quantitative and 23 qualitative morphological characters on plant, leaf and flower characters were evaluated. In molecular diversity studies, 27 RAPD primers and 10 ISSR primers were employed to establish phylogenetic relationships among the genotypes.

Analysis of variance revealed highly significant differences among the 104 genotypes of chrysanthemum for all the thirty quantitative traits, thus indicating wide variation among the genotypes. The chrysanthemum genotypes Pink Cascade, Beauty, Cloverlee Star and Yellow Gold were found to be the early flowering varieties. The genotypes Star Pink, Rose day and Jessie Hab Good exhibited maximum flower size. The high multiplication rate was observed in the genotypes Yellow Bonsai, PAU-B-107, Local Button, Chandrika and Aparajitha by producing more number of suckers per plant. The genotypes Local Button, Chandrika, PAU-B-107 and Rekha were adjudged as superior over other genotypes in terms of flower yield per plant.

Higher estimates for GCV and PCV were observed for all the characters studied except for plant height, days to flowering, spray length and duration of flowering showing ample scope for selection of those characters. High heritability coupled with high genetic advance as percentage of mean was recorded for all the quantitative characters studied except for duration of flowering indicating contribution of additive gene effects in the expression of these traits. A significant positive correlation both at genotypic and phenotypic levels was recorded between flower yield and plant attributes viz., plant spread at both the directions, number of primary branches per plant, spray length and number of flowers per spray. Path coefficient analysis revealed that plant spread in N-S direction recorded the highest direct effect on flower yield per plant followed by number of flowers per spray, days to 50%

flowering , number of primary branches per plant, average flower weight and duration of flowering.

Based on Mahalanobis D^2 analysis, the chrysanthemum genotypes were grouped into eleven divergent clusters. The highest inter cluster D^2 value was recorded between clusters VI and IX (195.87) indicating that crosses may be attempted between the genotypes of cluster VI (Apurva Singar and Local Button) and cluster IX (Miss India, Tambra, White Silver Cloud, Red Queen and Swan) to obtain new desirable recombinants in chrysanthemum. Among all the characters, the most important character contributing to divergence was number of ray florets per head followed by number of disc florets per head and flower yield per plant. With principal component analysis, the first eight principal components with eigen values more than one contributed to 73.23 per cent of cumulative variability.

RAPD analysis with 27 primers detected high diversity among the 37 chrysanthemum genotypes. A total of 278 amplified fragments were scored out of which 271 were found to be polymorphic (97.4%). Genotype-specific RAPD markers were detected only for few genotypes Meera(OPE-14), Salora(OPF-5), Terry(OPF-19), Punjab Anuradha(OPG-16), Anjali(OPG-19) and Asha(OPM-10) which could be exploited for DNA fingerprinting of these genotypes. The genetic similarity ranged from a coefficient of 0.174 to 0.600 with an average similarity coefficient of 0.387 indicating a high diversity among the group of genotypes studied. In the present study, Ratlam Selection and Snow Cem were found to be most genetically similar (0.60) followed by Akitha and Shintome with 55.3%. Cluster analysis led to the classification of genotypes into four major cluster and seven minor clusters with one genotype each in cluster V(Arka Ravi), VI(Meera), VII(Asha), VIII(Silper), IX(Autumn Joy), X (Lilith) and cluster XI (Chandrika).

With ten ISSR primers, 114 bands were amplified, of which 107 bands were polymorphic, accounting for 93.86% polymorphism. The similarity coefficient values ranged from 0.275 to 0.775 with a mean similarity matrix of 0.525. Among the 37 genotypes studied, the closest relationship was scored between Geetanjali and Red Stone with similarity level of 77.5%. The UPGMA dendrogram revealed that the chrysanthemum genotypes were grouped into ten clusters.

Both morphological and molecular characterization methods were found efficient in grouping the genotypes. Among these methods, molecular analysis grouped more accurately than morphological diversity. Among the two marker systems employed, 27 RAPD primers revealed 97.4% of polymorphism while 10 ISSR primers showed polymorphism of 93.86% among 37 genotypes. As per the clustering pattern of morphological, RAPDs and ISSRs, the genotypes Snow Cem and Ratlam Selection ; Akitha and Shintome as well were clustered in one group indicating the efficiency of three systems. Identification of markers associated with specific traits of interest, which could be converted into SCAR markers needs attention to speed up the crop improvement programme. Hybridization among promising genotypes of divergent clusters could yield heterotic effects for flower quality (in terms of colour, shape, size) and flower yield in addition to creation of new gene combinations and thus help in development of new promising cultivars of chrysanthemum.

CHAPTER - I

INTRODUCTION

Association of mankind and its emotional attachment with flowers dates back to centuries. Among the ornamental plants, chrysanthemum has special significance in the international cut flower trade. It is a national flower of Japan and is regarded as a symbol of royalty. In India, it occupies a place of pride both as commercial loose flower or cut flower and potted plant (pot mum). The utility and popularity of chrysanthemum have increased immensely with the introduction of novel trait of year round blooming based on scientific research in the field of photoperiodism and genetics. Because of its multifarious traditional uses, the crop has its own commercial value and a good number of varieties have been released. Keeping geographical advantage, India has 1,82,900 ha of land area marked for flower cultivation during 2009-10 with a production of 10,20,600 MT of loose flowers and 66,671 lakh number of cut flowers. Of these Andhra Pradesh accounts for 14% of total flower production. In Andhra Pradesh chrysanthemum is grown in an area of 3198 ha with an annual production of 36,777 MT (NHB, 2010).

The commonly grown chrysanthemums are hexaploids with average number of 54 chromosomes (Wolff, 1996). The modern, large, double and exquisitely flowered cultivars owe their origin to relatively small, single and non attractive types. This great transformation is the result of centuries of natural cross pollination, spontaneous and intentional hybridization coupled with mutation, chromosomal differentiation and polyploidy (Nazeer and Khashoo, 1982). Most extensive work has been done for developing novel chrysanthemum genotypes through induced mutation using physical and chemical mutagens (Broetjes and Van Harten, 1978).

Genetic variability, naturally existing or artificially created is an indispensable pre-requisite for any breeding programme. Genetic diversity is being used as source of genes in crop improvement for production of high yielding varieties, hybrids and to effect ecologically

suitable economic and social development. Gene identification and access to diverse germplasm pool is crucial for the successful incorporation of novel traits in commercial ornamental crops.

Since most ornamental plant improvement programs concentrate on aesthetic qualities such as flower and plant characteristics, the genetic base of the modern cultivars is becoming more and more narrow. Coupled with global marketing and adoption of these cultivars world wide, many heirloom varieties of these crops are being replaced by modern narrow genetic base cultivars, resulting in continuous loss of our traditional cultivars. Hence, characterization of germplasm is essential to provide information on the traits of accessions assuring the maximum utilization of the germplasm collection for the benefit of end user.

Characterization based on the important characteristics which distinguish accessions within a species enables an easy and quick discrimination among phenotypes. It allows simple grouping of accessions, development of core collections, identification of gaps and retrieval of valuable germplasm for breeding programme, resulting in better insight about an accession. Chrysanthemum cultivars exhibit wide range of diversity in morphological traits like flower number, size, colour and flowering duration. The emergence of new cultivars is closely connected with the problem of identifying and distinguishing the variation among the cultivars so developed. The registration and protection of newly released varieties in chrysanthemum is based on morphological characteristics as described in PPV & FRA (Protection of Plant Varieties and Farmers Rights Authority, 2001) guidelines. This method has its own drawback as the number of varieties to be tested increases and the genetic distances between varieties becomes smaller. Also the comparison of applicants with older varieties is difficult or even impossible as many varieties are not easily available and maintaining large reference collections is too expensive. With the advent of molecular biology techniques, DNA based markers played significant role in identification and

characterization of germplasm. A number of DNA based marker systems are now available. The basic premise is that variation in the nucleotide sequence of DNA can be exploited to produce characteristic finger prints. DNA based markers are classified into hybridization markers and polymerase chain reaction (PCR) based markers. Among the hybridization markers, RFLPs (Restriction Fragment Length Polymorphism) were the first to be used for plant genome mapping. RFLPs are co-dominant and can identify a unique locus; however, RFLP analysis is labour-intensive, time-consuming and expensive. The discovery of the Polymerase Chain Reaction (PCR) method of DNA amplification is an important milestone in molecular biology research. This method has provided geneticists with new ways of generating molecular markers for mapping and genome characterization. Some of the commonly used PCR-based marker systems are RAPDs (Randomly Amplified Polymorphic DNA), SCARS (Sequence Characterized Amplified Regions), ISSRs (Inter- Simple Sequence Repeats), SSRs (Simple Sequence Repeats) and AFLPs (Amplified Fragment Length Polymorphism). DNA markers for varietal identification and assessment of genetic diversity have been developed for several floricultural crops like Anthuriums (Ranamukhaarachchi *et al.*, 2001), rose (Debener *et al.*, 1996; Martin *et al.*, 2001), Alstromeria (Dubouzet *et al.*, 1998) and Bougainville (Chatterjee *et al.*, 2007). These markers are extremely useful for testing clonal fidelity as well as establishing hybridity. Among these, RAPD marker technology is one of the best available DNA – based tools for scoring variations between cultivars within species. It is convenient in performance and does not require any information about the DNA sequence to be amplified (Weder, 2002). The first study on the identification of chrysanthemums with the application of RAPD markers was carried out by Wolff and Peters-Van Rijn (1993). RFLP analysis (Wolff *et al.*, 1994) and RAPD finger printing (Wolff *et al.*, 1995) were used to study the chrysanthemums. However RFLP technique was found to be laborious and not well suitable for studies involving large number of samples. In contrast,

RAPD analysis was fast and relatively simple to perform and as such was used to assess genetic variability of species and closely related cultivars of chrysanthemum (Wolff *et al.*,1995).

Since, germplasm is a vital resource in any crop improvement programmes its proper description, characterization and evaluation, can have its long term utility. At Floricultural Research Station, Agricultural Research Institute, Hyderabad, Andhra Pradesh, a gene repository of chrysanthemum is maintained and identified as the lead centre for chrysanthemum at national level. Interestingly, most of these cultivars look alike to each other with respect to majority of the phenotypic characters except for few. Hence, detailed characterization and evaluation of chrysanthemum cultivars is necessary for identification and exploitation of useful varieties in crop improvement programmes and also for better conservation and management of genetic resources. The assessment of diversity based on morphological parameters has been often constrained by lack of precise data on distinguishable morphological characters and their weakness of environmental influence. However, DNA marker technologies largely overcome these limitations and are being extensively used in several crop plants not only for genetic diversity analysis but also for a wide range of molecular genetic studies.

In view of the above, the present study entitled, “**Assessment of genetic diversity in Chrysanthemum (*Dendranthema grandiflora* Tzvelev) cultivars based on phenotypic and molecular markers**” was carried out at Floricultural Research Station, Agricultural Research Institute, Hyderabad with the following objectives.

1. To document information on plant morphology including vegetative and floral characters of different chrysanthemum cultivars available at Floricultural Research Station, Agricultural Research Institute, Hyderabad, Andhra Pradesh.

2. To assess the extent of genetic diversity in chrysanthemum cultivars by using RAPD and ISSR markers.
3. To test the reliability of each marker technology i.e. RAPD and ISSR markers in finger printing of chrysanthemum cultivars and explore the possibility of using these markers for cultivar identification.
4. To estimate the extent of genetic divergence of chrysanthemum cultivars through Mahalanobis's D^2 statistics.
5. To study the character association among yield characters.

CHAPTER - II

REVIEW OF LITERATURE

Chrysanthemum, the “Queen of the East” is popular flower crop of commercial importance. Its distribution is seen almost throughout the world and the crop exhibits wide genetic variability in its growth and flower patterns. In the past, many studies were conducted on phenotypical characterization of chrysanthemum genotypes, however, the studies on molecular characterization are very limited till date. Studies on molecular diversity for identification of genetic variability helps in crop improvement programmes.

Hence, an attempt has been made in this chapter to review the available literature on the history, origin, taxonomy, distribution and genetic diversity using morphological and molecular markers in Chrysanthemum (*Dendranthema grandiflora* Tzvelev) and its related species and the review is documented under the following headings:

2.1 HISTORY, ORIGIN, TAXONOMY AND DISTRIBUTION

2.2 BIODIVERSITY AND GENETIC DIVERSITY

2.2.1 Significance of genetic diversity

2.2.2 Morphological characterization

2.2.3 Molecular characterization

2.2.4 Randomly Amplified Polymorphic DNA (RAPD) markers

2.2.5 Inter Simple Sequence Repeats (ISSR) markers

2.2.6. Comparison of morphological and molecular markers

2.1 HISTORY, ORIGIN, TAXONOMY AND DISTRIBUTION:

Chrysanthemum is native to northern hemisphere chiefly Europe and Asia. There are number of claims that it was originated in China. Modern cultivars with large, double and exquisite flowering owe their origin from relatively small, single and not so attractive flowering types.

Historically Confucious, the great Chinese philosopher made a mention about chrysanthemum as early as in 500 BC in his writings. Seeds of chrysanthemum reached Japan

via Korea more than thousand years back (Ackerson, 1967). In Japan, chrysanthemum underwent a great transformation and today Japan possesses the maximum diversity the largest number of cultivars. The first attempt to grow chrysanthemum in Europe was made in Holland with a Japanese cultivar. Introduction of chrysanthemum to the western world was from Netherlands in 1688 and they were cultivated as *Matricaria japonica*. Chrysanthemum later spread to Great Britain in 1754 and to United States in 1798 (Clark,1962). The National Chrysanthemum Society of America was founded in 1890 at New York with commercial growers as the members.

In India, it is difficult to say with certainty when chrysanthemum culture began. Saint Gyaneshwar 1290 AD mentioned about chrysanthemum in his famous Marathi exposition of Gita “Gyaneshwari” written in AD1290. The Hindi name Guldedi (meaning flower of Daud) suggests that it must have been grown during the Mughal period in India.

The genus once included a larger number of species, but was split several decades ago into several genera. The naming of the genera has been continuous but a ruling of the International Code of Botanical Nomenclature in 1999 resulted in defining species of the genus being changed to *Chrysanthemum indicum*, thereby restoring the economically important florists chrysanthemum to the genus Chrysanthemum. This genus contains many hybrids and thousands of cultivars developed for horticultural purposes. The most important hybrid is *Chrysanthemum x morifolium* (Syn. *Chrysanthemum x grandiflorum*) derived primarily from *Chrysanthemum indicum* but also involving other species. During the period between the splitting of the genus and the ICBN ruling, these species have customarily been included under the genus name Dendranthema. The other species previously included in the narrow view of the genus Chrysanthemum are now transferred to the genus Glebionis. The other genera separated from Chrysanthemum include Argyranthemum, Leucanthemopsis, Leucanthemum, Rhodanthemum and Tanacetum.

2.2 BIODIVERSITY AND GENETIC DIVERSITY

According to Zhu (1996), the word biodiversity was highlighted for the first time by Walter G. Rosen in 1985 in the “National Forum on Biodiversity” held in Washington. Two years, after the publication of the proceedings entitled “Biodiversity” edited by Wilson, the use of biodiversity became more common. The definition of “Biodiversity” has been accepted and signed by 156 nations in an official document, “United Nation Environment Programme, 1992” which is as follows:

“Biological diversity means the variability among living organisms from all sources including inter alia, terrestrial, marine and other aquatic systems or parts include diversity within species and of ecosystems.”

One of the components of biodiversity is genetic diversity. According to Brown (1989), genetic diversity is the raw material of evolution and to evolve a single population, there must be a number of forms present within the population with differences in the genetic makeup of individuals. In complexity it is a characteristic of a population in which individuals within that population possess different genetic traits (Bala Ravi, 2004).

2.2.1. Significance of Genetic diversity:

India is one of the major centers of diversity in the world and further continuous introduction of new crops from different parts of the world paved the way in enriching the genetic wealth. Collection, characterization and documentation of genetic resources enable their efficient utilization in the process of improvement and development of varieties. Genetic diversity is being used as source of genes in crop improvement for production of high yielding varieties, hybrids and to effect ecologically sustainable economic and social development. Gene identification and access to diverse germplasm pool is crucial for the successful incorporation of novel traits in commercial ornamental crops (Misra and Naveen Kumar, 2008).

Over exploitation and large scale replacement of present cultivars with improved cultivars, hybrids as well as introduction of exotic varieties caused serious threat to native genetic wealth. Unlike most food and other crops, the germplasm of ornamental plants is neither collected nor maintained anywhere except partly in Floricultural Institutes and Botanical Gardens. Further thousands of ornamental plant species are yet to be discovered, identified and documented. The processes such as genetic erosion, genetic vulnerability and genetic wipeout are largely responsible for loss of genetic diversity of cultivated crops. Frequent replacement of cultivars for value addition is also reported to be the main cause of genetic erosion around the world. Thus germplasm conservation is inevitable for crop security to meet any eventuality in future and characterization is also essential for the protection of varieties under the PPV & FR (Protection of Plant Varieties and Farmers Rights) Act.

2.2.2 Morphological characterization:

Assessment of genetic variation is the first step prospecting towards domestication and improvement of a species. For this purpose, several morphological, cytogenetic, biochemical and DNA markers have been used. Even though morphological variation may be related to genetic variation, they do not correlate perfectly. Much morphological variation depends on seasonal or developmental changes that affect many individuals in a population regardless of genotype. As per the study of Tang Chung-How (1963), during the evolutionary process, the primitive edible and medicinal chrysanthemums became an ornamental plant after the 3rd century, A.D. In the course of evolution, number of varieties with wide range of colour were developed by careful cultivation, selection and cross fertilization. According to the shape and characteristics of the petal, the Chinese chrysanthemums were classified into 30 types in the order of evolution process i.e from the primitive form to the advanced form.

Later on not only the petals, other floral characters were also considered for the classification of chrysanthemums by number of research workers.

Chang Shu-Lin (1965) studied morphological characters of 25 garden varieties of chrysanthemum and classified the chrysanthemum into four categories based on flower size, petal type, flower head type and flower colour. He also opined that, the formation of certain type of Chinese chrysanthemum flower is influenced by the type of petals, number of petals, ratio of length to width, proportion of 3 layers of petals and diversity of petals. About one third of the commercial varieties of chrysanthemum have originated as sports or somatic mutants (Dowrick and El-Bayoumi, 1966). Chromosomal studies showed that these new forms originated as a result of the loss or gain of chromosomal material during mitosis. Consequently there was a need to differentiate vegetatively derived accessions.

David Warren (1997) developed a machine vision system at National Institute of Agricultural Botany (NIAB) that assesses the shape of chrysanthemum leaves as part of the testing procedure applied to new chrysanthemum varieties before they are granted protection under the United Kingdom's plant Breeders Rights Scheme. At National Institute of Agricultural Botany (NIAB), chrysanthemums are described by a set of 64 morphological characters that describe features ranging from plant height to bloom colour. Fifteen of these characters were used to describe the size, shape and colour of the leaves. The manual and machine assessments of base shapes of 210 leaves from 21 varieties were compared and documented.

Fukai *et al.* (1998) studied morphological and physiological characteristics of 20 native species of *Dendranthema*, in Japan and reported unique and special plant forms in seven species (*D. boreale*, *D. indicum*, *D. japonicum*, *D. ornatum*, *D. occidentali-japonense* var. *ashizuriense*, *D. pacificum* and *D. yoshinaganthum*) with unique branching, flowering habits

and with considerable intra and inter specific variations. Among the large flowered chrysanthemum cultivars, the proportion of flat petaled group comprised of 42% and quilled petal group 33%, whereas anemone petal group (2%) had less number of cultivars. The quantitative characters like petiole length, leaf length, inflorescence diameter, peduncle diameter followed normal distribution while the other characters like floret length, internodal length, peduncle length, number of ray and disc florets followed skewed distribution. The variation coefficient of floral characters was higher than that of foliar.

In a study with fifty two clones of chrysanthemum cv. Shuho-no-chikara collected in Aichi, Japan significant differences were recorded for the characters viz., earliness in flowering, degree of appearance of red- purple stripes on white petals, frequency of change from incurving floret to tubular floret, stem length and the diameter of the head (Ohishi, 2000)

Fifty seven genotypes of chrysanthemum collected from different parts of the country were assessed by Behera *et al.*, (2002). Categorization of the genotypes was done based on the variation in flower size, number and growth habit. The genotypes DCS 29, DCS27 and DCS 36 with large and decorative type of inflorescence were reported to be suitable for garden display or exhibition purpose, while the genotypes DCS 24 and DCS 23 having medium size and more number of flowers per plant were found ideal for pot culture.

Fukai *et al.*, (2002) observed considerable variations in leaf and flower morphology and flowering time among 36 individuals of *Dendranthema yashinaganthum* collected from Japan. Three lines with large plants were octoploid ($2n = 72$) while others were found tetraploid ($2n=36$).

Under Assam conditions, 11 spray cultivars of chrysanthemum were examined by Madhumita *et al.*, (2003) to study the extent of genetic variation for growth and floral characters. Based on mean plant height, the cultivars tested were grouped into tall (>50cm in

height), medium (40-50cm in height) and short or dwarf types (<40cm in height). In case of flower characters, maximum genetic variation was observed for number of flowers/plant followed by number of ray florets and dry weight of flowers.

Balaji and Reddy(2004) performed variability studies in seventeen genotypes of chrysanthemum and observed wide variation with respect to morphological characters (plant height, stem girth, number of branches/plant and leaf area per plant) and floral characters (number of flowers/plant, flower yield/plant and flower diameter). The cultivar Harvest Home, Karnool, Saraval and Chandrika showed large sized flowers with more number of flowers/plant.

In addition to visual phenotypic characters, the microscopic characters like pollen morphology has also been used as a trait to identify chrysanthemum species(Akbar and Ajab Khan, 2006). He distinguished the chrysanthemum species by pollen size, exine thickness, spine length and number of spine rows. Among them, there is great range of variation in exine thickness which has proved useful at specific level. On the basis of this character four groups viz., Group-I: *Chrysanthemum tibeticum*, Group-II: *Chrysanthemum indicum*, *Chrysanthemum segatum*, Group-III: *Chrysanthemum leucanthemum*, *Chrysanthemum murifolium* and Group-IV: *Chrysanthemum stoliczkai*, *Chrysanthemum parthenium* were recognized.

Mishra *et al.*,(2006) carried out genetic variability studies in 27 cultivars of spray type chrysanthemum and revealed wide range of variability for flower yield, number of sprays/plant, number of flowers/spray and duration of flowering.

Based on vegetative and floral characters, twenty chrysanthemum genotypes were characterized by Simrat Singh *et al.*,(2008). Four accessions having better plant spread together with more number of flowers, synchronous flowering and comparatively long flowering duration were categorized as varieties for garden display. Four genotypes with

Korean double, Korean semi double and decorative flower types were categorized for loose flower purpose, while one accession with compact and dwarf growth habit was suitable for pot culture.

According to morphological variation in plant height, flower size and other characters, twenty genotypes like Thaichen queen, Tata Centenary, Snow ball, Poornima, Pink cloud, PC-39 and Maghi were grouped as cut flower; the genotypes such as Ajay, Snow Don, Flirt, Shyamal, Kundan and White Prolific for spray types; Flirt, Ajay and Neelima for bedding purpose; Ajay, Flirt, Shyamal for loose flower production. While remaining were grouped as pot mums and decorative types (Kishan Swaroop *et al.*, 2008).

Botanical characters of different cultivated types of medicinal species (*Chrysanthemum morifolium*) in China were observed by Liu *et al.* (2008) to study its genetic diversity. Based on botanical and morphological characters, the germplasm lines were divided into seven colonies, according to the study, the sawtooth number of lamina, leaf stalk length and leaf division number were the important laminae morphological characters which were of relatively independent characters and these traits varied considerably among the lines Datta *et al.* (2009) carried out palynological studies in original and gamma ray induced mutants of garden chrysanthemum cultivars and found change in size and shape of pollen grains. The shape of exine spines was variable with regard to base and tip. Also the shape of endocolpium varied from circular, square and indiscernible types among different cultivars. The study on petal epidermal micromorphology revealed considerable variation in cell boundaries, cell surface and papillae. The change in the flower colour due to mutations as associated with some changed micromorphology of petal surface.

Laxmi *et al.* (2009) conducted variability studies in seven spray type yellow coloured chrysanthemum varieties and observed high phenotypic and genotypic coefficient of variation for suckers per plant, yield per plant and number of flowers per plant. According to

the study, with in the single coloured group also significant variation was observed both in the vegetative and floral characters. Similarly, variation for number of flowers per plant (55.43 – 511.13) and flower yield per plant (26.47g – 598.9g) of 17 genetically diverse stocks of small flowered chrysanthemum was recorded(Raghava *et al.*,1992).

Four glass house and thirteen garden chrysanthemum cultivars from white, yellow, pink/purple and orange/red/bronze groups were analyzed by Anderson *et al.*,(2001) with thin layer chromatography for qualitative and quantitative pigment constituents. Different coloured groups recorded different pigment constituents. In white cultivars four phenyl propanoids were observed, two of which were caffeic and ferulic acids. Yin Dongmei *et al.*,(2010)correlated the morpho-anatomical features of two *Dendranthema* species *i.e Dendranthema nankingense* and *Dendranthem azawadskii* to their reaction to waterlogging. In *Dendranthema zawadskii*, adventitious roots were formed above the flooding level and the aerenchyma was developed in the root, stem and leaf. In the same species palisade parenchyma was thicker and larger intercellular spaces are developed in the spongy mesophyll which indicated the greater relative tolerance of this species to water logging.

2.2.3. Molecular characterization:

The most important role of conservation is to preserve the genetic diversity in viable populations of ecologically and commercially important species in order to prevent potential extinction. The analysis of genetic diversity and relatedness between or within different species, populations and individuals is a central task for many disciplines of biological science. Previously morphological characters, comparative anatomy, chemical constituents, physiology and cytological information have been used for classification of plants and evaluation of genetic variability. In the past, first modern system for classifying natural diversity into categories was devised by Linnaeus in 1753. Afterwards morphological markers have been exploited to estimate diversity of crop plants(Wettstein-Knowles, 1992).

Morphological traits are actually based on the visual observations, which are more prone to environmental effects and are representative of few loci only. However, in these studies life forms and growth patterns ultimately influencing the structure of the mature plant are often ignored or little emphasized because of the common opinion that all these characters are adaptive (Shulkina *et al.*, 2003). In case of ornamental plant cultivars, the identification has also been based on morphological characters and in particular on the colour of the inflorescence. But this method needs the plants to be examined when they are in flowering and is very subjective. On the other hand, a large number of phenotypic markers are required to estimate genetic relationships and genetic diversity to tag any variety. Moreover these are not distinctly viable enough to adequately distinguish and characterize genetic germplasm among elite lines (Melchinger, 1990). Hence the analysis of plant DNA allows the direct assessment of variation in genotypes.

Molecular markers have the virtues of high speed, efficiency and reliability and generate large amounts of information. They offer numerous advantages over the conventional phenotype based alternatives as they are stable and detectable in all tissues regardless of growth, differentiation and development. Basically, marker techniques can be classified into two categories: non-PCR based techniques or hybridization based techniques such as (Restriction Fragment Length Polymorphism (RFLP) and PCR based techniques like RAPD, SSR's etc. The publication of Botstein *et al.*, (1980) about the construction of genetic maps using restriction fragment length polymorphism (RFLP) was the first report on molecular marker technique in the detection of DNA polymorphism. After the invention of polymerase chain reaction (PCR) technology, a large number of approaches for generation of molecular markers based on PCR were detailed, primarily due to its apparent simplicity and high probability of success. These approaches include randomly amplified polymorphic DNAs (RAPD) (Williams *et al.*, 1990), Sequence Characterized Amplified Regions (SCAR)

(Williams *et al.*, 1991), Sequence-Tagged Sites (STS) (Fukuoka *et al.*, 1994), Inter-Simple Sequence Repeat (ISSR) (Zietkiewicz *et al.*, 1994), Amplified Fragment Length Polymorphic DNAs (AFLPs) (Vos *et al.*, 1995) and Simple Sequence Repeats (SSRs) (Hearne *et al.*, 1992). Of these RAPD and ISSRs have been commercially exploited in Chrysanthemums, hence literature has been collected on use of these techniques in molecular diversity studies.

2.2.4. Randomly Amplified Polymorphic DNA (RAPD) markers

With the advent of polymerase Chain Reaction(PCR) technology, the study of genetic differences in plants and animals was simplified. DNA fingerprinting, gene mapping and phylogenetic studies have tremendously benefited from PCR technology. RAPD analysis is a PCR-based molecular technique introduced by Williams *et al.*,(1990), where single short oligonucleotide primers are arbitrarily selected to amplify a set of DNA segments distributed randomly throughout the genome. As the approach requires no prior knowledge of the genome that is being analyzed, it can be employed across species using universal primers. Due to the speed and efficiency of RAPD analysis, it has got potential applications in population studies (Welsh *et al.*, 1991), biosystematics (Stiles *et al.*, 1993), gene tagging (Naqvi *et al.*, 1995) and finger printing (Virk *et al.*, 1995; Mackill, 1996). RAPD markers have also been successfully applied in molecular ecology (Hadrys *et al.*, 1992), genetic analysis (Williams *et al.*, 1993), assessing variation in plants (Newbury and Ford-Lloyd, 1993) combining ability in rice (Radhidevi *et al.*, 2002) and to study the extent of diversity within the plant germplasm (Virk *et al.*, 1995).

RAPD markers are considered to be a rapid tool for assessing genetic diversity at molecular level and this technique has further advantages over other systems of genetic documentation because it has universal set of primers and no preliminary work such as probe isolation, filter preparation or nucleotide sequencing is necessary in various plant species (Williams *et al.*, 1990,1993; Virket *et al.*, 1995). In addition, like any molecular marker, RAPD

profiles are independent of age, physiological status of tissues or environmental influences (Virket *al.*,1995).Because of the ease in using RAPD for assessing genetic diversity such as a relatively unbiased portion of the genome sampled, simplicity of use, low cost and use of small amount of template DNA, it has widely been used in detection of genetic diversity in closely related species, in different populations of a species, in individuals with in populations and for conservation genetics.

The first study of genetic variation in chrysanthemum was conducted by Wolff and Peters- Van Rijn (1993). The variation between cultivars was high enough and the cultivars could be distinguished from each other by using only two different primers. A family of cultivars derived from one original cultivar by vegetative propagation had identical fragment patterns. Genetic variability among related *Dendranthema* species was too high to study genetic distances either among cultivars with in chrysanthemum or among species related to chrysanthemum.

Sporting is a well known phenomenon in chrysanthemum by which new cultivars may arise vegetatively from the parental cultivar. In chrysanthemum there are three different cell layers that form different parts of the plant. Since the species is chimeric, these cell layers may be genetically different. Wolff (1996) observed differences at the DNA level among cultivars with in one family as well as between different cell layers of one cultivar using RAPD analysis.

Wolff *et al.*, (1995) applied several techniques of DNA analysis to identify both related and unrelated chrysanthemum cultivars. Unrelated cultivars could be distinguished by using RAPD's, ISSRs, hybridization based DNA finger printing as well as RFLPs. Whereas morphologically different cultivars having varied flower colour but belonging to one family i.e., vegetatively derived from one cultivar appeared to have the same DNA fragment

patterns, whichever technique was applied. The absence of polymorphism between different accessions of the same cultivar indicated a high stability of the observed patterns.

The RAPD markers generated by 22-20 mer primers, discriminated 7 wild *Dendranthema* species, 14 cultivars and 5 inter specific hybrids by banding profiles(Dai Si lan *et al.*,1998). Of 224 bands observed, 34 bands (15%) were polymorphic. Cluster analysis based on UPGMA method indicated that, of 7 wild species, *Dendranthema indicum*, *Dendranthema vestitum* and *Dendranthema nankingense* were closer to the cultivated groups while *Dendranthema zawadskii* was most distinct. Genetic distances between the former three species and cultivars were less than 0.4 but those between *Dendranthema zawadskii* and cultivars were more than 0.5. This indicated that the origin of Chinese cultivated chrysanthemum and its cultivars were derived from *Dendranthema indicum*, *Dendranthema vestitum* and *Dendranthema nankingense*.

Lee and Kim (2000) examined genetic diversity and genetic relationships of wild chrysanthemums in Korea. Three chrysanthemum cultivars of *Chrysanthemum zawadskii* complex using 8 RAPD primers produced 72 bands. Genetic variations of *Chrysanthemum zawadskii* was high enough to divide it into 3 large groups at subspecies level viz.,*latilobum* var *leiophyllum* (group-1), *lucidum* (group-II) and *acutilobum*(group-III). Genetic diversity in *Chrysanthemum* subtribe Asterinae, the so-called wild chrysanthemums of Korea was distinctly divided into 2 groups, comprising of *Chrysanthemum zawadskii* in one group and all other taxa in the other, with a very low similarity. The genotype of cultivated chrysanthemum seemed slightly closer to *Chrysanthemum boreale* and *Chrysanthemum indicum* than to *Chrysanthemum zawadskii*.

Huang *et al.*,(2000) studied genetic analysis of chrysanthemum hybrids using 45 random primers. Of these, 22 primers were selected to detect the molecular marker in three hybrid combinations of chrysanthemum. In this study, 34.4% to 48.9% of the RAPD markers

were found to reveal additivity among parents and offspring in three hybrid combinations of Chrysanthemum. However, 38% to 52.6% of RAPD markers were absent in offspring, but 11.6% to 13.1% of type VII markers were present in offspring. In two hybrid combinations, the parents were more similar to each other than either was to offspring.

Characterization of fifteen commercial varieties of chrysanthemum was carried out by Martin *et al.*, (2002) through RAPD analysis. A total number of 151 bands were obtained through the RAPD analysis with ten primers employed, of which, 113 were polymorphic. The analysis revealed a high diversity between the varieties and the level of similarity was in all the cases less than 80%. The most unrelated variety was found to be 'Monnete Blanche' with less than 60% similarity with others. Varieties were clustered into four well defined groups each containing three or four varieties and more or less at the same rate of similarity.

Eighteen cultivars of chrysanthemum were subjected to RAPD analysis by Qin Helan *et al.*, in 2002 and observed high polymorphism with three primers out of 80 random primers and the polymorphic amplification fragment length ranged from 600 bp to 1300 bp. The 1200bp fragment generated by primer OPD 15 was present only in "Dahongtuogui", while the 1100bp fragment generated by primer OPA 17 was only absent in "Yulingguan" among 18 cultivars. The cultivars with similar petal type have high similarity coefficient among 18 cultivars.

Sehrawat *et al.*, (2003) assessed genetic relatedness using 60 RAPD primers in thirteen commercial chrysanthemum cultivars representing standard, spray and no pinch no stake groups, of which 50% of primers amplified genomic DNA. The size of the DNA fragments ranged from 0.55 to 2 kb and 2 to 21 bands were obtained for each primer. And of a total of 257 clear and reproducible bands, 239 bands were polymorphic. Genetic variation amongst cultivars was high enough to divide them into two major groups. The 1st group consisted of

Snow Ball, Ajina Purple and Sonar Bangla cultivars, while the second group accounted for Nagpur Red, Haldighati, Cardinal, Puja, Jaya, Suneel, Vasantika, Gauri, Flirt and Baggi.

The relationship of 23 lines comprising of wild species, cultivars and inter specific hybrids in *Dendranthema* were studied by Xin lei and Chen Fadi (2004) with 15 random primers. Of the 153 bands produced, 77.1% were polymorphic. Each primer amplified an average of 10.2 bands. It was observed that the compatibility of hybridization was better, fecundity was higher and embryo rescue was easier in *Dendranthema* when the parents have closer relationship i.e with close genetic distance.

Lema-Ruminska *et al.*, (2004) applied RAPD markers to study the molecular characterization of 10 new radio mutants of chrysanthemum. Out of 20 primers used only eight resulted in polymorphic bands. The eight primers amplified a total of 79 bands including polymorphic fragments. One of the primers, RAPD-9 could able to identify all 10 cultivars. The number of polymorphic bands varied between four and 18 per primer with amplification products varying between 212 and 3019 bp. The original cultivar 'Richmond' differed in genetic distance from its lady group mutants. The cluster analysis separated radiomutants into two groups, but the genetic distance between them was very low.

Further, in 2005 they attempted in distinguishing chrysanthemum mutants of nero and wonder groups using 8 random primers. The number of polymorphic bands varied between 2 and 16 per primer with amplification products varying between 212- 2730 bp. The Ney similarity index values showed high similarity index(0.829) between the original cultivar Lilac wonder and its mutant Red wonder, while the lowest(0.511) values were observed between the mutants of nero and wonder groups. Relatively a high similarity index was recorded between the original cultivar Red Nero and its mutant and the accessions were separated into three groups based on cluster analysis.

Four miniature cultivars of *Chrysanthemum morifolium* Ramat. were screened by five selected RAPD primers which gave better profile among 40 random primers (Chatterjee *et al.*,2005). The primer P-1 was most effective to differentiate between the cultivars and reported high similarity coefficient of 72-88%. Shizuka, a collection from Japan did not show much divergence from the rest of the species which indicated that interpopulation genetic distance showed no association with geographical distance between population sites of origin.

Nianjun Teng *et al.*,(2006) used eighteen RAPD primers to assess the genetic variation of chrysanthemum plantlets regenerated from floret derived calli treated with 0,10,15, and 20 Gy gamma rays. These primers generated a total of 167 reproducible RAPD bands ranging in size from 0.3 to 2.0 kb of which 61.7% were polymorphic and 38.3% monomorphic. The number of bands per primer ranged from 4 to 14. The results showed that genetic variation among the generated plantlets was proportional to the dosage of gamma ray within a certain range only.

Wen-bin *et al.*,(2006) applied RAPD analysis to estimate the genetic diversity of 22 *Chrysanthemum morifolium* accessions. The 26 RAPD primers produced 233 bands of which 89.7% bands were found to be polymorphic. On an average 8.04 polymorphic bands were amplified by each primer. High polymorphism at the molecular level indicated high genetic diversity among the germplasm of *Chrysanthemum morifolium*.

Kumar *et al.*, (2006) studied the extent of diversity and relatedness in eleven radio mutants originated from two chrysanthemum cultivars Ajay and Thai chen Queen using RAPD primers. Out of 40 random primers screened, 21 gave reproducible polymorphic bands and the percentage of polymorphism varied from 20(OPR 2) to 100(OPS 2, OPK 3).The genetic distance between all thirteen cultivars varied from 0.43 to 0.96. Cluster analysis indicated that yellow and bright orange mutants derived from Cv Thai chen Queen were

placed in a separate group indicating their high genetic diversity from the rest of the mutants and parents.

Bhattacharya and Texeira da Silva (2006) subjected 10 original cultivars and their respective 11 mutant cultivars to RAPD analysis using 40 primers initially. Out of them 5 gave clear and reproducible profiles which were subsequently used for RAPD analysis. Bands were of very low molecular weight ranging from 400 to 1500 bp and bands for each primer ranged from 3 to 13. The similarity among the cultivars and mutants varied from 0.17 to 0.90.

According to Datta *et al.*, (2009) the variability between the original and induced mutants of chrysanthemum was achieved by RAPD analysis using 20 primers out of which 10 gave better profiles. Bands generated by RAPD fragments were of low molecular weight ranging from 500 bp to 1500 bp. The genetic similarity among the cultivars and mutants varied from 0.15 to 0.90 which indicated that mutants did not vary greatly in their morphology. Here only two primers P2 and P3 were able to distinguish some of the mutants from their parents.

Molecular characterization using 10 RAPD primers was carried out by Minano *et al.*, (2009) in eight cut flowers and two pot plant cultivars of chrysanthemum. A total number of 188 bands were obtained of which 81% were polymorphic. The amplification products ranged from 300bp to 1300bp. The dendrogram showed two main groups based mainly on type of inflorescence. The least related group with a level of similarity of 63.5% under one group included the two cultivars with micro/single inflorescence. The eight remaining cultivars clustered in the other group.

Barakat *et al.*, (2010) screened five RAPD primers to amplify the genomic DNA of *Chrysanthemum morifolium* cultivar 'Delistar white' and its mutants. The number of DNA fragments amplified ranged from 6 to 14 with a mean value of 9.6 bands per primer and the

size of the fragments ranged from 300 to 1200 bp. Out of 48 amplified fragments, 52.1% were polymorphic in one or other of fourteen genotypes. The genetic similarity among the fourteen genotypes ranged from 0.43 to 0.95 which suggested a broad genetic base for chrysanthemum genotypes. Based on UPGMA analysis of the parent and its thirteen somaclones, five clusters were observed, also the shortest genetic distance *i.e* with highest similarity value was observed between variant 12 and variant 13.

Thiago Luiz *et al.* (2009) studied genetic diversity using RAPD markers and morpho agronomic characters for a total of 42 accessions of gerbera consisting of 29 commercial and 13 wild accessions. According to him 6 clusters of genotypes were obtained where two of them include wild accessions and remaining four includes commercial accessions using RAPD markers. Shannon index(H) indicate the genetic variation among the gerbera accessions and showed higher values of 0.34 for commercial clusters in comparison to the values obtained for the individuals from the non-commercial clusters(0.27).

2.2.5. Inter Simple Sequence Repeat (ISSR) markers:

Inter simple sequence repeat (ISSR) technique is a PCR based method, which involves amplification of DNA segment present at an amplifiable distance in between two identical microsatellite repeat regions oriented in opposite direction. The technique uses microsatellites, usually 16-25 bp long, as primers in a single primer PCR reaction targeting multiple genomic loci to amplify mainly the inter- SSR sequences of different sizes. The microsatellite repeats used as primers can be di-nucleotide, tri-nucleotide, tetra- nucleotide or penta-nucleotide. The primers used can be either unanchored (Gupta *et al.*, 1994; Meyer *et al.*, 1993; Wu *et al.*, 1994) or more usually anchored at 3' or 5' end with 1 to 4 degenerate bases extended into the flanking sequences (Zietkiewicz *et al.*, 1994). The technique combines most of the benefits of AFLP and microsatellite analysis with the universality of RAPD. ISSRs have high reproducibility, possibly due to the use of longer

primers (16-25 mers) as compared to RAPD primers (10- mers) which permits the subsequent use of high annealing temperature (45-60°C) leading to higher stringency. As the need to protect proprietary germplasm is likely to increase in the future, ISSRs will have an important role in securing plant variety rights by virtue of its unique efficiency in distinguishing even closely related germplasm. To date, more polymorphism has been detected with the use of ISSRs than with any other assay procedure (Virk *et al.*, 2000).

Fukai *et al.*, (1998) conducted the genetic diversity studies of 150 cultivars of large flowered chrysanthemum using 50 ISSR primers. A total number of 229 bands were amplified of which 150 were polymorphic loci. The genetic variation in each floral types were quite high in which the flat petaled group was highest (PPB=95.2%) whereas the anemone petal group (PPB=86.9%) and the spoon petaled group (PPB=9.52%) were the lowest. The genetic relationship among the flat petaled group, the quilled petaled group and the spoon petaled group are closest followed by the filiform petal group.

Miao Heng-bin *et al.*, (2007) used ISSR analysis to detect the genetic diversity and relationship of 85 chrysanthemum cultivars. ISSR fingerprinting amplified by 22 ISSR primers revealed a total no. of 160 unambiguous bands, of which 148 were polymorphic and the polymorphic frequency was 92.5%. The similarity coefficient between cultivars ranged from 0.294 to 0.802. The 85 chrysanthemum cultivars were divided into 6 groups by UPGMA based on Jaccard coefficient and opined that genetic relationship of 85 cultivars was related to their petal type.

Miao Heng-bin *et al.*, (2008) carried out ISSR fingerprinting of 25 new cultivars of *Chrysanthemum morifolium* using 77 primers for cultivar identification and breeder's right protection of chrysanthemum. Of the 77 primers screened, 21 primers could generate 122 fragments and 93.4% of bands were polymorphic. The similarity coefficient of cultivars was between 0.282-0.757. All the cultivars were discriminated efficiently by primer ISSR 35.

Liu and Yang (2009) revealed genetic relationship among 11 wild species and 12 cultivated varieties of *Chrysanthemum* by ISSR-PCR technology. Out of 75 primers tested, he could identify 14 primers which were polymorphic. The results showed 142 distinguishable bands that accounted for 95.1% polymorphism. The germplasm of *Chrysanthemum nankingense* has the closest genetic relationship with all the tested cultivars and three of them *Chrysanthemum chanetii*, *Chrysanthemum japonicum* or *Chrysanthemum japonicum* var. *wakasaense* have secondly close relatives with other varieties. *Chrysanthemum indicum* var. *aromaticum* has genetic relationship far from other tested varieties.

With ISSR markers, the genetic relationship of 11 *Chrysanthemum indicum* varieties of different geographical distribution was studied by Zhou Jie and Chen (2009). With 13 ISSR primers, 166 polymorphic loci were detected out of 176 loci in total. Cluster analysis showed that genetic similarity value of cultivars in one habitat was 0.727 and 0.591 in another habitat.

Cai-hong *et al.*, (2010) subjected 25 chrysanthemum cultivars to 10 ISSR primers to reveal the genetic diversity in *Dendranthema X grandiflora* (Ramat) Kitamura. A total of 242 unambiguous bands were obtained of which 241 were polymorphic and polymorphism frequency was 99.6%. The similarity coefficient of cultivars was between 0.331~0.718. These cultivars were divided into four groups by UPGMA based on Jaccard coefficient.

While studying the origin of Chinese garden chrysanthemum, 86 germplasm lines were analyzed using 10 ISSR primers. The ratio of polymorphism was as high as 99.3% and from cluster tree analysis of ISSR-PCR, it was observed that *Chrysanthemum vestitum*, ornamental chrysanthemum and medicinal chrysanthemum had close genetic affinity followed by *Chrysanthemum zawadskii* and *Chrysanthemum nankingense*.

2.2.6. Comparision of Morphological and Molecular markers:

Each method of marker analysis would have its advantages and disadvantages. Several researchers compared the different methods in characterization and assessment of genetic diversity in *Chrysanthemum*. Some of them are given below:

Dendranthema pacificum and other *Dendranthema* species collected from Japan and US were subjected to morphological evaluation and RAPD analysis by Roh and Ikeda (2003). Both the morphological and the RAPD analysis indicated that *Dendranthema pacificum* accession 1-7 collected in the trade in the United States could be a hybrid between *Dendranthema pacificum* and other *Dendranthema* species collected from Japan.

The *Chrysanthemum indicum* polyploidy complex comprises morphologically differentiated diploids, tetraploids and hybrids between *Chrysanthemum indicum* and *Chrysanthemum lavandulifolium*. Yang *et al.* (2006) elucidated the genetic diversity and relationships of the *Chrysanthemum indicum* polyploidy complex. A total of 127 polymorphic bands were generated from the nine RAPD primers used. Of these 81 were shared among diploids and tetraploids and 39 were uniquely present in diploids. Analysis of all accessions with 9 ISSR primers identified a total of 163 reproducible polymorphic fragments. Among these 74 were shared among diploids and tetraploids and 79 were uniquely present in tetraploid genomes and 10 fragments were unique to diploid genomes. Both RAPD and ISSR analyses revealed a close relationship between diploid and tetraploid populations.

The morphological traits, RAPD and ISSR molecular markers were used by Ye *et al.*, (2008) to assess the levels of polymorphism across 20 inbred lines of *Zinnia elegans*. Thirty four morphological traits, 147 RAPD marker fragments as amplified by 12 arbitrary primers and 128 ISSR marker fragments as generated by 9 primers were scored. The number of polymorphic loci, the percentage of polymorphic loci, Shannon's Information index and the effective number of alleles calculated from RAPD were 100, 68.03%, 0.3559 and 1.4169 respectively. While from ISSR data the respective statistics were 97, 76.38%, 0.4013 and

1.4728. Finally he concluded that ISSR markers were considered slightly superior to RAPD markers for assessing genetic diversity between the accessions. However the mantel's test indicated significant correlation ($R=0.733$) of the RAPD and ISSR results. By contrast the morphological matrix showed low correlation with both RAPD and ISSR data matrices ($R=0.3814$ and 0.3765 respectively). Cluster analysis showed that grouping of the accessions according to all three methods correlated well with their geographic region of origin, but flower color was not strongly associated with the genetic classification of these inbred lines.

Qing-Song *et al.*, (2010) studied the diversity and genetic relationship among 29 populations of *Chrysanthemum morifolium*, one of *Chrysanthemum indicum* and one of *Chrysanthemum nankingense* from China using morphological traits and molecular markers. Twenty morphological traits as well as 182 ISSR marker fragments as generated by 22 primers were scored, of which 81.87% of bands were polymorphic. Mantel's test indicated low correlation of morphological trait with ISSR ($r = 0.246$). Cluster analysis revealed that the most populations from the south of China were classified into one cluster and the populations from the North of China were classified into another cluster.

In *Artemisia capillaris*, an ornamental potted plant belonging to family Asteraceae, RAPD and ISSR markers were used for comparative analysis of genetic diversity (Mohammad shafie *et al.*, 2011). Among these markers, RAPD primers generated 291 amplification products of which 108 were polymorphic and ISSR markers produced 283 amplification products, out of which 54 were polymorphic. For RAPD the similarity index values ranged from 0.1579 to 0.8189 whereas for ISSR the values ranged from 0.5686 to 0.9016. According to him, the RAPD fingerprinting detected more polymorphic loci (95.6%) than the ISSR finger printing (66.67%).

CHAPTER - III

MATERIALS AND METHODS

The present investigation on “**Assessment of genetic diversity in chrysanthemum (*Dendranthema grandiflora* Tzvelev) cultivars based on phenotypic and molecular markers**” was carried out at Floricultural Research Station, Agricultural Research Institute, Rajendranagar, Hyderabad during the period from 2010-11 and 2011-12.

3.1 LOCATION AND WEATHER DATA

The experimental site located at Floricultural Research Station, Agricultural Research Institute, Rajendranagar, Hyderabad falls under arid sub-tropical climate zone with an average rainfall of 800 mm. Experimental site is located at an altitude of 542.3m above sea level on 17.90⁰ North latitude and 78.23⁰ East Longitude. Mean monthly meteorological data on rainfall, average minimum and maximum temperature and relative humidity recorded during the period of experimentation was presented in Appendix-I. The details of materials used and the methods adapted in the experiments are described hereunder.

3.2 Experimental details

The investigation was carried out in two broad sequential experiments viz.,

1. Diversity studies on chrysanthemum genotypes through field evaluation
2. Diversity studies of chrysanthemum genotypes using molecular markers

3.2.1 Experiment 1: Diversity studies on chrysanthemum genotypes through field evaluation

3.2.1.1 Plant material used for genetic diversity analysis The experimental plant material for the present study comprised of 104 chrysanthemum genotypes planted at Floricultural Research Station, Agricultural Research Institute, Rajendranagar, Hyderabad during 2010-11 and 2011-12. The list of chrysanthemum genotypes selected for the study is presented in Table 1.

3.2.1.2 Experimental design

The experiment was laid out in an Augmented Block Design. The experimental design consisted of 10 randomized blocks in each of which four standard chrysanthemum

genotypes and ten entries were planted. The standard genotypes, namely Raichur, Silper, CO-1 and Punjab Anuradha were serving as checks. The positions of the four standard genotypes and the ten entries in each block were fully randomized and planted in rows. Each genotype was grown in a plot of 2 X 2 m consisting of 36 plants spaced at 30 x 30 cm.

3.2.1.3 Crop husbandry

The top shoot cuttings of all the genotypes were planted in lines on raised nursery beds for rooting. After thirty five days, the rooted cuttings were transplanted in the main field. Recommended package of practices were followed uniformly for successful raising of genotypes.

3.2.1.4 Observations recorded

Morphological characterization of chrysanthemum genotypes with respect to their plant, leaf and flower characters was done in each genotype. Morphological characters both qualitative (Annexure-II) and quantitative were recorded following PPV & FRA(2001) guidelines for chrysanthemum. The methodology adopted in recording each character and the observations recorded were as follows.

I. Plant characters:

1. Plant height(cm):

The height of the plant was measured with meter scale from the base of the plant to the top of the plant canopy at the time of first flower bud appearance. At random five plants were selected in each plot for recording the data.

2. Plant Spread(cm):

The maximum horizontal spread of the five randomly selected plants were measured in North-South and East-West direction at the time of first flower bud appearance in each plot and expressed in centimeters.

3. Plant type:

Plant type was recorded by visual observation with a scale of 1 to 2, considering 1 for non-bushy and 2 for bushy nature.

4. Plant Growth Habit:

Only in bushy varieties, the plant growth habit was recorded by visual observation with a scale of 1 to 7, considering 1 for upright, 3 for Semi – upright, 5 for spreading and 7 for trailing nature of the plants.

5. Number of primary branches/plant:

The total number of primary branches of five selected plants were recorded at 90 DAT and the averages were worked out.

6. Internodal length (cm):

The length between the two nodes from the middle portion of the plant was recorded from five randomly selected plants and expressed in centimeters.

II . Leaf Characters

7. Leaf length (cm):

Leaf length was considered from petiole base to lamina tip and average value of ten fully developed leaves taken from middle third of the stem from five random plants was recorded as leaf length and expressed in centimetres.

8. Leaf width (cm):

Leaf width was recorded at the widest point of the leaf and average of ten fully developed leaves from five randomly selected plants in each plot was taken from the middle third of the stem and expressed in centimetres.

9. Leaf margin:

Leaf lamina margin was recorded on 1 to 9 scale, 1 for serrated and 9 for entire.

10. Depth of lowest lateral sinus(cm):

At random ten leaves from the middle third of the stem from five plants in each plot were selected and depth of the lowest lateral sinus was measured from the base of the sinus to the apex of the lateral lobe and expressed in centimeters.

11. Margins of sinus between lateral lobes:

The directions of margins of sinus between lateral lobes was recorded on 1 to 3 scale considering 1 for diverging, 2 for parallel and 3 for converging.

12. Size of the stipule (cm) :

Stipule at the base of the leaf was recorded in the scale of 3 to 7, considering 3 for small, 5 for medium and 7 for large. Ten leaves were randomly selected from the middle third of the stem and observations were recorded.

13. Petiole length(cm):

Petiole length was measured with the meter scale from the base of the petiole to the base of the leaf lamina.

14 . Leaf colour:

Leaf colour on the upper and lower side was determined by comparing it with the RHS (Royal Horticultural Society, 2001) colour chart.

15. Length of terminal lobe(cm):

Length of the terminal lobe was measured from the base to the apex of the terminal lobe.

16. Shape of the base of sinus between lateral lobes:

The shape of the base of sinus between lateral lobes was recorded by 1 to 2 scale considering 1 for acute and 2 for round.

17. Shape of the base of the leaf:

The shape of the base of leaf was recorded in scale of 1 to 6, considering 1 for acute, 2 for obtuse, 3 for rounded, 4 for truncate, 5 for cordate and 6 for asymmetric.

III. Inflorescence characters:

18. Days to first flower bud initiation:

Number of days taken from transplanting to first flower bud appearance was recorded in each genotype.

19. Days to 50 percent flowering:

The data on number of days taken for 50 per cent flowering was recorded by counting the number of days taken from transplanting of the rooted cuttings to till 50 percent of plants in each plot showed flowering.

20. Variety class:

Based on the size of the flower and purpose of the grower, the genotypes are classified into standard, spray and mini types and represented by the scale of 1 to 3, considering 1 for standard, 2 for spray and 3 for mini types.

21. Inflorescence form:

Based on the shape of the inflorescence, the data was recorded in the scale of 1 to 3, taking 1 for corymbiform, 2 for pyramidal and 3 for cylindrical.

22. Inflorescence width (cm):

Inflorescence width was measured at the widest point with the meter scale from five randomly selected inflorescences in each plot.

23. Spray length (cm):

Ten flower sprays were selected at random and spray length was measured from the point of origin of the spray to the point of flower pedicel attachment and the mean was expressed in centimeters.

24. Bud width (mm):

The bud width was measured at the widest point with digital vernier callipers from five randomly selected buds in each plot and was expressed in millimeters.

25. Flower head type:

Based on number of ray floret rows, data was recorded in the scale of 1 to 5 considering 1 for without any ray florets, 2 for single row, 3 for semi double(more than one row of ray florets and a clearly defined central disc), 4 for daisy eyed(Double flower heads

where a disc is not visible in the early stages of flowering but can be seen as the flower head opens fully) and 5 for double types (Disc is not visible at any stage of flowering).

26. Flower weight (g):

Ten freshly harvested flowers from each plot were weighed and the average was expressed in grams.

27. Flower head diameter (cm):

The diameter of flower head was measured at full flower opening stage from five randomly selected flower heads in each plot.

28. Length of the peduncle (cm):

Peduncle length of the terminal flower head was recorded with meter scale from five randomly selected flower heads in each genotype.

29. Peduncle thickness (mm):

The thickness of the peduncle was measured with the digital vernier calipers from five randomly selected flower heads in each plot.

30. Longitudinal axis of majority of ray florets:

Based on orientation of ray floret the data was recorded in the scale of 1 to 5, considering 1 for reflexing, 2 for straight, 3 for incurving, 4 for twisted and 5 for broken.

31. Type of ray florets:

Based on the shape of the ray floret, the data was recorded in the scale of 1 to 5, considering 1 for ligulate, 2 for incurved, 3 for spatulate, 4 for quilled and 5 for funnel shaped.

32. Shape of tip of ray floret:

The shape of the tip of the ray floret was recorded by visual observation in the scale of 1 to 5, considering 1 for pointed, 2 for rounded, 3 for truncated, 4 for dentate and 5 for fringed.

33. Length of the ray floret (cm) :

Length of the outer ray floret was recorded at full flowering stage from five randomly selected flower heads in each genotype.

34. Width of the ray floret(cm):

Width of the outer ray floret was recorded at full flowering stage from five randomly selected flower heads in each genotype.

35. Ratio of ray floret length: width:

Ratio of ray floret length: width was computed by using the following formula.

$$\text{Ratio of ray floret length: width} = \frac{\text{Length of the ray floret}}{\text{Width of the ray floret}}$$

36. Colour of outer and inner side of ray florets:

Ray florets colour both for outer and innerside was determined by comparing it with RHS (Royal Horticultural Society,2001) colour chart.

37. Rolling of margins of ray floret:

In all the non quilled florets the rolling of margins of ray florets was recorded in the scale of 1 to 7, considering 1 for strongly involute, 2 for moderately involute, 3 for weakly involute, 4 for flat, 5 weakly revolute, 6 for moderately revolute and 7 for strongly revolute.

38. Number of ray florets/head :

Number of ray florets per flower at full flowering stage was recorded from fully opened flower from five randomly selected flower heads in each plot.

39. Diameter of the disc(cm) :

The diameter of the disc was measured at full flower opening stage from five randomly selected flower heads in each plot.

40. Number of disc florets:

Number of disc florets per flower at full flowering stage was recorded from fully opened flower.

41. Length of the disc floret (cm):

Length of the outer disc floret was recorded at full flowering stage from five randomly selected flower heads.

42. Disc profile in cross section:

The disc profile in cross section was recorded in the scale of 1 to 3, considering 1 for flat, 2 domed and 3 for conical.

43. Disc floret type:

Based on the shape of disc floret, the data was recorded in the scale of 1 to 5, considering 1 for needle shaped, 2 for quilled, 3 for funnel shaped , 4 for tubular and 5 for petaloid.

44. Disc floret colour:

The disc colour before anther dehiscence was determined by comparing it with RHS (Royal Horticultural Society,2001) colour chart.

45. Flower type:

Based on arrangement of ray and disc florets and shape of ray florets, Datta (1996) classified the chrysanthemum varieties based on International Chrysanthemum Society and different National chrysanthemum societies. As per his classification, the flower types of the genotypes under study were categorised as pompons, anemones, spider, Irregular incurved, spoon type, quilled, decorative, Single Korean, Double Korean, Single, Intermediate and button type.

46. Number of flowers per spray:

At random ten sprays were selected in each plot and number of flowers per spray were counted and the average was computed.

47. Number of flowers per plant:

The number of flowers from five tagged plants was recorded from each genotype and the average number of flowers per plant was worked out.

48. Duration of flowering:

It was recorded by counting number of days from first flowering to the final harvest of flowers.

49. Number of suckers per plant:

At thirty days after final harvest of flowers, the total number of suckers arising from the main stem was counted for five randomly selected plants from each genotype.

50. Economic use:

Based on the commercial use of the genotypes, the data was recorded in the scale of 1 to 6, considering 1 for garden display, 2 for cut flower, 3 for pot culture, 4 for loose flower, 5 for ground cover and 6 for others.

3.3 STATISTICAL ANALYSIS OF DIVERSITY STUDIES ON CHRYSANTHEMUM GENOTYPES

Analysis of variance and components of genetic variability such as mean, range, genotypic and phenotypic coefficient of variance (GCV and PCV respectively), heritability and genetic advance as per cent mean (GAM) were estimated using standard procedures. Multivariate analysis (D^2 statistics), phenotypic and genotypic correlations and Path-coefficient analysis were performed on the mean data obtained as detailed here under

3.3.1 Analysis of variance

Analysis of variance for each character was carried out by using the Augmented Design II method as per the procedure outlined by Federer (1956)

The significance test was carried out by referring to 'F' table value given by Fisher and Yates (1963).

3.3.2 Genotypic and phenotypic variances

The genotypic and phenotypic variances were computed based on the expected mean sum of squares as follows:

Phenotypic and genotypic variances were estimated using following formula,

$$\text{Genotypic variance } (\sigma^2 g) = \frac{\text{MSS (genotypes)} - \text{MSS (error)}}{\text{Number of replications}} = \frac{M_2 - M_3}{r}$$

$$\text{Phenotypic variance } (\sigma^2 p) = \sigma^2 g + \text{MSS error} = \frac{M_2 - M_3}{r} + M_3$$

Where, $\sigma^2 g$ = Genotypic variance (GV)

$\sigma^2 p$ = Phenotypic variance (PV)

M_2 = Treatment mean sum of squares

M_3 = Error mean sum of squares

3.3.3 Coefficient of variation

Both genotypic and phenotypic coefficients of variability were computed as per the method suggested by Burton and Devane (1953).

$$\text{Genotypic coefficient of variation (GCV)} = \frac{\sqrt{V_g}}{\bar{X}} \times 100$$

$$\text{Phenotypic coefficient of variation (PCV)} = \frac{\sqrt{V_p}}{\bar{X}} \times 100$$

Where,

V_g = Genotypic variance

V_p = Phenotypic variance

$\bar{X}$ = General mean of the character

PCV and GCV were categorized as low, moderate and high by following Sivasubramanian and Menon (1973). It is as follows

0 – 10 %	: Low
10 - 20%	: Moderate
20% and above	: High

3.3.4 Heritability

Heritability in broad sense refers to the proportion of genetic variance to the total observed variance in the population. It has been estimated as per the formula given by Lush (1940).

$$\text{Heritability (h}^2\text{)} = \frac{V_g}{V_p} \times 100$$

Where,

V_g = Genotypic variance

V_p = Phenotypic variance

Further, the range of heritability in broad sense was classified as suggested by Johnson *et al.*, (1955).

Less than 30%	: Low
30 – 60%	: Moderate
More than 60%	: High

3.3.5 Genetic Advance as per cent Mean (GAM)

Genetic advance as per cent mean was worked out for each character adopting the formula given by Johnson *et al.*, (1955)

$$\text{GA (as \% of mean)} = \frac{GA}{X} \times 100$$

Where,

GA : Genetic advance = $k \times \sigma^2_p \times h^2$

K : Selection differential which is equal to 2.06 at 5% intensity of selection (Lush, 1940)

σ^2_p	:	Phenotypic standard deviation
h^2	:	Estimated heritability, and
$\overline{X}$	:	General mean

The range of genetic advance as per cent of mean was classified according to Johnson *et al.*, (1955)

Low	:	Less than 10%
Moderate	:	10 – 20%
High	:	More than 20%

3.3.6 Correlation Coefficient Analysis

To determine the degree of association of characters with yield and also among the yield components in each environment, the correlation coefficients were calculated.

Both genotypic and phenotypic coefficients of correlation between two characters were determined by using the variance and covariance components as suggested by Al-Jibouri *et al.* (1958).

Genotypic coefficient of correlation	$r_g(xy) = \frac{\text{Cov}_g(xy)}{\sqrt{\sigma_g^2(x) \cdot \sigma_g^2(y)}}$
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Phenotypic coefficient of correlation	$r_p(xy) = \frac{\text{Cov}_p(xy)}{\sqrt{\sigma_p^2(x) \cdot \sigma_p^2(y)}}$
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Where,

$r_g(xy)$, $r_p(xy)$ are the genotypic and phenotypic correlation coefficients respectively.

Cov_g , Cov_p are the genotypic and phenotypic covariance of xy, respectively.

σ_g^2 and σ_p^2 are the genotypic and phenotypic variance of x and y, respectively.

The calculated value of 'r' was compared with table 'r' value with n-2 degree of freedom at 5% and 1% level of significance, where, n refers to number of pairs of observation.

3.3.7 Path Coefficient Analysis

Path coefficient analysis was carried out using phenotypic correlation values of yield components on yield as suggested by Wright (1921) and illustrated by Dewey and Lu (1959). Standard path coefficients which are the standardized partial regressing coefficients were obtained using statistical software packages called GENRES. These values were obtained by solving the following set of 'p' simultaneous equation using the above package.

$$P_{01} + P_{02} r_{12} + \dots + P_{0p} r_{1p} = r_{01}$$

$$P_{02} r_{21} + P_{02} + \dots + P_{0p} r_{2p} = r_{02}$$



$$P_{01} r_{p1} + P_{02} r_{p2} + \dots + P_{0p} = r_{0p}$$

Where, P_{01} , P_{02} , ..., P_{0p} are the direct effects of variables 1, 2, ..., p on the dependent variable 0 / r_{12} , r_{13} , ..., r_{1p} , ..., $r_{p(p-1)}$ are the possible correlation coefficients between various independent variables / r_{01} , r_{02} , r_{03} , ..., r_{0p} are the correlation between dependent and independent variables.

The indirect effects of the i^{th} variable via j^{th} variable is attained as $P_{0j} x r_{ij}$. The contribution of remaining unknown factor is measured as the residual factor, which is calculated and given below.

$$P^2_{0x} = 1 - [P^2_{01} + 2P_{01}P_{02}r_{12} + 2P_{01}P_{03}r_{13} + \dots + P^2_{02} + 2P_{02}P_{03}r_{23} + \dots + P^2_{0p}]$$

Residual effect, which measures the contribution of characters not considered was obtained as follows:

$$\text{Residual factor} = \sqrt{(P^2_{0x})}$$

Direct or indirect effects were categorized as suggested by Lenka and Mishra (1973) are given below:

Negligible	- 0.00 to 0.09;
Low	- 0.10 to 0.19;

Moderate	- 0.20 to 0.29;
High	- 0.30 to 0.99 and
Very high	- 1.00.

3.3.8 Genetic Divergence Analysis

The genetic divergence in one hundred and four genotypes for thirty characters were analysed through Mahalanobis's using D^2 statistic technique.

3.3.8.1 Mahalanobis distance (or) Generalised squared Distance (D^2)

Genetic diversity between genotypes was estimated by using D^2 analysis given by Mahalanobis (1936). The D^2 value between i^{th} and j^{th} genotypes for P characters was calculated as

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

Where

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

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

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

P = No. of characters

3.3.8.2 Test of Significance

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

The estimate of ‘ $\hat{\lambda}$ ’ (Wilk’s Criterion) was done using the following formula.

$$\hat{\lambda} = \frac{|E|}{|E+V|}$$

Where,

$|E|$ = Determinant of error matrix

$|E+V|$ = Determinant of error + varieties matrix.

Then the value of V statistic was worked out using Wilk’s lambda criterion

$$V_{(Stat)} = -m \log_e \hat{\lambda}$$

Where,

$m = n - [(P+Q+1)/2]$

n = Degrees of freedom for error + varieties

$\log_e \hat{\lambda} = 2.3026 \log_{10} \hat{\lambda}$

P = Number of variables or characters

Q = Number of genotypes

$V_{(Stat)}$ is distributed as χ^2 with PQ degrees of freedom

3.3.8.3 Transformation of Correlated Variables

In the present model, computation of D^2 value was reduced to simple summation values of differences in mean values of various characters of two genotypes i.e. $\sum d_i^2$. Therefore, transformation of correlated variables into standardized uncorrelated ones was done before working out the D^2 values. Transformation was done by using pivotal condensation method in computation of D^2 values.

3.3.8.4.1 Computation of D^2 values

For a given combination of i and j genotypes, the mean deviation i.e., $Y_{it} - Y_{jt}$

For $t = 1, 2, \dots, P$ variables were computed and D^2 values were calculated as sum of squares deviations.

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

$$D_{ij}^2 = \sum^P (Y_{it} - Y_{jt})^2$$

$t=1$

Where,

Y_{it} = Uncorrelated mean values of i^{th} genotype for t characters

Y_{jt} = Uncorrelated mean values of j^{th} genotype for t characters

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

3.3.8.5 Testing the significance of D^2 values

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

3.3.8.6 Grouping of genotypes into Various Clusters

Grouping of the genotypes into different clusters was done by using Ward's minimum variance method as described by Rao (1952). The criterion used in clustering by this method is that any two variables belonging to the same cluster should be at least on an average show a smaller D^2 value among themselves than those belonging to different clusters. The first step in grouping the genotypes into different clusters was to arrange the genotypes in the order of their relative distance from each other. For this purpose, the D^2 value of all combinations of each genotype were arranged in ascending order of their magnitude in a tabular form as described by Singh and Chaudhary (1977). To start with the two genotypes having the smallest distance with each other were considered first, to which a third population having smaller D^2 value from the first two genotypes was added. Similarly, the next nearest fourth genotype was considered and this procedure was continued. At certain stage, where it was felt that after adding a particular genotype if there was abrupt rise in the average D^2 value, then that population was not considered for inclusion in that cluster. The group of the first cluster was then excluded from the group and the rest were treated in a similar way. This process was continued till all the genotypes were included into one or the other cluster. After the

formation of the cluster, the average inter and intra cluster distances (divergence) were calculated.

3.3.8.7 Average intra - cluster distance

For the measurement of intra - cluster distances, the formula used was

$$\sum D_i^2 / n$$

Where,

$\sum D_i^2$ = Sum of distances between all possible combinations (n) of the populations included in a cluster.

n = Number of clusters

3.3.8.8 Average inter-cluster distance

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

$$\text{Average inter-cluster distance} = \frac{D^2}{(n_1 \times n_2)}$$

Where,

n_1 and n_2 are number of genotypes of cluster 1 and cluster 2 respectively.

3.3.8.9 Cluster Diagram

The clusters and their mutual relationships were presented diagrammatically. The square root of average D^2 value was an approximate measure of divergence between the clusters. Based on D^2 values (inter cluster distance) the scale given by Rao (1952) for rating of the distance was adopted and the cluster diagram was prepared.

3.3.8.10 Contribution of Individual Characters towards Divergence

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

$$\text{Percentage contribution of the character} = X = \frac{N \times 100}{M}$$

Where,

X = Per cent contribution of character.

N = Number of genotype contributions where the character was ranked first.

M = All possible combinations of the genotypes concerned.

3.3.9 Principal Component Analysis (PCA)

Principal component analysis was carried according to procedure described by Banfield (1978) using WINDOWSTAT software package Version 8.1. It is defined as a method of data reduction to clarify the relationships between two or more characters and to divide the total variance of the original characters into limited number of uncorrelated new variables. The main objectives of PCA are:

- i. To discover or to reduce the dimensionality of the data set.
- ii. To identify new meaningful underlying variables.

PCA can be performed on two types of data matrices viz., variance – covariance matrix and correlation matrix. With characters of different scale, a correlation matrix standardizing the original data set is preferred. If the characters are of same scale, a variance

– covariance matrix can be used. In the present study, PCA was performed on the correlation matrix of traits, thereby removing the effects of scale (Jackson, 1991). The main reasons for the use of the correlation matrix are :

1. The presence of original variables in different units.
2. Existence of differences in the variances, in case of original variables having the same units.

3.3.9.1 Eigen values and eigen vectors

The eigen values and eigen vectors were computed from data matrix. Eigen values define the amount of total variation that is displayed on principal components. The proportion of variation accounted for each principal component (PC) is expressed as the eigen value divided by the sum of the eigen values.

$$\text{Eigen value (PC1)} \\ \text{Per cent variance explained for PC}_1 = \frac{\text{Eigen value (PC1)}}{\text{Sum of eigen values}}$$

The eigen vector (loading) defines the correlation of each variable with the principal components.

The principal components were identified by the following procedure.

The j^{th} principal component (Y_j) of the observations X is the linear combination given as follows:

$$Y_j = A_{1j}X_1 + \dots + A_{pj}X_p$$

Where,

A_{ij} are found such that Y_j is uncorrelated $Y_1, Y_2, \dots, Y_{j-1}$ the j^{th} largest variance.

The A_{ij} are the elements of the normalized eigen vector associated with largest j^{th} eigen value.

The variance of the j^{th} principal component of the λ_j and the total system variance trace (S) = $\lambda_1 + \lambda_2 + \dots + \lambda_p$. The importance of the j^{th} principal component is given by

$$\frac{\lambda_j}{\text{Trace (S)}}$$

This is informative about the proportion of total variation that can be accounted for the j^{th} principal component. The correlation between the i^{th} original variable X_i and the j^{th} principal component Y_j is given by

$$\rho(X_i, Y_j) = \frac{A_{ij}}{\sqrt{S_i}} \sqrt{\lambda_j}$$

Where,

S_i is the standard deviation of X_i .

Thus, a principal component is a linear function of the test variables as described below.

$$\text{Principal component} = ax_1 + bx_2 + \dots + hx_8$$

Where, $a, b, \dots$ are coefficients and $x_1, x_2, \dots$ etc., are the variables in such a way that the principal component has a unit variance as reported by Ehrenberg (1985).

PCA scores for each accession under concerned PCs were computed and utilized to derive a 3D (dimensional) scatter plot of individuals.

3.3.9.2 Cluster analysis

Agglomerative hierarchical clustering technique was followed as given by Anderberg (1993).

1. Obtaining data matrix

PCA scores for 104 genotypes were used as input for clustering because principal component analysis provides variable independence and balanced weighting of traits, which leads to an effective contribution of different characters on the basis of respective variation.

2. Standardizing the data matrix

To compare the similarities among the genotypes the data matrix was standardized with a column standardizing function i.e., Q analysis. The data matrix was standardized in cluster analysis to make the characters contribute more equally to the similarities among genotypes and to nullify the arbitrarily affect of the units chosen for measuring the attributes among the accessions.

Column standardizing function CA-Q analysis was carried by the following formula.

$$Z_{ij} = \frac{\overline{X}_{ij} - \overline{X}_j}{s_{ij}}$$

Where

$$\overline{X}_j = \frac{\sum_{i=1}^n X_{ij}}{n}$$

Where

$$s_{ij} = \sqrt{\frac{\sum_{i=1}^n (X_{ij} - \overline{X}_j)^2}{n-1}}$$

For

n= total no.of accessions i.e., 84 in the case

i= ith accessions

j = jth variables

The resulting data after standardization is unitless and have mean zero and variance one.

3. Computing the resemblance matrix

A resemblance coefficient, which measures the over all resemblance (the degree of similarity or distance) between a pair of accessions was computed.

The data matrix was transformed to distance matrix (resemblance matrix) based on the dissimilarity coefficients using squared Euclidean distance method.

$$\text{Squared Euclidean distance } [d_{ij}] = \sum_{K=1}^P (X_{ik} - X_{jk})^2$$

Where,

P = number of characters

X_{ik} = value of i^{th} accession for k characters

X_{jk} = value of j^{th} accession for k characters

4. Execution of the clustering method

Distance matrix was converted into dendrogram by using Ward's method. Ward proposed a clustering procedure seeking to form the partitions $P_n, P_{n-1}, \dots, P_1$ in a manner that minimizes the loss associated with each grouping and to quantify that loss in a form that is readily interpretable. Information loss is defined by Ward's in terms of an error sum of squares criterion, ESS.

Cluster membership is assessed by calculated total sum of squared deviations from the mean of the cluster.

For the K^{th} cluster, Error sum of square is defined *i.e.*,

ESSK = Sum of squared deviations from the cluster centroid

If there are 'C' clusters, define the total error sum of squares as

ESS = Sum of ESSK,

$$\text{for } K = 1, \dots, C. \left(\sum_{k=1}^C ESSK \right)$$

Ward's minimum variance method considers the union of every possible pair of cluster and combine the 2 clusters whose combinations results in the smallest increase in ESS. In this method, the distance between two clusters is the ANOVA sum of squares between the two clusters added up over all the variables.

3.4 EXPERIMENT 2: DIVERSITY STUDIES OF CHRYSANTHEMUM GENOTYPES USING MOLECULAR MARKERS

Detection of molecular polymorphism among the 37 Chrysanthemum genotypes was performed by RAPD and ISSR analysis.

3.4.1.1 Plant material

The material used for the study comprised of 37 genotypes of chrysanthemum presented in Table 2. The plant material was maintained at Floricultural Research Station, Rajendranagar, Hyderabad.

3.4.1.2 Extraction of plant DNA

DNA was extracted by modified CTAB (Cetyl Trimethyl Ammonium Bromide) (Murray and Thompson, 1980) as detailed below.

- Healthy young leaves (5g) were collected from young plants and brought to laboratory on ice. The leaves were surface sterilized with 70 per cent ethyl alcohol and cut into pieces and immediately used for further processing.
- Approximately 1 g of fresh leaf sample was weighed and homogenized completely with liquid Nitrogen using mortar and pestle.

- Powdered leaf material was transferred to eppendorf tubes and suspended in a pre-heated (65°C) 15 ml of extraction buffer (containing 2% CTAB, 100mM Tris, 20 mM EDTA, 1.4M NaCl, 1% PVP and 1% β -mercapto ethanol.
- The contents were mixed by vortexing for few minutes and the reaction mixture was incubated for 45 minutes at 65°C in water bath.
- The tubes were centrifuged at 14,000 rpm for 10 minutes at 4°C.
- Supernatant was collected in a separate tube to which equal volume of Chloroform: Isoamyl alcohol (24:1) mixture was added and centrifuged at 14,000 rpm for 10 minutes at 4°C.
- Again the supernatant was collected in a separate tube to which 0.6 volume of ice cold isopropanol was added and incubated at -20°C for 10 min.
- Again the contents were centrifuged at 14,000 rpm for 10 min at 4°C.
- Pellet was collected and added two volume of 95 per cent ethanol and 0.5 vol of sodium acetate (pH 5.2) and left at -20°C overnight for precipitation of DNA.
- After incubation, the tubes were spun at 14,000 rpm for 10-15 minutes. Later the pellet obtained was washed with 70 per cent ethanol (100 μ l) and centrifuged at 14,000 rpm for 5 minutes.
- The pellet obtained was air dried and was dissolved in TE buffer and stored at -20°C for further use.

3.4.1.3 DNA quantity and quality estimation

The concentration of DNA was assessed electrophoretically by loading 2 μ l of isolated genomic DNA on 0.8% agarose gel along with known concentrations (50, 100, 200 ng/ μ l) of lambda uncut DNA (standard). After running the gel, the DNA concentration was detected by comparing with the standard and individual samples were diluted to required concentration for RAPD and ISSR analysis.

The concentration and quality of DNA was also estimated spectrophotometrically by using Nano Drop spectrophotometer at 260 nm.

3.4.1.4 RAPD reaction mixture

The RAPD reaction mixture consisted of 5 ng of template DNA, 1x PCR buffer (10 mM Tris pH 9.0, 50 mM KCl, 1.5 mM MgCl₂), 100 M of each of the four dNTPs, 0.4 M of RAPD primer and 0.3 Units of *Taq* DNA polymerase (Bangalore Genei, India) in a reaction volume of 10 µl.

3.4.1.5 Amplification conditions

PCR amplifications were performed in a Gene Amp 9700 Thermal Cyclor (Perkin Elmer Applied Biosystems) using 27decamer primers.

The amplification profile was as follows:

a.	Initial denaturation temperature	94 ⁰ C	3 minute
b.	Denaturation	92 ⁰ C	1 minute
c.	Primer annealing	37 ⁰ C	30 seconds
d.	Primer extension	72 ⁰ C	2 minute
Later step b to d were repeated		45 times	
e.	Complete primer extension	72 ⁰ C	7 minutes
f.	Soak temperature	4 ⁰ C	Till removal

3.4.1.6 Primer selection

Total 124 primers (Operon, USA) were screened out of which 5 primers from OPE series, 4 primers each from OPH series and OPI series, 3 primers each from OPF series, OPG series and OPK series, 2 primers each from OPJ series and OPL series, 1 primer from OPM series were selected for PCR amplification of genomic DNA of the *Chrysanthemum* genotypes. List of primers used in the study is furnished in Table 3.

3.4.1.7 Agarose gel electrophoresis

The PCR amplified products were separated on 1.0% agarose gel in 1x TAE buffer by electrophoresis at 100 V for 3 h and visualized with ethidium bromide staining. The microscopic pores in the agarose gel act as a molecular sieve. 5 µl of gel loading dye (Bromophenol blue) was added to 25 µl of PCR product and mixed well before loading into wells. The gel images were recorded using the Alpha Innotech Fluorchem gel documentation system and the sizes of amplification products were determined by comparison to *Eco* RI and *Hind* III

double digest (Bangalore Genei, India) as molecular weight standard. In general, RAPD markers suffer from a lack of reproducibility, but to check the consistency of the electrophoretic patterns and polymorphism detected, every PCR reaction was repeated twice and run in an Eppendorf master cycler and Gene Amp PCR system 9700 thermal cyclers. Regardless of the marker system used, all the PCR amplifications included a negative control (no DNA) to avoid erroneous interpretations.

3.4.2 ISSR (INTER SIMPLE SEQUENCE REPEAT) MARKER ANALYSIS

After quantification, the DNA was diluted to a concentration of 5 ng/µl for ISSR diversity analysis. A total of 10 ISSR primers were used for the analysis (Table 4).

3.4.2.1 PCR amplification for ISSR marker

The PCR reaction was carried out in a total volume of 10 µl containing 1.0 µl of 5 ng template DNA, 1.0 µl of 1x PCR buffer (10 mM Tris pH 9.0, 50 mM KCl, 1.5 mM MgCl₂), 0.2 µl of 25 mM MgCl₂, 0.6 µl of 200 M of each of the four dNTPs, 1.0 µl of 0.4 M ISSR primer and 0.2 µl of 0.6 Units *Taq* DNA polymerase (Bangalore Genei, India).

For ISSR analysis, a total of 46 UBC primers (UBC primer set No. 9, University of British Columbia, Canada) were screened out of which 10 primers were used. The PCR

amplification was empirically determined by testing different concentrations of genomic DNA and primer. The optimal annealing temperature was found to vary according to the base composition of the primers. A negative control that contained all PCR components except DNA was included in every experiment to test for DNA contamination of the reagents. PCR amplifications were performed in a Gene Amp 9700 thermal cycler (Perkin Elmer Applied Biosystems).

The amplification profile was as follows:

a.	Initial denaturation temperature	94 ⁰ C	4 minute
b.	Denaturation	92 ⁰ C	30 seconds
c.	Primer annealing	Based on primer	1 minute
d.	Primer extension	72 ⁰ C	1 minute
Later step b to d were repeated		35 times	
e.	Complete primer extension	72 ⁰ C	7 minutes
f.	Soak temperature	4 ⁰ C	Till removal

3.4.2.2 Agarose gel electrophoresis

The PCR amplified products were electrophoretically separated in 1.7% agarose (Bangalore Genei, India) gels buffered with 1x TAE at 100 V for 3 h and visualized with ethidium bromide staining. The gel images were recorded using the Alpha Innotech Fluorchem gel documentation system and the sizes of amplification products were determined by comparison to *Eco* RI and *Hind* III double digest (Bangalore Genei, India) as molecular weight standard.

3.5 SCORING AND STATISTICAL ANALYSIS FOR MOLECULAR ANALYSIS

Data was entered using a matrix in which all observed bands or characters were listed. The RAPD and ISSRs pattern of each genotype was evaluated; assigning character state ‘1’

to all the bands that could be reproducible and detected in the gel and '0' for the absence of band for each primer and used to

calculate a genetic similarity matrix using the Jaccard (J) coefficient, which is more appropriate for dominant markers as it does not count 0/0 matches in the calculation. The genetic distance between each pair of genotypes was calculated by SIMQUAL analysis of the NTSY-pc software package version 2.1 (Rohlf, 2000).

3.6 CLUSTER ANALYSIS

Cluster analysis was performed on molecular similarity matrices using the Unweighted Pair Group Method using Arithmetic means (UPGMA) algorithm, from which dendrograms depicting similarity among genotypes were drawn and plotted using NTSYS-pc. 2.1 Software (Rohlf, 2000).

CHAPTER – IV

EXPERIMENTAL RESULTS

The major emphasis of this study using phenotypic traits and molecular markers is to develop an appropriate technique for reliable measurement of genetic differences between the chrysanthemum genotypes for further use in breeding programmes and other economic uses as in landscaping, cut flowers. In the present experiment, one hundred genotypes in comparison with four checks were studied for their genetic diversity by morphological and 37 genotypes for molecular markers. The observations recorded are tabulated and enumerated as under.

Genetic diversity analysis at morphological level

One hundred and four genotypes of chrysanthemum maintained at Floricultural Research Station, Rajendranagar, Hyderabad were evaluated at the field level for studying their morphological diversity. Observations were recorded for thirty different quantitative traits and twenty three qualitative traits.

4.1 QUANTITATIVE TRAITS

The results of analysis of variance for 104 genotypes in chrysanthemum are furnished in Table 5. Highly significant differences among the genotypes were noticed for all the characters studied indicating the presence of sufficient amount of variability. The mean values calculated for the replicated data and range of variation among 104 chrysanthemum genotypes for thirty quantitative traits studied was presented in Table 6 & 7 and illustrated in fig 1-4. The variations observed for each quantitative character of genotypes under study was documented in this chapter.

4.1.1 Plant growth characters:

4.1.1.1 Plant height (cm) :

The chrysanthemum genotypes exhibited significant variation for the plant height. The overall mean height of plant at the time of first flower bud appearance was 40.61cm. The plant height among the collections ranged from a minimum of 23.22 cm in Yellow Bonsai to a maximum of 60.75cm in Chocolate Pompon Variegated. Out of 104 genotypes, sixty four were found to be significantly taller when compared to mean of check genotypes (38.08cm)

Perusal of the data indicated that maximum number of 45 genotypes showed short plant height in the range of 32.5 to 42.5cm followed by 34 genotypes with medium plant height of 42.5 to 52.5cm, While, only seven genotypes recorded highest plant height ranging from 52.5 to 62.5cm, While 18 genotypes were categorised as very short type recording the plant height less than 32.5cm.

4.1.1.2 Plant Spread (E-W) (cm)

The data on plant canopy spread in east-west direction was presented in Table -6. The genetic variation of plant spread was quite significant. Among the genotypes, the plant canopy spread ranged between 11.81cm and 57.03cm in the genotypes Ajay and Apurva Singar, respectively. The general mean was found to be 24.21 cm and fifty six genotypes exceeded the check mean (21.86 cm).

Of hundred and four genotypes, forty eight showed less plant spread in E – W direction with less than 22.5cm followed by forty four genotypes with medium plant spread within the range of 22.5 to 32.5cm. High plant spread in the range of 32.5 to 42.5cm was recorded in 10 genotypes. Only two genotypes Apurva Singar and PAU-B-107 had recorded very huge plant spread(>42.5cm) .

4.1.1.3 Plant Spread(N-S) (cm) :

The plant spread ranged from 13.34 to 69.99 cm. Among all the genotypes, Punjab Gold recorded minimum plant spread (13.34 cm) whereas, Local Button recorded the

maximum plant spread (69.99 cm). The mean plant spread of the genotypes was 24.23 cm and fifty eight genotypes were found to possess greater plant spread over the mean plant spread of check varieties (21.16cm).

The 52 genotypes exhibited a plant spread of less than 22.5cm and 42 genotypes exhibited a range of 22.5 to 32.5cm . While, 6 genotypes were categorised as high type with plant spread in the range of 32.5 to 42.5 cm. Only four genotypes were in the group with more than 42.5cm plant spread.

4.1.1.4 Number of Primary branches/ plant

Among 104 chrysanthemum genotypes, the highest number of primary branches was recorded in Pink Cascade (30.92) whereas the lowest was recorded in Red Fair Lady (3.73) with the general mean of 8.50. Ninety seven genotypes produced relatively higher number of branches compared to the mean of check varieties (4.92).

With the grouping of genotypes, it was found that maximum of 55 genotypes recorded medium number of branches with in the range of 6.25 to 11.25 followed by 30 genotypes with less than 6.25 primary branches per plant. While fifteen and four spray type genotypes were classified under high (11.25 to 16.25 branches per plant) and very high group (>16.25 branches per plant) respectively.

4.1.1.5 Internodal length (cm):

The maximum internodal length was recorded by the genotype Tata Centenary (4.01 cm), whereas the minimum was observed in Chocolate Pompon Variegated (0.42 cm). The mean internodal length was found to be 1.84 cm and thirty genotypes exceeded the mean value of the check varieties (2.15 cm).

Maximum number of fifty four genotypes were grouped under medium internodal length category (1.25cm – 2.25cm) followed by equal number of twenty two genotypes each

under short and lengthy internodal lengths category, whereas, very long internodal length was recorded in six genotypes.

II Leaf characters:

4.1.1.6 Length of the petiole (cm):

The petiole length ranged from 4.10cm (Red Queen) to 0.53 cm (Yokari) with the general mean of 1.86cm. The mean of check varieties was found to be 2.29cm and only twenty genotypes had lengthy petioles compared to the check mean.

Out of 100 genotypes and four checks evaluated, 73 genotypes recorded medium petiole length of 1.25 to 2.75cm followed by 20 genotypes with short petiole length less than 1.25cm. Whereas, 11 genotypes exhibited long petioles in the range of 2.75 to 4.25cm.

4.1.1.7 Length of the terminal lobe (cm):

Wide range of variation (0.53cm to 4.72cm) was observed for this character with an overall mean of the genotypes or the check mean of 1.93cm. Forty four genotypes showed length of the terminal lobe in excess of check mean of this character among the genotypes.

Based on the ranges, the genotypes showed four categories with maximum number of 56 genotypes with medium length of 1.25 to 2.25cm followed by 30 genotypes with long terminal lobe in the range of 2.25 to 3.25cm, while 15 genotypes showed short terminal lobe of less than 1.25cm. Only three genotypes exhibited very long leaf terminal lobe of more than 3.25cm.

4.1.1.8 Leaf length(cm):

Considerable genetic variation in length of leaf was noticed among the genotypes. It ranged from 1.80 cm (Yokari) to 8.70 cm (Red Queen) with the general mean of 5.07cm. Sixty genotypes had relatively higher leaf length compared to the mean leaf length of the check varieties (4.65cm). It can be inferred that eighty genotypes were found in the group

having medium sized leaves(3.25cm to 6.25cm) followed by 18 genotypes with large sized leaves (> 6.25cm) and only six genotypes in small leaf group(< 3.25cm).

4.1.1.9 Leaf width(cm):

The maximum leaf width was recorded in the cultivar Pride of Maid Fort (6.66 cm) whereas the minimum was observed in Yokari (1.52 cm). The mean leaf width was 3.94 cm and sixty genotypes exceeded the check mean value of the genotypes (3.53 cm).

Seventy five genotypes out of hundred genotypes along with the check were having leaves with medium leaf width (3.25cm – 6.25cm) followed by 27 genotypes with less than 3.25cm width, whereas, only two genotypes (Pride of Maid Fort and Red Queen) had recorded wider leaf with more than 6.25 cm.

4.1.1.10 Depth of the sinus (cm):

The genotypes exhibited considerable variation in depth of the sinus. It ranged between 0.23(Yokari) to 2.78 cm (Perfeeta) with the general mean of 1.26 cm. Forty nine genotypes recorded more sinus depth than the check mean of the genotypes (1.21 cm).

Only six genotypes fell under the group with shallow depth of the sinus, whereas 65 genotypes expressed medium sinus depth ranging from 0.5 cm to 1.5cm followed by 33 genotypes with deep sinus (> 1.5 cm).

III. Flower characters:

4.1.2.11 Days to 1st flower bud appearance:

Overall mean for days taken to first flower bud appearance in the genotypes was 78.71 days with a wide range of variation from as early as 48.99 days to as late as 133.59 days in Pink Cascade and Apurva Singar respectively. Eighteen genotypes have recorded less number of days required for flowering compared to the check mean of the genotypes (65.49 days).Among the genotypes evaluated, maximum number of 54 genotypes were mid type and showed first flower bud within the range of 65 to 85 days followed by 30 genotypes

under late varieties, whereas 20 genotypes fell under the group of early varieties (less than 65 days)

4.1.2.12 Days to 50% flowering:

The number of days taken to 50 per cent flowering ranged from 53.97 to 138.73. The cultivar Pink cascade took minimum number of days (53.97) to 50 per cent flowering, while maximum number of days were taken by the cultivar Apurva Singar (138.73). Twenty one genotypes have recorded early flowering compared to the mean of the check genotypes (82.48 days).

Based on mean number of days required to attain 50 per cent flowering maximum of sixty four genotypes were categorised into mid type (75 to 95 days) followed by thirty two genotypes under late type with 95 to 115 days, whereas only few genotypes viz., 3 and 5 genotypes were found to be early and very late varieties respectively.

4.1.2.13 Inflorescence width(cm):

Genotype Local Button exhibited the maximum width of inflorescence at widest point (71.79 cm) while, Red Fair Lady recorded the minimum width of 18.94 cm. The general mean width of inflorescence among the genotypes was 37.29 cm and about ninety one genotypes were found to possess greater width of inflorescence than the check mean (25.94 cm).

Eighteen genotypes fall under the group with narrow width of inflorescence while maximum number of 67 genotypes are with medium width of inflorescence in the range of 27.5cm to 47.5cm, whereas, nineteen genotypes showed broad inflorescence (>47.5cm).

4.1.2.14 Spray length (cm):

There was considerable genetic variation among the chrysanthemum genotypes for spray length, as it is evident from the observed range of variation between 7.29 cm (Coffee) to 31.84 cm (Apurva Singar). The mean spray length among the genotypes was 16.80 cm

and about fifty two genotypes were found to possess spray length exceeding the check mean spray length (16.37 cm).

It can be inferred that forty five genotypes were found in the group having medium spray length within the range of 11.25 to 16.25cm followed by forty four genotypes in long spray length category and ten genotypes in very long spray length group, while only five genotypes were having short spray length (6.25 to 11.25cm).

4.1.2.15 Bud width (mm):

This character ranged from 4.27 mm to 18.59 mm with the general mean of 9.92 mm. The cultivar Red Fair Lady recorded the maximum width of the bud whereas Ajay exhibited least bud width. Forty four genotypes presented more bud width than the mean bud width of the check varieties (1.03mm).

Among the hundred genotypes along with check, maximum of forty nine genotypes recorded medium bud within the range of 8 to 12mm followed by twenty eight genotypes with small bud width (4 to 8mm) and twenty six genotypes with large bud width (12-16mm). Exclusively only one genotype Red Fair Lady showed more than 16 mm width of the bud.

4.1.2.15 Average flower weight (g):

The average weight of the flower was characterized with variability among the genotypes, wherein, the cultivar Flirtation recorded the maximum weight of the flower (9.00 g), while Julie registered the minimum (0.61 g). Forty genotypes recorded relatively more flower weight than the check mean weight of the genotypes (2.29 g). Based on ranges, sixty seven genotypes were categorised under low flower weight group (0.5 to 2.5g), followed by twenty two genotypes with medium weight of 2.5 to 4.5g and nine genotypes within the range of 4.5 to 6.5g. Whereas, six genotypes recorded very high flower weight of more than 6.5g.

4.1.2.16 Flower diameter (cm):

There was considerable genetic variation among the chrysanthemum genotypes for flower diameter, as evident from the observed range of variation between 2.10cm (Local Button) to 8.89 cm (Star Pink) (Table 6). The mean flower diameter was found to be 5.00cm and fifty four genotypes exceeded the mean value of the check varieties (4.53 cm). Maximum number of 68 genotypes came under the group with medium flower diameter, followed by 32 genotypes under large flower diameter class, whereas only one accession (Star Pink) showed very large flower diameter of more than 8.75 cm. The mini varieties Dolly pink, Local Button and Sadbhavana exhibited the small flower diameter (< 2.75cm).

4.1.2.17 Length of the peduncle (cm):

The genetic variation of peduncle length among the genotypes ranged between 2.56 cm (Local Button and Mayuri) and 10.14 cm (Jessie Hab Good) and the general mean was found to be 5.56 cm. In comparison to check mean, thirty six genotypes exceeded the mean value of check varieties (6.10 cm).

Sixty six genotypes out of hundred and four were having flowers with medium peduncle length in the range of 3.25 to 6.25cm followed by thirty one genotypes with long peduncles and five genotypes with short type (>3.25cm), whereas, only two standard type genotypes (Jessie Hab Good and Star Pink) had recorded flowers with more than 9.25cm peduncle length.

4.1.2.19 Thickness of the peduncle (mm):

The genotype Robert Earn Shaw exhibited the more peduncle thickness (4.73 mm) whereas, Sukai Sport displayed the smallest peduncle thickness (0.88 mm) and the general mean was found to be 2.32 mm. Sixty nine genotypes had recorded greater peduncle thickness compared to mean peduncle thickness of the check genotypes (1.90 mm).

Amongst all genotypes medium peduncle thickness was observed in maximum number of 92 genotypes (1.25mm to 3.25mm), followed by thick peduncle in 9 genotypes and only three genotypes (Local Button, Sukai Sport and Co-1) fall under thin group based on peduncle thickness.

4.1.2.20 Number of ray florets/head:

The highest number of ray florets per head was recorded by the genotype Lilith (406.71), whereas the lowest was observed in Sukai Sport (10.98). More number of ray florets per head than the check mean (175.66) was recorded in forty eight genotypes. In general, medium number of ray florets per head in the range of 75 to 225 was recorded in forty five genotypes, followed by high number of ray florets in thirty two genotypes, less number of ray florets(<75) in twenty four genotypes and only three genotypes having double type flower head (Co-3, Lilith and Jaya) had comparatively very high number of ray florets per head.

4.1.2.21 Length of the ray floret(cm):

The genotype John webber had the longest ray florets in the outer whorl (5.39 cm) while, Sadbhavana had the shortest ray florets in the outer whorl (0.68 cm) with the general mean of 2.66 cm. With comparison to check mean, seventy genotypes had recorded greater ray floret length than the check mean of 2.05cm.

Among hundred genotypes along with the check varieties, maximum of 70 genotypes are having medium length of the ray florets (1.25 – 3.25cm) followed by 26 genotypes in the range of 3.25 to 5.25cm and 7 genotypes with less than 1.25 cm. Only one genotype John Webber, a standard variety exhibited very long ray floret with more than 5.25cm.

4.1.2.22 Width of the ray floret(cm):

The maximum width of the ray floret was observed in the genotype Rose Day (1.54 cm) whereas the minimum was recorded in the cultivar Swan (0.15cm) with a general mean

of 0.67cm. While, twenty six genotypes displayed more ray floret width when compared to the check mean of 0.73 cm.

Based on ranges, the medium ray floret width in the range of 0.5 to 1.0cm was observed in maximum of 71 genotypes followed by less width in twenty one accessions while twelve genotypes fell under the group of ray florets with high ray floret width.

4.1.2.23 Ratio of ray floret length /width

Among hundred genotypes and including the checks, the highest ratio of ray floret length/width was recorded in the cultivar Swan (24.00) whereas the lowest ratio was recorded in Shizuka (1.37). Sixty five genotypes displayed relatively higher ratio of ray floret length/width compared to the mean of the check genotypes (3.42).

It was clear that the maximum number of 95 genotypes belong to low group for ratio of ray floret length/width followed by 8 genotypes with medium range (6.25 to 11.25). Whereas, the high ratio of ray floret length/width was observed in only one genotype (Swan).

4.1.2.24 Disc diameter(cm)

The range of disc diameter varied from 3.34 to 0.00 cm with the general mean of 1.28 cm. Among all genotypes, Tandra and Tata Centenary had no disc whereas, Terry showed the maximum disc diameter of 3.34cm. Out of 104 genotypes, 78 genotypes had more disc diameter than the general mean of 0.83 cm.

As per the classification based on ranges, 62 genotypes showed medium disc diameter in the range of 0.75 to 1.75cm followed by 21 genotypes with less and 15 genotypes with high disc diameter and only six genotypes fell under very high disc diameter group.

4.1.2.25 Number of disc florets/head

The highest number of disc florets per head was recorded by the genotype Donald (300.64) whereas the double type of genotypes Tandra and Tata centenary had the flower head with only the ray florets without any disc florets. More number of disc florets per head

than the mean of check varieties (55.22) was recorded in seventy two genotypes. Maximum number of 46 genotypes were grouped under medium number of disc florets category (75 - 175) followed by 38 genotypes with less number group (<75). Whereas, high and very high groups for number of disc florets per head were accounted for total of 20 genotypes.

4.1.2.26 Length of the disc floret (cm)

Considerable genetic variation among the chrysanthemum genotypes for length of disc floret was observed with the range of variation between zero cm in double varieties (Tambra and Tata Centenary) to 1.76cm (Terry) with mean disc floret length of 0.70cm. Forty one genotypes were found to possess disc floret length exceeding the check mean (0.67 cm).

Among hundred genotypes along with check, maximum number of 52 genotypes are having medium disc florets in the range of 0.5 to 1.0 cm followed by twenty nine genotypes in short and twenty three genotypes in the long disc floret group.

4.1.2.27 Number of flowers/spray

The chrysanthemum genotypes displayed a higher level of genetic variation for the number of flowers per spray. The genotype Pink Cascade and Pusa Centenary recorded the lowest number of flowers per spray (2.95) whereas PAU -B-107 recorded the highest number of flowers per spray (23.30). The average number of flowers per spray over all the genotypes was found to be 7.60 and seventy genotypes produced more flowers per spray than the check mean(5.25)

Out of hundred genotypes and along with check varieties, 48 and 41 genotypes recorded low and medium number of flowers per spray respectively. While twelve genotypes fell under the group having sprays with high number of flowers and only three genotypes (PAU-B-107, Rekha and Beauty) with very high number of flowers per spray(>16.25).

4.1.2.28 Number of flowers/plant

The highest number of flowers per plant was recorded by the genotype Local Button (298.54) whereas the lowest was observed in Tambra (5.81) and the general mean of the genotypes was found to be 101.77. More number of flowers per plant than the mean of the check varieties (127.04) was recorded in thirty one genotypes. Maximum of 53 genotypes showed medium number of flowers per plant followed by thirty three genotypes under low yielder group and seventeen genotypes under high yield group. Only one cultivar, a mini type variety Local Button can be classified under very high yielder group.

4.1.2.29 Duration of the flowering (days)

Wide range of variation was observed in duration of flowering among the genotypes studied with an overall mean of 45.35 days. The range in the collection varied from as less as 36.22 days in mini variety Sadbhavana to as long as 70.35 days in the check variety Raichur. The mean duration of flowering among all genotypes was 45.35 days and out of hundred genotypes, 5 genotypes showed more duration of flowering than the mean duration of check varieties (55.85).

As per the classification based on ranges, 52 genotypes showed medium duration of flowering (42.5–52.5 days) (Table 7) followed by 39 genotypes with less and 12 genotypes with long duration of flowering (52.5 – 62.5 days) and only one genotype (Raichur) fell under the group with excess duration of flowering (80.35 days).

4.1.2.30 Number of suckers per plant

The chrysanthemum genotypes displayed a higher level of genetic variation for the average number of suckers per plant. The genotype Taichen Queen recorded no sucker development whereas, Yellow Bonsai recorded the highest number of suckers per plant (54.46). The average number of suckers per plant over all the accessions was found to be

15.00, and out of hundred genotypes evaluated, 25 genotypes showed more suckers than the mean of check varieties (18.53).

Out of hundred and four genotypes, maximum number of 59 genotypes recorded medium number of suckers per plant. While thirty one genotypes fell under the group having less number(< 7.5) and nine genotypes with high number of

suckers per plant. Whereas, only five genotypes produced very high number of suckers per plant(>47.5).

4.2.1 QUALITATIVE TRAITS

All the 104 chrysanthemum genotypes were visually assessed for 23 distinctive qualitative traits as listed in Table 8. Among 23 qualitative traits evaluated, the nature of the leaf margin viz., serrated character was observed to be similar in all 100 chrysanthemum genotypes, while the remaining qualitative traits observed were different among the genotypes. Classification of 104 chrysanthemum genotypes based on scoring of twenty three qualitative characters was furnished in Table 9.

4.2.1 Plant type:

Among 104 genotypes, eighty six genotypes (82.69%) exhibited bushy plant stature while eighteen accessions mostly the standard class (17.31%) exhibited non bushy nature (Plate 1)

4.2.2 Plant growth habit:

Out of 86 bushy types, 79 genotypes (75.95%) were of semi upright growth habit, while only seven genotypes (6.73%) recorded spreading growth habit.

4.2.3. Leaf margin: With respect to leaf margin, no significant variation was observed among the genotypes. All the genotypes evaluated (100.0%) exhibited the serrated nature of leaf margin (Plate 2).

4.2.4 Size of the stipule:

As per the classification based on scoring, 25 genotypes (24.04%) were of estipulated. Out of 79 stipulated genotypes, the variation in size was observed among them. Maximum of 43 genotypes (41.35%) had small stipules, 24 genotypes had medium stipules (23.08%) while only twelve genotypes (11.54%) had large stipules (plate 3).

4.2.5 Margins of sinus between lateral lobes

Out of 104 genotypes evaluated, 43 genotypes (41.34%) were grouped under parallel margin category followed by thirty seven genotypes (35.58%) under converging margin category and 24 genotypes with diverging margins of sinus between lateral lobes (23.08%) (plate 4).

4.2.6 Leaf colour (upper side)

The genotypes studied showed green colour on the upper side of the leaf with various intensities as represented by the RHS colour chart with different reference numbers. Maximum of 39 genotypes (37.5%) exhibited green colour with reference number N137A, followed by 23 genotypes with 137A(22.11%), 19 genotypes with N137B (18.27%), 16 genotypes with N137C (15.38%) and 7 genotypes with reference number 146A (6.73%).

4.2.7 Leaf colour (lower side)

All the genotypes showed green colour on the lower side of the leaf with slight variation in intensity of the colour. According to RHS colour chart, 93 genotypes (90.38%) exhibited colour with reference number 147B followed by five genotypes (4.81%) with reference number 146A, three genotypes with 146B (2.88%) and only two genotypes with colour reference number 148B (1.93%).

4.2.8 Shape of the base of the sinus between lateral lobes

Different shapes of the base of the sinus between lateral lobes were noticed among the chrysanthemum genotypes (plate 5). Sixty six genotypes (63.46%) were found to possess round base, while remaining thirty eight (36.54%) had acute sinus base between lateral lobes.

4.2.9 Shape of the base of the leaf

As per the classification based on scoring ,shape of the leaf base was classified as acute in 17 genotypes (16.35%), obtuse in 42 genotypes (40.38%), rounded in 26 genotypes (25.00%), truncate in 13 genotypes (12.50%), cordate in 5 genotypes (4.81%) and assymetric in only one genotype (plate 6).

4.2.10 Class of the variety

As per the classification based on the size of the flower and purpose of the grower, the varieties are classified into 19 standard types (18.27%),71 spray types (68.27%) and 14 mini types(13.46%) (plate 7).

4.2.11 Inflorescence form

As per the classification based on scoring, 102 genotypes (98.08%) had corymbiform inflorescence while only one genotype Poonam(0.96%)showed cylindrical form and another genotype(0.96%) Punjab Anuradha had Pyramidal form of inflorescence (plate 8).

4.2.12 Flower head type

Among 104 genotypes studied, 51genotypes (49.04%) exhibited semi double type of flower head followed by 34 genotypes (32.69%) with daisy eyed type. While, 17 genotypes (16.35%) were with single row of ray petals and only 2 genotypes were fully double type (1.92%) (plate 9).

4.2.13 Longitudinal axis of majority of ray florets

The longitudinal axis of majority of ray florets was straight in 83 genotypes (79.81%), while incurving in fourteen genotypes (13.46%) and reflexing in only seven genotypes (6.73%).

4.2.14 Type of ray florets

Among 104 genotypes of chrysanthemum, the ray florets in the flowers of 71 genotypes(68.27%) wereligulate type, 17 genotypes (16.35%)were incurved type, 14 genotypes (13.46%) werespathulate shaped, while only two genotypes viz., Perfeeta and Ravi Kiran (1.92%) observed quill type of ray florets (plate 10).

4.2.15 Shape of tip of ray florets

Ray florets with pointed tip was observed in forty three genotypes (41.35%) whereas with rounded tip in thirty nine genotypes (37.5%). The ray florets of only one genotype i.e Coffee (0.96%) had Truncated tip, while 20 genotypes (19.23%) recorded dentate tip of ray florets and only one genotype (0.96%) namely Flirtation had fringed tip of ray florets (plate 11).

4.2.16 Colour of ray florets (outer side)

Out of 104 genotypes studied, maximum of 37 genotypes (35.58%) exhibited yellow colour on the outer side of flowers followed by white colour with 23 genotypes (22.11%), pink (21.15%) and Reddish brown colour (21.15%) each in 22 genotypes.

4.2.17 Colour of ray florets (inner side)

The chrysanthemum genotypes were grouped into different categories based on colour of inner side of ray florets. According to RHS colour chart, 37 genotypes (35.58%) exhibited yellow colour with different intensity followed by 23 genotypes (22.11%) with white colour, 22 genotypes with reddish brown (21.15%) and twenty two genotypes with pink colour (21.15%) (plate 12)

4.2.18 Rolling of margins of ray florets

As per the classification based on scoring, 60 genotypes (57.69%) showed flat margins of ray florets followed by 25 genotypes (24.04%) with weakly revolute margins of ray florets, 14 genotypes (13.47%) showed weakly involute rolling and two genotypes each in moderately revolute (1.92%) and moderately involute (1.92%) category. However, rolling of margins of ray florets was strongly revolute in only one genotype i.e Silper (0.96%).

4.2.19 Disc in cross section

Among 104 chrysanthemum genotypes, maximum of 81 genotypes (77.88%) possessed flat shaped disc in the fully opened flower head followed by 20 genotypes

(19.23%) exhibiting dome shaped disc. On contrary, only one genotype Sadbhavana (0.96%) showed conical shaped disc in the flower head.

4.2.20 Type of disc floret

With regards to type of disc floret, 72 genotypes (69.23%) fell under the group having funnel shaped disc floret followed by 24 genotypes (23.08%) with petaloid shaped disc floret and six genotypes (5.77%) fell under the group of plants having tubular disc florets in their flower head.

4.2.21 Colour of disc floret

Almost all the genotypes (99.04%) showed yellow coloured to greenish yellow disc florets with slight variation in intensity of the colour except the variety Modella (0.96%) which showed light pink coloured disc florets with reference number 77C.

4.2.22 Type of the flower

As per the classification of Datta (1996), the chrysanthemum genotypes under study were categorised into 11 types. 8 genotypes were grouped as pompons (7.69%), 15 genotypes as Anemones (14.42%), 16 genotypes each under decorative (15.38%) and Irregular incurved types (15.38%), 9 genotypes fell under the group spoon type (8.65%), 2 genotypes under Quilled group (1.92%), 13 genotypes were of single Korean type (12.50%), 18 genotypes were considered as Double Koreans (17.31%), 3 genotypes were of Single type and one genotype each under Intermediate (0.96%) and Button type (0.96%) (plate 13).

4.2.23 Economic use

As per the economic use of the genotypes, the chrysanthemum genotypes are categorised into six types. 42 genotypes (40.38%) were suitable for loose flower cultivation followed by 23 genotypes (22.11%) suitable for garden display in landscaping, 22 genotypes (21.15%) for cut flower production, 13 genotypes (12.5%) suitable for pot culture and only 4 genotypes (3.86%) can be categorised under ground cover varieties.

4.3 GENETIC VARIABILITY STUDIES

The analysis of variance for thirty quantitative characters was carried out to partition the total variance due to genotypes and other sources. The variance due to known and unknown causes were worked out using the method suggested by Chaudhary and Prasad (1967). The results revealed highly significant differences among entries in respect of all the characters studied. The genetic parameters, viz., phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability in broad sense and genetic advance as

percent mean were estimated for all the characters under study and the results on variability among the 104 chrysanthemum genotypes were presented in Table 10. Further, the variability studied in different characters was presented character wise under the following heads:

4.3.1. Plant Height (cm)

The genotypes exhibited a wide range of variability for this trait with overall mean of 40.61 cm. The range in the collection varied from 23.22 cm to 60.75 cm. The per cent of genotypic and phenotypic coefficient of variations for this trait were 17.41 and 17.50 respectively (Table 10). This trait exhibited a high heritability (99.0%) with expected percent mean genetic advance (35.68).

4.3.2. Plant Spread in E –W direction (cm)

The overall mean of this character among all the entries was 24.21cm with the variability ranging from 11.81 to 57.03 cm. The estimate for phenotypic coefficient of variation (28.61 %) and genotypic coefficient of variation (28.51%) was found to be high for this character. The estimates for heritability (99.30 %) and genetic advance as per cent mean (58.54 %) were also found to be high for this character.

4.3.3. Plant Spread in N - S direction (cm)

A range of 13.34 to 69.99 cm was observed for plant spread in N – S direction with the mean value of 24.23 cm. The high phenotypic coefficient of variation and genotypic coefficient of variation of 28.62 per cent and 28.59percent, respectively were exhibited for this trait. The heritability observed was 99.8 per cent with high genetic advance of 58.84 as per cent of mean.

4.3.4. No. of primary branches/plant

Wide variation of 3.73 to 30.92 was noticed with the mean of 8.50 for this trait. A high phenotypic coefficient of variation (38.10 %) and genotypic coefficient of variation (38.08 %) was recorded. The estimates for heritability (99.9 %) and genetic advance as per cent mean (78.41 %) were also found to be high.

4.3.5. Length of the petiole (cm)

The mean petiole length was 1.86 and the observed minimum and maximum range was between 0.53 and 4.10. The character showed high genotypic coefficient of variation (31.65 %) and phenotypic coefficient of variation (33.23%) and it also showed the high heritability percentage (90.70%) with higher value of genetic advance over mean (62.08%).

4.3.6. Terminal lobe length (cm)

Average terminal lobe length of 1.93 with the range between 0.53 to 4.72 was recorded. The estimates of genotypic coefficient of variation (30.97 %) and phenotypic coefficient of variation (31.78 %) were high with high heritability of 95.00% and higher value of genetic advance over mean (62.17%).

4.3.7. Length of the leaf (cm)

The genotypes showed wide variability in the range of 1.80 to 8.70 cm with an overall mean of 5.07 cm. The estimates for phenotypic coefficient of variation (22.37%),

genotypic coefficient of variation (22.26 %), heritability (99.90 %) and genetic advance as per cent mean (45.63 %) were all high for this character.

4.3.8. Width of the leaf (cm)

Width of the leaf showed a wide variation, which ranged from 1.52 to 6.66 cm and mean value for this trait was 3.94 cm. High estimates of phenotypic coefficient of variation (22.13%), genotypic coefficient of variation (22.04 %) was recorded. The estimates for heritability (99.20%) and genetic advance as per cent mean (45.21%) were also found to be high.

4.3.9. Depth of the sinus (cm)

The mean of this character was 1.26 with wide range of variation between 0.23 and 2.78. The estimates for phenotypic coefficient of variation (34.85%), genotypic coefficient of variation (34.37 %), heritability (97.30 %) and genetic advance as per cent mean (69.83 %) were high for this character.

4.3.10. Internodal length (cm)

The minimum and maximum values for this trait were 0.42 and 4.01 with an overall mean of 1.84. The high phenotypic coefficient of variation and genotypic coefficient of variation of 34.47 per cent and 34.14 per cent, respectively were exhibited for this trait. The heritability observed was 98.10 per cent with high genetic advance of 69.66 as per cent of mean.

4.3.11. Days to first flower bud appearance

This character showed a wide range of variation varying from 48.99 to 133.59 days in the collection with an overall mean of 78.71 days. The phenotypic coefficient of variation and genotypic coefficient of variation were 15.89 and 15.72 per cent respectively. Heritability estimate was very high (97.90%) with high expected genetic advance (32.06%) as per cent of mean.

4.3.12. Days to 50% flowering

The variability observed for days to 50 per cent flowering was high, as reflected by its wide range from 53.97 days to 138.73 days with an average value of 92.51 days.

The phenotypic coefficient of variation and genotypic coefficient of variation were moderate being 12.24 per cent and 12.15 per cent, respectively. The magnitude of heritability estimated was very high (98.50%) with high expected genetic advance (GAM) of 24.83 as per cent of mean.

4.3.13. Inflorescence width (cm)

The range observed for width of inflorescence was 18.94 to 71.79 days with the average of 37.29 days. This trait showed high phenotypic coefficient of variation (25.55%) and genotypic coefficient of variation (25.50%) values and also high heritability percentage (99.60%) with high genetic advance as per cent of mean (52.41%).

4.3.14. Spray length (cm)

Spray length recorded wide variation in the range of 7.29 to 31.84 cm with an overall mean of 16.80 cm. Moderate estimates for phenotypic coefficient of variation (19.84 %) and genotypic coefficient of variation (19.79 %) were recorded for the trait, while the estimate for heritability (99.50%) and genetic advance as per cent mean (40.68 %) were found to be high for this character.

4.3.15. Bud width (mm)

The chrysanthemum entries showed high variability for flower bud width with a range of 4.27 to 18.59 cm and mean of 9.92 cm. All the genetic parameters viz., phenotypic coefficient of variation (28.25%), genotypic coefficient of variation (27.98 %) the estimate for heritability (98.00 %) and genetic advance as per cent mean (57.06 %) were high for this character.

4.3.16. Average flower weight (g)

Average flower weight ranged between 0.61 to 9.0 g with the mean value of 2.49 g for the chrysanthemum genotypes studied. The character recorded high estimates for all variability parameters studied, viz., phenotypic coefficient of variation (66.69 %), genotypic coefficient of variation (66.55 %), heritability (99.60 %) and genetic advance as per cent mean (136.81 %).

4.3.17. Flower diameter (cm)

The flower diameter exhibited a wider variation, which ranged from 2.10 to 8.89 cm with an overall mean of 5.0 cm. The trait revealed high phenotypic coefficient of variation (28.44 %) and high genotypic coefficient of variation (28.39 %). The estimates for heritability recorded was high (99.7 %) with high genetic advance as per cent mean (58.38 %).

4.3.18. Peduncle length (cm)

The overall mean of the character was 5.56 cm with the variability ranging from 2.56 to 10.14 cm. The estimate for phenotypic coefficient of variation (25.34%) and genotypic coefficient of variation (25.29%) was found to be high for this character. The estimates for heritability (99.60 %) and genetic advance as per cent mean (51.99%) were also found to be high for this character.

4.3.19. Peduncle diameter (mm)

The peduncle diameter among the chrysanthemum genotypes showed wide variation ranging from 0.88 to 4.73 mm with the average value of 2.32 mm. The genetic parameters viz., phenotypic coefficient of variation (29.09 %), genotypic coefficient of variation (28.82 %), heritability (98.20 %) and genetic advance as per cent mean (58.82 %) were found to be high for this character.

4.3.20. Number of ray florets/head

Average total number of ray florets per head was 170.70 with a range of 10.98 to 406.71 ray florets per head. The genotypic and phenotypic coefficients of variation were 55.74 and 55.70 per cent respectively. This trait exhibited a high heritability (99.80%) with high expected genetic advance as per cent of mean (114.65).

4.3.21. Length of the ray floret (cm)

Wide range of variations (0.68 to 5.39 cm) was observed for this character with an overall mean of 2.66 cm in the chrysanthemum germplasm. The phenotypic coefficient of variation and genotypic coefficient of variation were 33.28 and 32.89 per cent respectively. This trait exhibited a very high heritability (97.70%) with high per cent mean genetic advance (66.97).

4.3.22. Width of the ray floret (cm)

The genotypes exhibited a wide range of variability for this trait with overall mean of 0.67 cm. The range in the collection varied from 0.15 to 1.54 cm. The per cent of genotypic and phenotypic coefficient of variations for this trait were 31.65 and 31.73 respectively. This trait exhibited a high heritability (99.5%) with expected per cent mean genetic advance (65.03).

4.3.23. Ratio of ray floret length : width

The variability observed for ratio of ray floret length : width was high, as reflected by its wide range from 1.37 to 24.0 with an average value of 4.23. The Phenotypic coefficient variation and genotypic coefficient variation were high being 45.78 per cent and 45.54 per cent, respectively. The magnitude of heritability estimated was very high (99.0 %) with high expected genetic advance of 93.32 as per cent of mean.

4.3.24. Disc diameter (cm)

The mean disc diameter of chrysanthemum flower was 1.28 cm with a range of 0.00 to 3.34 cm. The Phenotypic coefficient variation (51.38%) and genotypic coefficient variation (51.37%) values were high. The trait had a high heritability of 100 per cent and high expected per cent mean genetic advance (105.80 %).

4.3.25. Number of disc florets/head

Overall mean for number of disc florets/head among the genotypes was 109.32 with a wide range of variation from zero number of disc florets to as high as 300.64. Genotypic and phenotypic coefficient of variations were 60.50 and 60.51 per cent respectively . This trait showed very high heritability (100 %) with a high per cent mean of genetic advance (124.61).

4.3.26. Length of disc floret (cm)

Average length of disc floret was 0.70 with a range of zero to 1.76 cm. The phenotypic and genotypic coefficient of variation were 48.96 and 48.94 per cent respectively. This trait exhibited a high heritability (99.90%) with high expected genetic advance as per cent of mean (100.78).

4.3.27. Number of flowers/spray

The number of flowers per spray showed high variability with the range of 2.95 to 23.30 and with the average value of 7.60. The estimate for phenotypic coefficient of variation (41.23 %) and genotypic coefficient of variation (40.34 %) was found to be high for this character. The estimates for heritability (95.70 %) and genetic advance as per cent mean (81.32 %) were also found to be high for this character.

4.3.28. Number of flowers/plant

The genotypes exhibited a wide range of variation for this character with a mean of 101.77. The range extended from the minimum of 5.81 to maximum of 298.54 flowers per plant. The estimates for phenotypic and genotypic coefficient of variation were 55.21 and

54.87 respectively. The magnitude of heritability for this trait was very high (98.80%) with high genetic advance as per cent mean (112.35).

4.3.29. Duration of flowering

The duration of flowering ranged from as less as 36.22 to as high as 70.35 days with a mean of 45.35 days. The estimate for phenotypic coefficient of variation (9.58 %) and genotypic coefficient of variation (9.23%) was found to be low for this character. However, the estimates for heritability (92.90 %) was very high and genetic advance as per cent mean (18.33%) was found to be moderate for this character.

4.3.30. Number of suckers/plant

The range observed for number of suckers per plant was zero to 54.46 with a mean of 15.0. The character showed high phenotypic coefficient of variation (69.85 %) and high genotypic coefficient of variation (69.7 %) with very high heritability percentage (99.6 %) and high genetic advance as per cent mean (143.28 %).

4.4 CORRELATION STUDIES:

The study of relationship between the chrysanthemum flower yield and its components is very important for selection of yield improvement. In general, the values of correlation at genotypic level were higher than the phenotypic correlations indicating that there is strong inherent association between the various characters studied. The analysis of phenotypic (P) and genotypic correlation(G) of yield and yield components were worked out for the fourteen quantitative characters using mean data generated from 104 genotypes raised during *kharif* 2010-11 and 2011-12 which are presented in Table 11.

4.4.1. Correlation of yield components with flower yield per plant

A significant positive correlation both at genotypic and phenotypic levels was recorded between flower yield and plant attributes viz., plant spread at both the directions, number of primary branches per plant, spray length and number of flowers per spray (Table

11 and Fig. 5). The magnitude of correlation with flower yield was highest in case of number of flowers per spray with correlation values of ($r = 0.5903$ and $r = 0.6017$) at phenotypic and genotypic levels respectively followed by characters viz., plant spread in the North- South direction ($r_p = 0.5638$, $r_g = 0.5676$) , plant spread in the East - West direction ($r_p = 0.4594$, $r_g = 0.4645$) , number of primary branches per plant ($r_p = 0.4365$, $r_g = 0.4387$) and length of the spray ($r_p = 0.2267$, $r_g = 0.2301$).

Other characters like days to first flower bud appearance, days to 50% flowering and duration of flowering recorded positive but non-significant correlation with flower yield both at genotypic and phenotypic levels. On the other hand characters like bud width ($r_p = -0.4716$, $r_g = -0.4737$) , average flower weight ($r_p = -0.2903$, $r_g = -0.2917$) and flower diameter ($r_p = -0.4839$, $r_g = -0.4857$) showed significant but negative correlations with flower yield both at phenotypic and genotypic levels.

4.4.2. Association among yield components:

4.4.2.1. Plant height

At both phenotypic and genotypic levels, plant height recorded a positive significant correlation with plant spread East-West in direction ($r_p = 0.2877$, $r_g = 0.2935$), plant spread in North-South direction ($r_p = 0.2405$, $r_g = 0.2410$), leaf length ($r_p = 0.2470$, $r_g = 0.2526$), days to first flower bud appearance ($r_p = 0.1755$, $r_g = 0.1759$), days to 50% flowering ($r_p = 0.1876$, $r_g = 0.1895$), spray length ($r_p = 0.3128$, $r_g = 0.3166$) and bud width ($r_p = 0.4559$, $r_g = 0.4621$), average flower weight ($r_p = 0.3763$, $r_g = 0.3789$) and flower diameter ($r_p = 0.3205$, $r_g = 0.3232$). While it showed negative association with number of primary branches per plant and number of flowers per spray.

4.4.2.2. Plant spread in E-W direction

The character under consideration had high significant positive association with plant spread in N-S direction ($r_p = 0.8158$, $r_g = 0.8179$), number of primary branches per plant ($r_p =$

0.4033, $r_g=0.4040$), days to commencement of flowering ($r_p= 0.2886$, $r_g=0.2927$), days to 50% flowering ($r_p= 0.3140$, $r_g=0.3190$), spray length ($r_p= 0.5028$, $r_g=0.5055$), number of flowers per spray ($r_p= 0.5226$, $r_g=0.5338$) and flower yield ($r_p= 0.4594$, $r_g=0.4645$) at both phenotypic and genotypic levels. It exhibited positive but non-significant association with leaf length, leaf width, average flower weight and duration of flowering. Non-significant and negative correlation was observed only with bud width and diameter of flower at both the levels.

4.4.2.3. Plant spread in N-S direction

Highly significant positive correlations were observed between plant spread in N-S direction and number of primary branches per plant ($r_p= 0.4425$, $r_g=0.4435$), days to first flower bud appearance ($r_p= 0.2427$, $r_g=0.2433$), days to 50% flowering ($r_p= 0.2373$, $r_g=0.2384$), spray length ($r_p= 0.4112$, $r_g=0.4134$), number of flowers per spray ($r_p= 0.4948$, $r_g=0.5055$) and flower yield per plant ($r_p= 0.5638$, $r_g=0.5676$). However, this character had non-significant but positive correlation with leaf length ($r_p= 0.0241$, $r_g=0.0241$) and duration of flowering ($r_p= 0.0295$, $r_g=0.0297$). Results further revealed that it showed non-significant and negative correlation with leaf width ($r_p= -0.0608$, $r_g= -0.0624$), bud width ($r_p= -0.1384$, $r_g= -0.1418$), flower weight ($r_p= -0.0543$, $r_g= -0.0548$) and flower diameter ($r_p= -0.1499$, $r_g= -0.1504$).

4.4.2.4. Number of primary branches/ plant

The relationship of number of primary branches per plant with number of flowers per spray ($r_p= 0.2787$, $r_g=0.2853$) and flower yield per plant ($r_p= 0.4365$, $r_g=0.4387$) was positive and highly significant at 0.5% level of significance as revealed from the correlation coefficients values at both phenotypic and genotypic levels. The character exhibited positive but non-significant association with spray length ($r_p= 0.0921$, $r_g=0.0924$) and duration of flowering ($r_p= 0.0566$, $r_g=0.0607$).

The phenotypic and genotypic correlation showed negative and highly significant association with bud width ($r_p = -0.2866$, $r_g = -0.2890$), average flower weight ($r_p = -0.2309$, $r_g = -0.2316$) and flower diameter ($r_p = -0.3066$, $r_g = -0.3067$). Further it also showed non-significant and negative correlation with leaf length ($r_p = -0.0511$, $r_g = -0.0518$) and leaf width ($r_p = -0.0877$, $r_g = -0.0884$), days to commencement of flowering ($r_p = -0.0094$, $r_g = -0.0096$) and days to 50% flowering ($r_p = -0.0644$, $r_g = -0.0651$).

4.4.2.5. Leaf length

At both phenotypic and genotypic level, leaf length showed positive and highly significant correlation with leaf width ($r_p = 0.8618$, $r_g = 0.8713$), bud width ($r_p = 0.3293$, $r_g = 0.3322$), average flower weight ($r_p = 0.3384$, $r_g = 0.3405$), flower diameter ($r_p = 0.4069$, $r_g = 0.4095$) and duration of flowering ($r_p = 0.2237$, $r_g = 0.2429$). It exhibited positive and non-significant relationship with days to 50% flowering ($r_p = 0.0192$, $r_g = 0.0214$) and spray length ($r_p = 0.0288$, $r_g = 0.0298$). Further, the results revealed that days to first flower bud appearance ($r_p = -0.0135$, $r_g = -0.0137$) number of flowers per spray ($r_p = -0.0985$, $r_g = -0.1003$) and flower yield per plant ($r_p = -0.0756$, $r_g = -0.0779$) showed non-significant and negative association with this trait.

4.4.2.6. Leaf width

Leaf width expressed highly significant positive correlation with average flower weight ($r_p = 0.2753$, $r_g = 0.2780$) and flower diameter ($r_p = 0.2984$, $r_g = 0.3014$), while it was significant with bud width ($r_p = 0.2165$, $r_g = 0.2198$) and duration of flowering ($r_p = 0.1843$, $r_g = 0.1871$) at both phenotypic and genotypic levels. Non-significant and negative correlation was noticed with days to first flower bud appearance ($r_p = -0.0798$, $r_g = -0.0833$), days to 50% flowering ($r_p = -0.0696$, $r_g = -0.0721$), spray length ($r_p = -0.0425$, $r_g = -0.0429$), number of flowers per spray ($r_p = -0.1145$, $r_g = -0.1173$) and flower yield per plant ($r_p = -0.0985$, $r_g = -0.0988$).

4.4.2.7. Days to first flower bud appearance:

The character expressed highly significant positive association with days to 50% flowering ($r_p = 0.9172$, $r_g = 0.9312$), moderately significant with spray length ($r_p = 0.2395$, $r_g = 0.2454$) and number of flowers per spray ($r_p = 0.2232$, $r_g = 0.2320$) and low significant association with average flower weight ($r_p = 0.2029$, $r_g = 0.2037$).

Further, bud width ($r_p = 0.1277$, $r_g = 0.1346$) and flower yield per plant ($r_p = 0.1396$, $r_g = 0.1468$) showed non-significant correlation with this trait in positive direction whereas for flower diameter ($r_p = -0.0148$, $r_g = -0.0173$) and duration of flowering ($r_p = -0.0544$, $r_g = -0.0743$) it was negatively correlated.

4.4.2.8. Days to 50% flowering

Positive and highly significant correlation of days to 50% flowering was observed with spray length ($r_p = 0.2752$, $r_g = 0.2765$) and moderate association with average flower weight ($r_p = 0.2626$, $r_g = 0.2651$) and number of flowers per spray ($r_p = 0.2280$, $r_g = 0.2356$) and low significant correlation with bud width ($r_p = 0.1967$, $r_g = 0.1989$). However, it showed non-significant correlation with flower diameter ($r_p = 0.0359$, $r_g = 0.0376$) and flower yield per plant ($r_p = 0.1245$, $r_g = 0.1281$) in positive direction, while it was in negative direction with duration of flowering ($r_p = -0.0640$, $r_g = -0.0725$).

4.4.2.9 Spray length

Spray length displayed positive and significant correlation with number of flowers per spray ($r_p = 0.4174$, $r_g = 0.4289$), flower yield per plant ($r_p = 0.2267$, $r_g = 0.2301$) and average flower weight ($r_p = 0.2156$, $r_g = 0.2157$). It also showed positive but non-significant association with bud width ($r_p = 0.1034$, $r_g = 0.1043$), flower diameter ($r_p = 0.0092$, $r_g = 0.0093$) and duration of flowering ($r_p = 0.1003$, $r_g = 0.1067$).

4.4.2.10. Bud width

Both genotypically and phenotypically bud width showed positive and highly significant correlation with average flower weight ($r_p = 0.6361$, $r_g = 0.6409$) and flower diameter ($r_p = 0.6460$, $r_g = 0.6491$), while highly significant and negative correlation with number of flowers per spray ($r_p = -0.2323$, $r_g = -0.2448$) and flower yield per plant ($r_p = -0.4716$, $r_g = -0.4737$). It exhibited positive non-significant correlation only with duration of flowering ($r_p = 0.0951$, $r_g = 0.0971$).

4.4.2.11. Average flower weight

The association between average flower weight and flower diameter was positive and highly significant ($r_p = 0.5738$, $r_g = 0.5753$), while it was significant and negatively correlated with number of flowers per spray ($r_p = -0.2034$, $r_g = -0.2077$) and flower yield per plant ($r_p = -0.2903$, $r_g = -0.2917$). It exhibited positive non-significant correlation only with duration of flowering ($r_p = 0.0291$, $r_g = 0.0298$).

4.4.2.12. Flower diameter

Flower diameter exhibited a negative and highly significant correlation at both phenotypic and genotypic levels with number of flowers per spray ($r_p = -0.3385$, $r_g = -0.3449$) and flower yield per plant ($r_p = -0.4839$, $r_g = -0.4857$) whereas positive significant correlation with duration of flowering ($r_p = 0.1726$, $r_g = 0.1773$) at 5% level of significance.

4.4.2.13. Number of flowers per spray

Highly significant positive correlation was noticed between number of flowers per spray and flower yield per plant ($r_p = 0.5903$, $r_g = 0.6017$), while the positive association of this trait with duration of flowering was non-significant ($r_p = 0.0608$, $r_g = 0.0625$).

4.4.2.14. Duration of flowering :

Duration of flowering observed positive and non-significant relationship with flower yield per plant ($r_p = 0.0256$, $r_g = 0.0368$).

4.5. PATH COEFFICIENT ANALYSIS

Path coefficient analysis was carried out to partition the correlation coefficients into direct and indirect effects. This analysis was carried out at phenotypic and genotypic level considering flower yield per plant as dependent character and its attributes viz., plant height, plant spread in both the directions, number of primary branches per plant, leaf length and width, days to first flower bud appearance, days to 50% flowering, spray length, bud width, average flower weight, flower diameter, number of flowers per spray and duration of flowering as independent characters. The residual effect was considered as influence of other variables that were not studied and the results were presented in Table 12 & 13 and illustrated in fig 6 & 7. In general genotypic path effects were higher than phenotypic effects.

4.5.1. Direct effects

Among the fourteen characters studied, eight characters showed positive direct effects on the flower yield per plant at both phenotypic and genotypic levels. Plant spread in N-S direction (0.3916, 0.3918) recorded the high direct effect on flower yield per plant followed by number of flowers per spray (0.3176, 0.3216), while low effect by leaf length (0.1787, 0.1879), days to 50% flowering (0.1152, 0.1218, number of primary branches per plant (0.1066, 0.1081) and negligible effect by average flower weight (0.0781, 0.0798) and duration of flowering (0.0337, 0.0423). Plant height exhibited positive negligible direct effect (0.0010) at phenotypic level, while it had a negative negligible direct effect (-0.0047) at genotypic level. Plant spread in E-W direction (-0.1397, -0.1410), leaf width (-0.0762, -0.0835), days to flower commencement (-0.0694, -0.0735), spray length (-0.0202, -0.0217), bud width (-0.2759, -0.2804) and flower diameter (-0.2275, -0.2318) showed negative direct effect on the flower yield per plant at both phenotypic and genotypic levels.

4.5.2. Indirect effects:

4.5.2.1. Plant height

Plant height showed positive negligible indirect effect on flower yield per plant through all other traits studied except the number of primary branches per plant which showed negative effect at phenotypic level (-0.0001). Whereas at genotypic level, it had negative negligible indirect effect on flower yield per plant via other characters except the number of primary branches per plant (0.0003) and number of flowers per spray (0.0001) which showed positive indirect effect.

4.5.2.2. Plant spread in E-W direction

The negative and negligible indirect effect was showed by this trait on flower yield per plant through the quantitative characters except bud width (0.0077, 0.0082) and flower diameter (0.0182, 0.0185) at both phenotypic and genotypic levels. While its indirect effect via plant spread in N-S direction was low and negative (-0.1140, -0.1156) at both the levels.

4.5.2.3. Plant spread in N-S direction

A high indirect positive effect of plant spread in N-S direction both at phenotypic and genotypic level was observed on flower yield per plant via plant spread in E-W direction (0.3196, 0.3210). It had low positive indirect effect via number of flowers per spray (0.1938, 0.1979), number of primary branches per plant (0.1734, 0.1737) and spray length (0.1611, 0.1619) and negligible positive effect via plant height, leaf length, days to flowering and duration of flowering at both the levels. However it showed negative indirect effect through leaf width, bud width, average flower weight and flower diameter.

4.5.2.4. Number of primary branches per plant:

Number of primary branches per plant exerted negligible positive indirect effect on flower yield per plant via plant spread in both E-W and N-S directions (0.0431, 0.0436 and 0.0473, 0.0479 respectively), spray length (0.0099, 0.0100), number of flowers per spray (0.0301, 0.0304) and duration of flowering (0.0061, 0.0065). At both the levels it

noticed negligible negative indirect effect through plant height, leaf length and width, days to first flower bud appearance and days to 50% flowering, bud width, average flower weight and flower diameter.

4.5.2.5. Leaf length:

At both phenotypic and genotypic levels leaf length showed high positive indirect effect on dependent character flower yield per plant only through leaf width (0.1540, 0.1637). While the positive effect via plant height, plant spread in both the directions, days to 50% flowering, spray length, bud width, average

flower weight, flower diameter and duration of flowering was found to be negligible. The negative indirect effect on flower yield per plant was through number of primary branches per plant, day to first flower bud appearance and number of flowers per spray.

4.5.2.6. Leaf width

The highest positive indirect effect of this trait on flower yield was observed via number of flowers per spray at both phenotypic and genotypic levels (0.0087, 0.0098) followed by number of primary branches per plant (0.0067, 0.0074), days to first flower bud appearance (0.0061, 0.0070), days to 50% flowering (0.0053, 0.0060), plant spread in N-S direction (0.0046, 0.0052) and spray length (0.0032, 0.0036). Further it influenced negatively through plant height, plant spread in E-W direction, leaf length, bud width, average flower weight, flower diameter and duration of flowering.

4.5.2.7. Days to first flower bud appearance:

Days to first flower bud appearance contributed negligible positive indirect effect via number of primary branches per plant (0.0007, 0.008), leaf length (0.0009, 0.0010), leaf width (0.0055, 0.0061), flower diameter(0.0010, 0.0013) and duration of flowering

(0.0038, 0.0055). The other quantitative characters showed negligible and negative indirect effect on flower yield per plant.

4.5.2.8. Days to 50% flowering:

A low positive indirect influence on flower yield per plant was noticed via days to first flower bud appearance (0.1056, 0.1134) at both phenotypic and genotypic level. It had contributed negligible positive indirect effect by all other quantitative characters except number of primary branches per plant, leaf width and duration of flowering which showed negative effect.

4.5.2.9. Spray length

At both phenotypic and genotypic level, the indirect effect of spray length to flower yield via all characters was negative and negligible including the direct effect except the leaf width. At both the levels the indirect effect via leaf width was positive and low (0.0009).

4.5.2.10. Bud width

The indirect effect via plant spread in both the directions, number of primary branches per plant and number of flowers per spray was positive and negligible. A low negative indirect effect was through plant height, average flower weight and flower diameter while the effect was negligible via leaf length and width, days to first flower bud appearance, days to 50% flowering, spray length and duration of flowering.

4.5.2.11. Average flower weight

The indirect contribution of average flower weight to flower yield per plant was positive and negligible through all the growth and floral characters except the plant spread in N-S direction, number of primary branches per plant and number of flowers per spray whose effect is negative at both phenotypic and genotypic levels.

4.5.2.12. Flower diameter:

Flower diameter exerted negligible positive indirect effect via plant spread in both the directions, number of primary branches per plant (0.0697, 0.0711), days to first flower bud appearance (0.0034, 0.0040) and number of flowers per spray (0.0770, 0.0779) while the other traits showed negative indirect effect to flower yield per plant.

4.5.2.13. Number of flowers per spray:

Along with the high direct positive effect, a low positive indirect effect via plant spread in E-W direction (0.1660, 0.1717) and N-S direction (0.01571, 0.1626) and spray length (0.1330, 0.1374) contributed to flower yield followed by number of primary branches per plant, days to flowering and duration of flowering. While the characters plant height, leaf length and width, bud width, average flower weight and flower diameter showed negative effect.

4.5.2.14. Duration of flowering:

At both phenotypic and genotypic levels, duration of flowering influenced the flower yield indirectly through all the characters by positive and negligible means except the days to first flower bud appearance and days to 50% flowering which showed negative effect.

4.6 GENETIC DIVERGENCE

The genetic divergence among 104 genotypes of chrysanthemum was quantitatively assessed by adopting Mahalanobis's D^2 statistics and Principal Component Analysis, while the divergence at molecular level was studied using different markers.

4.6.1 MAHALANOBIS D^2

The mean values for different quantitative characters were utilized for working out genetic distance between pairs of genotypes. The D^2 values between any two varieties were calculated as the sum of squares of the differences between the mean values of all the thirty

quantitative characters and used for final grouping of the genotypes. Estimation of D^2 values was carried out for 104 genotypes.

4.6.1.1 Grouping of genotypes into different clusters (D^2 analysis)

In Chrysanthemum, 104 genotypes were grouped into eleven clusters by treating estimated D^2 values as the square of the generalized distance using Ward's minimum variance method. The distribution of genotypes into eleven clusters is shown in Table 14 and illustrated in figure 8. The clustering pattern revealed, cluster VII was the largest with 31 genotypes, followed by cluster III with 15 genotypes and cluster I having 12 genotypes. Clusters IV, VIII and X each had nine genotypes in them, while the XI cluster had six genotypes followed by clusters V, IX each bearing five genotypes. The remaining cluster VI had two genotypes whereas the cluster II was solitary with only one genotype.

4.6.1.2. Mean intra and inter cluster distances

The average D^2 values of intra and inter cluster distances among eleven clusters are given in Table 15 and illustrated in Fig 9. The intra and inter cluster D^2 values among the eleven clusters showed that, the intra cluster D^2 values ranged from 0.00 to 92.10. The cluster VI comprised of two genotypes revealed the maximum genetic diversity with intra cluster distance of 92.10 among themselves, followed by cluster V ($D^2 = 60.71$) and cluster IX with intra cluster divergence of 51.75. Since cluster II is represented by single genotype, the intra cluster divergence was found to be zero.

The inter cluster D^2 values ranged from 38.92 to 195.87. As indicated by the inter cluster D^2 values of eleven clusters, the lowest inter cluster D^2 value was recorded between cluster III and IV (38.92) while the highest inter cluster D^2 value was recorded between clusters VI and IX (195.87).

The nearest and farthest clusters from each cluster based on D^2 values was given in the Table 16 which revealed that Cluster III was nearest to cluster I ($D^2 = 40.13$) and

cluster VI was distant to cluster I ($D^2 = 113.05$). Cluster III exhibited intimate relation with cluster I ($D^2 = 80.02$) and wide diversity with cluster VI ($D^2 = 176.14$). While, cluster III showed close proximity with cluster IV ($D^2 = 38.92$) and similar to cluster I and II it also exhibited maximum divergence with cluster VI (89.77). In respect of cluster IV, the cluster II, with inter cluster D^2 value of 104.71 was the farthest and cluster III was nearest with the D^2 value of 38.92. Cluster V was closely related with cluster III (63.10) and was diverse from cluster II (138.73). Cluster VI showed close proximity with cluster III (89.77) and exhibited maximum divergence with cluster IX ($D^2 = 195.87$). Nearest cluster to cluster VII was cluster III (42.97) and farthest was cluster VI (128.99). Cluster VIII was closely related to cluster VII (45.41) and widely related to cluster VI (151.03). Cluster IX was nearest to cluster VIII ($D^2 = 62.66$) and was distant to cluster VI ($D^2 = 195.87$). With regards to cluster X, it was nearest to cluster VII (50.08) and farthest to VI (172.54). Finally, for cluster XI, the nearest and farthest clusters were cluster X (59.52) and cluster VI (146.10) respectively.

4.6.1.3.1. Relative contribution of different characters towards divergence

Difference in proportion of contribution of each character and relative ranking of various characters to total divergence was furnished in Table 17. Among all the characters, number of ray florets per head contributed the maximum of 51.81% to the diversity by taking first rank *i.e* 2775 times out of 5356.21 total number of combinations, followed by number of disc florets per head (26.10 %

with 1398 times ranked first) and flower yield per plant (21.06 % with 1128 times ranked first). The characters *viz.*, days to first flower bud appearance, days to 50 per cent flowering, number of suckers per plant, inflorescence width, duration of flowering and plant height contributed 34.0, 9.0, 7.0, 2.0, 2.0 and 1.0 per cent respectively to the genetic divergence in decreasing order, while other characters like plant spread in both directions, number of branches per plant, petiole length, terminal lobe length, leaf lamina

length and width, depth of sinus, internodal length, spray length, bud width, mean flower weight, flower diameter, peduncle length and diameter, ray floret length and width, ratio of ray floret length and width, disc diameter, disc floret length and number of flowers per spray did not contribute towards diversity.

4.6.1.3.2. Mean performance of characters in clusters

The mean cluster values of different traits for eleven clusters were summarized in Table 18. A large variation in mean performance of genotypes in different clusters was observed. The genotypes of cluster XI recorded mean maximum plant height (49.90 cm), while cluster X observed minimum mean plant height of 29.99 cm. With regard to plant spread in both the directions, the highest plant spread in E-W (49.16 cm) and N-S (56.68 cm) directions was noticed in cluster VI and lowest plant spread was recorded in cluster IX. The cluster means for number of primary branches per plant ranged from 5.57 to 30.92. The highest number of primary branches per plant was recorded in the genotype in cluster II (30.92). The lowest mean value for number of primary branches per plant was registered by the cluster X (5.57).

The genotypes of cluster IX (3.14 cm) exhibited maximum petiole length, whereas, the lowest values were noticed in cluster VI (1.27 cm). Cluster V and cluster II recorded highest and the lowest terminal lobe length of 3.15 cm and 1.00 cm respectively. The lines belonging to cluster XI exhibited the highest leaf lamina length (6.85 cm) and leaf lamina width (5.08 cm), while the cluster VI exhibited the lowest leaf lamina length (3.82 cm) and width (2.26 cm). The cluster means for internodal length ranged from 2.62 to 1.16 cm recorded by the clusters VIII and IV respectively. In case of days to first flower bud appearance and days to 50 % flowering, the solitary genotype in cluster II were earliest with cluster mean of 48.99 and 53.97 respectively, while the genotypes of cluster VI were late in flowering. With respect to inflorescence width, cluster VI had the

highest mean value (65.46 cm) while, cluster II had lower mean value of 18.99 cm. Similarly the genotypes in cluster VI registered maximum spray length (26.05 cm) and lowest mean value for the same was recorded by the only genotype in cluster II (13.26 cm).

The highest mean bud width (1.42 cm) was shown by the genotypes in the cluster X, while cluster I covered the genotypes bearing lowest bud width (0.53 cm). Regarding average flower weight, cluster XI and cluster I recorded highest (6.49 g) and lowest (0.95 g) average flower weight respectively. The cluster means for flower diameter ranged from 7.66 to 2.59 cm. The highest flower diameter was recorded by cluster X (7.66 cm), while the lowest mean value for flower diameter was registered by the cluster VI (2.59 cm). In case of peduncle length and peduncle diameter the highest cluster mean values of 8.11 and 3.60 respectively were recorded by the genotypes in cluster X. The lowest peduncle length (2.82 cm) and diameter (1.56 cm) were recorded by the cluster II bearing single genotype. The highest total number of ray florets per head (243.92) was shown by the cluster X, whereas, the genotypes in cluster IV recorded the lowest number of ray florets per head (77.97). Regarding the ray floret length, the cluster means ranged from 4.03 to 1.06cm which were recorded by the genotypes of cluster IX and cluster VI respectively. When the width of ray florets were observed, the genotypes in cluster X showed the highest width (1.16 cm) and genotypes in the cluster VI (0.39cm) recorded the lowest values for the same. The lines in cluster IX (10.48cm) and cluster I (2.58cm) exhibited the highest and lowest ray floret length width ratio respectively. The maximum cluster mean values for disc diameter and number of disc florets per head were observed in cluster IV with the values of 2.44 cm and 182.17 respectively. While the lowest mean values for the same disc characters were noticed in the clusters IX (0.65 cm) and cluster II bearing

single genotype (80.85). With regards to disc floret length, it ranged from 1.20 to 0.26 cm which were noticed in the cluster IV and cluster IX respectively.

The important yield contributing characters viz., the number of flowers per spray and number of flowers per plant were maximum in cluster VI with the mean values of 14.29 and 203.59 respectively. The solitary genotype in cluster II recorded lowest number of flowers per spray (2.95) while, the minimum number of flowers per plant in cluster X was 22.82. With respect to duration of flowering, cluster VIII had the highest mean value of 51.85, while the cluster II had lower mean value for duration of flowering (40.35 days).

Finally, the highest and the lowest suckering habit was noticed in the genotypes included in cluster VI (29.78) and cluster II (2.86) respectively.

4.6.2 PRINCIPAL COMPONENT ANALYSIS

In principal component analysis, the number of variables was reduced to a linear expression called the principal components and these accounted for most of the variation produced.

Principal component analysis (PCA) for quantitative morphological characters were performed adopting correlation matrix method. The character loading values for principal components represented the weights defining the contribution of different characters for the respective principal components. Further, the loadings signs (+ / -) are indicative of the direction of contribution, similar to that of regression coefficients.

The principal components, eigen values, per cent variability, cumulative per cent of variability and component loading of different morphological characters studied were furnished in Table 19. The principal components with eigen values less than one were considered to be non-significant as per the procedure. In the present investigation, the first eight principal components with eigen values more than one contributed to 73.23 per cent of cumulative variability among the 104 genotypes of chrysanthemum evaluated for 30

morphological characters. The first principal component accounted for 22.71 per cent of variability while the second and third accounted for 15.91 and 9.04 per cent of total variability, respectively. The percent of variability from fourth to ninth principal component accounted for 8.02, 5.91, 4.38, 3.73, 3.53 and 2.85 in decreasing order respectively. All the nine principal components accounted for 76.08 per cent of cumulative variability.

Contribution of vectors towards divergence:

4.6.2.1 PC I vector:

The first principal component (PC I) contributed highest variability of 22.71%. Characters like flower diameter (0.312) and length of the ray floret (0.312) contributed maximum to the diversity followed by bud width (0.275), peduncle diameter (0.270), peduncle length (0.266), average flower weight (0.231), ray floret width (0.211), leaf length (0.200), number of ray florets per head (0.186), leaf width (0.185), petiole length (0.179), plant height (0.152), ratio of ray floret : width (0.130), terminal lobe length (0.122), internodal length (0.110), depth of the sinus (0.107) and duration of the flowering (0.056). The number of flowers per plant (-0.262), number of flowers per spray (-0.206), number of suckers per plant (-0.193), number of primary branches per plant (-0.187), plant spread in N-S direction (-0.157), inflorescence width (-0.136), plant spread in E-W direction (-0.116), disc diameter (-0.100), number of disc florets per head (-0.087), length of the disc floret (-0.080), spray length (-0.030), days to first flower bud appearance (-0.027) and days to 50% flowering (-0.014) contributed negatively towards genetic divergence.

4.6.2.2 PC II vector:

The second principal component (PC II) noticed to explain 15.91% of the variability and among the characters, inflorescence width (0.345) contributed towards maximum diversity. The plant height in E-W direction (0.324), plant height in N-S direction (0.295), depth of the sinus (0.267), days to 50% flowering (0.246), terminal lobe length (0.242), spray

length (0.236), days to first flower bud appearance (0.228), plant height (0.226), leaf length (0.217), number of flowers per spray (0.205), disc diameter (0.197), number of disc florets per head (0.170),

number of flowers per plant (0.170), leaf width (0.152) average flower weight (0.150), disc floret length (0.144), petiole length (0.136), bud width (0.115), number of primary branches per plant (0.110), number of suckers per plant (0.109), ray floret length (0.091) flower diameter (0.078), peduncle diameter (0.072), ratio of ray floret length: width (0.047), ray floret width (0.040) and duration of flowering (0.039) in descending order positively contributed towards diversity. While, only three characters i.e. number of ray florets per head (-0.081), intermodal length (-0.031) and peduncle length (-0.010) showed negative contribution towards divergence.

4.6.2.3 PC III vector:

The third principal component contributed 9.04 % variability and showed high loadings for days to 50% flowering (0.249), days to first flower bud appearance (0.239), spray length (0.215), average flower weight (0.212), ray floret width (0.187), bud width (0.182), peduncle length (0.140), plant height (0.143), peduncle diameter (0.157), disc floret length (0.100), flower diameter (0.085), plant spread in N-S direction (0.080), length of the ray floret (0.080), disc diameter (0.077), inflorescence width (0.063), number of ray florets per head (0.059), plant spread in E-W direction (0.076), number of flowers per spray (0.039) and intermodal length (0.028) in decreasing order of the elements. The characters which have contributed negatively to genetic divergence were depth of the sinus (-0.369), leaf width (-0.359), terminal lobe length (-0.324), leaf length (-0.323), petiole length (-0.285), duration of flowering (-0.163), ratio of ray floret length: width (-0.091), number of flowers per plant (-0.071), number of primary branches per plant (-0.067), number of suckers per plant (-0.060) and number of disc florets per head (-0.048).

4.6.2.4 PC IV vector

The fourth principal component explained 8.02% of variability and number of ray florets per head(0.358) and plant spread in E-W direction (0.223) contributed maximum to the diversity, followed by plant spread in N-S direction (0.212), internodal length (0.192), number of flowers per spray (0.159), duration of flowering (0.149), inflorescence width (0.119), spray length (0.119), number of flowers per plant (0.116), ratio of ray floret length: width (0.100), number of primary branches per plant (0.090), plant height (0.089), peduncle length (0.053), days to first flower bud appearance (0.021), days to 50% flowering (0.017), terminal lobe length (0.008) and peduncle diameter (0.001). While the characters, disc diameter(-0.474), number of disc florets per head (-0.434), disc floret length (-0.409), ray floret width (-0.161), average flower weight (-0.083), petiole length (-0.070), ray floret length (-0.037), bud width (-0.032), depth of the sinus (-0.019), flower diameter (-0.016), leaf width (-0.012), leaf length(-0.011) and number of suckers per plant(-0.008) contributed negatively towards the genetic divergence.

4.6.2.5 PC V vector:

The percent variability contributed by the fifth vector of principle component analysis was 5.91%. The characters that showed positive contribution to genetic divergence in descending order of contribution were ray floret width (0.359), number of suckers per plant (0.221), duration of flowering (0.211), number of flowers per plant (0.177), peduncle length (0.145), plant spread in N-S direction (0.138), disc floret length (0.127), peduncle diameter (0.122), number of primary branches per plant (0.120), spray length (0.117), flower diameter (0.111), plant spread in E-W direction (0.097), plant height (0.092), number of disc florets per head (0.091), number of flowers per spray (0.085), average flower weight (0.070), number of ray florets per head (0.040), inflorescence width (0.025), ray floret length (0.021),

bud width (0.016), leaf length (0.004) and leaf width (0.003). However, the plant characters like days to first flower bud appearance (-0.492), days to 50% flowering (-0.461), ratio of ray floret length: width (-0.325), petiole length (-0.135), disc diameter (-0.080), internodal length (-0.062), terminal lobe length (-0.046) and depth of the sinus (-0.055) showed negative contribution towards the genetic variability.

4.6.2.6 PC VI vector:

The sixth principal component explained 4.38 % of variability and showed high positive correlation for internodal length (0.532) followed by ray floret width (0.241), plant height (0.237), terminal lobe length (0.150), depth of the sinus (0.140), days to first flower bud appearance (0.089), number of primary branches per plant (0.076), spray length (0.065), leaf width (0.064), disc diameter (0.048), days to 50% flowering (0.043), plant spread in E-W direction (0.019), disc floret length (0.014) and leaf length (0.007) in decreasing order of the elements. While the other character viz., ray floret length : width (-0.512), peduncle diameter(-0.276), number of suckers per plant (-0.216), ray floret length (-0.187), number of disc florets per head (-0.175), number of flowers per spray (-0.159), number of ray florets per head(-0.125), peduncle length (-0.107), flower diameter (-0.106), number of flowers per plant (-0.098), inflorescence width (-0.069), duration of flowering (-0.055), plant spread in N-S direction (-0.040), petiole length (-0.023), bud width (-0.017) and average flower weight (-0.001) were observed to contribute negatively towards genetic divergence.

4.6.2.7 PC VII vector:

In the seventh principal component (PC 7 vector), ratio of ray floret length: width contributed the maximum (0.446) to the diversity among the characters followed by internodal length(0.386), plant height (0.284), number of disc florets per head (0.212), plant spread in N-S direction(0.185),disc diameter (0.127), duration of flowering (0.125), bud width (0.121), ray floret length (0.107), number of primary branches per plant (0.096), plant

spread in E-W direction (0.087), disc floret length (0.078), inflorescence width (0.073), peduncle length (0.062) and spray length (0.044). Negative contribution to genetic divergence was displayed by days to 50% flowering (-0.260), ray floret width (-0.245), average flower weight (-0.208), days to first flower bud appearance (-0.204), number of ray florets per head (-0.204), number of suckers per plant (-0.201), number of flowers per spray (-0.182), leaf width (-0.181), leaf length (-0.112), number of flowers per plant (-0.109), peduncle diameter (-0.080), petiole length (-0.074), depth of the sinus (-0.058), terminal lobe length (-0.022) and flower diameter (-0.015).

4.6.2.8 PC VIII vector:

The third principal component contributed 3.53 % variability and showed high loadings for number of primary branches per plant (0.433) followed by plant spread in E-W direction (0.185), plant spread in N-S direction (0.180), leaf length (0.165), leaf width (0.155), inflorescence width (0.099), peduncle diameter (0.094), average flower weight (0.070), ray floret width (0.067), ray floret length (0.059), flower diameter (0.059), petiole length (0.046), bud width (0.044), ratio of ray floret length: width (0.016) and plant height (0.009). While the characters viz., duration of flowering (-0.634), spray length (-0.275), peduncle length (-0.201), terminal lobe length (-0.172), number of suckers per plant (-0.154), depth of the sinus (-0.128), days to 50% flowering (-0.125), length of the disc floret (-0.114), days to first flower bud appearance (-0.109), number of flowers per spray (-0.094), number of flowers per plant (-0.091), internodal length (-0.076), disc diameter (-0.046), number of disc florets per head (-0.036) and number of ray florets per head (-0.023) exhibited negative contribution to genetic variability.

4.6.2.9 PC IX vector:

The percent variability contributed by the fifth vector of principle component analysis was 2.85%. The characters that showed positive contribution to genetic divergence were length of disc floret(0.419,)number of ray florets per head (0.318), spray length(0.278), peduncle diameter(0.211), leaf width(0.156), peduncle length(0.124), spread in E-W direction(0.121), number of flowers per spray (0.120), plant height(0.116), leaf length(0.101), disc diameter(0.084), petiole length(0.022), depth of the sinus(0.007) and number of primary branches per plant(0.003). The other characters that contributed negatively to genetic divergence were number of suckers per plant(-0.405), ray floret length(-0.328), width of the ray floret(-0.215), internodal length(-0.203), flower diameter(-0.186), number of flowers per plant(-0.178), average flower weight(-0.161), plant spread in N-S direction(-0.113), number of disc florets per head(-0.101), days to 50% flowering(-0.088), days to first flower bud appearance (-0.085), duration of flowering (-0.068), terminal lobe length(-0.048), ratio of ray floret length: width(-0.047), inflorescence width(-0.041) and bud width(-0.039).

A three dimensional plot is illustrated in the fig.10 depicting the spatial distribution of 104 chrysanthemum genotypes using principal component analysis score (PCA). Wider distribution of the genotypes was observed indicating the presence of considerable diversity in the germplasm lines.

4.6.3 GENETIC DIVERSITY AT MOLECULAR LEVEL

In the present study 37 chrysanthemum genotypes were studied for their genetic diversity at molecular level using 27 Random Amplified Polymorphic DNA (RAPD) markers and 10 Inter Simple Sequence Repeat (ISSR) markers.

4.6.3.1 DNA yield and purity assessment

The young, fully developed, healthy leaves of 37 chrysanthemum genotypes were used for the isolation of DNA by using the modified CTAB method. This protocol resulted

in white translucent DNA pellets that was easily solubilised in TE (Tris EDTA) buffer. The DNA obtained by this method was homogeneous and not degradable. The quality of DNA was verified by Nano-drop spectrophotometer and also by agarose gel electrophoresis. The results indicated that the isolated DNA was of good quality and had high molecular weight (Plate 14).

The DNA yield ($\eta\text{g}/\mu\text{l}$) and the OD ratio (260/280) for all the genotypes collected are evident in Table 20. The yield of genomic DNA extracted from 37 chrysanthemum genotypes ranged from 303.5 to 1556.3 $\eta\text{g}/\mu\text{l}$. The purity as determined by OD ratio varied from 1.66 to 1.94 indicating that the DNA was relatively of high purity and was suitable for PCR amplification. The highest content of DNA i.e. 1556.3 $\eta\text{g}/\mu\text{l}$ was obtained with the genotype Chandrika, while the lowest amount of DNA (303.5 $\eta\text{g}/\mu\text{l}$) was realized with Meera.

4.6.3.1.1 RAPD analysis

Thirty seven genotypes of chrysanthemum were scanned by 27 selected RAPD primers which gave better profile among 124 primers screened (Table 21) (Plate 15-28). The number of DNA fragments amplified per primer ranged from 7(OPE-15, OPG- 9, OPG-16, OPH-13, OPH-20) to 17 (OPK-19) with a mean value of 10.3 bands per primer. Similarly, a total of 278 amplicons were produced by examining the 37 genotypes of chrysanthemum with 27 RAPD primers. The number of scored polymorphic bands ranged from 6 to 17 in different primers with an average of 10.11 polymorphic fragments per primer. The amplicon sizes ranged from 350 bp to 3500 bp. A total of 278 amplified fragments were scored out of which 271 were found to be polymorphic (97.4%). All the primers except OPE-14, OPE-15, OPE-18, OPF-3, OPF-5 and OPI-18 gave highest polymorphism (100%). Maximum number of polymorphic bands in the present study were obtained with the primer OPK-19 which gave 17 polymorphic bands while the primer OPE-15 produced only 6 polymorphic bands.

The characteristic fragments generated by an array of 27 RAPD primers employed as single arbitrary primers are summarized in Table 22. The polymorphic fragments present in only one genotype were considered to be unique fragments. Out of the total 271 polymorphic fragments, six were unique to particular genotypes viz; Meera, Salora, Terry, Punjab Anuradha, Anjali and Asha (Table 22).

4.6.3.2.1 Similarity index

Banding profiles obtained with 27 primers for thirty seven genotypes of chrysanthemum were analyzed on the basis of presence(1) or absence (0) of the band. Monomorphic and minor polymorphic bands were excluded from the data sets, because monomorphic bands do not contribute to the genetic distance calculation and minor polymorphic bands may be artefacts due to differences in genomic DNA quality and other factors. Only distinct, reproducible polymorphic bands were considered for the genetic analysis. Jaccard's similarity coefficients among these genotypes were calculated to establish the genetic relationships and are presented in table 23. Genetic similarity based on Jaccard's coefficient revealed considerable level of diversity among the genotypes under the study. The genetic similarity among thirty seven chrysanthemum genotypes ranged from a coefficient of 0.174 to 0.600 with an average similarity coefficient of 0.387 among the group of genotypes studied.

In the present study, Ratlam Selection and Snow Cem were found to be most genetically similar (60.0%) followed by Akitha and Shintome with 55.3% and Terry and Salora with 55.1%. On contrary, Aparajitha and Lilith were found to be the least genetically similar (17.4%).

Among the spray type, yellow coloured varieties i.e., Aparajitha, Punjab Gold, CO-3, Raichur, Silper, Yellow Gold, Punjab Anuradha, Rekha and Chandrika, the genotype Chandrika is having least average similarity index value of 0.253 with other yellow coloured

genotypes. Among these, the genotypes Punjab Anuradha is having similarity coefficient value of 0.482 with Punjab Gold indicating the existence of high genetic similarity among these cultivars because they were selected from the same geographical region.

4.6.3.2.2 Cluster analysis

The genetic relationships of chrysanthemum genotypes were further evaluated by UPGMA cluster analysis, on RAPD data set, using a minimum variance algorithm (Fig11). It resulted in eleven clusters at a genetic similarity coefficient of 0.23 with Chandrika and Aparajitha at the extreme ends of the dendrogram.

All the 37 genotypes were grouped into four major clusters of which three genotypes were grouped in cluster I, five genotypes in cluster II, nineteen genotypes in cluster III, three genotypes in cluster IV and seven minor clusters with one genotype each in cluster V(Arka Ravi), VI(Meera), VII(Asha), VIII(Silper), IX(Autumn Joy), X(Lilith) and cluster XI (Chandrika). Genotypes in these clusters were diverged at similarity value of 0.23.

The cluster I included the three yellow coloured genotypes Aparajitha, Basanthi and Local button, while the other genotypes of this class *i.e.*, Punjab Gold, Yellow Gold, CO-3, Punjab Anuradha and Rekha which includes decorative and double Korean types were included in the cluster II.

The genotypes Shaffoli, Shintome, Akitha, Anjali, Terry, Salora, PAU-B-107, Red Stone, Geetanjali, Neelima, Red Gold, IIHR-13, Flirtation, Jaya, Kalyani Mauve, Farr, Harvest House, Mother Teresa and Pusa Centenary were assigned to cluster III. In this group Shaffoli and Pusa Centenary were straddling the extremes. The similarity ranged from a lowest of 0.42 between Mother Teresa and Pusa Centenary to the highest of 0.56 between Akitha and Shintome and Terry and Salora.

The three genotypes in the cluster IV includes Raichur, Snow Cem and Ratlam Selection with the similarity index of 0.43. The white coloured, decorative type of genotypes

Snow Cem and Ratlam Selection are included in one group with the similarity index of 0.6 indicating that they are closely related to each other.

As shown in the dendrogram, the Clusters V, VI, VII, VIII, IX, X and XI had one genotype each with Arka Ravi, Meera, Asha, Silper, Autumn Joy, Lilith and Chandrika respectively. Among these solitary clusters, Chandrika and Lilith were found to be more diverse with low similarity index value of 0.23. Asha, Silper and Autumn Joy constitutes three separate clusters at more or less same level of similarity (0.32) .

4.6.3.2.3 Principal component analysis for RAPD analysis

Principal Component Analysis was carried out based on the Jaccard's similarity matrix (Table 24). The First component accounts for 38.64% of all changes and second component accounted for 3.35% of principal changes. In this manner, totally 5 principal components accounted for 50.68% of changes. The principal component analysis was performed based on molecular data of 27 RAPD markers to visualize the genetic relatedness among the chrysanthemum genotypes in detail. The description of the data was done using two dimensional and three dimensional pictorial graph and the same is represented in Fig.12 & 13. From the graph, it is evident that the chrysanthemum genotypes were dispersed on the PC plot, which is a reflection of moderate genetic base. The results of PCA show a clear cut separation. However, as depicted in figure some of the genotypes appear to be overlapping with each other depicting high similarity in these genotypes. It was clear from the analysis that the results obtained from the dendrogram were repeated in the PCA, which strengthened the ability and accuracy of the RAPD analysis applied to chrysanthemum genotypes in the present study.

According to the PCA, Chandrika, Lilith, Autumn Joy and Silper were separated from rest of the genotypes and quite distinct from others forming

separate entities and the results obtained from PCA are in agreement with the dendrogram generated by UPGMA method.

4.6.3.3 ISSR ANALYSIS

The 37 genotypes were also studied for their genetic diversity at molecular level using Inter Simple Sequence Repeat (ISSR) markers. Forty six ISSR oligonucleotides were used for amplification of all the thirty seven genotypes. Among the forty six ISSR primers used in the preliminary analysis, only ten primers produced the polymorphic bands. The details of the 10 ISSR primers used for assessing the molecular diversity among 37 genotypes of chrysanthemum are furnished in table 25. All the primers were initially subjected to gradient PCR technique in order to identify the optimum annealing temperatures. These ISSR primers produced bright, distinguishable, scorable and unambiguous bands. A total of 114 bands were produced across 37 genotypes, of which 107 bands were polymorphic, accounting for 93.86% polymorphism. The number of bands varied from seven (ISSR-825) to fifteen (ISSR-808 and ISSR-857) depending on the primer with a mean value of 11.4 bands per primer. The amplicon sizes obtained with the ISSR primers ranged from 220bp to 2000bp. The percentage of polymorphism exhibited by ISSR primers ranged from 71.43% (ISSR-825) to 100% (ISSR-808, 810, 812, 840 and 842). A comparison of the repeat motifs within the ISSR primers and the number of bands obtained indicated that primers with AG repeats (ISSR 808) and those with AC repeats (ISSR 857) produced more number of bands (15). The primers that were based on the GA and AG repeat motifs produced more polymorphism(100%) than the primers based on other motifs. The banding patterns obtained with these ISSR markers in all the 37 genotypes of chrysanthemum are illustrated in plate 29.

4.6.3.3.1 Similarity index

The banding pattern of ISSR markers scored in the form of binary data was used for computing Jaccard's similarity index values obtained for each pair wise comparison among the 37 genotypes of chrysanthemum and are presented in the table 26. The similarity coefficient based on 10 ISSR markers ranged from 0.275 to 0.775. Among the 37 genotypes studied, the lowest similarity index (0.275) was recorded between Autumn Joy and Flirtation. The genotypes Geetanjali and Red Stone recorded the highest similarity index value of 0.775.

The similarity level of more than 60% was recorded in the following pair wise genotypes viz., Punjab Anuradha and Silper, Shaffoli and Silper, Ratlam Selection and Shaffoli, Mother Teresa with Terry and Neelima, Akitha with Shintome and Terry, Salora with Shaffoli, Terry and Neelima, IIHR-13 with Terry, Ratlam Selection and Jaya, PAU-B-107 with Farr, Red Stone with Snow Cem, Terry, Ratlam Selection and Anjali.

The yellow coloured varieties Aparajitha, Punjab Gold, CO-3, Raichur, Silper, Yellow Gold, Punjab Anuradha, Rekha and Chandrika were having 50% level of similarity on an average among themselves.

4.6.3.3 2 Cluster analysis

The similarity values obtained for each pair wise comparison of ISSR markers among the 37 chrysanthemum genotypes were used to construct dendrogram based on UPGMA analysis and the results are presented in Fig 14. Jaccard's similarity coefficient ranged from 0.35 to 0.78. The 37 genotypes were clustered into ten clusters at 0.35 similarity coefficient value, on the basis of their similarity calculated as proportion of shared alleles. Cluster pattern revealed that, cluster IV was the largest one consisting of 11 genotypes followed by cluster III with six genotypes. Cluster V included five genotypes, cluster VIII with four genotypes, cluster I and II with three genotypes each, cluster VI with two genotypes and cluster VII, IX and X with one genotype each.

The first cluster (cluster I) contained three genotypes Aparajitha, Chandrika and Meera sharing more common alleles with 53% similarity. In cluster II, the genotypes Punjab Gold and CO-3 were found more closer with 58% similarity than with genotype Lilith (48% similarity). The cluster III had five genotypes with Silper and Yellow Gold straddling the extremes. It was further divided into two sub clusters of which Silper and Punjab Anuradha; Shaffoli and Salora were identical at 0.63 and 0.65 similarity coefficient values respectively. This cluster also contains a genotype Neelima which was distantly related to Shaffoli and Salora sub cluster (60% similarity level).

The cluster IV contains 11 genotypes of which Snow Cem, Ratlam Selection, IIHR-13 forms one sub group while Terry and Mother Teresa are assigned to one sub group at 63% of similarity level. Another sub group includes Red Stone and Geetanjali which were more identical among all the genotypes in ISSR clustering pattern with 78% of similarity level. While the genotypes Rekha, Anjali and Basanthi and Harvest House were formed into separate groups as shown in dendrogram with the similarity index of 0.53.

In the fifth cluster, five genotypes are included in which Akitha and Shintome were identical with 68 % similarity level and PAU-B-107 and Farr formed into one group with 62% similarity level and also distantly related to genotype Jaya at 53% similarity level. The two genotypes Pusa Centenary and Red Gold were identical with 0.53 similarity coefficient value in the cluster VI. The local collection Raichur forms a distinct genotype under cluster VII which was distantly related to other clusters at 0.43 similarity coefficient value.

In the cluster VIII, the genotypes Arka Ravi and Asha were found to be identical with 54% similarity having common alleles among them. Similarly, Kalyani Mauve and Local Button forms a group under same cluster VIII, also exhibited resemblance among themselves with 54% similarity. Both the groups have displayed similarity at 45% level.

The cluster IX (Flirtation) and cluster X (Autumn Joy) are having one genotype each which forms a separate entities from rest of the genotypes. Flirtation of cluster IX and Autumn Joy of cluster X showed similarity coefficient values of 0.40 and 0.35 respectively.

4.6.3.3.3 Principal component analysis for ISSR analysis

Principal Component Analysis was done based on the Jaccard's similarity matrix obtained (Table 27). The First component accounts for 48.89% of all changes and second component accounted for 3.42% of principal changes. In this manner, totally 4 principal components accounted for 57.98% of changes. The Principal Component Analysis was performed based on molecular data of 10 ISSR primers to visualize the genetic relatedness among the chrysanthemum genotypes in detail.

The description of the data was done using two and three dimensional pictorial graph and is represented in Fig.15 & 16. From the graph, it is evident that the chrysanthemum genotypes were dispersed on the PC plot, which is a reflection of its genetic base. The results of PCA showed a clear cut separation. However, as depicted in figure some of the genotypes appear to be overlapping with each other depicting high similarity in these genotypes. It was clear from the analysis that the results obtained from PCA were in agreement with the dendrogram generated by UPGMA cluster analysis. This strengthened the ability and accuracy of the ISSR analysis applied to chrysanthemum genotypes in the present study.

Table 1: Particulars of Chrysanthemum genotypes studied

S. No.		S. No.	Genotypes	S. No.	Genotypes
1	Autumn Joy	36	Lohagin	71	Rose Day
2	Ajay	37	Mallika	72	Royal Purple
3	Akitha	38	Mayuri	73	Sadbhavana
4	Anjali	39	Meera	74	Salora
5	Anupam	40	Miss India	75	Seedling Red
6	Anupama (L)	41	Modella	76	Shaffoli
7	Apurva Singer	42	Moharaj	77	Shintome
8	Arka Ravi	43	Mother Teresa	78	Sizuka
9	Asha	44	Mr. Whiteney	79	Skater's Waltz
10	Aparajitha	45	N-13	80	Snow Ball
11	Basanthi	46	Naintara	81	Snowcem
12	Beauty	47	Neelima	82	Sony
13	Chandrika	48	Pankaj	83	Star Pink
14	Chocolate Pompon Variegated	49	Paragaon	84	Statesman
15	Cloverlee Star	50	PAU-B-107	85	Sukai Sport
16	CO-2	51	Perfeeta	86	Swan
17	CO-3	52	Peet Singar	87	Taichen
18	Coffee	53	Pink Cascade	88	Tambra
19	Dolly Orange	54	Plants & Seeds Choice	89	Tata Centuary
20	Dolly Pink	55	Pride of Maid Fort	90	Terry
21	Donald	56	Priya	91	Vasantika
22	Farr	57	Prof. Harris	92	White White Silver
23	Flirtation	58	Poonam	93	Cloud
24	Freedom	59	Punjab Gold	94	White Snow
25	Garden Beauty	60	Pusa Centenary	95	Winter Queen
26	Geetanjali	61	Raja	96	Yellow Bonsai
27	Harvest House	62	Rajahmundry Selection	97	Yellow Cherub
28	IIHR 13	63	Ratlam Selection	98	Yellow Gold
29	Jaya	64	Ravikiran	99	Yellow Star
30	Jessie Hab Good	65	Red Fair Lady	100	Yokari
31	John Webber	66	Red Stone	101	Raichur
32	Julie	67	Red Gold	102	Silper
33	Kalyani Mauve	68	Red Queen	103	CO-1
34	Lilith	69	Rekha	104	Punjab Anuradha
35	Local Button	70	Robert Earn Shaw		

Table 3:List of Random Primers along with their nucleotide sequences used for DNA amplification of Chrysanthemum genotypes

S.No.	RAPD primer	Nucleotide sequence(5'-3')
1	OPE-14	TGCGGCTGAG
2	OPE-15	ACGCACAACC
3	OPE-16	GGTGACTGTG
4	OPE-18	GGACTGCAGA
5	OPE-19	ACGGCGTATG
6	OPF-3	CCTGATCACC
7	OPF-5	CCGAATTCCC
8	OPF-19	CCTCTAGACC
9	OPG-9	CTGACGTCAC
10	OPG-16	AGCGTCCTCC
11	OPG-19	GTCAGGGCAA
12	OPH-13	GACGCCACAC
13	OPH-16	TCTCAGCTGG
14	OPH-17	AAGCAGCAAG
15	OPH-20	CACCGTTCTG
16	OPI-6	AAGGCGGCAG
17	OPI-18	TGCCCAGCCT
18	OPI-19	AATGCGGGAG
19	OPI-20	AAAGTGCGGG
20	OPJ-14	CACCCGGATC
21	OPJ-15	TGTAGCAGGG
22	OPM-10	TCTGGCGCAC
23	OPK-8	GAACACTGGG
24	OPK-18	CCTAGTCGAG
25	OPK-19	CACAGGCGGA
26	OPL-1	GGCATGACCT
27	OPL-18	ACCACCCACC

Source: Operon Technologies, INC. 1000 Atlantic Avenue Suite 108, Alameda, CA 94501

Table 4: List of ISSR Primers along with their nucleotide sequences used for DNA amplification of Chrysanthemum genotypes

S.No	Primer	Nuceotide sequence(5'-3')	DNA repeats
1	808	AGAGAGAGAGAGAGAGC	(AG) ₈ C
2	810	GAGAGAGAGAGAGAGAT	(GA) ₈ T
3	812	GAGAGAGAGAGAGAGAA	(GA) ₈ A
4	825	ACACACACACACACACT	(AC) ₈ T
5	836	AGAGAGAGAGAGAGAGYA	(AG) ₈ YA
6	840	GAGAGAGAGAGAGAGAYT	(GA) ₈ YT
7	842	GAGAGAGAGAGAGAGAYG	(GA) ₈ YG
8	846	CACACACACACACACART	(CA) ₈ RT
9	855	ACACACACACACACACYT	(AC) ₈ YT
10	857	ACACACACACACACACYG	(AC) ₈ YG

Table 7: Classification of 104 genotypes of chrysanthemum based on ranges of thirty quantitative morphological characters

S.No.	Group	Range	No. of accessions in each group
I	Plant Characters		
1.	Plant height (cm)		
	Very short	<32.5	18
	Short	32.5 – 42.5	45
	Medium	42.5 – 52.5	34
	Tall	52.5 – 62.5	7
2.	Plant spread(E – W) (cm)		
	Less	<22.5	48
	Medium	22.5 – 32.5	44
	High	32.5 – 42.5	10
	Very high	>42.5	2
3.	Plant spread(N – S) (cm)		
	Less	< 22.5	52
	Medium	22.5 – 32.5	42
	High	32.5 – 42.5	6
	Very high	>42.5	4
4.	No. of primary branches/plant		
	Low	<6.25	30
	Medium	6.25 – 11.25	55
	High	11.25 – 16.25	15
	Very high	> 16.25	4
5.	Petiole length (cm)		
	Short	< 1.25	20
	Medium	1.25 – 2.75	73
	Long	2.75 – 4.25	11
6.	Terminal lobe length (cm)		
	Short	< 1.25	15
	Medium	1.25 – 2.25	56
	Long	2.25 – 3.25	30
	Very long	> 3.25	3
7.	Leaf length (cm)		
	Short	< 3.25	6
	Medium	3.25 – 6.25	80
	Long	> 6.25	18
8.	Leaf width (cm)		
	Short	< 3.25	27
	Medium	3.25 – 6.25	75
	Long	> 6.25	2
9.	Depth of the sinus (cm)		
	Short	< 0.5	6
	Medium	0.5 – 1.5	65
	Long	> 1.5	33

10.	Internodal length (cm)		
	Short	< 1.25	21
	Medium	1.25 – 2.25	54
	Long	2.25 – 3.25	22
	Very long	> 3.25	7
II.	Flower Characters		
11.	Days to 1st flower bud appearance		
	Early	< 65	20
	Mid	65 - 85	54
	Late	> 85	30
12.	Days to 50% flowering		
	Early	< 75	3
	Mid	75 - 95	64
	Late	95- 115	32
	Very late	> 115	5
13.	Inflorescence width (cm)		
	Narrow	< 27.5	18
	Medium	27.5 – 47.5	67
	Broad	> 47.5	19
14.	Spray length (cm)		
	Short	6.25 – 11.25	5
	Medium	11.25 – 16.25	45
	Long	16.25 – 21.25	44
	Very long	> 21.25	10
15.	Bud width (mm)		
	Small	4.0 – 8.0	28
	Medium	8.0 – 12.0	49
	Large	12.0 -16.0	26
16.	Average flower weight (g)		
	Low	0.5- 2.5	67
	Medium	2.5 – 4.5	22
	High	4.5 – 6.5	9
	Very high	> 6.5	6
17.	Flower diameter (cm)		
	Small	< 2.75	3
	Medium	2.75 – 5.75	68
	Large	5.75 – 8.75	32
	Very large	> 8.75	1
18.	Peduncle length (cm)		
	Short	< 3.25	5
	Medium	3.25 – 6.25	66
	Long	6.25 – 9.25	31
	Very long	> 9.25	2

19.	Peduncle thickness (mm)		
	Thin	< 1.25	3
	Medium	1.25 – 3.25	92
	Thick	> 3.25	9
20.	Number of ray florets/head		
	Less	< 75	24
	Medium	75 – 225	45
	High	225 – 375	32
	Very high	> 375	3
21.	Length of the ray floret(cm)		
	Low	< 1.25	7
	Medium	1.25 – 3.25	70
	High	3.25 - 5.25	26
	Very high	> 5.25	1
22.	Width of the ray floret(cm)		
	Less	< 0.5	21
	Medium	0.5 – 1.0	71
	High	> 1.0	12
23.	Ratio of ray floret length/width		
	Low	< 6.25	95
	Medium	6.25 – 11.25	8
	High	11.25	1
24.	Disc diameter (cm)		
	Small	< 0.75	21
	Medium	0.75 – 1.75	62
	Large	1.75 – 2.75	15
	Very large	> 2.75	6
25.	No. of disc florets/head		
	Less	< 75	38
	Medium	75 – 175	46
	High	175 – 275	17
	Very high	> 275	3
26.	Length of the disc floret (cm)		
	Short	< 0.5	29
	Medium	0.5 – 1.0	52
	Long	> 1.0	23
27.	No. of flowers/spray		
	Low	1.25 - 6.25	48
	Medium	6.25- 11.25	41
	High	11.25 – 16.25	12
	Very high	> 16.25	3
28.	No. of flowers/plant		
	Low	< 62.5	33
	Medium	62.5 – 162.5	53
	High	162.5 – 262.5	17
	Very high	> 262.5	1

S.No.	Group	Range	No. of accessions in each group
29.	Duration of the flowering(days)		
	Less	32.5 – 42.5	39
	Medium	42.5 – 52.5	52
	More	52.5 – 62.5	12
	Excess	> 62.5	1
30.	Number of suckers/plant		
	Low	< 7.5	31
	Medium	7.5 – 27.5	59
	High	27.5 – 47.5	9
	Very high	> 47.5	5

**Table 20: Yield and absorbance ratio of DNA for
37 chrysanthemum genotypes**

S.No	GENOTYPES	OD ratio (260/280)	DNA yield ($\mu\text{g}/\mu\text{l}$)
1.	Aparajitha	1.90	626.0
2.	Punjab Gold	1.92	877.0
3.	CO-3	1.94	745.3
4.	Raichur	1.85	697.8
5.	Silper	1.66	504.2
6.	Yellow Gold	1.90	722.2
7.	Punjab Anuradha	1.80	778.5
8.	Rekha	1.92	474.5
9.	Chandrika	1.70	1556.3
10.	Snow cem	1.72	517.5
11.	Meera	1.67	303.5
12.	Shaffoli	1.90	864.5

13.	Terry	1.92	838.5
14.	Shintome	1.90	819.5
15.	Arka Ravi	1.94	744.6
16.	Ratlam Selection	1.92	591.5
17.	Neelima	1.94	741.5
18.	Flirtation	1.94	791.5
19.	Mother Teresa	1.90	770.0
20.	Autumn Joy	1.87	534.5

**Table 20: Yield and absorbance ratio of DNA for
37 chrysanthemum genotypes**

S.No	GENOTYPES	OD ratio (260/280)	DNA yield ($\eta\text{g}/\mu\text{l}$)
21	Anjali	1.92	735.4

22	Akitha	1.90	779.0
23	PAU-B-107	1.93	676.5
24	Farr	1.93	636.8
25	Jaya	1.93	529.0
26	Harvest House	1.80	550.8
27	Lilith	1.66	421.3
28	Asha	1.93	1021.0
29	Pusa Centenary	1.94	1021.0
30	Salora	1.92	816.5
31	IIHR-13	1.90	894.5
32	Basanthi	1.92	1124.0
33	Red Stone	1.92	876.5
34	Geetanjali	1.90	906.5
35	Red Gold	1.94	1021.0
36	Kalyani Mauve	1.80	535.5
37	Local Button	1.91	943.5

Table 21: List of RAPD primers used and number of bands generated in 37 genotypes of Chrysanthemum

S.No.	RAPD primer	Number of bands	Total no. of polymorphic bands	Percentage polymorphism (100%)	Size of amplified product(bp)
1	OPE-14	10	9	90.00	450-3300
2	OPE-15	7	6	85.71	830-2000
3	OPE-16	11	11	100.0	420-2600
4	OPE-18	11	10	90.90	600-3300
5	OPE-19	12	12	100.0	440-3000
6	OPF-3	9	8	88.89	500-2200
7	OPF-5	8	7	87.50	750-3000
8	OPF-19	10	10	100.0	450-2500
9	OPG-9	7	7	100.0	750-2000
10	OPG-16	7	7	100.0	800-1600
11	OPG-19	11	11	100.0	350-2027
12	OPH-13	7	7	100.0	600-3000
13	OPH-16	11	11	100.0	350-1900
14	OPH-17	11	11	100.0	400-2000
15	OPH-20	7	7	100.0	420-2000
16	OPI-6	14	14	100.0	560-2000
17	OPI-18	12	10	83.33	600-3500

18	OPI-19	12	12	100.0	450-2200
19	OPI-20	11	11	100.0	450-2500
20	OPJ-14	13	13	100.0	350-2000
21	OPJ-15	13	13	100.0	564-3000
22	OPM-10	9	9	100.0	500-1900
23	OPK-8	11	11	100.0	750-2200
24	OPK-18	9	9	100.0	550-3300
25	OPK-19	17	17	100.0	450-2500
26	OPL-1	8	8	100.0	450-2200
27	OPL-18	10	10	100.0	600-2000

Table 24: Principal Component Analysis showing the contribution of 27 RAPD primers among 37 Chrysanthemum genotypes

S. No.	Genotypes	Eigen value	Percentage variation explained	Cumulative variation (%)
1.	Aparajitha	14.30	38.64	38.64
2.	Punjab Gold	1.24	3.35	41.99
3.	CO-3	1.12	3.03	45.02
4.	Raichur	1.07	2.90	47.92
5.	Silper	1.02	2.76	50.68
6.	Yellow Gold	0.94	2.54	53.22
7.	Punjab Anuradha	0.85	2.29	55.51
8.	Rekha	0.82	2.22	57.73
9.	Chandrika	0.78	2.11	59.85
10.	Snow Cem	0.76	2.06	61.91
11.	Meera	0.75	2.03	63.94
12.	Shaffoli	0.73	1.96	65.90
13.	Terry	0.72	1.95	67.85
14.	Shintome	0.69	1.86	69.71
15.	Arka Ravi	0.67	1.82	71.53
16.	Ratlam Selection	0.64	1.72	73.25
17.	Neelima	0.63	1.70	74.95
18.	Flirtation	0.63	1.70	76.65
19.	Mother Teresa	0.61	1.64	78.30
20.	Autumn Joy	0.58	1.57	79.87
21.	Anjali	0.56	1.52	81.38
22.	Akitha	0.55	1.48	82.86
23.	PAU-B-107	0.51	1.39	84.25
24.	Farr	0.51	1.38	85.63
25.	Jaya	0.50	1.35	86.98
26.	Harvest House	0.48	1.30	88.28
27.	Lilith	0.47	1.27	89.55
28.	Asha	0.46	1.24	90.79
29.	Pusa Centenary	0.45	1.22	92.01
30.	Salora	0.42	1.14	93.15
31.	IIHR-13	0.41	1.09	94.24
32.	Basanthi	0.39	1.06	95.30
33.	Red Stone	0.39	1.04	96.35
34.	Geetanjali	0.37	0.99	97.34
35.	Red Gold	0.35	0.95	98.29
36.	Kalyani Mauve	0.34	0.92	99.21
37.	Local Button	0.29	0.79	100.0

Table 25 : Details of ISSR primers and amplified bands of all the DNA samples as obtained from thirty seven genotypes of Chrysanthemum

[illegible]

Table 27: Principal Component Analysis showing the contribution of ten ISSR primers among 37 Chrysanthemum genotypes

S.No.	Genotypes	Eigen value	Percentage variation explained	Cumulative variation (%)
1.	Aparajitha	18.09	48.89	48.89
2.	Punjab Gold	1.26	3.42	52.31
3.	CO-3	1.08	2.93	55.24
4.	Raichur	1.02	2.75	57.98
5.	Silper	0.91	2.46	60.44
6.	Yellow Gold	0.91	2.45	62.89
7.	Punjab Anuradha	0.82	2.21	65.10
8.	Rekha	0.74	1.99	67.09
9.	Chandrika	0.73	1.97	69.06
10.	Snow Cem	0.71	1.91	70.97
11.	Meera	0.67	1.80	72.77
12.	Shaffoli	0.66	1.78	74.55
13.	Terry	0.63	1.71	76.26
14.	Shintome	0.61	1.65	77.91
15.	Arka Ravi	0.55	1.49	79.40
16.	Ratlam Selection	0.53	1.44	80.83
17.	Neelima	0.51	1.39	82.22
18.	Flirtation	0.50	1.36	83.59
19.	Mother Teresa	0.48	1.31	84.89
20.	Autumn Joy	0.47	1.28	86.17
21.	Anjali	0.46	1.24	87.41
22.	Akitha	0.42	1.13	88.55
23.	PAU-B-107	0.40	1.09	89.64
24.	Farr	0.39	1.05	90.69
25.	Jaya	0.36	1.03	91.72
26.	Harvest House	0.34	0.93	92.65
27.	Lilith	0.32	0.87	93.52
28.	Asha	0.32	0.86	94.38
29.	Pusa Centenary	0.28	0.75	95.13
30.	Salora	0.27	0.74	95.87
31.	IIHR-13	0.26	0.71	96.59
32.	Basanthi	0.25	0.67	97.25
33.	Red Stone	0.23	0.63	97.89
34.	Geetanjali	0.22	0.60	98.49
35.	Red Gold	0.21	0.58	99.07
36.	Kalyani Mauve	0.19	0.51	99.58
37.	Local Button	0.16	0.42	100

Fig 1 Mean performance of chrysanthemum genotypes for plant characters

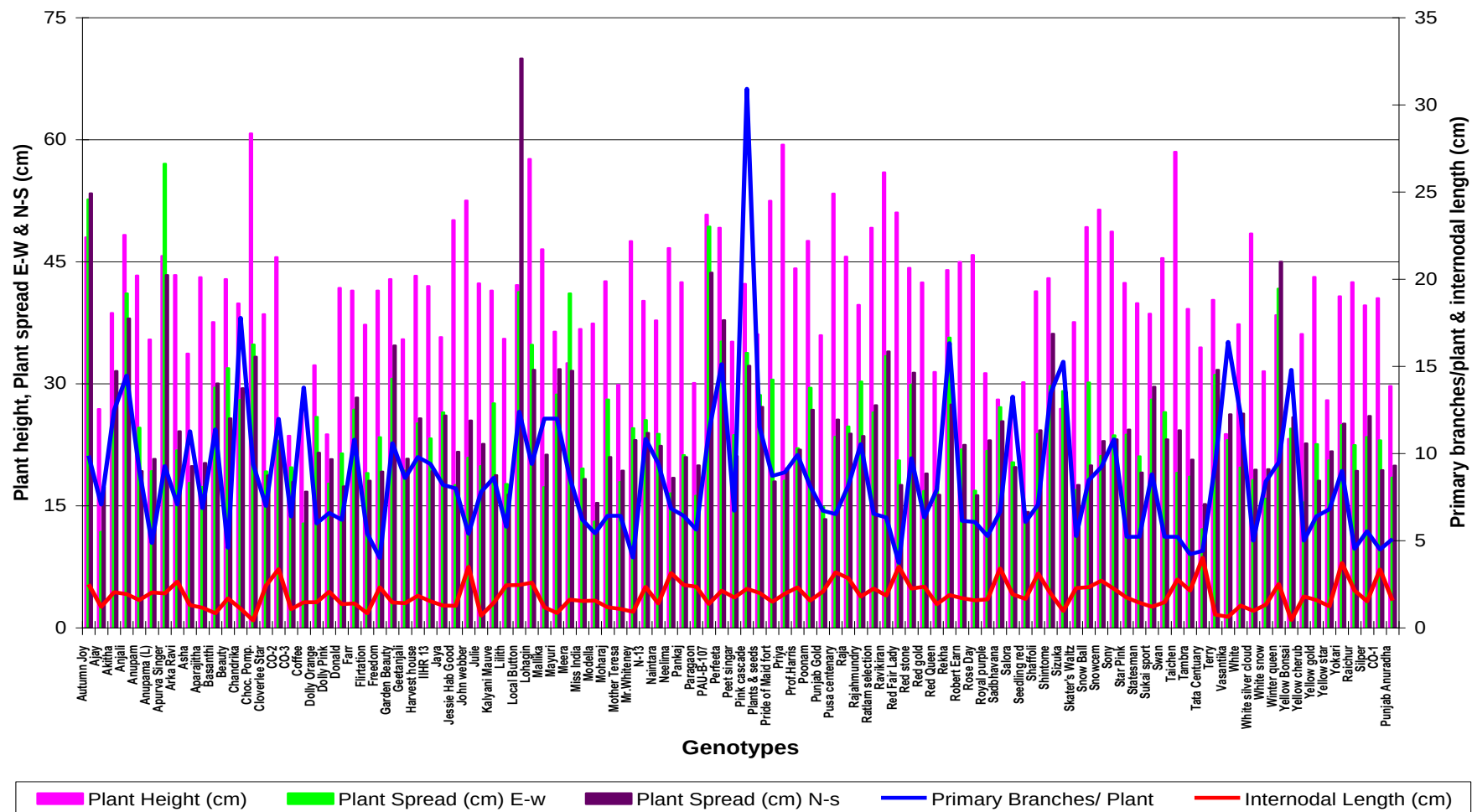


Fig 2 Mean performance of chrysanthemum genotypes for leaf characters

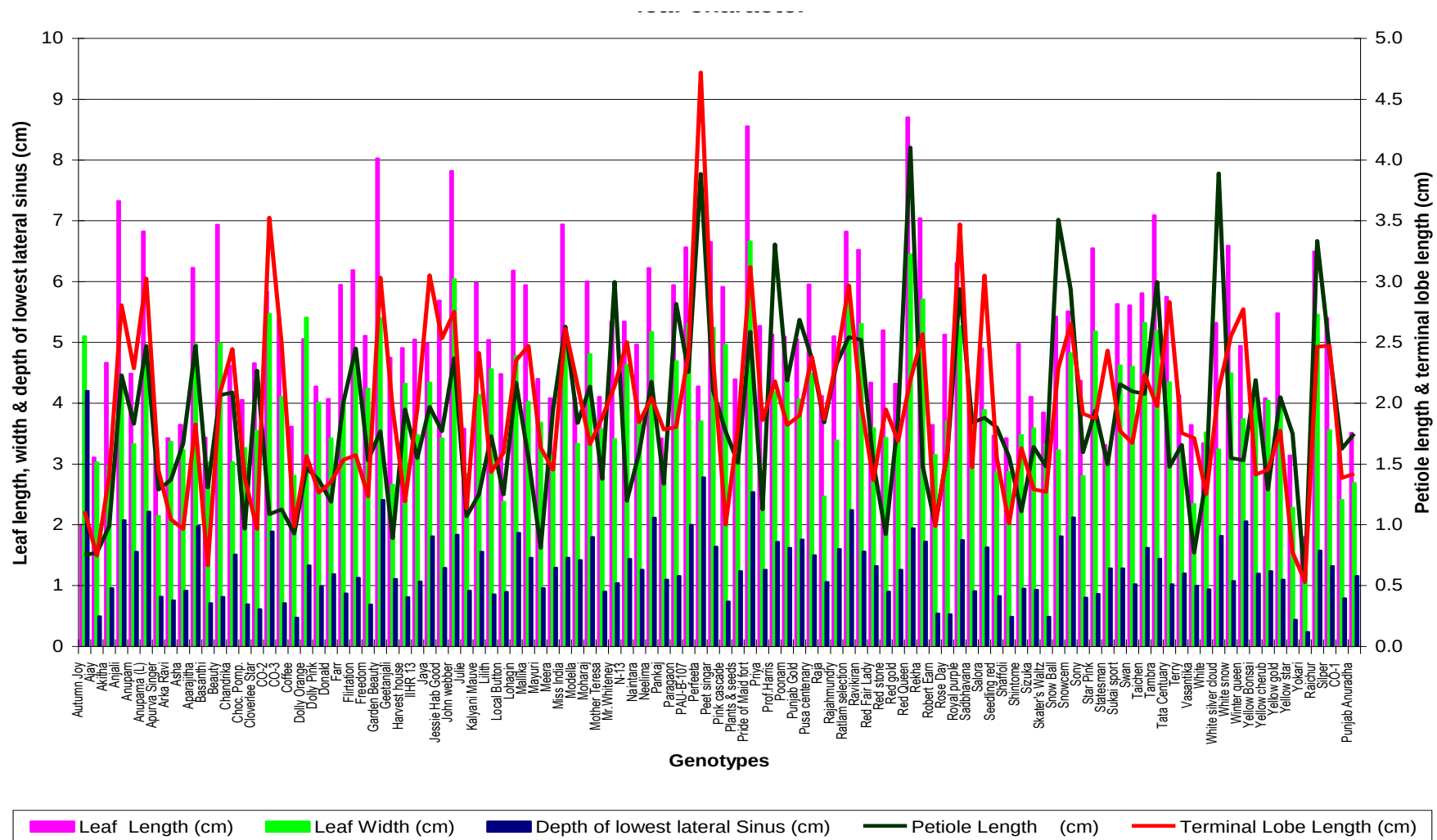


Fig 3 Mean performance of chrysanthemum genotypes for Inflorescence characters

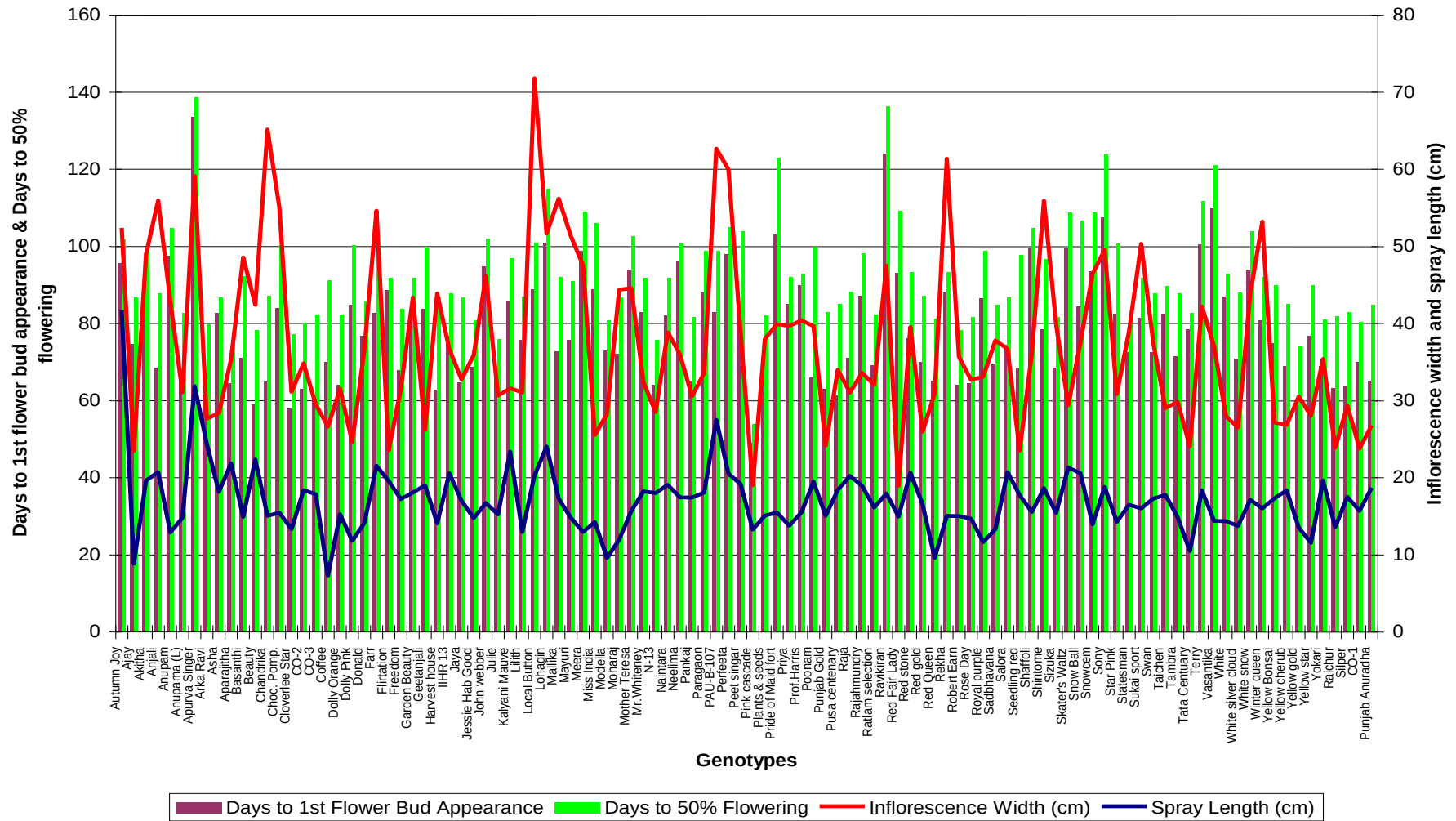


Fig 4Mean performance of chrysanthemum genotypes for floral characters

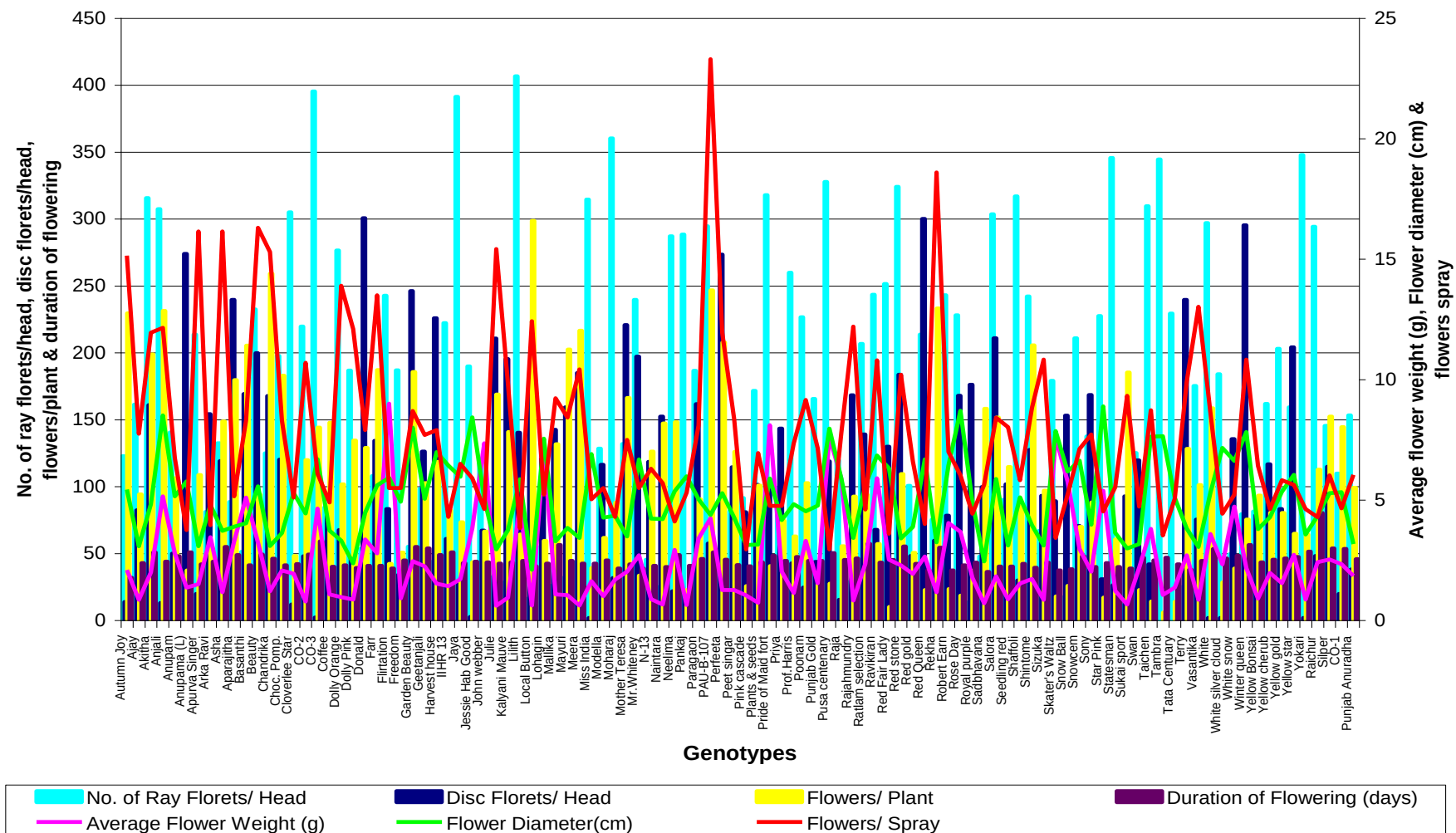


Fig 5 Comparison of Phenotypic and Genotypic correlation coefficient for flower yield per plant in chrysanthemum

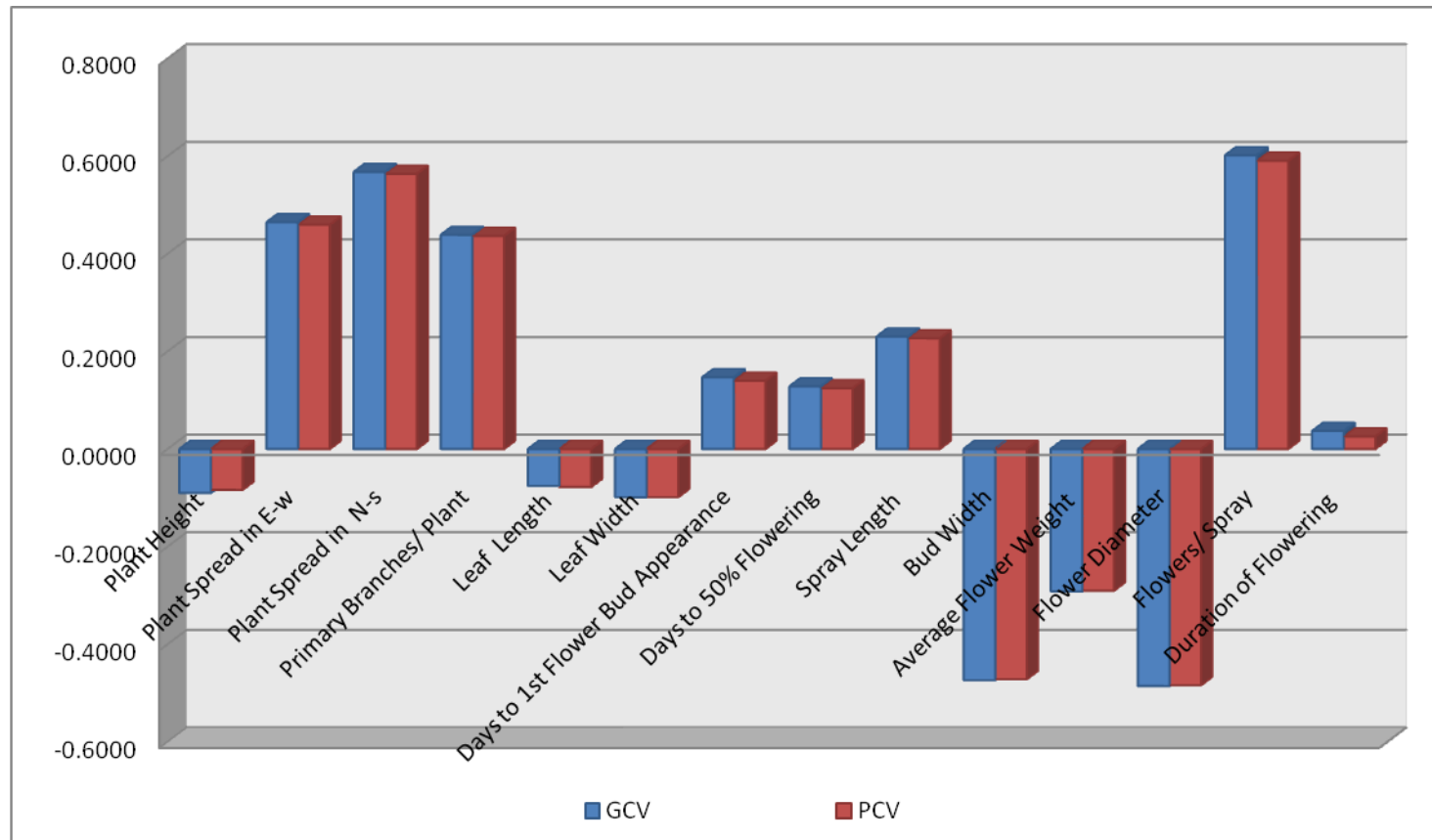


Fig 7: Genotypic path diagram for Number of flowers per plant

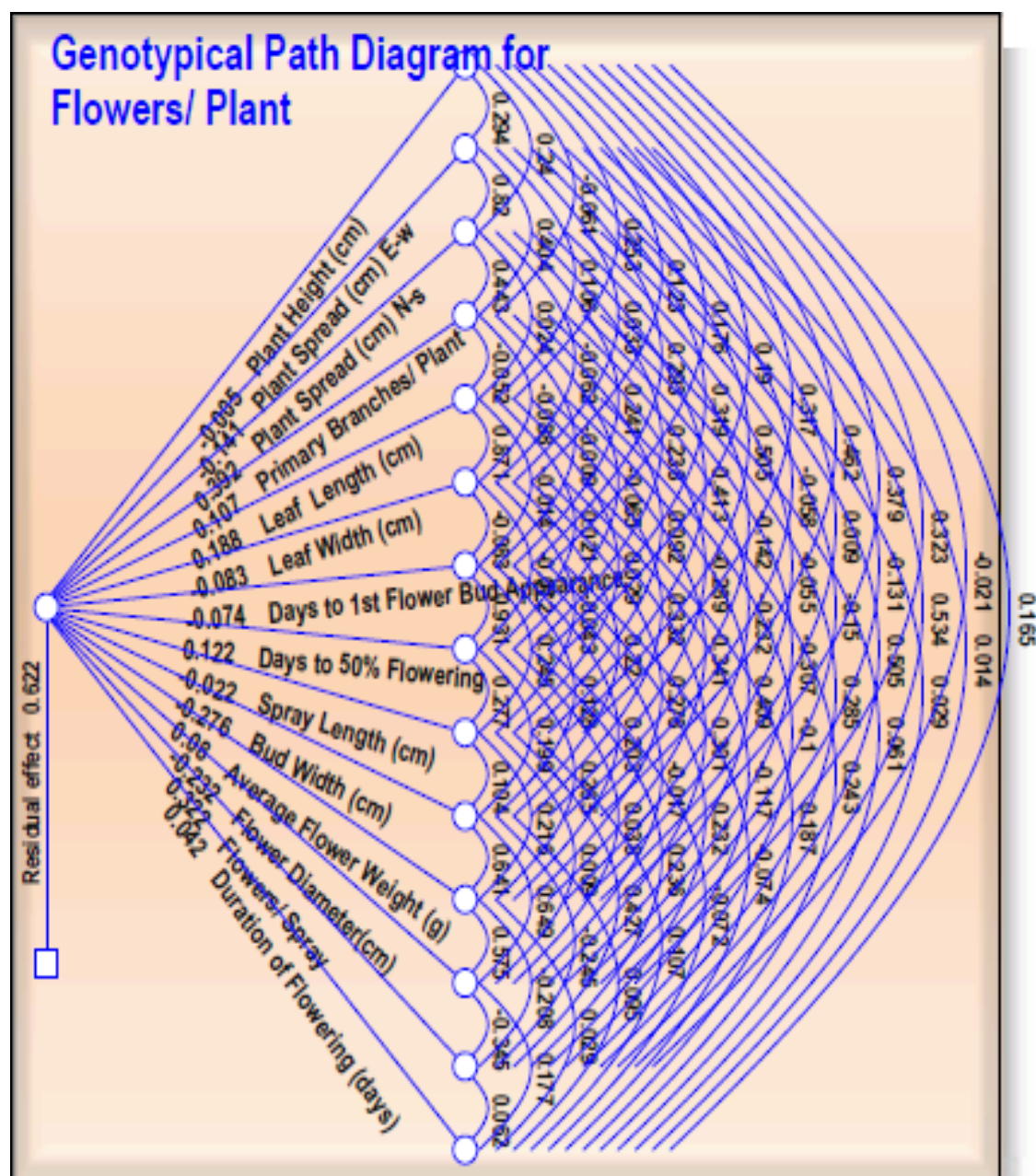
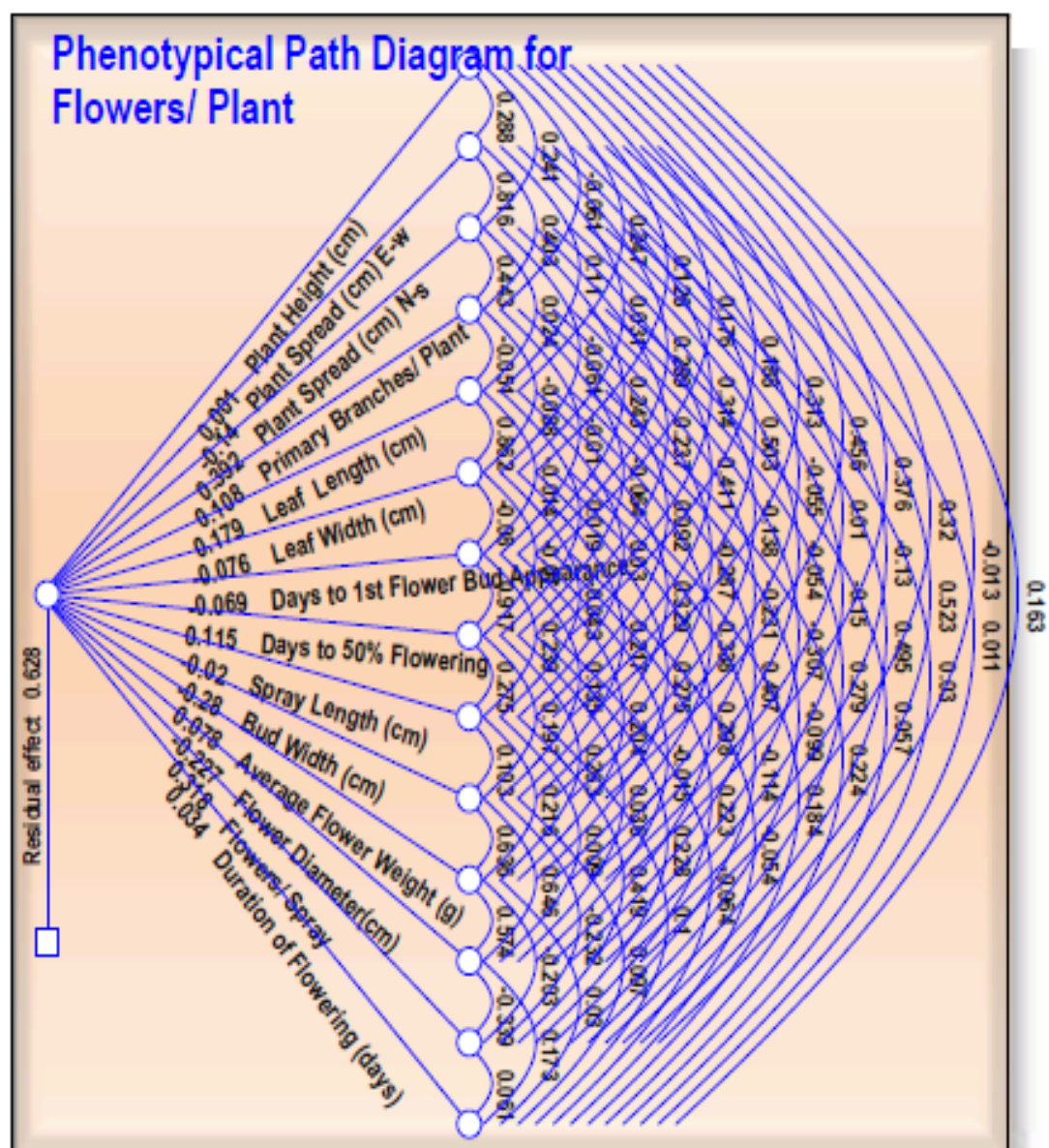


Fig 6: Phenotypic path diagram for Number of flowers per plant



The diagram shows 11 nodes arranged in a circle, labeled 1 through 11. Each node is a blue circle with a black number. Each edge is a blue line connecting two nodes, with a black numerical value. The values on the edges are: 30.48 (1-2), 86.26 (2-3), 38.91 (3-4), 63.1 (4-5), 83.86 (5-6), 109.05 (6-7), 129. (7-8), 45.41 (8-9), 64.03 (9-10), 80.05 (10-11), 59.52 (11-1), 103.6 (1-2), 143.32 (1-3), 40.13 (2-4), 43.94 (3-5), 138.13 (4-6), 89.77 (5-7), 62.56 (6-8), 73.25 (7-9), 42.97 (8-10), 176.14 (9-11), 101.81 (10-1), 77.36 (11-2), 70.51 (1-3), 127.78 (1-4), 88.26 (1-5), 55.88 (1-6), 63.12 (1-7), 129.74 (1-8), 95.88 (1-9), 77.99 (1-10), 32.14 (10-11), 32.28 (7-8), 40.02 (8-9), 51.75 (9-10), 63.96 (10-11), 68.39 (11-1), 61.93 (1-2), 99.29 (2-3), 104.71 (3-4), 101.19 (4-5), 104.08 (5-6), 92.14 (6-7), 90.1 (7-8), 113.05 (8-9), 83.2 (9-10), 83.89 (10-11), 102.58 (11-1), 146.1 (1-2), 43.44 (2-3), 68.52 (3-4), 172.54 (4-5), 101.32 (5-6), 79.02 (6-7), 151.03 (7-8), 195.87 (8-9), 151.03 (9-10), 129. (10-11).

Euclidean² Distance (Not to the Scale)

Fig 8: Clustering pattern in chrysanthemum genotypes based on morphological characters

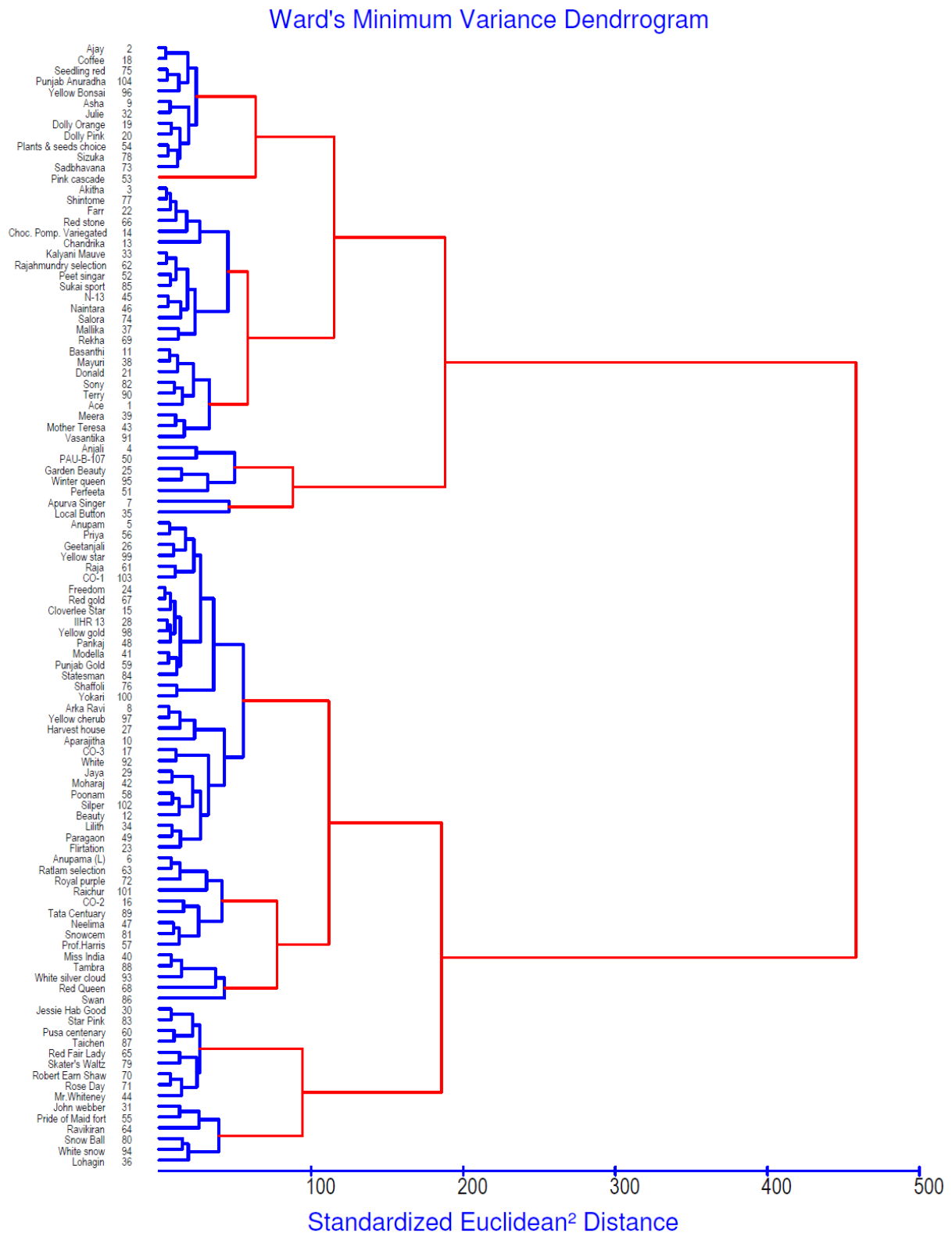
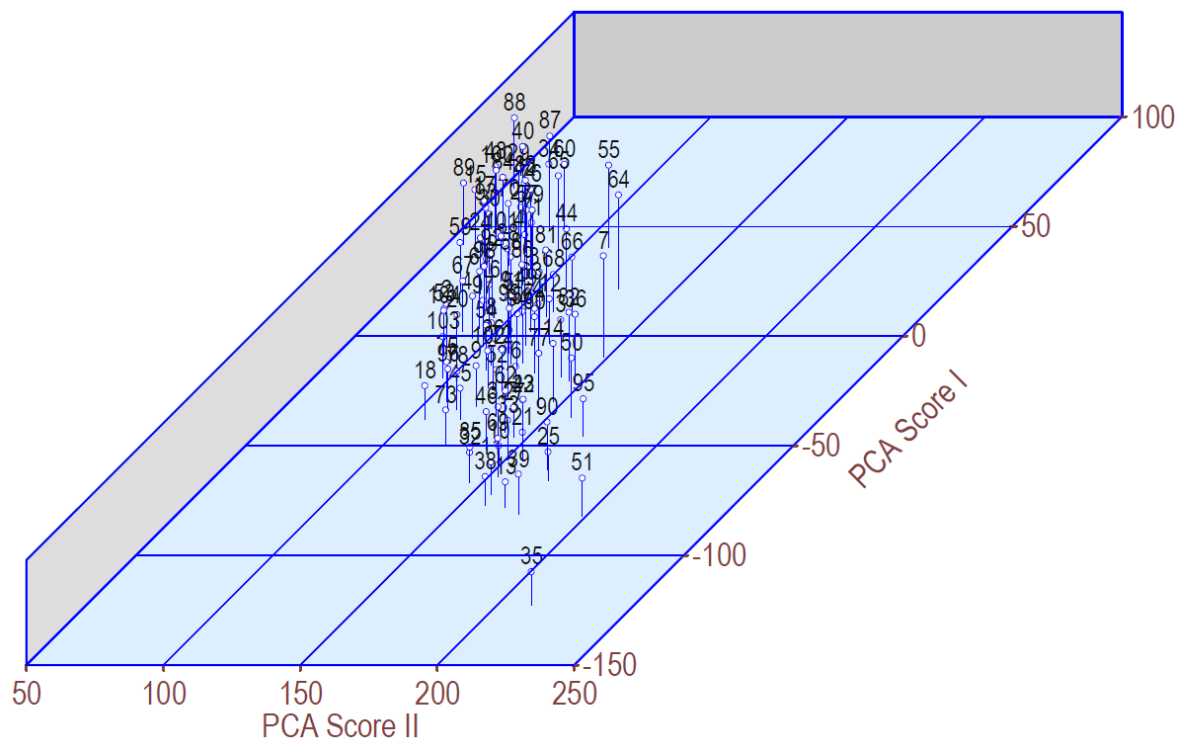


Fig 10: The relative position of Chrysanthemum genotypes based on PCA scores of morphological characters (Three dimensional)



Names of the genotypes:

1 Autumn Joy	22 Farr	43 Mother Teresa	64 Ravikiran	85 Sukai sport
2 Ajay	23 Flirtation	44 Mr. Whiteney	65 Red Fair Lady	86 Swan
3 Akitha	24 Freedom	45 N-13	66 Red stone	87 Taichen
4 Anjali	25 Garden Beauty	46 Naintara	67 Red gold	88 Tambra
5 Anupam	26 Geetanjali	47 Neelima	68 Red Queen	89 Tata Centuary
6 Anupama	27 Harvest house	48 Pankaj	69 Rekha	90 Terry
7 Apurva Singer	28 IIHR 13	49 Paragaon	70 Robert Earn Shaw	91 Vasantika
8 Arka Ravi	29 Jaya	50 PAU-B-107	71 Rose Day	92 White
9 Asha	30 Jessie Hab Good	51 Perfeeta	72 Royal purple	93 White silver cloud
10 Aparajitha	31 John webber	52 Peetsingar	73 Sadbhavana	94 White snow
11 Basanthi	32 Julie	53 Pink cascade	74 Salora	95 Winter queen
12 Beauty	33 Kalyani	54 Plants & seeds	75 Seedling red	96 Yellow Bonsai
13 Chandrika	34 Lilith	55 Pride of Maid fort	76 Shaffoli	97 Yellow cherub
14 Choc. Pompon Variegated	35 Local Button	56 Priya	77 Shintome	98 Yellow gold
15 Cloverlee Star	36 Lohagin	57 Prof. Harris	78 Sizuka	99 Yellow star
16 CO-2	37 Mallika	58 Poonam	79 Skater's Waltz	100 Yokari
17 CO-3	38 Mayuri	59 Punjab Gold	80 Snow Ball	101 Raichur
18 Coffee	39 Meera	60 Pusa centenary	81 Snowcem	102 Silper
19 Dolly Orange	40 Miss India	61 Raja	82 Sony	103 CO-1
20 Dolly Pink	41 Modella	62 Rajahmundry	83 Star Pink	104 Punjab Anuradha
21 Donald	42 Moharaj	63 Ratlam selection	84 Statesman	

Fig: 11 Dendrogram generated using UPGMA analysis showing the genetic relationship among Chrysanthemum genotypes using RAPD data

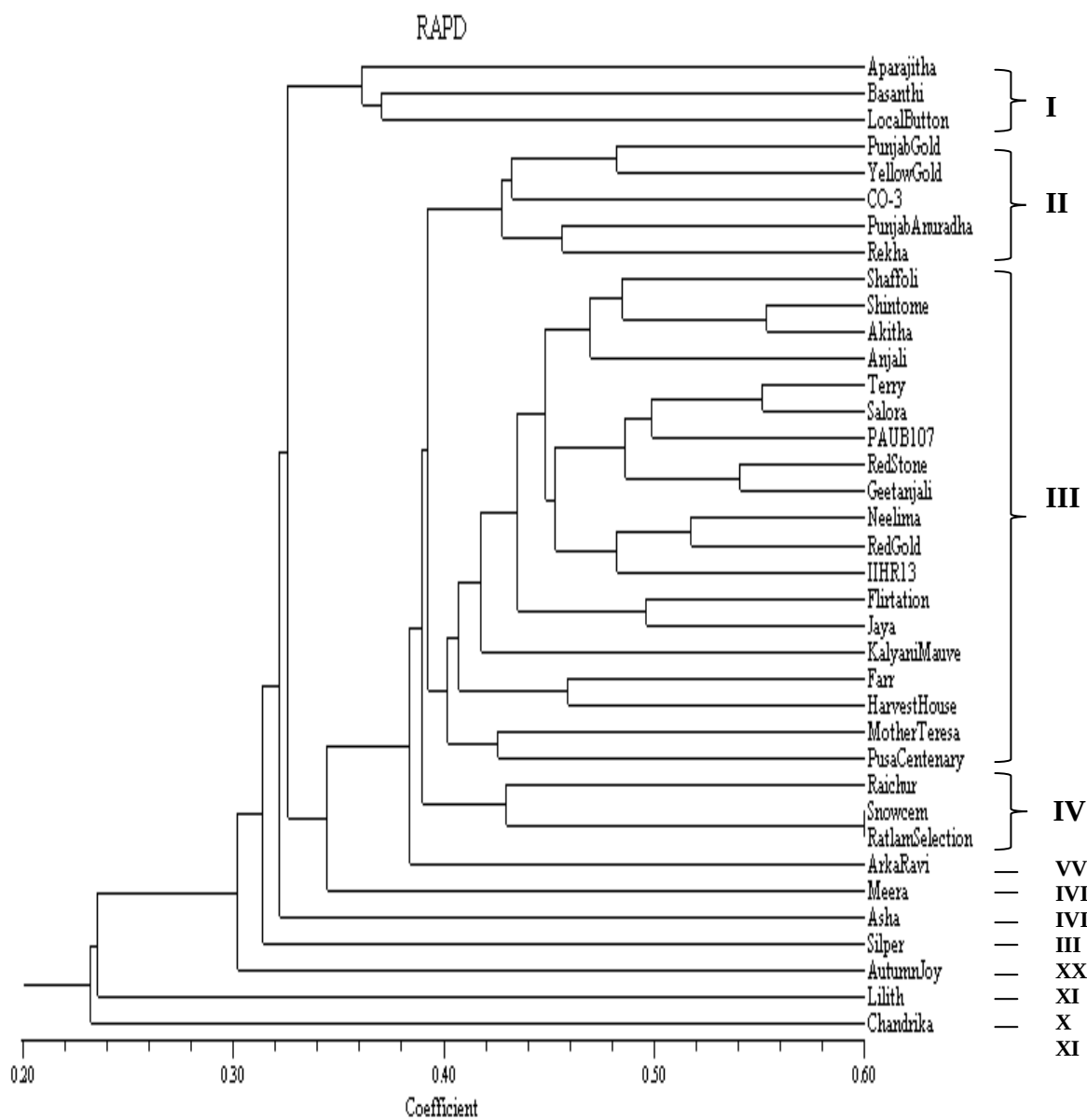
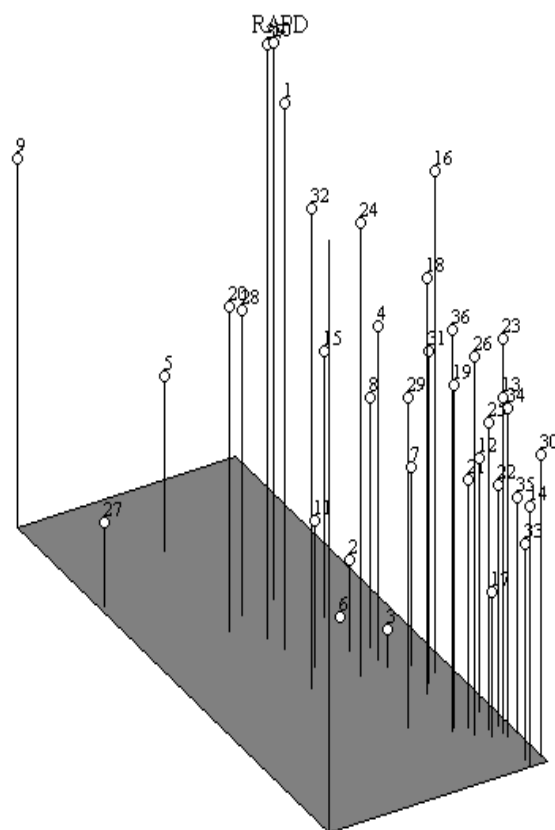


Fig 13 The relative position of Chrysanthemum genotypes based on RAPD markers(Three dimensional)



Name of the genotypes:

- | | | |
|--------------------|----------------------|--------------------|
| 1. Aparajitha | 13. Terry | 25. Jaya |
| 2. Punjab Gold | 14. Shintome | 26. Harvest House |
| 3. CO-3 | 15. Arka Ravi | 27. Lilith |
| 4. Raichur | 16. Ratlam Selection | 28. Asha |
| 5. Silper | 17. Neelima | 29. Pusa Centenary |
| 6. Yellow Gold | 18. Flirtation | 30. Salora |
| 7. Punjab Anuradha | 19. Mother Teresa | 31. IIHR-13 |
| 8. Rekha | 20. Autumn Joy | 32. Basanthi |
| 9. Chandrika | 21. Anjali | 33. Red Stone |
| 10. Snow Cem | 22. Akitha | 34. Geetanjali |
| 11. Meera | 23. PAU-B-107 | 35. Red Gold |
| 12. Shaffoli | 24. Farr | 36. Kalyani Mauve |
| | | 37. Local Button |

Fig: 12 Two dimensional scaling of 37 genotypes of Chrysanthemum by principal component analysis using the Jaccard's similarity coefficients based on RAPD markers.

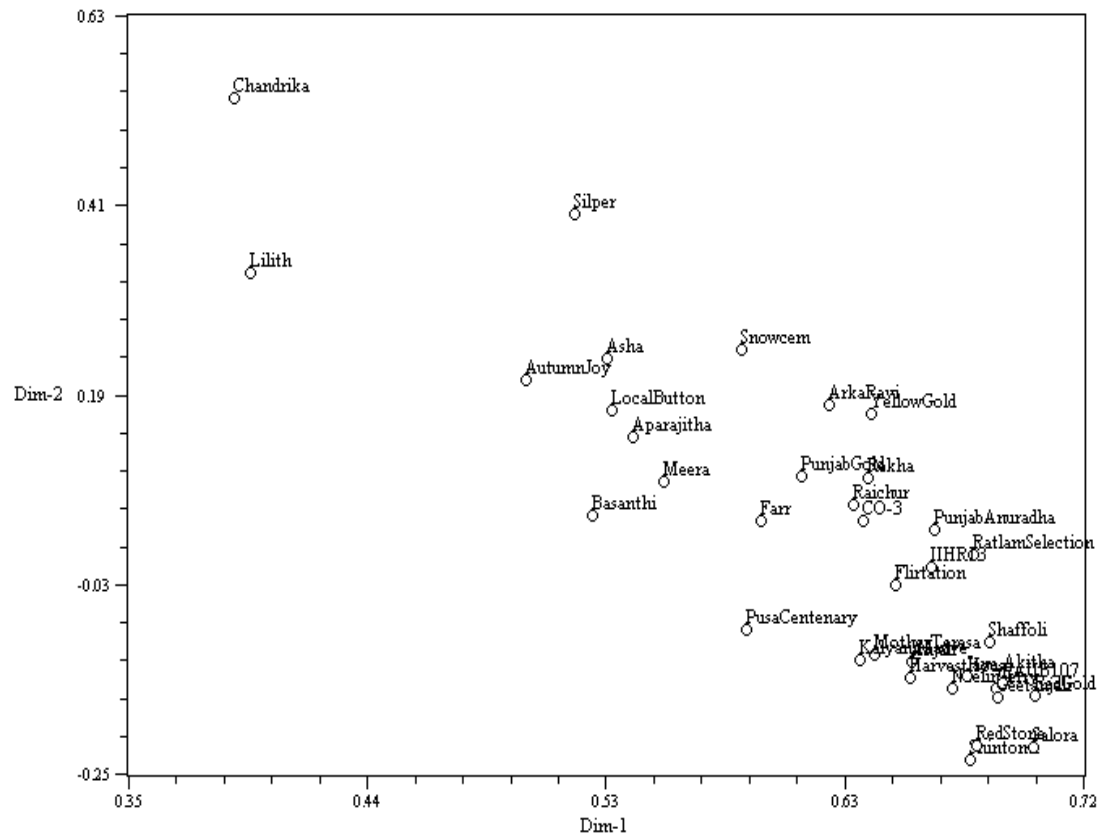


Fig: 14 Dendrogram generated using UPGMA analysis showing the genetic relationship among Chrysanthemum genotypes using ISSR data

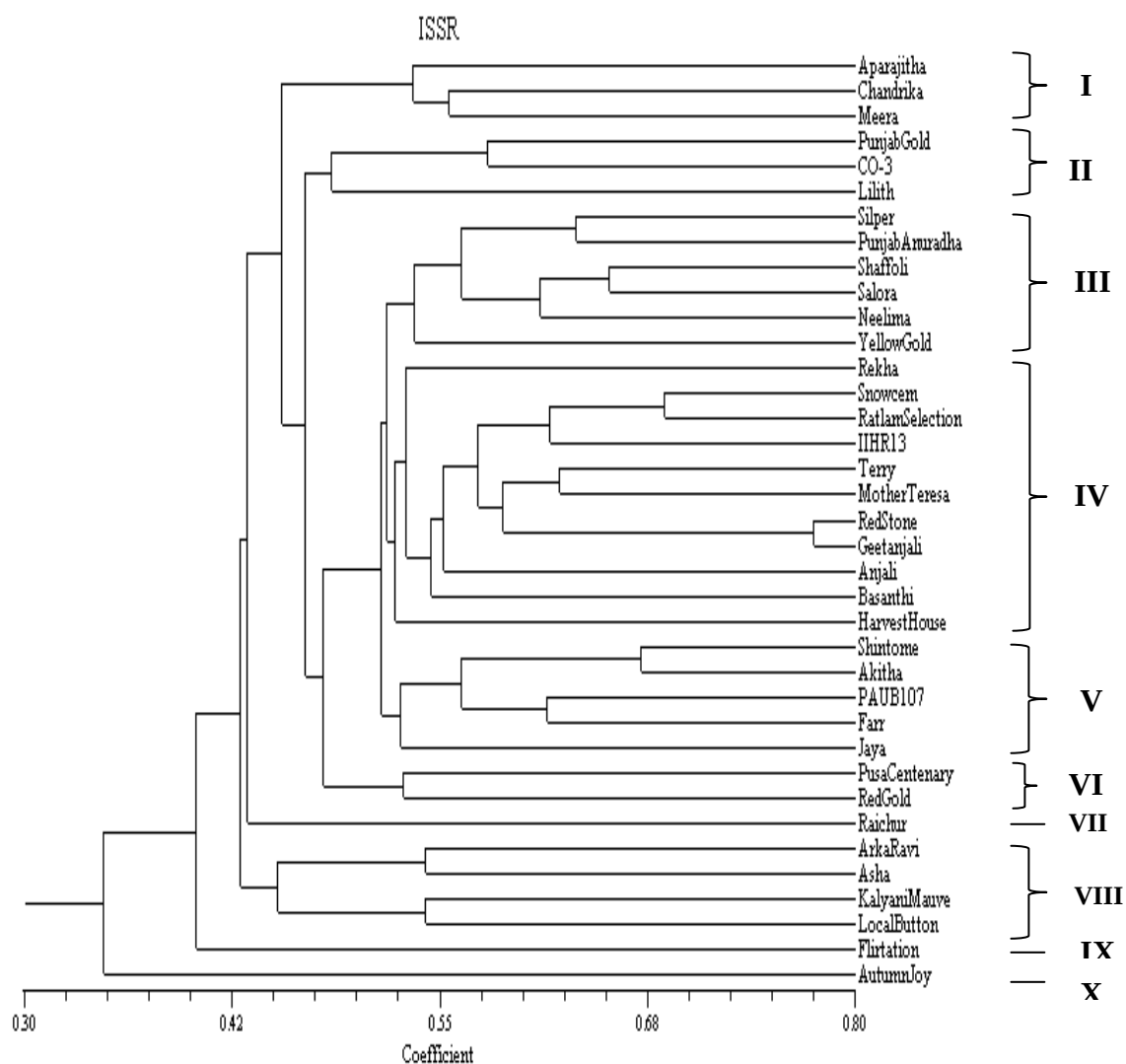


Fig: 15 Two dimensional scaling of 37 genotypes of Chrysanthemum by principal component analysis using the Jaccard's similarity coefficients based on ISSR markers.

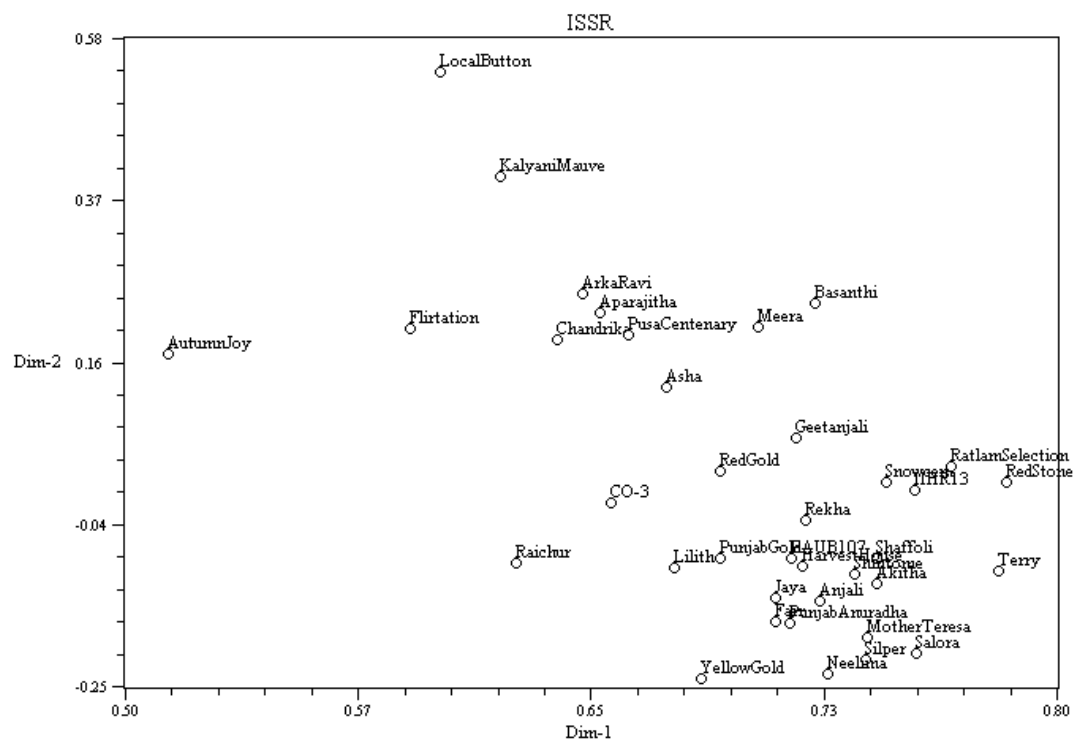
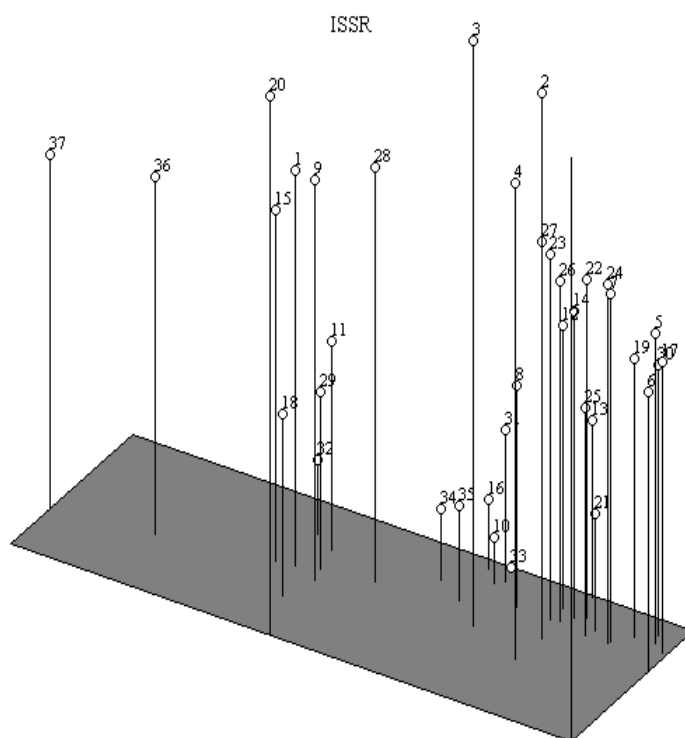


Fig :16 Principal component analysis of 37 chrysanthemum genotypes based on ISSR markers (Three dimensional)



- | | | | |
|--------------------|----------------------|-------------------|--------------------|
| 1. Aparajitha | 9. Chandrika | 18. Flirtation | 27. Lilith |
| 2. Punjab Gold | 10. Snow Cem | 19. Mother Teresa | 28. Asha |
| 3. CO-3 | 11. Meera | 20. Autumn Joy | 29. Pusa Centenary |
| 4. Raichur | 12. Shaffoli | 21. Anjali | 30. Salora |
| 5. Silper | 13. Terry | 22. Akitha | 31. IIHR-13 |
| 6. Yellow Gold | 14. Shintome | 23. PAU-B-107 | 32. Basanthi |
| 7. Punjab Anuradha | 15. Arka Ravi | 24. Farr | 33. Red Stone |
| 8. Rekha | 16. Ratlam Selection | 25. Jaya | 34. Geetanjali |
| 9. Chandrika | 17. Neelima | 26. Harvest House | 35. Red Gold |
| | | 36. Kalyani Mauve | |
| | | 37. Local Button | |

CHAPTER - V

DISCUSSION

Chrysanthemum ((*Dendranthema grandiflora* Tzvelev) comes in a wide variety of colors, including white, off-white, yellow, gold, bronze, red, burgundy, pink, lavender and purple and often possess a wide range of varietal characters. For commercial floriculture, there is always a demand for newer varieties and all the present-day colourful chrysanthemums are the result of selections, spontaneous and induced mutations and hybridization. Traditional breeding, more recently, together with genetic, molecular techniques, has focused on the enhancement of the plant's ornamental value through the improvement of flower colour, size and form, plant height, growth form and sensitivity to light quality which resulted in huge number of cultivars in chrysanthemum.

Sustained progress in improvement of the crop through plant breeding rests on the availability of genetic diversity, which refers to the variations in genetic makeup and is reflected in morphological, physiological and behavioural differences between individuals and populations (Frankham *et al.*, 2002). Assessment of genetic variability has a significant impact both in terms of crop improvement by broadening the base of varieties, as well as for the efficient conservation and management of genetic resources. Classical methods of estimating genetic diversity and / or relatedness among plants have relied on phenotypic traits. But it is difficult to measure variability in natural populations because these traits are influenced by the environmental factors to a large degree. Since the beginning of the 1990s, the development of appropriate tools has resulted in a leading role for molecular markers in the characterization of genetic diversity. In the last decade, the use of DNA markers for the study of crop genetic diversity has gained importance, and has revolutionized the biological science. Increasingly, techniques are being developed to more precisely, quickly and cheaply assess genetic variation.

The knowledge of nature and extent of genetic variation available in the germplasm or breeding material helps the breeder for planning sound breeding programmes. Further, preservation of germplasm is a worldwide concern and conservation of specific diverse genepools will be useful to breeders to ensure the effectiveness of the crop improvement programmes. Several studies have shown that in contrast to their high morphological diversity, cultivated chrysanthemum varieties show an extremely low level of polymorphism when genotyping systems like isozymes are used.

Thus, an attempt was made to study the diversity among chrysanthemum germplasm using qualitative and quantitative traits and with molecular markers such as RAPD and ISSR in order to complement morphological data and to throw more light on the genetic diversity of chrysanthemum. In the light of available literature and explanation wherever possible, results of the present investigation are discussed in this chapter.

5.1 GENETIC DIVERSITY AT MORPHOLOGICAL LEVEL

The morphological characterization includes measuring the variability between chrysanthemum genotypes using morphological characters, both quantitative and qualitative which were recorded following the PPV & FRA guidelines for chrysanthemum. The analysis of variance for thirty quantitative morphological characters revealed highly significant differences among the genotypes.

5.1.1 Quantitative traits

With regards to Chrysanthemum, significant variations were recorded for vegetative characters studied. The growth phenomenon of genotypes was characteristically different for plant height, plant spread, production of primary branches, leaf size and internodal length. In general, variations in growth traits of the genotypes is considered to be a varietal character linked to the genetic constitution of the genotypes (Kanamadi and Patil, 1993 and Behera *et al.*, 2002)

Maximum plant height was noticed with the genotype Chocolate Pompon Variegated (60.75cm) and maximum number of genotypes showed short plant height within the range of 32.5 to 42.5cm. The difference in the plant height is considered an important criterion for the selection of chrysanthemums for their cultivation intended for different purposes (Shanker and Tiwari, 1993). Generally, the growth pattern of the plant is also reflected through its spread in different directions. In the present study, the genotype Apurva Singar showed maximum plant spread (57.03cm) in E-W direction, while it was the genotype Local button which recorded maximum plant spread (69.99cm) in N-S direction. Most of the genotypes (55) had medium number of primary branches per plant ranging between 6.25 and 11.25. Among the genotypes, Pink cascade produced maximum number of (30.92) branches per plant.

With respect to leaf and petiole length, the genotype Red Queen recorded maximum length of 8.70cm and 4.10 cm respectively. While the leaf width was maximum with the genotype Pride of Maid Fort (6.66 cm) followed by Red Queen. However, maximum number of 73 genotypes recorded medium petiole length ranging from 1.25 to 2.75cm. The genotype Yokari showed very small leaf in terms of both length and width and also the depth of sinus in the leaf. The genotypes included in the present study also expressed significant variation for internodal length, where in highest internodal length was recorded for Tata Centenary (4.01cm). Fifty four genotypes were grouped under medium internodal length category (1.25cm – 2.25cm).

Considerable variation was observed among the genotypes for the floral characters. Pink cascade in addition to having maximum number of branches per plant it flowered early by taking 48.99 days for first flower bud appearance and 53.97 days for 50% flowering. Forty five genotypes in the group were having medium spray length with in the range of 11.25 to 16.25cm. The cultivar Red Fair Lady, though recorded the maximum width of the

bud (18.59mm), it showed minimum inflorescence width(18.94cm).Maximum weight of the individual flower (9.00 g) was observed in the cultivar Flirtation, while majority of the genotypes (67) are categorized under low flower weight group (0.5 to 2.5g) followed by twenty two genotypes with medium weight of 2.5 to 4.5g. The flower size in terms of diameter was maximum with Star Pink (8.89cm) followed by Rose day (8.71cm) and Jessie Hab Good(8.43cm). Maximum number of 68 genotypes showed medium sized flower in the range of 2.75 to 5.75cm. The highest values for peduncle length and width was recorded by the genotypes Jessie Hab Good (10.14cm) and Robert Earn Shaw(4.73cm) respectively. With regard to number of ray florets per head which indicates the type of the flower whether single or double is an important character. Number of ray florets were observed maximum in the genotype Lilith (406.71). The maximum length and width of ray floret was recorded in the genotypes John Webber (5.39cm) and Rose Day (1.54cm) respectively. Among all genotypes, Tambra and Tata Centenary had no disc, whereas Terry showed the maximum disc diameter (3.34cm) and length of disc floret(1.76 cm). The above genotypes thus are worthy for utilization in improvement of the respective characters mentioned.

With respect to duration of flowering 52 genotypes showed medium duration in the range of 42.5 to 52.5 days, while the variety Raichur showed long period of flower availability up to 80.35 days. The variation for the blooming period among the varieties can be attributed to differences in their genetic make-up (Gaikwad and Dumbre Patil, 2001 and Kavita Kand Pal *et al.*, 2003).

The genotype Local button recorded highest flower yield per plant (298.54) followed by Chandrika (259.14)though the genotypes PAU -B-107, Rekha produced highest number of flowers per spray (23.30 and 18.60 respectively) (Table 6 and fig 4). Variation in yield potential of flowers is linked to the genetic make up of the genotype. Thus the genotypes Local Button, Chandrika, PAU-B-107 and Rekha appeared to be promising donors for flower

yield and other economic traits. Similar variation as reflected by genetic behavior of plants for yield of chrysanthemum flowers was reported by Behera *et al.* (2002) and also by Manohar Rao and Pratap (2003).

The number of suckers per plant, an important character showing the multiplication capacity of a genotype was found to be maximum in Yellow Bonsai (54.46). Similar finding for suckering capacity of chrysanthemum varieties was reported by Bhaskaran (2001) and Jayanthi and Vasanthachari (2003)

5.1.2 Qualitative traits

Among 104 accessions, eighty six genotypes (82.69%) were of bush type, while remaining 18(17.31%) genotypes are of non bushy nature. Within the 86 bushy plants 79 genotypes (75.95%) exhibited semi upright plant growth habit and seven genotypes (6.73%) exhibited spreading growth habit. In all the genotypes the leaf margin was serrated. The stipule was absent in 25 genotypes (24.04%) and out of 79 genotypes possessing stipules, the size variation was observed among them.

Maximum of 43 genotypes (41.35%) had small size, 24 genotypes showed medium size (23.08%) while only twelve genotypes (11.54%) possessed large stipules. The appreciable variation in margins of sinus between lateral lobes was observed with 43 genotypes (41.34%) having parallel margin followed by thirty seven genotypes (35.58%) under converging margin category and 24 (23.08%) genotypes with diverging margins (Table 8).

All the genotypes were with green colour on both lower and upper side of the leaf with slight variation in intensity of the colour as represented by colour reference number of RHS colour chart. Two types of shapes of the base of the sinus between lateral lobes were noticed, viz., 68 genotypes (63.46%) with round base, while remaining thirty eight (36.54%) had acute sinus base between lateral lobes. With regards to leaf base, maximum of 42

genotypes (40.38%) recorded obtuse leaf base followed by rounded base in 26 genotypes (25.00%), while only one genotype Paragaon Yellow (0.96%) observed asymmetric leaf base.

Based on the size of the flower and purpose of the grower, the 19 genotypes (18.27%) of chrysanthemum were classified as standards, maximum of 71 genotypes (68.27%) as spray types and 14 genotypes (13.46%) as miniatures. Majority of the genotypes (98.08%) had corymbiform form of inflorescence while only one genotype each, cylindrical form and Pyramidal form of inflorescence was noticed in Poonam and Punjab Anuradha respectively. The flower head varied from single (16.35%) to semi double type (45.19%) and double type (38.46%) and majority of genotypes (79.81%) showed straight axis of ray florets. The type of the ray florets is an important character for classifying the chrysanthemum varieties. Considerable variation for this character was recorded with 68.27% of genotypes showing ligulate type of ray florets, 17 genotypes (16.35%) with incurved nature, 14 genotypes (13.46%) recorded spatulate shaped, while only two genotypes Perfeeta and Ravi Kiran (1.92%) observed quilled type of ray florets. Fukai *et al.* (1998) in a study grouped the chrysanthemum cultivars in accordance with the type of ray florets. The evaluated cultivars were grouped as flat-petaled (42%) and quilled-petaled (33%) Anemone-petaled (2%) groups.

The results on flower colour based on ray floret colour revealed that 37 genotypes (35.58%) exhibited yellow colour with different intensity followed by 23 genotypes (22.12%) with white colour, 22 genotypes with reddish brown (21.15%) and twenty two genotypes with pink colour (21.15%). As per findings of Fukai *et al.* (1998) the white flowered (226; 23%) and the yellow flowered cultivars (213; 21%) were the two dominating, while the red (29), green (29) and orange (15) cultivars were much fewer. Maximum of 60 genotypes (57.69%) expressed flat margins of ray florets and 77.88% of genotypes possessed flat disc profile in cross section. The shape of the disc floret varied from funnel shaped (69.23%) to

petaloid nature (23.08%) and only six genotypes (5.77%) fell under the group of plants having tubular disc florets in the flower head. Almost all the genotypes showed yellow coloured to greenish yellow coloured disc florets with slight variation in intensity of the colour except the variety Modella with light pink coloured disc.

Evaluating the genotypes for their economic use, maximum of 42 genotypes (40.38%) were suitable for loose flower cultivation followed by 23 genotypes (22.11%) suitable for garden display, 22 genotypes (21.15%) for cut flower production, 13 genotypes (12.5%) suitable for pot culture and only 4 genotypes (3.86%) can be categorised under ground cover varieties. Similar categorization of chrysanthemum genotypes based on the variation in flower size, number and growth habit was done by Behera *et al.*, (2002) and Kishan Swaroop *et al.*, (2008).

5.2 GENETIC PARAMETERS

The success of any crop improvement programme depends on the magnitude of genetic variability and the extent to which the desirable trait is heritable (Singh and Chaudhary, 1977). The estimates of variability of yield and yield contributing characters and their heritable components in the material are more important in any crop breeding programme. The genotypic coefficient of variation measures the range of variability available in crop species and also enables to compare the amount of variability present in different characters. Whereas, the phenotypic expression of a character is the result of interaction between the genotype and environment. Hence, the total variance needs to be partitioned into heritable and non-heritable components so as to assess the true breeding nature of a particular trait (Falconer, 1964).

The heritability is of interest for a plant breeder primarily as a measure of selection for a particular character and as an index of transmissibility. Randhawa *et al.*, (1975) was of opinion that higher the heritability value of a character, less will be the environmental

influence, thereby indicating an opportunity for selecting a genetically good individual. As per Escuro *et al.*, (1963) the genetic advance indicate the potentiality of selection at a particular level of selection intensity. Thus heritability estimate along with genetic advance are more valuable than heritability alone in predicting the response to selection. Johnson *et al.*, (1955), Robinson (1963), Burton (1952) and Swarup and Chaugle (1962) indicated that genetic variability with heritability estimates would give a better idea on the amount of genetic advance expected out of selection. Panse (1957) reported that magnitude of heritable variability is the most important aspect of the breeding material which has close relationship on its response to selection.

5.2.1 Genetic Variability

Critical estimates of the genetic parameters, viz., phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability in broad sense and genetic advance as per cent mean were estimated for all the characters under study (table 10). The genotypes exhibited a wide range of variability for all the characters. These genotypes under study showed considerable variation in their mean performance for plant, leaf, flower and yield characters. It might be due to difference either in the genetic constitution of the individual plants or in the environment in which they are grown. Similar kind of high degree of genetic variability in qualitative and quantitative morphological characters was reported earlier by Chattopadhyay *et al.*, (1991) in eleven chrysanthemum genotypes and Talukadar *et al.*, (2003) in spray chrysanthemums.

Breeders cannot depend only on the knowledge of variability alone to improve the crop but variance has to be partitioned into phenotypic and genotypic coefficient of variation, which are more reliable for effective selection. It is interesting to note that the differences between GCV and PCV values were minimum implying least influence of environment and

additive gene effects indicating that the genotypes can be improved and selected for these characters.

The characters such as Plant Spread in both the directions, No. of primary Branches/Plant, Petiole Length, Terminal lobe length, Leaf Length, Leaf width, Depth of the sinus, Internodal length, Inflorescence width, Bud width, Average flower weight, Flower diameter, Peduncle length, Peduncle diameter, Ray florets/head, Length and Width of ray floret, Ratio of ray floret length : width, Disc diameter, Number of disc florets/head, Length of disc floret, Number of flowers/spray, Number of flowers/plant and Number of suckers/plant showed higher

estimates for GCV and PCV (Table 10). Singh and Sen (2000), Nandkishore and Raghava(2001) in marigold, Chaugule (1985), Chattopadhyay *et al.*,(1991) in chrysanthemum and Ravi Kumar and Patil (2003) in Chinaster also reported higher GCV and PCV for number of flowers per plant. Comparatively high estimates of variability observed in the above characters especially number of suckers per plant and average flower weight showed that there is ample scope for selection.

The estimates for phenotypic coefficient of variation and genotypic coefficient of variation were moderate for plant height, days to first flower bud appearance , days to 50% flowering and spray length indicating moderate to high variability available in the germplasm for selection. GCV and PCV values for duration of flowering was low. The selection is difficult for these characters because of low variation among genotypes. Similar low GCV and PCV for flowering duration was observed by Namita *et al.*, (2008), Singh and Sen (2000) in marigold and Sirohi and Behera (1999) and Barigidad *et al.*, (1992) in chrysanthemum.

5.2.2. Heritability and Genetic Advance

The coefficient of variation reveals the extent of variability present for different characters and it does not indicate the extent of heritable portion of total variation observed in the character. Hence, information on heritability and genetic advance will be of immense value to breeders to know about the gene effects involved in the inheritance of characters. Burton (1952) suggested that genetic variation along with heritability estimates would give a better idea about the efficiency of selection. High estimates of heritability were observed for all thirty quantitative characters viz., plant height, plant spread in both the directions, No. of primary Branches/ Plant, Petiole Length, terminal lobe length, leaf length, leaf width, depth of the sinus, intermodal length, days to first flower bud appearance, days to 50% flowering,

inflorescence width, spray length, bud width, average flower weight, flower diameter, peduncle length, peduncle diameter, ray florets/head, length of ray floret, width of ray floret, ratio of ray floret length : width, disc diameter, number of disc florets/head, length of disc floret, number of flowers/spray, number of flowers/plant, duration of flowering and number of suckers/plant.

The heritability value alone can't provide any indication of the amount of genetic progress that would result from the selection of the best individuals. The heritability and genetic advance, when calculated together are most useful for predicting the resultant effect, thus, selecting the best individual than considering heritability or genetic advance alone. Since, magnitude of genetic advance is influenced by units of measurement, genetic advance as percentage of mean was computed.

High heritability coupled with high genetic advance as percentage of mean was recorded for all the quantitative characters studied except duration of flowering, indicating contribution of additive gene effects in the expression of these traits. Therefore improvement in these characters can be done through direct selection to select better genotypes of chrysanthemum. Similar kind of high estimates of heritability coupled with high genetic advance as percentage of mean were reported by Chaugule (1985) for suckers per plant, yield

per plant, number of flowers per plant and number of branches per plant, Sheela *et al.*, (2007) for number of flowering shoots/year in heliconias and Barigidad *et al.*, (1992) for number of petals per flower including both number of ray and disc florets in chrysanthemum. On contrary, high heritability coupled with moderate genetic advance as per cent of mean was noticed by Mishra *et al.*, (2006) for days to first flower bud appearance in chrysanthemum.

High estimates of heritability coupled with moderate genetic advance as per cent of mean was recorded for duration of flowering suggesting that the character is governed by dominant and epistatic gene action and needs further improvement of genotypes for this character for further selection and subsequent use in breeding programme. On contrary, high heritability coupled with high genetic advance as percentage of mean was noticed by Laxmi *et al.*, (2009) for duration of flowering indicating the role of additive gene effects in its expression.

5.3 CHARACTER ASSOCIATION

5.3.1 Correlation coefficient analysis

The phenotype of the plant is the result of interaction of a large number of factors. The yield is the end product of interactions of many factors known as contributing components, hence, it is a complex trait. Understanding of the interaction of characters among themselves and with the environment has been of great use in the plant breeding. The influence of these characters can be known through correlation studies. Correlation coefficients measure the magnitude and direction of association among the characters. Genetic correlation among different characters of a plant often arises because of either linkage or pleiotropy (Harland, 1939).

Grafius (1959) opined that there may not be any gene for yield as such but operates only through its components. Hence, the study of character association through correlation will help in selecting the yield attributes. From this it could be possible to bring about genetic up gradation in one character by selection of the other pair. The correlation pattern is expected to differ with material that is studied, since it is a reflection of the genetic make up of the population.

The correlation studies primarily aim to know the suitability of various characters for indirect selection, because selection of any particular trait may bring about undesirable changes in other associated characters. In general, in the present study the genotypic correlation was higher than phenotypic correlation indicating less influence of environmental factors and relative stability of the accessions. It simultaneously helps to improve different characters (Falconer, 1964).

The correlation between flower yield per plant with different yield attributes and the attributes themselves are presented in table 11. A significant positive correlation both at genotypic and phenotypic levels was recorded between flower yield and plant attributes viz., plant spread at both the directions, number of primary branches per plant, spray length and number of flowers per spray. The magnitude of correlation with flower yield was highest in case of number of flowers per spray followed by characters viz., plant spread in both the directions, number of primary branches per plant and length of the spray, indicating that the association between yield and these characters was positive and high. These results are in consonance with those reported by Dar and Chattopadhyay (2003) in *Zinnia*, Reena *et al.*, (2005), Deepti Singh and Santosh Kumar (2008), Singh and Singh (2005) in marigold. This indicates that flower yield in chrysanthemum can be improved by direct selection of these characters. The positive correlation between the desirable characters is favourable to the plant breeder because it helps in simultaneous improvement of all the characters.

Other characters like days to first flower bud appearance, days to 50% flowering and duration of flowering recorded positive but non-significant correlation with flower yield both at genotypic and phenotypic levels. On the other hand, characters like bud width, average flower weight and flower diameter showed

negative correlation with flower yield both at phenotypic and genotypic levels indicating that the association between these two traits was negative and high. These results are in consonance with those reported by Raghava *et al.*, (1992), Sirohi and Behera (1999), Pal and George (2002), Deka and Paswan (2002) and Bhaskaran *et al.*, (2004).

Plant height exhibited high significant positive association with plant spread in both the directions, leaf length, days to first flower bud appearance, days to 50% flowering, spray length, bud width, average flower weight and flower diameter. Raghava *et al.*, (1992) also reported a positive association of plant height with flower size in chrysanthemum. These results are in agreement with those of Negi *et al.*, (1983), Aswath and Parthasarathy (1993) who reported significantly positive correlation of plant height with flower size in china aster.

Janakiram and Rao (1993) observed significant positive association of plant spread with flower yield in French marigold. Similarly in the present study, plant spread in both the directions had significant positive association with number of primary branches per plant, days to commencement of flowering, days to 50% flowering, spray length, number of flowers per spray and flower yield at both phenotypic and genotypic levels. Hence, for improving the flower yield in chrysanthemum the selection can target this character.

The relationship of number of primary branches per plant with number of flowers per spray and flower yield per plant was positive and highly significant. This is in agreement with the earlier findings of Jhon *et al.*, (1994) who observed significant correlation between number of branches per plant and flower yield in Zinnia. Leaf length and width expressed significant positive correlation with average flower weight, flower diameter, bud width and duration of flowering at both phenotypic and genotypic levels.

With regards to earliness in flowering, significant positive association was showed with spray length, number of flowers per spray and average flower weight. Spray length displayed positive and significant correlation with number of flowers per spray, flower yield

per plant and average flower weight. Bud width showed positive and highly significant correlation with average flower weight and flower diameter. The association between average flower weight and flower diameter was positive and highly significant, while it was significant and negatively correlated with number of flowers per spray and flower yield per plant. Flower diameter exhibited a negative and highly significant correlation at both phenotypic and genotypic levels with number of flowers per spray and flower yield per plant whereas positive significant correlation with duration of flowering at 5% level of significance. Highly significant positive correlation was noticed between number of flowers per spray and flower yield per plant

5.3.2 Path coefficient analysis

The computed correlation coefficient values are partially useful in explaining the nature and extent of association existing between pairs of characters. The economic character like flower yield is dependent on several component characters, which are mutually related. Correlation explains the true association existing between the component characters with dependent character (flower yield). Slight change in any one component will ultimately disturb the complex. Hence character has to be analyzed for its action which is done through path analysis as devised by Wright (1921), where the two types of action namely direct effect of component character on flower yield and the indirect effects through other component characters on flower yield are obtained, which cannot be realised by the correlation studies. Further, Li (1956) emphasized the importance of path diagram which facilitates the understanding of the nature of cause and effect system.

If the correlation coefficient between causal factor and the effect is almost equal to its direct effect, this correlation explains the true relationship and direct selection through this trait will be useful. If the correlation coefficient is positive, but its direct effect is negative or negligible, the indirect effect appears to be the cause of that positive correlation. In such

situation, the other factors are to be considered simultaneously for selection. Sometimes, correlation coefficient may be negative, but the direct effect may be positive and high, under such circumstances, a restricted simultaneous selection has to be followed *i.e.*, restriction has to be imposed to nullify the undesirable indirect effect in order to make use of the direct effect.

Based on the above, the characters subjected to correlations were also subjected to path coefficient analysis for estimating the direct and indirect effects, so as to formulate more authentic basis for selection in chrysanthemum.

In the present study, path coefficient analysis was performed for flower yield per plant taking it as a dependent variable and fourteen other characters *viz.*, plant height, plant spread in both the directions, number of primary branches per plant, leaf length and width, days to first flower bud appearance, days to 50% flowering, spray length, bud width, average flower weight, flower diameter, number of flowers per spray and duration of flowering as independent characters. Both phenotypic and genotypic paths were worked out and are presented in table 12, 13 and fig 6, 7. Since the phenotypic path will have a greater influence of environmental factors, the genotypic path was considered with a greater weightage.

Plant spread in N-S direction recorded the highest direct effect on flower yield per plant followed by number of flowers per spray, leaf length, days to 50% flowering, number of primary branches per plant, average flower weight and duration of flowering. The high direct effect of these traits appeared to be the main factor for their strong association with flower yield per plant. Hence, direct selection for these traits would be highly effective in improving the per plant flower yield.

Plant spread in N-S direction showed a high indirect positive effect on flower yield per plant *via* plant spread in E-W direction followed by number of flowers per spray, number of primary branches per plant and spray length. As plant spread in N-S direction influences

flower yield per plant through these characters, indirect selection for plant spread in N-S direction *via* the aforesaid characters may be useful in improving the yield. Since this trait is showing high positive correlation and high direct effect on flower yield per plant, one can improve the flower yield in chrysanthemum by making selection for this character during yield improvement programme.

Along with the high direct positive effect, number of flowers per spray exhibited a positive indirect effect to flower yield via plant spread in both the directions and spray length. Hence, selection based on number of flowers per spray may be useful. This suggests that emphasis must be given on such traits while exercising selection to improve the flower yield in chrysanthemum. Therefore, it would be rewarding to stress on plant spread in N-S direction and number of flowers per spray while making selections. These traits also had high correlation and high direct effect on flower yield per plant.

The results of the study indicated that the characters with positive correlation have shown high direct effects. Hence, the perusal of correlation and path analysis studies of the present investigation revealed that plant spread in N-S direction and number of flowers per spray are the highly important yield attributing components in the order of having direct bearing on the improvement of flower yield per plant. The residual effect in the present study was found to be high in path analysis indicating that there is a need to include other characters in order to derive a much clear picture of the causal relationship.

5.4 GENETIC DIVERGENCE

Quantification of genetic diversity existing within and between groups of germplasm is important and particularly useful in proper choice of parents for realizing higher heterosis and obtaining useful recombinants. Several methods have been advocated by various workers to estimate the genetic divergence in crop plants (Murthy and Arunachalam, 1966; Bhatt, 1970; Hussaini, 1973). According to Singh (1998), it is desirable to select suitable and

genetically divergent parents, based on information about the genetic variability and genetic diversity present in the available germplasm. Hence, the present study was undertaken to assess the nature and the magnitude of genetic diversity to identify suitable donors having wider genetic base in chrysanthemum.

5.4.1 D^2 analysis

The multivariate analysis by means of Mahalanobis D^2 statistics is found to be an useful tool in quantifying the degree of divergence between biological population at genotypic level and also to assess the relative contribution of different components to the total divergence both at inter and intra cluster level (Mischner and Sokal, 1957 ; Morashima and Oka, 1960 ; Nair and Mukherjee, 1960 and Murthy and Quadri, 1966). The magnitude of variability and its quantitative estimation for each character would only indicate the potentiality of the collection and the scope for improving the studied character through selection. It also provides a quantitative measure of association between geographic and genetic diversity based on generalized distance (Mahalanobis, 1936). Further, the problem of selecting diverse parents for hybridization programme can be narrowed, if one can identify the characters responsible for the discrimination between the populations (Rao, 1952).

The genetic divergence among 104 genotypes of chrysanthemum was quantitatively assessed by adopting Mahalanobis's D^2 statistics and the mean values of thirty quantitative characters were utilized for working out genetic distance between pairs of genotypes and used for final grouping of the genotypes. The magnitude of D^2 values suggested that there was considerable variability in the material studied, which led to genetic diversity.

Based on D^2 values the Chrysanthemum genotypes were grouped into eleven clusters and were shown in Table 14 and illustrated in figure 8. Among the clusters, cluster VII was the largest with 31 genotypes, followed by cluster III with 15 genotypes and cluster I having 12 genotypes. Clusters IV, VIII and X each had nine genotypes in them. While the XI cluster had six genotypes followed by clusters V, IX each bearing five genotypes. The remaining

clusters VI had two genotypes whereas the II cluster was solitary. The present findings are in accordance with Bihari *et al.*, (2009) and Swaroop (2010) who assessed genetic divergence in 50 and 28 gladiolus genotypes and grouped them into six and eight clusters respectively. The pattern of distribution of accessions from different eco-geographical regions into different clusters with different divergence values was at random supporting that geographical diversity is not related to genetic diversity. The main forces other than geographical origin responsible for this genetic diversity may be natural/artificial selection, exchange of breeding material, genetic drift and environmental variation.

Similar conclusions were drawn by Kavitha and Anburni (2009), Tyagi (1978) and Shanmugam (1973) who did not observe association between geographical distribution of accessions and genetic divergence.

The intra cluster D^2 values among the eleven clusters showed that, the intra cluster D^2 values ranged from 0.00 in cluster II which is represented by single genotype to 92.10 in cluster VI having two genotypes (Table 15). It revealed the presence of divergent genotypes within different clusters. The inter cluster D^2 values ranged from 38.92 to 195.87 wherein, the lowest inter cluster D^2 value was recorded between cluster III and IV (38.92) while the highest inter cluster D^2 value was recorded between clusters VI and IX (195.87) indicating that crosses may be attempted between the genotypes of cluster VI (Aparna Singar and Local Button) and cluster IX (Miss India, Tambra, White Silver Cloud, Red Queen and Swan) to obtain new desirable recombinants in chrysanthemum. On the other hand, minimum distance observed between cluster III and cluster IV (38.92) indicated almost parallel diversity among the genotypes included in this cluster.

The two genotypes in cluster VI had highest plant spread in both directions, maximum inflorescence width, maximum spray length, highest number of flowers per spray and number of flowers per plant and finally the highest suckering habit, while the 5 genotypes in cluster

IX exhibited maximum petiole length, highest leaf length and width and maximum ray floret length. From this it can be suggested that the genotypes present in these clusters with high mean performance for majority of the important quantitative traits can be utilized in breeding programme.

Among all the characters, the most important character contributing to divergence was number of ray florets per head followed by number of disc florets per head and flower yield per plant. The characters viz., days to first flower bud

appearance, days to 50 per cent flowering, number of suckers per plant, inflorescence width, duration of flowering and plant height contributed 34.0, 9.0, 7.0, 2.0, 2.0 and 1.0 per cent respectively to the genetic divergence in decreasing order. The other characters like plant spread in both directions, number of branches per plant, petiole length, terminal lobe length, leaf length and width, depth of sinus, internodal length, spray length, bud width, mean flower weight, flower diameter, peduncle length and diameter, ray floret length and width, ratio of ray floret length and width, disc diameter, disc floret length and number of flowers per spray showed no or negligible contribution towards divergence in the present material studied. The present study clearly indicated that progenies expected from crossing diverse parents would reveal greater diversity for crop improvement. These results are in good agreement with the findings of Aswath (1994) in gerbera, Desh raj and Mishra (2000) in gladiolus, Prasad *et al.*, (1998) and Nimbalkar *et al.*, (2006) in dahlia.

Based on multivariate analysis, the cluster-VI had scored high mean values for important economic attributes. As the magnitude of heterosis depends largely on the degree of genetic diversity of parental lines, the genotypes Apurva Singar and Local Button can be used to derive a broad spectrum of genetic variability in the segregating generations for flower yield per plant.

5.4.2 Principal Component Analysis

PCA is a method of data reduction to clarify the relationships between two or more characters and to divide the total variance of the original characters into a limited number of uncorrelated new variables (Wiley, 1981). It is used to identify multi-dimensional relationship existing among characters for the definition of groups and determines the relative contribution of each trait to the total variation in accessions. The proportion of variation accounted for by each principal component is expressed as the Eigen value divided by the sum of the Eigen values. The importance of component is evaluated by means of the percentage of the total variation explained by it and the first component is defined as the most important, since it accounts for the greatest part of the total variation found in the original data. Cruz and Carneiro (2003) reported that if the first component accumulates a relatively high percentage of the total variation, generally determined as over 80%, then it is presumed that they satisfactorily explain the variability expressed in the evaluated plants.

In the present investigation, among the 104 genotypes of chrysanthemum evaluated for 30 morphological characters, the first eight principal components with eigen values more than one contributed to 73.23 per cent of cumulative variability. The first principal component accounted for 22.71 per cent of variability while the second and third accounted for 15.91 and 9.04 per cent of total variability, respectively. The percent of variability from fourth to ninth principle component accounted for 8.02, 5.91, 4.38, 3.73, 3.53 and 2.85 in decreasing order respectively. All the nine principal components accounted for 76.08 per cent of cumulative variability.

The first principal component (PC1) accounted for 22.71 per cent of total variance was loaded with maximum contributing variables like flower diameter followed by length of the ray floret, bud width, peduncle length and diameter, average flower weight, ray floret

width, leaf length and width, number of ray florets per head, petiole length, plant height, terminal lobe length, intermodal length, depth of the sinus and duration of the flowering.

A three dimensional plot is illustrated and the same is presented in fig. 10 depicting the spatial distribution of chrysanthemum genotypes using principal

component analysis score (PCA). Wider distribution of the genotypes was observed indicating the presence of considerable diversity in the germplasm lines.

5.4.2 GENETIC DIVERSITY AT MOLECULAR LEVEL

Genetic variation is a pre-requisite for successful crop improvement programme. Study of the diversity among available varieties or breeding lines is very important in agricultural species, and is particularly interesting in chrysanthemum as in most of the cases the origin of varieties is unknown. Traditionally, genetic diversity studies have been based on morphological characters. However, the development of new techniques has allowed these analysis to be based on DNA information. Though a range of plant characters are currently available for distinguishing between closely related individuals, their sensitivity to environment and less genome coverage hinders their usage. DNA based molecular markers acted as versatile tools to study variability and diversity in different plant species. Molecular markers clearly allow the comparison at DNA level, among different genotypes avoiding any environmental influence on gene expression. Molecular markers enable the assessment of genetic similarity between accessions in the early stages of development (non stage dependent) and are advantageous for diversity studies (Nicolosi *et al.*, 2000). One of them is the PCR based technique RAPD (Random Amplified Polymorphic DNA) (Williams *et al.*, 1990) that has been widely used for plant germplasm characterization (Wilde *et al.*, 1992; Wolff *et al.*, 1994).

In the present study, apart from using the morphological traits, RAPD and ISSR markers were also used to estimate the extent of molecular diversity among chrysanthemum genotypes. The diversity among 37 chrysanthemum genotypes based on the results of RAPD and ISSR data are discussed hereunder.

5.5.1 DNA yield and purity

The purity of DNA is determined by OD 260/280 ratio where in the ratio of 1.65 or less indicates protein contamination while values exceeding 1.95 indicated the presence of RNA (Sambrook *et al.*, 1989). In the present investigation the purity of DNA of chrysanthemum genotypes was found to be in the range of 1.66 to 1.94 indicating that the DNA obtained from this protocol was of good quality (Table 20).

5.5.2 Genotyping of Chrysanthemum genotypes using RAPD markers

One hundred and twenty four primers were screened for their ability to amplify the genomic DNA of the 37 genotypes of Chrysanthemum. Of these, 27 RAPD primers yielded good number of amplicons with molecular size ranged from 350bp to 3500 bp. This was comparable with results generated by polymorphic bands ranging from 300 bp to 1200 bp (Barakat *et al.*, 2010) in *Chrysanthemum morifolium*, 400bp to 1900 bp (Chatterjee *et al.*, 2005) in four miniature cultivars of chrysanthemum, 212 to 2730 bp (Lema-Rumińska *et al.*, 2005) in radio mutants of chrysanthemum, 550 to 2000 bp (Sehrawat *et al.*, 2003) in commercial cultivars of *Chrysanthemum morifolium*, 600 bp to 1300 bp (Qin Helan, *et al.*, 2002) in 18 cultivars of Chrysanthemum.

In the present study, a total number of 271 polymorphic bands were produced by the use of 27 RAPD primers which amplified 10.11 bands per primer and appears to be reasonable number to make meaningful statements about the diversity or relatedness among the different chrysanthemum genotypes. Similar kind of amplification of 9.6 bands per primer

in *Chrysanthemum morifolium* cultivar "Delistar White" and its mutants was reported earlier by Barakat *et al.* (2010). On the other side, these results are considered rather high for RAPD amplification, compared to the average number of amplified bands recorded in other crops such as three fragments in *Triticum turgidum* (Joshi and Nguyen, 1993), 4.3 fragments in *Solanum tuberosum* (Masuelli *et al.*, 1995).

A total of 278 fragments were amplified with 27 RAPD primers. Of which 271 were found to be polymorphic (97.4%) which indicated the efficiency of the selected primers to characterize the germplasm. Maximum polymorphism in the present study was obtained by the primer OPK-19 with 17 polymorphic bands while the primer OPE-15 produced only 6 polymorphic bands. This value was higher than those reported by Xin lei and Chen Fadi (2004) with 77.1 % polymorphism in 23 lines comprising of wild species, cultivars and inter specific hybrids in *Dendranthema*, Nianjun Teng *et al.*, (2006) with 61.7% polymorphism among chrysanthemum plantlets regenerated from callus treated with 0,10,15, and 20 Gy gamma rays, Wen-bin *et al.*, (2006) with 89.7% polymorphism among 22 *Chrysanthemum morifolium* accessions. This high level of polymorphism expressed by the primers indicated the importance of these primers in DNA fingerprinting studies.

The highest percentage of polymorphism (100%) was observed with majority of the RAPD primers except OPE-14, OPE-15, OPE-18, OPF-3, OPF-5 and OPI-18. The high polymorphism observed in the present study confirms that much diversity exists within this germplasm. The total number of amplified fragments generated per primer had no correlation with proportion of polymorphic bands. Similar pattern was observed by Williams *et al.*, (1993). Martin *et al.*, (2002) used RAPD analysis with ten primers to characterize fifteen commercial varieties of chrysanthemum. Kumar *et al.*, (2006) also noticed a high degree of polymorphism in chrysanthemum cultivars using RAPD markers. Their results indicated that

out of forty primers screened, twenty-one were selected on the basis of robustness of amplification, reproducibility and scorability.

Williams *et al.*, (1990) reported that polymorphism among individuals could arise through nucleotide change that prevented amplification by introducing either a mismatch at one priming site, deletion of a priming site, insertions that rendered priming sites too distant to support amplification and insertions or deletions that change the size of the amplified product. Bhat *et al.*, (1995) suggested that the number of polymorphisms might be more important than the number of primers for the generation of stable phenogram and it would vary with plant material used for investigation and the sequences that are amplified. RAPD has proved to be a very useful and rapid method to detect variation between different chrysanthemum varieties, even with less number of markers. Wolff (1996) reported that the choice of the primers may be an important factor in obtaining a rapid discrimination between samples.

Genotype-specific RAPD markers were detected (Table 22) only for few genotypes Meera (OPE-14), Salora (OPF-5), Terry (OPF-19), Punjab Anuradha (OPG-16), Anjali (OPG-19) and Asha (OPM-10) which could be exploited for DNA fingerprinting of these genotypes by converting RAPD markers into STS (Sequence Tagged Site) markers and this is useful for detecting mixtures between cultivars (Fernandez *et al.*, 2002 and Chimote *et al.*, 2007). Cultivar specific RAPD markers were also identified by Sehrawat *et al.*, (2003) in *Chrysanthemum morifolium* cultivars and Qin Helan *et al.* (2002) who observed 1200 bp fragment generated by primer OPD15 only in 'Dahongtuogui' cultivar of chrysanthemum. However, to have more authentic, reliable and durable fingerprints it is suggested to have a range of primers to distinguish a variety. Mutants with different flower colour could be identified at the molecular level using RAPD technique holding promise for the development of robust SCAR markers. The results of Badr *et al.*, (2006), El-Nashar (2006) and Kumar *et*

al., (2006) indicated that RAPD markers could be effectively used for genetic diversity studies among radio mutants.

The data in the present study confirmed that RAPD methods are useful tools for identifying closely related genotypes. Their advantage principally lies in detecting simultaneously many randomly distributed loci within the genome, in a simple, cost-effective manner, requiring no previous knowledge of genome sequence as other methods like SSRs. Also RAPD primers quickly scan the whole genome detecting point mutations and insertion-deletion events (Milbourne *et al.*, 1997).

However, some doubts have been expressed regarding the reproducibility of the RAPD technique and the suitability of the RAPD markers for genetic diversity studies (Rafalski *et al.*, 1991, Wilkie *et al.*, 1993 and Hallden *et al.*, 1996). The reproducibility of RAPDs can be achieved by using optimized PCR conditions and by scoring only reproducible bands (Aliyev *et al.*, 2007). According to the measurements of genetic similarity there are no redundant genotypes in the collection used in the present study indicating that the genetic purity of all genotypes analyzed have been well maintained.

5.5.2.1 Genetic diversity and genetic relatedness using RAPD markers

Cluster analysis revealed that all cultivars were not clustered on the same branch, suggesting variations within them. The association among different genotypes may be attributed to the broad genetic base, which enables them to maintain and exist in different gene combinations. The similarity coefficients ranged from 0.174 to 0.600, suggested a broad genetic base for chrysanthemum genotypes. Cluster analysis of chrysanthemum genotypes employing the UPGMA method led to the classification of genotypes into four major clusters having multiple genotypes and seven minor clusters with one genotype each in cluster V(Arka Ravi), VI(Meera), VII(Asha), VIII(Silper), IX(Autumn Joy), X (Lilith) and

cluster XI (Chandrika). The reason for the separation of these genotypes as individual clusters is due to their different genetic backgrounds.

At genetic similarity index of 0.23, the two yellow coloured, spray type genotypes Chandrika and Aparajitha were found to span the extremes of the dendrogram with the remaining genotypes distributed in between them. This clearly indicates diversity is mainly because of inherent genetic differences at DNA level.

The cluster I included the three yellow coloured genotypes Aparajitha, Basanthi and Local button. The five yellow coloured genotypes (Punjab Gold, Yellow Gold, CO-3, Punjab Anuradha and Rekha) belonging to spray class, decorative and double Korean types were included in the cluster II. Sharing of genetic similarity, among the genotypes originating from widely divergent locations was also evident as Punjab genotypes were grouped together with one genotype from Coimbatore in cluster II. Though they differed morphologically, they were similar at the DNA level. This suggested a distinct genetic identity and rather large genetic divergence from most plant selections of the geographically closer ones (Das *et al.*, 2004). The other possible reason might be sharing a similar gene pool before their geographical separation (Jaiswal and Amin, 1992). This could be a reason for less genetic distance observed between these genotypes in this study.

The genotypes Shaffoli, Shintome, Akitha, Anjali, Terry, Salora, PAU-B-107, Red Stone, Geetanjali, Neelima, Red Gold, IIHR-13, Flirtation, Jaya, Kalyani Mauve, Farr, Harvest House, Mother Teresa and Pusa Centenary were assigned to cluster III. In this group Shaffoli and Pusa Centenary were straddling the extremes.

The similarity ranged from a lowest of 0.42 between Mother Teresa and Pusa Centenary to the highest of 0.56 between Akitha and Shintome ; Terry and Salora.

Except Geetanjali, Kalyani Mauve and Pusa Centenary, all other genotypes are pink and red coloured ones with few white coloured genotypes clustered in one group suggesting

that they either originated from a common cultivar or that the technique needs further improvement to detect cultivar variation, such as point mutations which can not be detected by RAPD (Dettori and Palombi, 2000). These results are in agreement with Coletta Filho *et al.*, (1998) and Shaaban *et al.*, (2006). This could be overcome by using more number of primers such that they cover largest portion of genome which would be possible to elucidate the diversity more precisely.

Induced mutagenesis through irradiation or chemical treatment has been successfully used for the development of new flower colour/shape mutants in *Dendranthema*. India has a particular impressive share as it has commercially released 46 mutant cultivars in chrysanthemum alone in year 2004 (Chopra, 2005). They reported that the role of hybridization in genetic diversification is low, while the frequent mutation and subsequent selections are the major factors of diversification. This study showed that differences observed between commercial and exotic cultivars and other local selections could have been produced through mutations and subsequent propagation from mutant part of mother plant. This can be assigned to be a major reason for clustering of genotypes into one cluster though they are morphologically different. This process will result in different progenies from the mother plant through time. Results of several studies performed by Motahashi *et al.* (1992), Sugarawa and Oowada (1995), Asins *et al.* (2001), Domingues *et al.* (2004) and Dehesdtani *et al.* (2007) support these findings.

Intra-location genetic divergence among the plants was quite evident from the grouping of plants collected from Punjab in cluster II (Punjab Gold and Punjab Anuradha) and cluster III (PAU-B-107), sub cluster IV (Ratlam Selection) and Cluster IX (Autumn Joy).

The dendrogram (Fig 11) based on RAPD analysis showed that most of the chrysanthemum genotypes were distantly related with low genetic similarity index of 0.23 and the genotypes have been grouped into different clusters. High level of genetic variability

was observed among chrysanthemum varieties. Wolff & Peter-Van Rijn (1993) in their study involving other varieties of chrysanthemum reported that high level of variability is quite logical in out crossing plant species like chrysanthemum. However, they could not detect differences in RAPD pattern among somaclonal mutant cultivars. They reported that the varieties derived vegetatively from one original cultivar could not be distinguished from each other using this technique. Similar pattern of high genetic diversity with RAPD markers were observed in 15 commercial varieties of chrysanthemum (Martin *et al.*, 2002) in which level of similarity was in all the cases less than 80%. A high genetic distance among the different chrysanthemum cultivars showed a possibility of introgressing new and novel genes from the chrysanthemum gene pool. On contrary, Chatterjee *et al.*, (2005) reported high similarity among four chrysanthemum mini cultivars showing low genetic diversity. It might be after mutation breeding or crossing with exotic parents which are genetically similar thus narrowing the genetic pool of the species (Corazza-Nunes *et al.*, 2002).

From the foregoing discussion, it can be concluded that RAPD markers could be employed for finger printing and characterization of genotypes at molecular level, assessment of molecular genetic divergence and relatedness among chrysanthemum genotypes independent of any environmental influence. Being more efficient than morphological markers these could be an useful tool in cultivar identification and protecting them from possible infringements in future.

5.5.3 Genetic diversity and relatedness using ISSR markers

In the present study, apart from using morphological traits and RAPD markers, ISSR markers were also used to estimate the extent of molecular diversity among chrysanthemum genotypes. ISSR markers seem to produce more reliable and reproducible bands than RAPDs because of longer length of the primer and their high annealing temperature (Nagaoka and Ogihara, 1997; Wolfe and Liston, 1998; Qian *et al.*, 2001; Bornet

and Branchard, 2001). Like RAPDs (Williams *et al.*, 1990; Welsh and McClelland, 1994), ISSRs are simple and quick method to check and estimate diversity level because of no prior sequence data is needed and a large number of markers can be generated (Zietkiewicz *et al.*, 1994). Forty six ISSR oligonucleotides were used for amplification of all the thirty seven genotypes out of which only 10 primers yielded reproducible amplicons, while 36 primers failed to amplify.

The ten primers produced 114 bands across 37 genotypes of which 107 bands were polymorphic, accounting for 93.86% polymorphism. The ability of ISSR primers to reveal available polymorphism more efficiently offers great potential to determine intra- and inter genomic diversity as compared to other arbitrary primers like RAPD as has been reported by Zietkiewicz *et al.*, (1994). However, the number of total polymorphic and discriminate fragments recorded in the present study was higher for RAPD than ISSR. The number of bands varied from seven (ISSR-825) to fifteen (ISSR-808 and ISSR-857) with a mean value of 11.4 bands per primer and the PCR product size obtained ranged from 220bp to 2000bp. The percentage of polymorphism exhibited by different ISSR primers ranged from 71.43% (ISSR-825) to 100% (ISSR-808, 810, 812, 840 and 842). Though, the number of bands produced were more with the poly AG primers (15) and with the poly AC primers (15), the primers that were based on the poly GA and poly AG motif produced more polymorphism than the primers based on any other motifs used in the present investigation. This is in accordance with the findings of Shwetha Gupta *et al.*, (2008) among different genotypes of *Jatropha Curcus*.

The similarity coefficient based on 10 ISSR markers ranged from 0.275 to 0.775 with a mean similarity matrix of 0.525. Miao Heng-bin *et al.*, (2008) have also reported the same range of similarity coefficient (0.282-0.757) while studying the genetic diversity in 25 cultivars of *Chrysanthemum morifolium* using ISSR primers. Among the 85 cultivars of

Chrysanthemum kitamura, Miao Heng-bin *et al.*, (2007) observed the similarity coefficient range of 0.294 to 0.802 with 22 ISSR primers. Among the 37 genotypes studied, the closest relationship was scored between Geetanjali and Red Stone with similarity level of 77.5% although they exhibit considerable morphological variation with yellow and red coloured flowers respectively. This conflict between molecular and morphological markers in the absence of intermediates may be the result of strong disruptive selection that preserved typical floral morphologies after secondary contact and gene exchange. Other studies have documented the retention of morphological differentiation despite a lack of genetic differentiation (Streisfeld and Kohn, 2005 ; Currat *et al.*, 2008), and a recent study describing a pattern similar to that seen here demonstrates that infrequent hybridization can lead to genomic homogenization while not blurring morphological boundaries (Barreto and McCartney, 2008). Among the genotypes, Autumn Joy and Flirtation gave the lowest similarity level of 27.5% which were considered distantly related genotypes.

The similarity level of more than 60% was recorded in the following pairwise genotypes viz., Punjab Anuradha and Silper; Shaffoli and Silper; Ratlam Selection and Shaffoli; Mother Teresa with Terry and Neelima; Akitha with Shintome and Terry; Salora with Shaffoli, Terry and Neelima; IIHR-13 with Terry, Ratlam Selection and Jaya; PAU-B-107 with Farr; Red Stone with Snow Cem, Terry, Ratlam Selection and Anjali.

The spray type, yellow coloured varieties Aparajitha, Punjab Gold, CO-3, Raichur, Silper, Yellow Gold, Punjab Anuradha, Rekha and Chandrika are having 50% level of similarity on an average among themselves.

Cluster and PCA analysis were employed to observe the genetic relationship in thirty seven genotypes of *Chrysanthemum*. The UPGMA dendrogram has ten clusters in which cluster IV was the largest one consisting of 11 genotypes followed by cluster III with six genotypes, cluster V with five genotypes, cluster VIII with four genotypes, cluster I and II

with three genotypes each, cluster VI with two genotypes and cluster VII, IX and X with one genotype each. The first cluster (cluster I) contains three genotypes Aparajitha, Chandrika and Meera sharing more common alleles with 53% similarity. In the II nd cluster, the genotypes Punjab Gold and CO-3 were found identical with 58% similarity, as also evident from RAPD primers in one cluster and with phenotypically similar traits with respect to number of primary branches per plant, leaf characters, bushy and semi upright growth habit of the plant, yellow coloured and spray type of flowers. The cluster III had five genotypes with Silper and Yellow Gold straddling the extremes. It was further divided into two sub clusters of which Silper and Punjab Anuradha; Shaffoli and Salora were identical at 0.63 and 0.65 similarity coefficient value respectively. This cluster also contains a genotype Neelima which was distantly related to Shaffoli and Salora sub cluster at 60% similarity level. The two genotypes Pusa Centenary and Red Gold were identical with 0.53 similarity coefficient value in the cluster VI. The local collection Raichur forms a distinct genotype under cluster VII as reflected by its unique nature of extended duration of flowering. The genotype Flirtation forms the most diverse genotype lying in cluster IX which also showed unique trait of fringed tip of ray floret and cluster X is having one genotype Autumn Joy which forms a separate entity from rest of the genotypes as evident in RAPD clustering pattern. Akitha showed close relationship with Shintome in all the cases i.e, RAPD, ISSR and phenotypic diversity studies. It may be due to the seedlings drift with the migrating people from place to place.

5.5.4 Comparision of clustering pattern based on morphological diversity and molecular diversity

Morphological characterization is an important first step in the assessment of genetic diversity, but has certain limitations due to morphological plasticity and parallel evolution (Prakash and He, 1996). It is being used for various purposes including

identification of duplicates, studies of genetic diversity patterns and correlation with characteristics of agronomic importance (CIAT, 1993). Morphological characters of plants may be affected by environmental conditions and the plants grown in different environmental conditions may be different morphologically. Thus the use of morphological characters for the classification may result in discrepancy. Therefore, use of molecular marker tools may give precise information for characterization of the crop genotypes. Efficiency of a molecular marker technique depends on the amount of polymorphism it can detect among the set of genotypes under study. However, the genetic differences exhibited as presence/absence of polymorphisms that exist between genotypes can be combined with phenotypic analyses to augment germplasm characterization.

The comparison of morphological cluster and marker clustering pattern will give information on the amount of variability found between the genotypes and the best method of assessing the diversity. The evolution of varieties in distinct agro climatic zones demonstrates significant levels of variation in response to the selection pressure in the zones (Singh, 1998). It is, therefore, not surprising to find significant levels of polymorphism among the 37 genotypes of Chrysanthemum with RAPD and ISSR markers. This shows that there is no correlation between genetic make up and geographical distances. The genetic uniformity or non-uniformity is reflected by the genetic distance value as described by Das and Mukherjee (1997.)

Genetic distances between the Chrysanthemum genotypes were estimated using both morphological and molecular markers (RAPD and ISSRs). These methods were able to distinguish all the genotypes used in the study. Clustering of 104 genotypes based on the morphological traits distributed into eleven clusters. Within each cluster few genotypes with identical qualitative characters numbering to thirty seven genotypes were selected for further diversity studies at molecular level. Both morphological and molecular characterization

methods were found efficient in grouping the genotypes. The clustering patterns of both morphological and molecular systems were partially similar in the present study. The genotypes Punjab Gold, CO-3 and Lilith grouped in cluster II of ISSR were similarly grouped in cluster VII as per morphological data. As per the clustering pattern of morphological, RAPDs and ISSRs, the genotypes Snow Cem and Ratlam Selection ; Akitha and Shintome as well were clustered in one group indicating the efficiency of three systems. However, molecular analysis grouped the genotypes more accurately. Although clustering based

on morphological traits and RAPDs divided the genotypes into eleven clusters, the data of ISSRs differed from the other two. The results obtained from cluster analysis based on RAPD and ISSR data sets were different. This was also reflected in the correlation coefficient calculated based on similarity matrices of RAPD and ISSR by using Mantel test. Although the value of correlation coefficient between RAPD and ISSR markers was significant as $r = 0.3906$, indicating that there is a faint concordance between RAPDs and ISSRs, according to its interpretation ($0.7 \leq r < 0.8$ poor fit; $r < 0.7$ very poor fit). This inferred that the two sets of markers explore genetic variation differently.

Among the two marker systems employed, 27 RAPD primers revealed 97.4% of polymorphism while 10 ISSR primers showed polymorphism of 93.86% among 37 genotypes. The molecular markers (RAPD and ISSR) yielded 378 polymorphic markers that discriminated the 37 genotypes into 11 and 10 clusters, respectively. High capacity of ISSR primers to reveal polymorphism offers great potential to determine intra- and inter genomic diversity as compared to other arbitrary primers like RAPD as has been reported by (Zietkiewicz *et al.*, 1994). However, the number of total polymorphic and discriminate fragments recorded in the present study was higher for RAPD than ISSR. The differences found among the dendrograms generated by RAPD and ISSR could be partially explained

by different number of PCR products analyzed (278 for RAPD and 117 for ISSR) and marker sampling error and or the level of polymorphism detected, reinforcing the importance of the number of loci and their coverage of the overall genome in obtaining reliable estimates of genetic relationships among the cultivars (Lorance *et al.*, 1996). ISSR markers are directed at multiple microsatellite loci distributed across the genome while RAPD markers scrutinize the whole genome facilitating the detection of genome wide genetic variation. Both the markers resulted in polymorphic loci, but of the two marker systems RAPD yielded more polymorphic fragments; Similar observation has been made by (Basha and Sujatha, 2007) where the number of total polymorphic fragments was higher for RAPDs than ISSRs. This might be because of higher number of RAPD primers used (27) than that of ISSR (10).

Furthermore, in the analysis with RAPD markers, the genotype Chandrika of cluster XI, Lilith of cluster X, Autumn Joy of cluster IX, Silper of cluster VIII, Asha of cluster of VII, Meera of VI and Arka Ravi of cluster V were the most outliers when compared with cluster III comprising of 19 genotypes. With regard to ISSR markers, the genotype Autumn Joy of cluster X, Flirtation of cluster IX and Raichur of cluster VII were found to be diverse when compared to cluster IV with 11 genotypes. Despite the distinctness of these genotypes in terms of its molecular profile, analysis of phenotypic traits revealed that they found to possess unique phenotypic trait of fringed type of ray floret and maximum flower weight in Flirtation and long duration of flowering in Raichur. Similarly, the genotypes which emerged as diverse by RAPD primers were characterized by unique phenotypic traits of maximum number of ray florets (Lilith), strongly revolute margin of ray floret (Silper). Hence, unique phenotypic traits like type of ray floret (fringed), flower weight, duration of flowering, number of ray florets, margin of ray floret (strongly revolute) among the qualitative and quantitative traits can guide in the identification of diverse genotypes.

However, a more detailed study with more number of molecular markers and a larger set of germplasm may provide further understanding regarding the quantitative traits influencing the grouping of genotypes and the cause of diversity. Focused study on such traits, which aid in identification of diverse genotypes during germplasm collection and evaluation, expedite results in the crop improvement programme of this potential flower crop.

The genotypes Terry and Salora with 58% of similarity based on RAPDs grouped in cluster III fell into different clusters (cluster III and cluster IV) based on morphological cluster pattern. Similarly, Red Stone and Geetanjali placed in one group at maximum of 78% similarity as per ISSRs data, are distributed in two different clusters (cluster III and Cluster VII) based on morphological cluster pattern which indicates that though genotypes are genotypically similar they may not be phenotypically similar. The identification of chrysanthemum lines based on morphological characters in large number of germplasm lines is difficult because it is the product of genotype and environmental interaction.

Higher efficiency of molecular markers (RAPDs and ISSRs) over morphological characterization was well demonstrated. This kind of higher efficiency of molecular markers over morphological markers was reported in sweet potato by Komaki *et al.* (1998) and in *Solanum* spp by Jacoby *et al.* (2003).

Consideration of all genotypes without preliminary screening may lead to a lesser weightage for typical characters leading to erroneous clustering when different genotypes got similar morphological characters. The genotypes which can not be assigned to definite categories could be subjected to molecular marker analysis. Thus, for estimation of genetic distances within a specific category, the present methodology of morphological characterization is highly useful and molecular markers could be used for definitive conclusions.

In this study, the genotypes were mainly grouped according to plant, leaf and flower characters which are complex and polygenic. Such characters are environmentally influenced and therefore liable to subjective evaluation. Hence, the molecular characterization is more efficient in the generation of an unbiased picture of diversity than a morphological approach. However, the morphological characterization is still important in germplasm management, as it is the predominant method of characterization. Determination of molecular diversity should not be seen as replacement for traditional characterization but rather as a complement to it.

Low correlation between molecular and morphological traits had been reported in other studies in European barley varieties (Scout *et al.*, 1997), Synthetic hexaploid wheat and their parents (Lage *et al.*, 2003) and squash germplasm (Ferriol *et al.*, 2004). It suggested that morphological and molecular differences are apparently independent.

Although the correlation between the morphological and molecular data was low, both methods allowed grouping the chrysanthemum genotypes analyzed in this work. Despite the fact that morphological traits were relatively less efficient for precise discrimination of closely related genotypes, the cost and time invested were lower than the molecular analysis. Therefore, based on cost, efficiency and information gained, both techniques appeared complementary to study the characterization and diversity among chrysanthemum genotypes. Use of more number of markers in future could give further precise results.

In morphological studies, among all the characters number of ray florets per head followed by number of disc florets per head and flower yield per plant which contributed maximum towards diversity should be given due consideration for chrysanthemum crop improvement. The identified traits could be effectively harnessed as a handy tool for selection of diverse germplasm at the time of germplasm collection or short listing of accessions for the crossing programmes. The two genotypes Apurva Singar and Local Button which showed high mean values for important economic traits like plant spread in both directions, inflorescence width, spray length, number of flowers per spray and number of flowers per plant can be used to derive a broad spectrum of genetic variability in the segregating generations for flower yield per plant. The data generated in the present investigation provide information useful not

only for selection programmes for further improvement of chrysanthemum, but also for the establishment of relationship among genotypes worldwide.

Identification of markers associated with specific traits of interest, needs attention to speed up the crop improvement programme. Hybridization among promising genotypes from divergent clusters could yield heterotic effects for flower quality (in terms of colour, shape, size) and flower yield in addition to creation of new gene combinations and thus help in development of new promising cultivars of chrysanthemum.

This study is first of its kind on morphological and molecular characterization of chrysanthemum genotypes with the genotypes available at Floricultural Research Station, Rajendranagar, Hyderabad in particular employing the different genotypes grown in state of Andhra Pradesh.

The results of present investigation suggest the need of further molecular characterization of chrysanthemum gene pool with large number of markers in order to get an insight into molecular complexity involved in the gene pool as well as to derive an accurate phylogenetic relationships existing among them.

5.12 FUTURE LINE OF WORK

- This study is not exhaustive in terms of sample size and area coverage. Hence, more survey and sample collection has to be carried out so as to cover other agro ecologies where probably more diversified lines occurs for future use in chrysanthemum improvement programmes.
- Identification of markers associated with specific traits eg. Dwarf nature and variable flower colour is of great interest. The markers associated with specific traits could be converted into SCAR markers which will speed up the chrysanthemum improvement.
- Further, research work involving QTL mapping for identification of markers linked to important traits and improvement of chrysanthemum through marker assisted breeding would speed up the breeding programme in this crop.

- There is also a need to resort to extensive hybridization in breeding programme to produce more variants with superior qualities like flower colour, dwarfness and disease resistance for wilt and leaf spot. Hybridization among members belonging to divergent clusters could yield heterotic effects in addition to the creation of new gene combinations.

Future attempts in chrysanthemum improvement programme could, therefore, be planned based on the consideration of the findings of the present investigation, as these have direct implications in chrysanthemum improvement programme.

CHAPTER VI

SUMMARY

The present investigation was carried out in chrysanthemum (i) to characterize plant morphological *i.e* both qualitative and quantitative characters and to ascertain the nature and amount of variability (ii) to determine the relationship between different characters through correlation analysis and to study the direct and indirect effects of yield components on flower yield through path coefficient analysis (ii) to assess the magnitude of genetic divergence as well as molecular diversity (RAPD and ISSR markers) prevalent among chrysanthemum genotypes (iii) to test the reliability of each marker technology in finger printing of chrysanthemum genotypes (iv) to compare the efficiency of both the markers, RAPD and ISSRs and (v) to identify the implications of the findings for future breeding and conservation programmes of chrysanthemum.

The material for the study comprised of 104 chrysanthemum genotypes in which four checks *viz.*, Punjab Anuradha, Raichur, Silper and CO-3 were included. These were evaluated in Augmented Block design during 2010 and 2011, at Floricultural Research Station, Rajendranagar, Hyderabad. Observations were recorded on 30 quantitative and 23 qualitative characters of plant, leaf and flower characters. Twenty seven RAPD primers and ten ISSR primers were tested to study the genetic diversity in Chrysanthemum genotypes. The experimental results are summarised below.

6.1 CHARACTERIZATION OF CHRYSANTHEMUM GENOTYPES USING MORPHOLOGICAL MARKERS

- Analysis of variance revealed highly significant differences among the 104 genotypes of chrysanthemum for all the thirty quantitative traits thus indicating wide variation among the genotypes.

- The chrysanthemum genotypes Pink Cascade, Beauty, Cloverlee Star and Yellow Gold were found to be the early flowering varieties. The genotypes Star Pink, Rose day and Jessie Hab Good exhibited maximum flower size.
- The high multiplication rate was observed in the genotypes Yellow Bonsai, PAU-B-107, Local Button, Chandrika and Aparajitha by producing more number of suckers per plant.
- The genotypes Local Button, Chandrika, PAU-B-107 and Rekha were adjudged as superior over other genotypes in terms of flower yield per plant.
- The characters such as plant spread in both the directions, no. of primary branches/plant, petiole length, terminal lobe length, leaf length, leaf width, depth of the sinus, internodal length, inflorescence width, bud width, average flower weight, flower diameter, peduncle length, peduncle diameter, ray florets/head, length of ray floret, width of ray floret, ratio of ray floret length : width, disc diameter, number of disc florets/head, length of disc floret, number of flowers/spray, number of flowers/plant and number of suckers/plant showed higher estimates for GCV and PCV .
- High heritability coupled with high genetic advance as percentage of mean was recorded for all the quantitative characters studied except duration of flowering indicating contribution of additive gene effects in the expression of these traits. Therefore improvement in these characters can be done through direct selection to select better genotypes of chrysanthemum.
- A significant positive correlation both at genotypic and phenotypic levels was recorded between flower yield and plant attributes viz., plant spread at both the directions, number of primary branches per plant, spray length and number of flowers per spray. This indicates that flower yield in chrysanthemum can be improved by direct selection of these characters.

- Path coefficient analysis revealed that plant spread in N-S direction recorded the highest direct effect on flower yield per plant followed by number of flowers per spray, days to 50% flowering, number of primary branches per plant, average flower weight and duration of flowering. Hence, it would be rewarding to lay stress on these characters in selection programme for increasing yield.
- Based on Mahalanobis D^2 analysis, the chrysanthemum genotypes were grouped into eleven divergent clusters with maximum of 31 genotypes in cluster VII followed by cluster III with 15 genotypes and cluster I having 12 genotypes. Clusters IV, VIII and X each had nine genotypes in them. While the XI cluster had six genotypes followed by clusters V, IX each bearing five genotypes. The remaining clusters VI had two genotypes whereas the II cluster was solitary.
- In the present study the highest inter cluster D^2 value was recorded between clusters VI and IX (195.87) indicating that crosses may be attempted between the genotypes of cluster VI (Apurva Singar and Local Button) and cluster IX (Miss India, Tambra, White Silver Cloud, Red Queen and Swan) to obtain new desirable recombinants in chrysanthemum.
- Among all the characters, the most important character contributing to divergence was number of ray florets per head followed by number of disc florets per head and flower yield per plant.
- Based on multivariate analysis, the cluster-VI had scored high mean values for important economic attributes. As the magnitude of heterosis depends largely on the degree of genetic diversity of parental lines, the genotypes Apurva Singar and Local Button can be used to derive a broad spectrum of genetic variability in the segregating generations for flower yield per plant.

- Among the 104 genotypes of chrysanthemum evaluated for 30 morphological characters, the first eight principal components with eigen values more than one contributed to 73.23 per cent of cumulative variability.
- The first principal component (PC1) accounted for 22.71 per cent of total variance was loaded with maximum contributing variables like flower diameter followed by length of the ray floret, bud width, peduncle length and diameter, average flower weight, ray floret width, leaf length and width, number of ray florets per head, petiole length, plant height, terminal lobe length, internodal length, depth of the sinus and duration of the flowering.

6.2 CHARACTERIZATION OF CHRYSANTHEMUM GENOTYPES USING MOLECULAR MARKERS

- The yield of genomic DNA extracted from 37 chrysanthemum genotypes ranged from 303.5 to 1556.3ng/μl (Table 20). The purity as determined by OD ratio varied from 1.65 to 1.94 indicating that the DNA was relatively of high purity and was suitable for PCR amplification.

6.2.1 RAPD Analysis

- RAPD analysis detected high diversity among the 37 chrysanthemum genotypes. A total of 278 amplified fragments were scored out of which 271 were found to be polymorphic (97.4%) with 27 random primers.
- The highest percentage of polymorphism (100%) was observed with all the RAPD primers except OPE-14, OPE-15, OPE-18, OPF-3, OPF-5 and OPI -18.
- Genotype-specific RAPD markers were detected only for few genotypes Meera (OPE-14), Salora (OPF-5), Terry (OPF-19), Punjab Anuradha (OPG-16), Anjali (OPG-19) and Asha (OPM-10) which could be exploited for DNA finger printing of these genotypes.

- The genetic similarity among thirty seven chrysanthemum genotypes ranged from a coefficient of 0.174 to 0.600 with an average similarity coefficient of 0.387 indicating a high diversity among the group of genotypes studied.
- Ratlam Selection and Snow Cem were found to be most genetically similar (0.60) followed by Akitha and Shintome with 55.3% of similarity and Terry and Salora with 55.1%. On contrary, Aparajitha and Lilith were found to be the least genetically similar (0.174).
- Cluster analysis of chrysanthemum genotypes employing the UPGMA method led to the classification of genotypes into four major cluster and seven minor clusters with one genotype each in cluster V (Arka Ravi), VI (Meera), VII (Asha), VIII (Silper), IX (Autumn Joy), X (Lilith) and cluster XI (Chandrika).
- Sharing of genetic similarity, among the genotypes originating from widely divergent locations was also evident as Punjab genotypes are grouped together with one genotype from Coimbatore in cluster II.
- Intra-location genetic divergence among the plants was quite evident from the grouping of plants collected from Punjab in cluster II (Punjab Gold and Punjab Anuradha) and cluster III (PAU-B-107), cluster IV (Ratlam Selection) and Cluster IX (Autumn Joy).
- The dendrogram based on RAPD analysis showed that most of the chrysanthemum genotypes were distantly related with low genetic similarity index of 0.23 and the genotypes have been grouped into different clusters.

6.2.2 ISSR Analysis

- Genetic diversity among 37 chrysanthemum genotypes was also assessed using ten ISSR primers. On an average the ISSR primers produced 114 bands of which 107 bands were polymorphic, accounting for 93.86% polymorphism.
- The percentage of polymorphism exhibited by different ISSR primers ranged from 71.43% (ISSR-825) to 100% (ISSR-808, 810, 812, 840 and 842).
- The Jaccard's similarity coefficient values based on 10 ISSR markers ranged from 0.275 to 0.775 with a mean similarity matrix of 0.525. This range showed moderate to high diversity among genotypes.
- Among the 37 genotypes studied, the closest relationship was scored between Geetanjali and Red Stone with similarity level of 77.5% although they exhibit considerable morphological variation with yellow and red coloured flowers respectively.
- The UPGMA dendrogram constructed using Jaccard's similarity coefficient, revealed that the chrysanthemum genotypes were grouped into ten clusters in which cluster IV was the largest one consisting of 11 genotypes followed by cluster III with six genotypes, cluster V with five genotypes, cluster VIII with four genotypes, cluster I and II with three genotypes each, cluster VI with two genotypes and cluster VII, IX and X with one genotype each.

6.2.3 Comparison of clustering pattern based on morphological diversity and molecular diversity

- Both morphological and molecular characterization methods were found efficient in grouping accessions, and formed separate groups. Among these methods, molecular analysis grouped more accurately than morphological diversity.

- Although clustering based on morphological traits and RAPDs divided the genotypes into eleven clusters, the data of ISSRs did not do the same. The results obtained from cluster analysis based on RAPD and ISSR data sets were different which was reflected in the correlation coefficient value of $r = 0.3906$ by using Mantel test. This inferred that the two sets of markers explore genetic variation differently.
- Among the two marker systems employed, 27 RAPD primers revealed 97.4% of polymorphism while 10 ISSR primers showed polymorphism of 93.86% among 37 genotypes.
- Both the markers resulted in polymorphic loci but of the two marker systems RAPD yielded more compared to ISSR. The differences found among the dendrograms generated by RAPD and ISSR could be partially explained by different number of PCR products analyzed (278 for RAPD and 117 for ISSR).
- The genotypes Punjab Gold, CO-3 and Lilith grouped in cluster II of ISSR were similarly grouped in cluster VII as per morphological data. As per the clustering pattern of morphological, RAPDs and ISSRs, the genotypes Snow

Cem and Ratlam Selection ; Akitha and Shintome as well were clustered in one group indicating the efficiency of three systems.

- Identification of markers associated with specific traits of great interest, which could be converted into SCAR markers needs attention to speed up the crop improvement programme. Hybridization among promising genotypes of divergent clusters could yield heterotic effects for flower quality (in terms of colour, shape, size) and flower yield in addition to creation of new gene combinations and thus help in development of new promising cultivars of chrysanthemum.

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Annexure-I

Meteorological data recorded during 2010-11

Month	TEMPERATURE (°C)		R.H. (%)		RAIN	RAINY	Temperature Mean (°C)
	MAX	MIN	I	II	FALL	DAYS	
					(mm)		
June'10	35.2	24.7	81.6	60.2	113.7	6.0	30.0
July'10	29.4	22.5	89.3	76.8	278.9	16.0	26.0
August'10	30.3	22.6	91.3	74.6	203.1	13.0	26.5
September'10	29.4	22.2	89.3	71.1	243.8	13.0	25.8
October'10	30.1	20.6	86.3	63.4	108.0	7.0	25.4
November'10	28.7	19.4	92.8	65.8	26.8	4.0	24.0
December'10	27.8	13.2	86.3	44.1	18.2	2.0	20.5
January'11	29.5	10.4	84.2	29.3	0.0	0.0	19.9
February'11	31.2	15.0	82.8	35.2	6.0	1.0	23.1
March'11	35.6	17.7	64.9	26.2	0.0	0.0	26.7

Meteorological data recorded during 2011-12

Month	Temperature (°C)		R.H. (%)		Rain fall (mm)	Rainy days	Temperature Mean (°C)
	MAX	MIN	I	II			
June'11	35.0	24.4	74	45	40.7	4	29.7
July'11	31.2	22.7	86	62	191.0	11.0	26.9
August'11	30.2	22.7	90	71	147.3	9.0	26.4
September'11	30.7	22.0	88	74	68.1	5.0	26.3
October'11	32.1	20.3	90	69	47.9	4.0	26.2
November'11	30.1	15.4	82	45	27.5	1.0	22.8
December'11	29.9	12.6	83	40	-	-	21.3
January'12	30.1	14.2	77	37	-	-	22.2
February'12	31.8	20.2	82.7	54.2	-	-	26.0
March'12	31.6	19.5	82	53	-	-	25.6

Annexure-II

1. Plant growth habit (Only bushy varieties):



1

Upright



3

Semi-upright



5

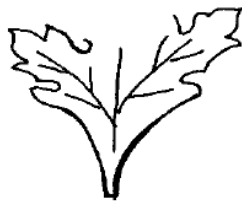
Spreading



7

Trailing

2. Shape of Leaf base :



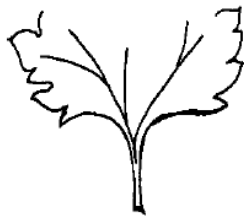
1
Acute



2
Obtuse



3
Rounded



4
Truncate

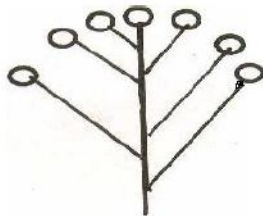


5
Cordate



6
Asymmetric

3. Inflorescence form:



1
Corymbiform



2
Pyramidal



3
Cylindrical

4. Longitudinal axis of majority of ray florets:



1
Reflexing



2
Straight



3
Incurving

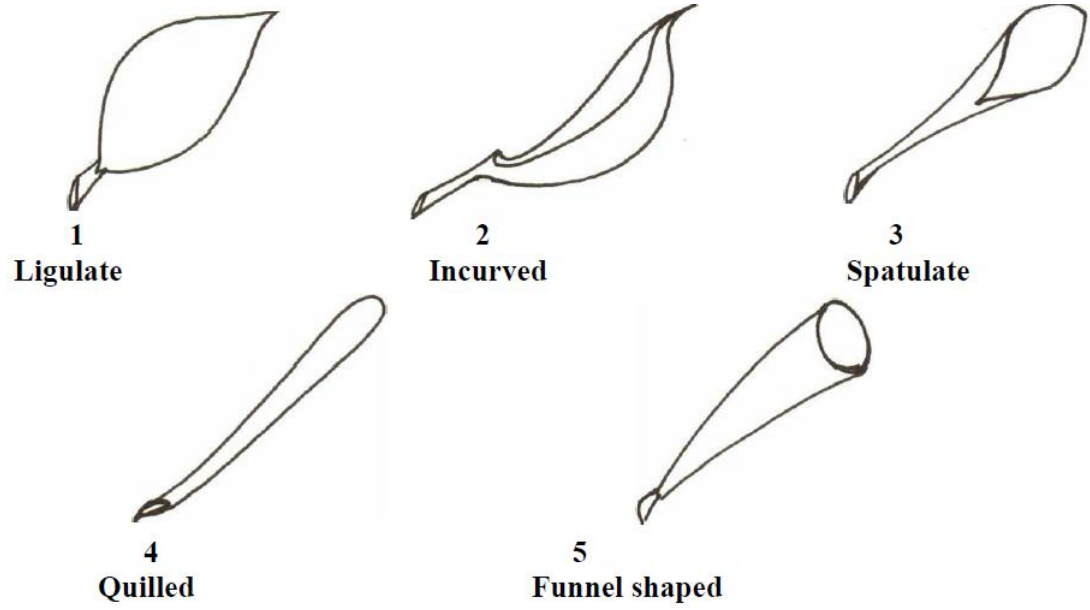


4
Twisted

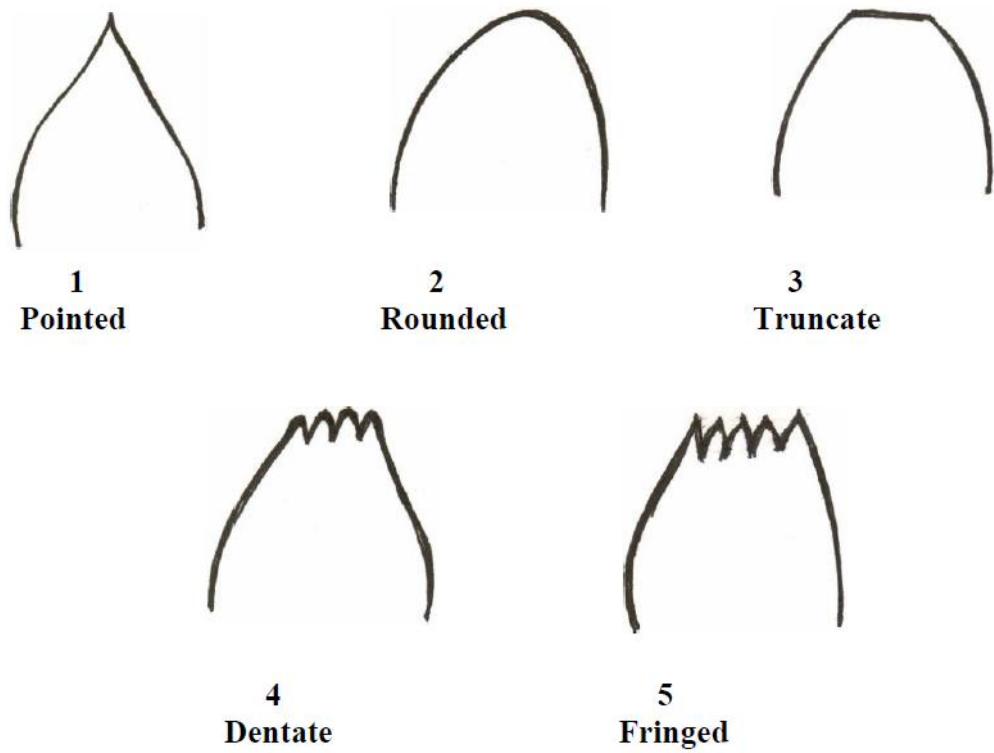


5
Broken

5. Type of ray floret:



6. Shape of tip of ray floret:



7. Rolling of margins of ray floret:



1
Strongly
Involute



2
Moderately
involute



3
Weakly
involute



4
Flat
(not rolled)



5
Weakly revolute



6
Moderately revolute



7
Strongly revolute

8. Disc profile in cross section:



1
Flat



2
Domed



3
Conical

9. Type of disc floret:



1
Needle shaped



2
Quilled



3
Funnel shaped



4
Tubular



5
Petaloid