MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION IN GLADIOLUS (*Gladiolus hybridus* Hort.) VARIETIES

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By

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C E R T I F I C A T E

This is to certify that the thesis entitled **"MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION IN GLADIOLUS (*Gladiolus hybridus* Hort.) VARIETIES"** submitted by **MISS GEETA VENKATAPUR,** for the degree of **MASTER OF SCIENCE (HORTICULTURE)** in **FLORICULTURE AND LANDSCAPE ARCHITECTURE,** of K. R. C. College of Horticulture, University of Horticultural Sciences, Bagalkot, is a record of research work carried out by her during the period of her study in this university, under my guidance and supervision, and the thesis has not previously formed the basis of the award of any degree, diploma, associateship, fellowship or other similar titles.

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Date: July, 2013

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Affectionately dedicated
To
Mummy, Pappa,
Brother and Teachers

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1. INTRODUCTION

Gladiolus (*Gladiolus hybridus* Hort.) is an important bulbous ornamental prized for its beauty of spikes as well as longer vase-life and said to be “Queen of bulbous flower crops”. It is commonly known as Sword lily due to its sword- shaped foliage. In Europe it is called as Corn flag because *Gladiolus illyricus* was found to be weed in corn field.

It is the leading geophytes grown worldwide. It is one of the most attractive and popular bulbous flowers, known for its majestic spikes possessing attractive, elegant and delicate florets. There is no flower to surpass its beauty due to its long lasting spikes occurring in striking colors as unicoloured, bicoloured or multicoloured.

The genus *Gladiolus* is a member of family *Iridaceae* and sub-family *Ixioideae.* South Africa, particularly the Cape of Good Hope, is considered to be the centre of diversity for this genus. The current number of species in the genus is 255 (Pragya *et al.*, 2010b) and all the species are herbaceous perennial in nature. The modern cultivars of *G. grandiflora* are believed to be originated from a number of wild species viz., *G. cruentus, G. natalensis, G. oppositiflorus, G. papilio and G. saundersii*.

Gladiolus occupies a pristine place in the garden for its magnificent inflorescence, wide array of colours, fascinating varieties of shapes, different shades, varying number of florets, size, and wide range of keeping quality and adaptability to different seasons. Gladiolus is ideal for garden display and floral arrangement for table and interior decoration as well as making high quality bouquets. Other than decoration, the flowers of *Gladiolus saundersi*, *G. ecklonii* and *G. cruentus* are used as uncooked salad after nipping off the anthers. Flowers dipped in butter and fried until crispy or stuffed with a savoury hamburger, vegetable filling and preparation of gladiolus cake and others are recommended recipes. Cooked corms of *Gladiolus saundersii* mixed with food is effective against diarrhoea.

The major countries producing gladiolus as cut flowers are USA, Holland, Italy, France, Bulgaria, Brazil, Australia and Israel. In India major gladiolus growing states are West Bengal, Maharashtra, Uttar Pradesh, Punjab, Haryana, Andhra Pradesh, Karnataka, Delhi and Nagaland. In Karnataka it is grown in an area of 264 ha and the production is 518 lakh spikes (Anon., 2011). Major cities which possess demand for gladiolus are Delhi, Chennai, Kolkata, Mumbai, Bangaluru and Pune.

As the number of gladiolus cultivars is continuously increasing, the newly constituted cultivars need to be more intensively monitored for novelty, distinctness, uniformity, stability and molecular markers useful for the protection of Plant Breeder’s Rights. This would make protection of new gladiolus cultivars more specific and effective. Moreover, characterization of accessions is vital for their conservation and management as well as in understanding the genetic relationships between them, which could further be very useful for breeding in

supporting the selection of cross combinations from large sets of parental genotypes, thus broadening the genetic base of breeding programmes.

Morphological traits have long been used to estimate systematic relationships in crops and ornamentals. Though simple and irreplaceable, these descriptors suffer from many drawbacks, such as influence of environment on trait expression, epistatic interaction and pleiotropic effects. Furthermore, paucity of sufficient number of stable morphological markers for unequivocal identification of increasing number of reference collection of varieties enforces to look for alternatives.

Molecular approaches collectively represent a potential goldmine of important information that can be applied as an efficient tool for effective characterization of germplasm. The prime advantage of the use of morphological markers is that they are simple, fast and inexpensive. Then biochemical markers came into existence, but these markers account for a very small fraction of genetic variability, and some are likely to be influenced by environment. Characterization by methods that directly utilize DNA can potentially address the limitations associated with morphological and biochemical methods since all genetic differences between individuals are laid down in the primary sequence of their genomic DNA. To overcome these environmental influences there is a need to use recently developed DNA based markers like Restriction Fragment Length Polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Sequence-Related Amplified Polymorphism (SRAP), Simple Sequence Repeat (SSR) and Inter-Simple Sequence Repeat (ISSR) which provide excellent tools to study the genetic diversity.

As one of the DNA-based markers, Sequence-Related Amplified Polymorphism (SRAP) is designed to amplify open reading frames (ORFs) based on specially designed primer pairs (Li and Quiros, 2001). The forward primers preferentially amplify exonic regions and the reverse primers preferentially amplify intronic regions and regions with promoters. Compared with other marker systems, SRAP markers have been successfully used to study genetic diversity and relationships because of its simplicity, reproducibility and disclosure of multiple markers from a single two-primer reaction, when compared with other marker systems.

Keeping these points in view, the present investigations were therefore, undertaken with following specific objectives.

1. Morphological characterization of gladiolus varieties.
2. Molecular characterization of gladiolus varieties by molecular markers (SRAP).
3. To estimate the genetic variability and diversity between the selected varieties.

2. REVIEW OF LITERATURE

Information on nature and magnitude of variability existing in the plant material and association among the various characters is a prerequisite for improvement in the yield. A knowledge of the available variability within the species for the desired characters enables the breeder in determining the most potential parents. Hence, the genetic potentialities for yield and its contributing characters and their relationship should be properly assessed. In crop breeding programme there is a constant need for diverse genetic resources for improving the quantity and quality. In this chapter a review of the existing literature on the molecular markers and their use in genetic diversity analysis, phylogenetic relationship, cultivars identification and classification, has been done.

2.1 Morphological markers

Traditionally, cultivars have been identified by morphological, physiological or horticultural descriptions. These descriptions are largely subject to environmental conditions and human judgment. Genetic variation is the raw material on which all plant improvement programmes depend. Evaluation of genetic diversity among elite lines of germplasm can provide predictive estimate of genetic variation among segregating progenies for cultivar development. The diversity in the germplasm of any crop species is estimated by using morphological characters. But these morphological markers are not very suitable to use in genetic improvement strategies because they are influenced by environment to modify the phenotypic features either in desirable or undesirable ways. However, the prime advantage of the use of morphological markers is that they are simple, fast and inexpensive. But in order to get a meaningful assessment of the genetic diversity, a large number of polymorphic markers are required. Use of morphological markers in the study of genetic diversity is limited by the availability of only few morphological markers. However, these characters are still extremely useful for initial genetic evaluation studies.

Morphological traits are the oldest and most widely used markers and they may still be considered as optimal for identification of certain germplasm and cultivars on the basis of leaf, fruit size, shape, color of flesh and skin and other physical characteristics. However, these characters may change with environmental conditions. Now it is feasible to fingerprint all the collections present in India using molecular markers to supplement the phenotypic markers which will help to plan breeding programmes.

2.1.1 Variability

Genetic variability studies will help the plant breeder to make an effective and efficient selection of genotypes from the available material which can be utilized for further crop improvement. The success of any breeding programme mainly depends on the extent of genetic variability available in the population. Genotypic coefficient of variation indicate the

relative magnitude of genetic diversity present in the material and helps to compare the genetic variability for different characters.

Studies on assessment of genetic variability for its morphological and yield parameters in gladiolus have been carried out by several workers in the past. A brief review on extent of variability for different quantitative and quality traits in gladiolus is presented

Balamurugan *et al.* (2002) reported that the genotypic coefficient of variation in gladiolus were highest for the number of cormels produced per plant (105.34%) followed by number of corms (103.94%) and number of side shoots (54.67%). Similar trend was also observed for phenotypic coefficient of variation. Highest GCV and PCV were observed for number of cormels produced per plant. Lowest GCV was noted for longevity of individual florets (6.10%) followed by duration of first floret opening (10.46%) and leaf length (10.98%).

Bichoo *et al.* (2002) studied the genetic variability in forty one gladiolus genotypes for seventeen characters. The estimates of variability range and mean revealed significant differences among all the characters. Data indicated the existence of wide range of phenotypic variability among the genotypes of gladiolus. A comparison of genotypic co-efficient of variation among the characters revealed high value for number of cormels per plant followed by weight of ten cormels.

Twenty two genotypes of gladiolus were assessed for genetic variability for twenty characters. The phenotypic coefficient of variance value ranged from 7.56 per cent for diameter of first floret to 71.27 per cent for average weight of cormels per plant, while their corresponding genotypic variance value ranged from 4.77 per cent for diameter of first floret to 58.61 per cent for average weight of cormels per plant. Higher GCV and PCV estimates were found for number of cormels produced per plant and average weight of cormel per plant (Nazir *et al*., 2004).

Genetic variability was observed in forty diverse lines of gladiolus during *rabi* season. The magnitudes of phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) were similar, indicating the little role of environment on the expression of various characters. The phenotypic and genotypic variance estimates were highest for number of cormels and lowest for number of spikes. The estimates of GCV were lower than PCV for all the characters studied (Patil *et al*., 2004)

Genetic variability among thirty five gladiolus genotypes for twenty characters was determined. The genotypes exhibited a wide range of variation for number of days to spike emergence, floret size, number of florets open at a time, number of florets per spike, spike length and rachis length. Differences between phenotypic and genotypic variation was narrow for all the characters, indicating less environmental effects (Balaram and Janakiram, 2004).

Sheikh and John (2005) studied the variation among the genotypes of iris. The phenotypic co-efficient of variations were higher than the genotypic co-efficient of variations for all the characters. High phenotypic and genotypic co-efficient of variations were observed for plant spread (53.16% and 50.00%, respectively) followed by stalk length (45.34% and 44.77%, respectively) and plant height (38.04% and 37.34%, respectively), thus indicating the presence of considerable variability in these characters.

The genetic variation in ninteen characters of thirty one genotypes of gladiolus were reported by Verma *et al.* (2006). The range of variation was high for 5 cormel weight per plant (4.00-23.3 g), number of cormels/plant (4.00-13.00), corm weight (32.33-73.43 g), plant height (73.48-146.51cm) and number of florets/spike (8.09-20.40). A narrow difference between phenotypic and genotypic coefficients of variation was observed for days to germination, rachis length, floret width, corm diameter and 5 cormel weight per plant, indicating low environmental influence on these parameters. High phenotypic and genotypic coefficients of variation were observed for number of cormels per plant, 5 cormel weight per plant, corm weight, rachis length and corm diameter. Low phenotypic and genotypic coefficients of variation were observed for days to last floret bud opening, days to first floret bud opening, days to first floret bud emergence and days to spike emergence.

Nimbalkar *et al.* (2007) evaluated thirty six gladiolus genotypes to study the variability among them. The result showed that phenotypic co-efficient of variations (PCV) were found higher than the genotypic co-efficient of variations (GCV) for all the characters. Higher values of GCV and PCV were obtained for number and weight of corms and cormels per plant, internodal length and rachis length had moderate variability.

Lepcha *et al.* (2007) observed the performance of thirteen gladiolus genotypes for vegetative, floral, corm and cormel character under rainfed conditions of Uttarakhand hills. A large variability was observed for number of cormels per plant (9.75-56.50), size of cormel (0.82-3.32 cm), weight of cormel (4.61-21.33 g), plant height at spike emergence (31.0-62.25 cm), spike length (48.0-87.75 cm) and number of florets (10.25-19.50 per spike).

Variation among thirty two gladiolus genotypes for all fifteen characters. High genotypic coefficient of variation and phenotypic coefficient of variation were observed for corm weight (29.59 and 29.58 g), plant height at 30 days after planting (16.10 and 15.34) as revealed by Archana *et al.* (2008).

Balaram and Janakiram (2009) studied variability among 35 gladiolus genotypes for seven corm characters revealed that, all the genotypes exhibited wide range of variation for the number of cormels per corm. The differences between phenotypic and genotypic co-efficients of variation were narrow indicating less environmental influences on their expression. High phenotypic and genotypic co-efficients of variations, heritability and genetic advance recorded for number of daughter corm, number of cormels per corm and 25 cormel weight.

Kumar *et al*. (2011a) estimated genetic variability, heritability and genetic advance of 15 quantitative characters in forty four gladiolus cultivars. Significant difference was observed for most of the characters, the estimated phenotypic coefficient of variation (PCV) was higher than the genotypic coefficient of variation (GCV). The estimated range of PCV and GCV were ranging from 8.41 (PCV) and 6.83 (GCV) for diameter of floret to 127.33 (PCV) and 126.33 (GCV) for number of florets per spike.

Twenty nine genotypes of gladiolus were evaluated for fifteen different characters by Kumar *et al*. (2011b). The phenotypic coefficient of variation (PCV) was higher than genotypic coefficient of variation (GCV) for all the fifteen characters studied. The higher PCV and GCV estimates were found for number of daughter corm/mother corm and cormel production.

2.1.2 Heritability and genetic advance

The effectiveness of selection for any character does not depend on the amount of phenotypic variability alone. It is necessary that breeder should determine how much of the phenotypic variability present in the character is heritable. Heritability estimates provide information on the degree of inheritance of characters from parent to progeny. Knowledge on the heritability of different characters is a pre-requisite for an efficient breeding programme.

Genetic advance estimates the amount of improvement that can be achieved over the base population. Heritability values alone may not provide clear predictability of breeding values. The estimation of heritability along with genetic advance is usually more useful in predicting the resultant effect for selecting best individuals (Johnson *et al*., 1955). This is due to the fact that a character may have very high heritability but very less phenotypic variation, thus giving low values of genetic advance. Since, the estimates of heritability gives the indication of the amount of progress expected from the selection, they are most meaningful when accompanied by estimates of genetic advance. Genetic advance is affected by factors like intensity of selection, heritability and phenotypic variance.

Hegde and Patil (1995) revealed on twenty five genotypes of gladiolus revealed that high heritability with high genetic advance were observed for the characters like number of shoots, rachis length and dormancy period. These characters help for further selection and crop improvement.

Sheikh *et al.* (1995) found genetic variability, heritability and expected genetic advance for nine characters of thirty varieties of gladiolus. Maximum heritability (99.0%) was recorded for cormels per plant and minimum (13.33%) for weight of corm. High heritability was associated with a high genetic advance in characters like cormels per plant (99.00% and 125.89%, respectively), weight of corm (85.72% and 81.82%) and corms per plant (89.19% and 53.66%), indicating influence of additive genes.

Sharif *et al.* (2000) estimated the genetic variability of seventeen quantitative traits of thirty five indigenous and exotic cultivars of gladiolus. Estimates of broad sense heritability for different traits ranged from 18.30 to 99.80 per cent. High heritability with high expected genetic gain was observed for spike weight, corm weight, corm size and number of corms and cormels per plant.

Genetic advance was maximum for number of cormels per plant (207.97%) followed by weight of corms per plant (59.42%), weight of cormels per plant (43.50%), indicating the heritability due to additive gene effects. Number of leaves per plant (0.64%), number of corms per plant (2.03%) and days required for sprouting (2.28%) exhibited low genetic advance indicating non additive gene effect and high genotype environment interaction in gladiolus (Katwate *et al*., 2002).

High heritability for twenty characters in twenty two genotypes of gladiolus was reported by Nazir *et al.* (2004). Heritability estimates were high (> 80%) for days to 50 per cent heading, first floret colour showing and complete opening of first floret. Whereas, number of cormels per plant, average weight of cormels per plant showed moderate to high heritability along with genetic advance as per cent mean showing additive gene effects.

Balaram and Janakiram (2004) studied the genetic variability among thirty five gladiolus genotypes for twenty characters. High values were recorded for heritability and genetic advance for number of shoots per plant and number of spikes per corm, suggesting selection of genotypes based on these traits for further improvement through breeding programmes.

High heritability with high genetic advance was observed in hybrids of gladiolus for spike weight, number of cormels per plant, weight of daughter corm and dormancy period of corm (Patil *et al.*, 2004).

The phenotypic coefficients of variation were generally higher than the genotypic coefficients of variation for all characters studied. Spike length, plant height and leaf length exhibited high heritability coupled with high genetic advance, whereas number of florets per spike showed low heritability but high genetic advance. This indicates the importance of additive gene effects in the control of these characters and non-additive gene effects were evident for the other characters (Ghimiray, 2005).

High heritability along with high genetic advance as per cent of mean was observed maximum for number of cormels per plant (98.93% and 197.26%, respectively) followed by weight of cormels per plant (88.58% and 110.86%) and average weight of corms (91.47% and 72.40%), while respective minimum values were observed for number of spikes per plant (90.90% and 27.16%) and duration of flowering (78.08% and 14.66%) in gladiolus (Neeraj *et al*., 2005).

The maximum heritability (97.53%) was recorded for stalk length and minimum (62.09%) for length of petals in iris. The high heritability was associated with a genetic advance for characters like stalk length, plant height and plant spread, indicative of influence of additive genes. High heritability was associated with low values of genetic advance, indicating non-additive genetic control for other characters (Sheikh and John, 2005).

Pratap and Rao (2006) evaluated ten gladiolus genotypes for six characters. High heritability estimates were recorded for days to flowering (94%), plant height (89%) and number of florets per spike (84%). Low heritability values were recorded for spike length and vase life. High genetic advance as per cent of mean coupled with high variability was observed for plant height, number of florets per spike and days to flowering. High heritability and genetic advance as per cent mean was observed plant height (89% and 27.16), days to flowering (94% and 16.58),

Higher heritability was observed for five cormel weight per plant, spike length, days to first floret bud opening, days to spike emergence and number of flowers per spike, compared to the other parameters in gladiolus (Verma *et al.,* 2006).

A study was conducted by Nimbalkar *et al.* (2007) on the performance of thirty six different genotypes of gladiolus. Number of corms per plant showed higher estimates of broad sense heritability, indicating high degree of environmental influence. Genetic advance was high for number of cormels per plant (141.01%) followed by weight of corms per plant (61.17%) and weight of cormels per plant (35.41%), indicating the heritability is due to additive gene effects. Number of corms per plant (0.60%), number of leaves per plant (1.10%), intermodal length (1.87%) exhibited low genetic advance, indicating non-additive gene effect.

Archana *et al.* (2008) revealed the variation among the genotypes of gladiolus for all the fifteen characters. High heritability and genetic advance as per cent mean was observed for corm weight (93.6% and 60.92%), corm diameter (96.3% and 30.47%) and plant height at 30 days after planting (90.7% and 29.90%).

Heritability and genetic advance determines the heritable portion of variation. High heritability was associated with high genetic advance for the characters weight of daughter corm and number of cormels per corm indicates additive gene effects. High heritability was associated with low values of genetic advance, indicating non-additive genetic control for other characters (Balaram and Janakiram, 2009).

Kumar *et al*. (2011b) evaluated twenty nine genotypes of gladiolus for fifteen different characters. High heritability with high genetic advance was observed for plant height, days to first floret show colour, weight of corm and cormel production.

That range of heritability values and genetic advance as percent mean ranged from 8.6 to 99.6%. and 8.11 to 261.22% respectively for all the traits. High heritability coupled with high genetic advance was obtained for days to 50% sprouting, number of leaves per plant, length of leaves, number of florets per spike, diameter of corm, weight of corms per plant, number of cormels per plant and cormels weight per plant indicating the contribution of additive gene effect in expression of these traits were reported by Kumar *et al*. (2011c).

2.1.3 Correlation and path co-efficient analysis

The performance of any crop or variety largely depends on genotypic and environmental interaction. Correlation coefficient analysis measures the mutual relationship between various plant characters and determines the component characters on which selection is based for genetic improvement for a particular character. A positive correlation between desirable characters is favorable because it helps in simultaneous improvement of both the characters. Such knowledge of correlation will help the plant breeder in planning more efficient breeding programme.

There are three types of correlations *viz.*, phenotypic, genotypic and environmental correlations. Phenotypic correlation is the observable correlation between two variables and includes both genotypic and environmental effects. Genotypic correlation on the other hand, is the inherent association between two variables may be either due to pleiotropic action of genes or linkage, more likely both or developmentally induced relationships. A brief review of the nature of association of character in gladiolus investigated by several workers is presented.

The idea of correlation was presented by Galton (1889) and later elaborated by Fisher (1918) and Wright (1921). The direct observation of phenotypic correlations does not indicate the magnitude or direction of genetic correlation, which presents a true genetic picture of relationship between the genes controlling the characters.

The technique of path coefficient analysis was developed by Wright (1921), as a means of separating direct and indirect contribution of various factors; path coefficient analysis is a standardized partial regression coefficient analysis and as such measures the direct influence of one variable upon the other and permits the separation of correlation coefficients into components of direct and indirect effects. Use of this technique requires a cause and effect situation among the variables. It is a technique used to find the relative contribution of component characters directly on the main characters and indirectly through other characters to increase the efficiency in selection programmes. Among the correlation between dependent and independent characters is due to the direct effect of the characters, it reflects a true relationship between them and selection can be practiced for such a character in order to improve dependent variable. Otherwise, broadly speaking a breeder has to select for the later through which the indirect effect is exerted.

Misra and Saini (1990) reported that number of florets per spike in gladiolus had positive significant correlation with plant height, number of leaves per shoot, durability of spike, number of capsule per spike, weight of daughter corm and cormels, whereas number of shoots per plant had negative correlation with days to flowering. Anuradha (1990) revealed the positive and significant association of spike length with rachis length, plant height, number of florets per spike, floret diameter, floret length, leaf area , size and weight of corm at both phenotypic and genotypic levels.

Correlation indicating the relationship between vegetative characters and floral characters of gladiolus. Total leaf area was positively correlated with plant height, blooming period and diameter of spike but it was negatively correlated with the duration of flowering. Blooming period and length of spike showed positive correlation with number of florets and diameter of spike (Rajeevan *et al.*, 1994).

Correlation and path coefficient analysis for seventeen quantitative characters in thirty five exotic and indigenous cultivars of gladiolus were done by Lone *et al.* (1999). Number of florets per spike and spike length exhibited significant and positive phenotypic and genotypic correlation with flowering duration, number of florets open at one time, floret size, distance between two florets, plant height, spike weight, vase life, size and weight of corm. Whereas, significant negative association existed between number of corms and weight of corms per plant.

Hussain *et al.* (2001) in a study with twenty five genotypes of gladiolus indicated that characters like durability of whole spike, number of florets remaining open at a time, rachis length, plant height and number of cormels per plant showed a significant positive correlation with number of florets per spike. Path analysis studies revealed that, days to 50 per cent heading recorded maximum positive direct effect towards number of florets per spike, while days to first floret showing colour showed maximum indirect positive effect.

Jhon *et al.* (2002) revealed that, the spike length exhibited significant positive correlation with plant height, number of florets per spike, floret size, corm weight and size of corm and strong negative correlation with days to sprout, days to basal floret opening and ten cormels weight at both phenotypic and genotypic levels. The investigation revealed that spike length, number of florets and floret size were important characters which can be utilized in gladiolus breeding programme.

Anuradha *et al.* (2002) estimated path coefficient analysis in gladiolus showed that spike length had the maximum direct effect with plant height followed by rachis length, floret length, number of floret per spike and weight of daughter corm. Direct effects of leaf area, floret diameter and number of leaves exhibited negative and low but their phenotypic correlation were positive and significant indicating high indirect effects through other characters. Residual effect of 1.5 per cent indicates that the studied characters contributed

98.5 per cent of variability towards spike length. Thus improvement in plant height and rachis length directly increases spike length.

Path analysis in were carried out among twenty two genotypes of gladiolus for twenty characters studied by Nazir *et al.* (2005). Days to first floret color showing and average weight of cormels per plant showed positive effects. These characters also showed positive significant correlation with number of florets/spike, so it is suggested that single plant selection for these traits would be useful for improvement in number of florets per spike trait.

Nimbalkar *et al.* (2007) studied the correlation and path analysis in gladiolus and reported that number of days required for corm sprouting was positively and significantly correlated with days taken to flowering, number of corms and cormels per plant, plant height with length of spike, internodal length, weight of corms and cormels, rachis length and number of florets per spike. The character like rachis length exhibited the magnitudinally highest direct effect (0.819) followed by number of leaves per plant (0.211), weight of cormels per plant (0.205) on the number of florets per spike. The residual effect was 0.349 indicating that the contributing characters explain 63.1 per cent of variability in the yield.

Significant and positive correlations in gladiolus were observed for corm weight, corm diameter, plant height, number of leaves at 60DAP, number of days taken for full emergence of spikes and number of days taken for first flower initiation (Archana *et al.,* 2008).

Balaram and Janakiram (2009) revealed that plant height, spike length, number of florets open at one time, floret length, weight of daughter corm and rachis length had positive and significant correlation with maximum direct effect on number of florets per spike. While leaf number, florets diameter and spike length had positive significant correlations, they exhibited maximum indirect effects in gladiolus.

Balaram and Janakiram (2009) studied the association of various morphological traits through path coefficient analysis in gladiolus showed that spike length had the maximum direct effect with positive and significant correlation with plant height and rachis length. Direct effects of number of florets per spike, spike girth, floret diameter and weight of daughter corm exhibited negative and low but their phenotypic correlation were positive and significant indicating high indirect effects through other characters. Residual effect of 1.5 per cent indicates that the studied characters contributed 98.5 per cent of variability towards spike length. Thus improvement in plant height and rachis length directly increases spike length.

The correlation analysis in gladiolus revealed that plant height exhibited highly significant and positive correlation with weight of corm, corm diameter, rachis length and number of leaf per plant. There exists a significant and positive relationship of number of leaf per plant with weight of corm, corm diameter and rachis length; spikes length with number of leaves per plant. Floret diameter also exhibited significant and positive correlation with marketable spike per corm and number of daughter corm per mother corm (Kumar *et al*., 2011c)

Choudhary *et al*. (2011a) carried out experiment on the association of various morphological traits through correlation analysis in gladiolus and showed that spike length had significant positive correlation with plant height, rachis length, duration of flowering and number of florets per spike, whereas it had negative correlation with number of shoots per plant, size of the floret and number of corms per plant at both phenotypic and genotypic levels. The investigation revealed that spike length, number of florets per spike and floret size are important spike quality characters. Hence, these characters may be considered as selection indices in gladiolus breeding programme.

Path coefficient analysis was carried out using phenotypic correlation coefficient for number of florets per spike. The number of florets per spike was directly positive influenced by the characters plant height, rachis length, spike length, cormels weight, floret diameter, number of leaves, leaf area, number of florets open at a time and spike per plant. Though, diameter and weight of corm exhibited negative direct effects, their phenotypic correlation were positive and significant indicating that, they had high indirect effects through other traits. Thus improvement in spike length, rachis length and plant height directly increases number of florets per spike as reported by Choudhary *et al*. (2011b).

Path coefficient analysis was worked out for spike length and number of florets per spike in twelve genotypes. Plant height and rachis length exhibited direct effects on spike length, while spike length, rachis length and plant height had direct influence on number of florets per spike. Improving plant height and rachis length can bring about improvement in spike length. Similarly, improvement in spike length, rachis length and plant height directly increase number of florets per spike. (Choudhary *et al*., 2011b) .

Pal and Singh (2012) reported the association between different characters and the direction and magnitude of different characters towards the yield, total number of florets per spike in twenty two genotypes of gladiolus. Characters like days taken to sprouting, plant height, spike length, rachis length, weight of corm, number of corms per plant and propagation coefficient showed a significant positive correlation with number of florets per spike.

2.2 Biochemical markers (Protein markers)

The most commonly used protein markers are isozymes. Functional enzymes that are multimeric in nature, normally exist in different molecular forms. It has been recognized that, while retaining substrate specificity these forms are distinguishable based on electrophoretic mobility.

Though isozymes are useful for varietal identities, the protein based markers account for very small fraction of genetic variability and some are likely to be influenced by environmental and management practices.

2.3 DNA based markers

A molecular marker is any measurable molecular characteristic that is inherited in a simple Mendelian fashion. Molecular markers are powerful tool to assess the genetic diversity and population structure, because they are plentiful, independent of tissue or environmental effects. Presently the term molecular marker invariably points to DNA based markers using a variety of techniques to assay variation at the DNA level. The DNA markers can detect differences in genetic material between two or more individuals. Such information is of tremendous importance in studying genetic diversity, phylogenic relationships, marker assisted selection and construction of linkage maps in crops. DNA based markers are superior to other markers because these are greater in number and are highly polymorphic.

There are two DNA based markers a) DNA-DNA hybridization based markers and b) Polymerase chain reaction (PCR) based markers. Hybridization based markers involves use of radioactive labeled probes e.g. Restriction Fragment Length Polymorphism (RFLP). The PCR based marker involves amplification of DNA using thermocycler and is relatively easier, cheaper and more widely used e.g. Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Sequence-related amplified polymorphism (SRAP), Simple Sequence Repeat (SSR), Inter-Simple Sequence Repeat (ISSR) etc.

2.3.1 Sequence Related Amplified Polymorphism (SRAP)

Sequence-related amplified polymorphism is a novel molecular marker which combines simplicity, reliability, moderate throughput ratio and facile sequencing of selected bands. The SRAP marker system which is based on open reading frames (ORFs) developed from genome sequence data of *Arabidopsis.* SRAP marker is based on two-primer amplification. It provides a unique combination of forward and reverse primers which can be selected arbitrarily giving a large number of primer combinations. The primers are 17 or 18 nucleotides long and consist of the following elements. Core sequences, which are 13 to 14 bases long, where the first 10 or 11 bases starting at the 5' -end, are sequences of no specific constitution (“filler” sequences), followed by the sequence CCGG in the forward primer and AATT in the reverse primer. The core is followed by three selective nucleotides at the 3' -end. The filler sequences of the forward and reverse primers must be different from each other and can be 10 or 11 bases long.

Since this is an ORF-based marker system, it targets functional genes and results in a moderate number of co-dominant markers which has potential for their application in crop breeding. This marker system was first used and demonstrated by Li and Quiros in *Brassica oleracea* in 2001, SRAP marker have been several reports in different plant species ranging from field crops to tree species for assessing genetic diversity, mapping and tagging of genes, hybrid identification and sex determination.

2.4 Use of molecular markers in genetic diversity

2.4.1 Gladiolus

Takatsu *et al.* (2001) revealed the information on the genetic relationship in wild gladiolus species through randomly amplified polymorphic DNA (RAPD) analysis. Out of the 140 tested primers, 32 amplified a total of 133 RAPD bands in 33 gladiolus species. The genetic distances among the species analyzed ranged from 0.842 for the most related species to 0.564 for the more distant ones. The genetic distance was calculated from the data of these RAPDs, and dendrogram was separated into four major cluster.

Gang *et al.* (2008) applied RAPD analysis in the classification and genetic relationship of 26 cultivars of *G. hybridus* Hort. Thirty-three arbitrary primers screened from 520 primers were used and a total of 206 RAPD sites were detected with a mean of 6.24 fragment amplified for each primer. A total of 185 polymorphic DNA fragments were detected among all the 206 amplified fragments, which accounted for a high level of 89.8% of all and could be used for identification of different cultivars. The result revealed that the germplasm resource of *G. hybridus* Hort cutting flower cultivar had a narrow genetic base on molecular level, some genetic relationship existed in summer large-flowers of *G. hybridus* Hort varieties.

Wang *et al.* (2008) studied the genetic relationship of 26 cultivars of *Gladiolus hybridus* Hort. by using ISSR molecular marker. Total 19 arbitrary primers screened from 100 primers were used for further PCR and diversity analysis. A total of 110 ISSR sites were detected with a mean of 5.63 fragments amplified for each primer. A total of 103 polymorphic DNA fragments were detected among all the 110 amplified fragments, which accounted for a high level of 93.6% of all and could be used to identify different cultivars. The cluster analysis revealed that the idioplasm resource of *Gladiolus hybridus* Hort. Cutting flower cultivar had a narrow genetic basis on molecular level, and certain genetic relationship existed in summer large-flowers of *Gladiolus hybridus* Hort. varieties.

Pragya *et al.* (2010a) studied on analysis of diversity and characterize 54 *Gladiolus* cultivars using morphological traits and RAPDs and to estimate the genetic relationships between the selected genotypes. Nine morphological traits and 225 random amplified polymorphic DNA (RAPD) markers amplified with 25 arbitrary primers were employed to discriminate between the cultivars and to evaluate the relatedness between them. A total of 225 RAPD bands (250–3000 bp) were obtained of which 211 (93.78%) were polymorphic. In both the cluster analyses, cultivar ‘Pusa Lohit’ branched out from the dendrograms, confirming that it is quite different from all other genotypes.

Genetic relationships of gladiolus cultivars inferred from fluorescence based AFLP markers reported by Pragya *et al.* (2010b). A total of 24 AFLP primer pairs with three samples were initially screened, from which 9 primer sets that showed clear scorable and highly polymorphic bands were selected for AFLP reactions. Fluorescence-labeled amplification

products were subjected to electrophoresis and then analyzed using an automated sequencer. The number of AFLP fragments generated per primer set ranged from 10 to 151 with fragment sizes varying from 50 to 450 bp. A total of 660 AFLP fragments were detected, of which 658 (99.70%) were polymorphic. A wide range of degree of relatedness between cultivars was reported in this study; some are closely relatedwhile others were found quite distinct from each other. The greatest similarity was found between Pusa Lohit and Pusa Swarnima (Jaccard coefficient = 0.788), while Pusa Gunjan was found to be the most distinct genotype.

2.4.2 Ornamental crops

Wolff and Peters-van (1993) studied the genetic variation in chrysanthemum using a technique to RAPDs. The variations between cultivars were identified by using only two different primers. A family of cultivars derived from one original cultivar by vegetative propagation, had identical fragment patterns. The presence of high level of polymorphism and clonal stability, RAPD fragments were useful for cultivar identification.

In order to study genetic variability at the DNA level in chrysanthemum, *Pst*I and *Hind* III genomic libraries were constructed. Probes from both libraries were tested for the presence of RFLPs. Of the probes from the *Pst*I library, 91 per cent appeared to hybridize the low copy genes, while only 31 per cent of these from the *Hind* III library appeared to do so. Genetic analysis was simplified by using locus specific polymerase chain primers to obtain simple polymorphic patterns in a number of cases. The RFLP probes and primers developed were used in the marker-assisted selection (Wolff *et al*., 1994).

Jong *et al*. (1996) studied the 23 *Lilium* sp by RAPD analysis with three primers and produced distinctive polymorphism. The UPGMA analysis indicated that all the Asiatic hybrids were grouped with SI of 0.56 and could be separated from other *Lilium* sp.

Scott *et al*. (1996) recommended the use of DNA amplification fingerprinting (DAF) to study genetic relationships between closely related chrysanthemum cultivars using arbitrary octamer primers. The phenotypic patterns were established by unweighed pair group cluster analysis using arithmetic means (UPGMA) and principal coordinate analysis (PCO) and the average distance between series. DNAs from all cultivars belonging to a series were bulked together to generate profiles containing unique amplified products for each series. Thus DAF technique can be utilized for distinguishing clonal materials along with patent production, phylogenetic study for identifying useful markers in breeding applications.

Small differences within population were observed in *Rhododendron simsii* by AFLP analysis with 3 primer combination. Large variation was observed within *R*. *simsii* species and between different species from the *Tsutsui* subgenus (De-Riek *et al*., 2000).

Genetic relationships of 22 *Alstroemeria* species including one interspecific hybrid (*A. aurea* X *A. inodora*) and distinctly related species *Bomarea salsilla* and *Leontochir* sp. using AFLP marker by cluster analysis and PCA could separate all the three groups (Han *et al*., 2001).

Genetic variation in chrysanthemum was examined by Sehrawat *et al*. (2003) using random amplified polymorphic DNA within the thirteen commercial cultivars representing standard, spray and no pinch- no stake. Genetic variation was studied using 60 decamer primers. Of these, 31 primers amplified genomic DNA. The genetic variation was high enough to divide them into two major groups. These groupings were in consistent with their morphological differences and geographical distribution. The first group consisted of Snow Ball, Ajina Purple and Sonar Bangala cultivars, while the second group accounted for Nagpur Red, Haldighati, Cardinal, Puja, Jaya, Suneet, Vasantika, Gauri, Flirt and Baggi.

Prakash *et al.* (2005) assessed the level of genetic variations among 24 cut-flower *Anthurium andraeanum* Hort. Cultivars using the RAPDmarker. Eight decamer primers produced a total of 98 reproducible PCR bands that were used to calculate the Nei and Li's genetic distance (GDNL) coefficients amongst the cultivars. GDNL values ranged from 0.018 to 0.163 with an average of 0.09 (representing an average genetic similarity of 91.34%). This significantly low average genetic distance among the various cultivars indicated that genetic variation among the cultivars was low. A dendrogram, produced using unweighted pair group method using arithmetic averages (UPGMA), grouped the cultivars into four main clusters. Cultivar 'Antartica' was genetically distinct from all the others. 'Midori' and 'Bourgogne' together formed a cluster whereas the remaining 21 cultivars grouped into two clusters and were closely related to each other.

Sheela *et al.* (2006) analyzed Seventeen *Heliconia* species and varieties using RAPD markers. Eight primers, which produced the highest number of bands, were used for DNA amplification. The genetic similarity matrix constructed with Jaccard's coefficient using RAPD marker scores showed that the highest value was between Petra Orange and Parakeet, while the lowest was between Golden Torch and *H. humilis*. The 17 species and varieties of *Heliconia* formed nine distinct clusters at similarity coefficient value of 0.42, implying a strong parallelism between genetic and morphologic/taxonomic variability of *Heliconia* genotypes.

Khan and Pankajaksan (2010) reported the genetic diversity among commercial varieties of *Anthurium andreanum* Linden using RAPD markers. A total of 12 Anthurium varieties were subjected to Random Amplified Polymorphic DNA (RAPD) marker analysis. Genetic relationships were examined among twelve accessions of anthurium varieties along with a hybrid as an out group check. A high degree of polymorphism was observed. UPGMA (Unweighted Pair-Group Method using Arithmetic average) cluster analysis of genetic similarity indices grouped all the accessions into two major clusters. Intra-clustering within the two clusters grouped the accessions as per genetic background.

Xue *et al*. (2010) reported the genetic map construction of two *Dendrobium* species with a double pseudo-testcross strategy using random amplified polymorphic DNA (RAPD) and sequence-related amplified polymorphism (SRAP) markers. A F_1 mapping population of 90 individuals was developed from a cross between *D. officinale* and *D. hercoglossum*. A total of 307 markers, including 209 RAPD and 98 SRAP were identified and used for genetic linkage group analysis. The maps constructed in this study covered 92.7% and 82.7% of the *D. hercoglossum* and *D. officinale* genomes respectively providing an important basis for the mapping of horticultural and medicinal traits and for the application of marker-assisted selection.

Shen *et al*. (2011) established SRAP (sequence-related amplified polymorphism) marker system in *Salvia splendens* and 17 cultivars were identified using the SRAP markers. The concentrations of primers, dNTP, DNA template, Taq DNA polymerase and annealing temperature were optimized in order to distinguished completely by 17 varieties only using a single primer combination.

The markers were applied to assess the level and pattern of genetic diversity in seven populations of *Dendrobium loddigesii*. Seventeen SRAP primer combinations generated a total of 231 clear amplification bands encompassing 187 (80.95%) polymorphic bands. A high level of genetic diversity was detected (PPB = 80.52%, H = 0.2743, I = 0.4113) at the species level. There was a moderate genetic differentiation (Gst = 0.304) among populations. Two main clusters were detected by cluster analysis using the unweighted pair-group method with arithmetic average (UPGMA). Mantel test revealed that no significant positive correlation was found between genetic distances and geographic distances. Determining the level of genetic diversity and pattern of population genetic structure of this species would be helpful for its conservation and management (Cai *et al.* 2011).

Genetic diversity and population structure of an endangered Orchid (*Dendrobium loddigesii* Rolfe) from China revealed by SRAP markers was described by Xiaoyan *et al.* (2011). *Dendrobium loddigesii* Rolfe is an endangered perennial herb with ornamental and medicinal value. Determining the level of genetic diversity and pattern of population genetic structure of this species would be helpful for its conservation and management. Sequence-related amplified polymorphism (SRAP) markers were applied to assess the level and pattern of genetic diversity in seven populations of *Dendrobium* loddigesii. Seventeen SRAP primer combinations generated a total of 231 clear amplification bands encompassing 187 (80.95%) polymorphic bands. A high level of genetic diversity was detected (PPB = 80.52%, H = 0.2743, I = 0.4113) at the species level. There was a moderate genetic differentiation (Gst = 0.304) among populations. Two main clusters were detected by cluster analysis using the unweighted pair-group method with arithmetic average (UPGMA). Mantel test revealed that no significant positive correlation was found between genetic distances and geographic distances

Lirenwei *et al.* (2012) studied the association analysis of phenotypic traits with SRAP markers in chrysanthemum. In order to provide a genetic basis for studies on complex quantitive traits and for molecular assisted breeding of chrysanthemum, the SRAP markers associated with important horticulture traits were screened. The genomic regions with selection sweep were detected through scanning 58 representative chrysanthemum cultivars using 19 SRAP markers. Population structure was firstly analyzed, then association analysis between SRAP markers and 18 important phenotypic traits were performed using TASSEL GLM. Genetic structure analysis showed that the selected cultivar population was composed of 5 subpopulations, namely flat type subgroup, tube type subgroup, irregular type subgroup, anemone type subgroup and Japanese subgroup. There were 6 SRAP loci associated with 5 quantitative characters among which 3 flower traits were associated with 5 loci, while 1 stem and 1 leaf traits were associated with 1 locus, respectively. The rate of explanation on the phenotype of related locus ranged from 0.0738 to 0.4791. It is feasible to estimate and differentiate chrysanthemum population's structure effectively using SRAP markers, and the markers obtained in this study are promising in molecular assisted breeding.

Genetic relationships among 42 cultivars of canna were determined by using amplified fragment length polymorphism (AFLP) marker was examined by Astha Gupta *et al*. (2013). A total of 1607 DNA fragments was produced with 25 AFLP primer combinations and out of which 1491 (92.78%) were found polymorphic and 116 (7.22%) monomorphic. The number of polymorphic fragments varied from 33 (E-ACA/M-CAA) to 86 (E-ACT/M-CAA) with an average of 59.6 per primer combination and percent polymorphism varied from 81.7% (E-AAG/M-CAA) to 100% (E-ACC/M-CTT) with an average of 92.8% per primer combination. The polymorphism information content (PIC) value ranged from 0.24 to 0.35 with an average of 0.30 per fragment and the highest PIC value (0.35) was noticed for primer combination E-AAG/M-CAC followed by E-ACT/M-CTA, E-ACA/M-CAA, EAAG/ M-CAA (0.34). Jaccard's similarity coefficient varied from 0.33 to 0.72 with an average of 0.49 ± 0.03. The maximum genetic similarities (72%) were noticed between the cultivar NBC 1 and NBC 2; NBC 16 and NBC 19 followed by NBC 19 and NBC 30 (70%). Based upon genetic similarity coefficient the cultivars NBC 43, NBC 24, NBC 38, NBC 22, NBC 29 and NBC 36 were found to be most divergent among all the cultivars. The UPGMA clustering revealed four major groups accommodating 93% cultivars and three cultivars each of different species i.e. *C. argentina* (NBC 24), *C. latifolia* (NBC 43) and *C. generalis* (NBC 13) did not grouped with any clusters.

3. MATERIAL AND METHODS

The present investigations on “Morphological and molecular characterization in gladiolus (*Gladiolus hybridus* Hort.) varieties” was carried out at the Department of Floriculture and Landscape Architecture, Kittur Rani Channamma College of Horticulture, (University of Horticultural Sciences, Bagalkot), Arabhavi, Gokak taluk, Belagavi district of Karnataka during the period of September 2012 to April 2013. The details of the materials used and the methods adopted during the course of the investigation are presented in this chapter.

3.1 Geographical location of the experimental site

Arabhavi is situated in the Northern dry zone (zone-3) of Karnataka state, geographically lies at 16^0 15’ North latitude, 94^0 45’ East longitude with an altitude of 640 m above mean sea level.

3.2 Climatic conditions of the experimental site

Arabhavi comes under Zone-3 of Region -2 of agro- climatic zones of Karnataka and it has the benefit of both South- West and North- East monsoons. The total rainfall of this area is about 387.60 mm, distributed over a period of seven to eight months (March to October) with peak during June to July. This area receives irrigation water from Ghataprabha Left Bank Canal (GLBC) from mid July to mid March. The maximum temperature during the period of experimentation 38.34^0C and relative humidity 88.59 per cent. The meteorological data recorded during experimentation period at meteorological observatory of the Agricultural Research Station, Arabhavi is presented in Appendix – I

3.3 Experimental Details

3.3.1 Planting materials

For the experiment fifteen varieties of gladiolus were used**.** Among these**,** ten varieties were procured from Department of Floriculture, Govt. of Jammu and Kashmir, two varieties from Allahabad, one varieties from Indian Institute of Horticultural Research, Hessarghatta, Bangalore and remaining two varieties from Department of FLA, K.R.C. College of Horticulture, Arabhavi. The corms were stored for three months after lifting from the field of the previous crop and the uniform corms were planted after the dormancy period. The pass port data of fifteen gladiolus varieties is presented in Table 1.

3.3.2 Design and experimental layout

Experimental design	: Randomized Complete Block Design
Number of replications	: Two
Number of varieties	: Fifteen
Number of plants per treatment	: 30
Spacing	: 30 x 20 cm
Plot size	: 1.8 m X 1.5 m
Planting method	: Flat bed

3.4 Cultural Practices

3.4.1 Preparation of experimental site

Land was thoroughly ploughed to a depth of 30 cm and brought to fine tilth. All the weeds, stubbles, stones were removed and well decomposed farm yard manure @ 25 t/ha was applied and mixed well. The plots were prepared with a dimension of 2.7 m^2, a distance of 30 cm was kept between two replications for laying irrigation channel and for working.

3.4.2 Planting

Corms of uniform size (4 – 5 cm diameter) were selected for planting. Before planting the corms were dipped in Carbendazim (0.1%) solution for 10 minutes and dried in shade. These treated corms were planted at a spacing of 30 x 20 cm at 5-6 cm depth and light irrigation was given immediately after planting. The crop was raised and maintained by following standard cultural practices.

Plate 1. General view of the experimental plot

Table 1. Passport data for the 15 gladiolus varieties

Sl. No	Varieties	Description	Origin	Selection /cross	Source of collection
1	American Beauty	Mid season variety, spike length 70-100 cm, 10 to 13 florets per spike, reddish pink petals with whitish throat.	USA	Sylvia X Patrica	Jammu and Kashmir
2	Candy man	Early season variety, spike length about 60-85 cm, 12-16 florets per spike, florets dark maroon colour, petals ruffled and thickly arranged on the spike.	USA	Jr. Prom X Lucky Star	Jammu and Kashmir
3	Charms Flow	Mid season variety, spike length 100-120 cm, 11to 15 florets per plant, pink colour florets with whitish central blotch.	Holland	Wind Song X Pink Frost	Jammu and Kashmir
4	Delhi Local	Early season cultivar. Spike length 40-60 cm, 10-12 florets per spike. Florets reddish orange in colour with orange blotch.	India	____	Allahabad
5	Green Bay	Late season variety, spike length 60- 90 cm, 10 to 14 florets per spike, florets with greenish yellow colour.	USA	Mazere X Melody	Jammu and Kashmir
6	Her majesty	Mid season variety, spike length 60-70 cm, 11to 14 florets per plant, purple colour florets with whitish central blotch.	Holland	Sylvia X Pancy	Jammu and Kashmir
7	Jester Gold	Late season variety, spike length 75 - 85 cm, 12-15 to florets per spike, yellow color florets with reddish blotch.	Holland	Erusioun X Lucky Star	Jammu and Kashmir
8	Punjab Morning	Early season variety, spike length 70-75 cm, 15 to 17 florets per spike, florets light pinkish colour with red central blotch.	PAU, Ludiana India	Sancerrie X Snow Prince	Allahabad

Cont…….

Sl. No	Varieties	Description	Origin	Selection /cross	Source of collection
9	Eighth Wonder	Late season variety, spike length 100-130 cm, 16 to 20 florets per spike having orange colour florets.	Australia	Friendship X *G. eckloni*	Jammu and Kashmir
10	Red Majesty	Early season variety, spike length 100-120 cm, 13 to 16 florets per spike, florets reddish colour with whitish central blotch.	USA	Friendship X *G. tristis*	Jammu and Kashmir
11	Summer Sunshine	Early to mid season variety, spike length 100-120 cm, 10 to 13 florets per spike, florets yellow colour with reddish central blotch.	Holland	Open pollinated	Jammu and Kashmir
12	Copper King	Late season variety, spike length 80-100 cm, 10 to 14 florets per spike, florets dark red colour with yellow central throat.	USA	____	KRCCH, Arabhavi
13	White Prosperity	Mid season variety, spike length 90-120 cm, 14 to 17 florets per spike, florets white in colour.	USA	Brum X Luck Star	Jammu and Kashmir
14	Vedanapoli	Mid season variety, spike length 70-100 cm, 11 to 15 florets per spike, florets light to dark pink colour with whitish throat.	India	American Beauty X White Prosperity	KRCCH, Arabhavi
15	Darshan	Mid season variety, spike length cm, 12 to 15 florets per spike, Red-purple with white blotch.	India	Watermelon pink X Shirley(pink)	IIHR, Bangalore

American Beauty

Candyman

Charms Flow

Delhi Local

Green Bay

Plate 2a. Gladiolus varieties used in the experiment.

Her Majesty

Jester Gold

Punjab Morning

Eighth Wonder

Red Majesty

Plate 2b. Gladiolus varieties used in the experiment

Summer Sunshine

Copper King

White Prosperity

Vedanapoli

Darshan

Plate 2c. Gladiolus varieties used in the experiment

3.4.3 Irrigation

Maintained the optimum soil moisture condition by irrigating the crop once a week.

3.4.4 Fertilizers

Recommended dose of fertilizers N,P and K @ 150, 80 and 60 kg/ha were applied as per the recommendations of the package of practices, UAS, Dharwad (Anon.2007) was applied in the form of urea, single super phosphate and muriate of potash, respectively. Fifty per cent of nitrogen and full dose of phosphorus and potash were applied as basal dose and the remaining fifty per cent of nitrogen was applied 45 days after planting.

3.4.5 Intercultural operations

Hand weeding was taken up as and when weeds were emerged. Stalking with wooden sticks was given to the plants to avoid falling and to provide support to the spike. Earthing up was practiced for better development of corms.

3.4.6 Plant protection measures

Alternaria leaf spot disease noticed during flowering stage. The disease was controlled by spraying Mancozeb (3g/lit). Fusarium wilt disease was noticed during vegetative and flowering stage. Corms were dipped in Carbendazim (0.1%) solution for 10 minutes and dried in shade and planted. At monthly intervals the plot was drenched with Carbendazim (2g/lit). The pest incidence was not noticed during crop duration.

3.4.7 Harvesting

The spikes were harvested at color showing stage with at least four leaves left on the plant intact. The harvested spikes were used for quality parameter studies. after

3.5 Biometrical observations

The observations on vegetative, flowering and corm characters were recorded from five randomly tagged plants in each replication.

3.5.1 Vegetative parameters

Observations on vegetative parameters were recorded at three different stages, i.e., 30, 60 and 90 days after planting.

3.5.1.1 Plant height (cm)

The height of the tagged plants was measured from the base of the plant to the tip of the longest leaf in centimeters and average was worked out. This was done at all the three stages of plant growth i.e., at 30, 60 and 90 days.

3.5.1.2 Stem girth (cm)

Stem girth was measured by leaving 5 cm from the ground level with the help of vernier calipers at 30, 60 and 90 days after planting from all the tagged plants and average was worked out. It was expressed in centimeters.

3.5.1.3 Number of leaves per plant

Number of leaves produced in the tagged plants was recorded by counting the number of leaves at 30, 60 and 90 days after planting and average was worked out.

3.5.1.4 Leaf length (cm)

Leaf length three leaves was recorded from the base to tip of the leaf in the tagged plants at 30, 60 and 90 DAP and average was worked out and expressed in centimeters.

3.5.1.5 Leaf width (cm)

Width of three leaves from the middle was recorded at 30, 60 and 90 days after planting in the tagged plants by using scale. The average was worked out and expressed in centimeters.

3.5.1.6 Leaf area (cm^2)

The leaf area was computed by multiplying the leaf length, width and correction factor (Appendix – II) to arrive at the actual leaf area. The conversion was computed by dividing the actual leaf area recorded on the graph sheet by computed leaf area (length and width) and expressed in square centimeters.

3.5.2 Flowering parameters

3.5.2.1 Days taken for spike initiation

The number of days taken from planting of corms to spike emergence in the tagged plants was counted and average was worked out.

3.5.2.2 Days to first three florets color showing stage (Commercial stage)

The number of days taken from planting of corms to the first three florets colour showing stage was recorded in tagged plants and the average was worked out.

3.5.2.3 Duration of flowering (days)

Number of days from the first flower harvested to the last flower harvested in the plot was recorded as the total duration of flowering in each treatment.

3.5.3 Quality and Yield Parameters

3.5.3.1 Spike length (cm)

Length of spike was measured in the cut flowers obtained from tagged plants from base of the stem upto the tip of the inflorescence and it was expressed in centimeters.

3.5.3.2 Rachis length (cm)

Length of the rachis was measured in centimeters from basal floret to the last floret in the cut flowers obtained from tagged plants.

3.5.3.3 Number of florets per spike

Number of florets per spike was counted in each cut flower harvested from the tagged plants and average was worked out.

3.5.3.4 Floret diameter (cm)

Diameter of the floret was measured by using measuring scale and average floret diameter was expressed in centimeters.

3.5.3.5 Weight of the spike (g)

Spike weight was recorded by weighing the spikes when the first one to two basal florets opened and the average was worked out and expressed in grams.

3.5.3.6 Vase life (days)

Vase life was determined in plain tap water. Spikes were harvested at colour showing stage and they were kept in tap water at room temperature for the study. Number of days was counted until the florets lost their visual marketable value. Total vase life was worked out and expressed in days.

3.5.3.7 Number of spikes per plant

Number of spikes produced per plant was recorded from the tagged plants and the average number of spikes produced per plant was worked out.

3.5.4 Corm and cormel parameters

3.5.4.1 Number of daughter corm per plant

Number of corms produced per plant was recorded from the tagged plants and their average was worked out.

3.5.4.2 Diameter of daughter corm (cm)

Corm diameter was recorded by using scale in the corms obtained from the tagged plants and average was worked out and expressed in centimeters.

3.5.4.3 Weight of corm (g/ plant)

The harvested daughter corms from the tagged plants were weighed individually and average weight of corms produced by plants was worked out and expressed in grams.

3.5.4.4 Number of cormels per plant

Cormels produced per plant were counted from the tagged plants and average was worked out.

3.5.4.5 Weight of cormels (g/plant)

Weight of the harvested cormels from the tagged plants was recorded and expressed in grams from each treatment.

3.5.4.6 Weight of ten cormels (g)

Weight of the ten harvested cormels from the tagged plants was recorded and expressed in grams from each treatment.

3.5.4.7 Fusarium wilt

Fusarium wilt disease caused by *Fusarium oxysporum* was observed at flowering stage. Intensity of disease was recorded by using 0 to 3 point rating scale. Observation was taken from randomly selected plants in each variety at normal condition.

Disease severity was assessed with a 0 – 3 visual scale,

Where,

0 = no symptoms,

1 = yellowing of leaves

2 = drying of leaves

3 = dead or almost dead plants.

To assess the degree of susceptibility or resistance in gladiolus genotypes, the following rating and cultivar reaction table was used (Riaz *et al*, 2010).

Score obtained	**Cultivar reaction**
0	Resistance (R)
0-1	Moderately resistance (MR)
1-2	Moderately susceptible (MS)
2-3	Susceptible (S)

3.6 Statistical analysis for morphological traits

The standard statistical methods were followed as detailed here under. Statistical analysis of the data was carried out by using Statistical Package for Agricultural Research (SPAR) and Generous

3.6.1 Analysis of variance

Variance is the measure of variability and is defined as the average of the square deviation from the mean. It helps in working out the variance due to different source and also provides the basis for test of significant (Singh and Choudhary, 1979).

Analysis of variance was carried out as per the procedure given by Panse and Sukhatme (1967) using the mean values of random plant in each replication from all treatments to find out the significance of treatment effect.

Source of variation	Degree of freedom	Sum of squares	Mean sum of squares	'F' ratio
Replication	(r-1)	RSS	Mr	Mr/Me
Treatment	(t-1)	TSS	Mt	Mt/Me
Error	(r-1) (t-1)	ESS	Me	
Total	(rt-1)		Mr+Mt+Me	

Where,

r = Number of replications

t = Number of genotypes

RSS, TSS and ESS = Sum of squares of replications, genotypes and error respectively

Mr, Mt and Me = Mean squares of replications, genotypes and error respectively

Variation due to genotype was tested by comparing calculated values to Table 'F' value at five per cent.

3.6.2 Critical difference

In order to compare the means of entries, critical difference (CD) was calculated by using the following formula.

CD = SE x't' value at error degrees of freedom.

Where SE = $\sqrt{\frac{2 \text{ X Error MSS}}{r}}$

t = Tabulated 't' value at 5 per cent or 1 per cent probability level.

3.6.3 Estimation of genetic parameter

3.6.3.1 Genotypic, phenotypic and environmental variances

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

Environment variance ($\sigma^2 e$) = Error mean sum of squares

Phenotypic variance ($\sigma^2 p$) = Genotypic variance + Environment variance

3.6.3.2 Coefficient of variation

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

Genotypic coefficient of variation (%) = $\sqrt{\frac{\sigma^2 g}{\bar{X}}}$ X 100

Phenotypic coefficient of variation (%) = $\sqrt{\frac{\sigma^2 p}{\bar{X}}}$ X 100

Where,

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

$\sigma^2 g$ = Genotypic variance

$\sigma^2 p$ = Phenotypic variance

PCV and GCV were classified as suggested by Shivasubramanian and Menon (1973) as follows.

0 – 10% - Low

10-20 - Moderate

20% and above - High

3.6.3.3 Heritability (h^2)

In broad sense, heritability was calculated as the ratio of genotypic variance to the phenotypic variance and expressed in percentage.

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

Where,

$\sigma^2 g$ = Genotypic variance

$\sigma^2 p$ = Phenotypic variance

Heritability percentage was categorized as demonstrated by Robinson *et al.* (1949)

0 – 10% - Low

10-20% - Moderate

>20% - High

3.6.3.4 Genetic advance (GA)

This was calculated using formula given by Robinson *et al.* (1949)

$$GA = i \times h^2 \times \sigma p$$

Where,

i = Selection of differential (i=2.06) at 5 per cent selection intensity.

h^2 = Heritability in broad sense

σp = Phenotypic standard deviation of the trait

3.6.3.5 Genetic advance over mean (GAM)

Genetic advance as per cent over mean was worked out as suggested by Johnson *et al.* (1955).

$$\text{GAM (\%)} = \frac{\text{GA}}{\bar{\text{X}}} \text{ X } 100$$

Where,

GA = Genetic advance

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

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

0 – 10% - Low

10-20% - Moderate

>20% - High

3.6.3.6 Correlation

Genotypic (rg) and phenotypic (rp) coefficient of correlation were estimated as suggested by Al-Jibourie *et al.* (1958)

$$\text{Genotypic correlation} = \frac{\text{Co V xy (G)}}{\sqrt{\text{Vx (G)} \times \text{Vy(G)}}}$$

$$\text{Phenotypic correlation} = \frac{\text{Co V xy (P)}}{\sqrt{\text{Vx (P)} \times \text{Vy(P)}}}$$

Where,

CoVxy (G) = Genotypic covariance between x and y

CoVxy (P) = Phenotypic covariance between x and y

Vx (G) = Genotypic variance of character x

Vx (P) = Phenotypic variance of character x

Vy (G) = Genotypic variance of character y

Vy (P) = Phenotypic variance of character y

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

3.6.3.7 Path coefficient analysis

The concept of path coefficient analysis developed by Wright (1921) and illustrated by Dewey and Lu (1959) was carried out separately to know the direct and indirect effects of the important components, which are the standardized partial regression. Coefficients were obtained by solving the following set of 'p' simultaneous equations through the use of 'Doo-little techniques' as given by Goulden (1959)

$P_{01} + P_{02}r_{12} + \ldots\ldots\ldots\ldots\ldots\ldots + P_{op}r_{1p} = r_{01}$

$P_{01}r_{12} + P_{02} + \ldots\ldots\ldots\ldots\ldots\ldots + P_{op}r_{2p} = r_{02}$

$P_{01}r_{1p} + P_{02}r_{2p} + \ldots\ldots\ldots\ldots\ldots\ldots + P_{op} = r_{op}$

Where P_{01}, P_{02}…….P_{0p} are the direct path effects of 1,2,……P variables between dependent variable and independent variable and r_{01}, r_{02}…….r_{0p} are the correlation coefficients between dependent variable and independent variable. The indirect effect of 'i' th variable through 'j' th variable was worked out as $P_{0j} \times r_{ij}$.

The contribution of the remaining unknown factors is measured as the residual factor and calculated as

$P^2_{0x} = 1 (P^2_{01} + 2P_{o1} P_{02}r_{12} + 2P_{01}P_{03}r_{13} + \ldots\ldots..P^2_{02} + 2P_{02} P_{03}r_{23} + \ldots\ldots P^2_{0}p)$

Residual factor = $\sqrt{P_{ox^2}}$

The direct and indirect effects were classified based on the scale given by Lenka and Misra (1973).

More than 1.0 - Very high

0.30 to 0.99 - High

0.20 to 0.29 - Moderate

0.10 to 0.19 - Low

0.00 to 0.009 - Negligible

3.6.4 Transformation of morphological data to 0-1 scale

Both qualitative and quantitative data were simultaneously used for the principal component cluster analysis using morphological data and are used in the form of values. Though the genetic variation between the genotypes contributing for the variation in qualitative aspect is high, comparatively larger numerical nature of quantitative data

smothered qualitative characters. Genotypes with higher values for qualitative characters were found to show a higher genetic distance from the other genotypes, making the qualitative observations insignificant. To overcome this and to give equal weightage for all the characters, morphological values (qualitative and quantitative) were transformed to 0-1 scale using the formula. The values < 0.5 are taken as 0 and > 0.5 taken as 1.

$$V_t = \frac{(V - V_{min})}{(V_{max} - V_{min})}$$

V - value of a particular character of any accession

Vt – transformed value

V_{min}. – minimum value of that character among all accession

V_{max}. - maximum value of that character among all accession

3.7 DNA extraction

3.7.1 Sample preparation

The samples for DNA extraction were collected from all the fifteen varieties of gladiolus. Two hundred mg of young leaves was collected. The leaves were first washed with distilled water and these leaf samples were used immediately for extraction or stored at -20°C for short term, for future use.

3.7.2 Buffers and solutions prepared for extraction of DNA

Stock solution used (Appendix III and IV)

C-TAB (n Cetyl- N, N, N, Trimethyl Ammonium Bromide)	:	2 per cent
NaCl (Sodium Chloride)	:	2M
Tris- HCl (p^H 8.0)	:	100mM
EDTA (Ethylene Diamine Tetra Acetic Acid) (p^H 8.0)	:	25mM
Chloroform/ Isoamylalcohol	:	24: 1 (v/v)
Poly vinyl pyrolidone (PVP)	:	2 per cent
β- Mercaptoethanol	:	0.2 per cent
Isopropanol	:	100 per cent

Ethanol	:	70 per cent
Tris- EDTA (TE) buffer	:	10 mM Tris-base and 1 mM EDTA, p^H 8.0

3.7.3 DNA extraction protocol

The C-TAB method of genomic DNA extraction was followed as per the protocol mentioned by Mishra *et al* (2011) with minor modifications. Following protocol was used for extracting of DNA from all the samples.

Genomic DNA isolation

1. Genomic DNA was extracted from fresh young leaves using a modified CTAB method.
2. Two hundred mg of young leaves were taken and they were placed into mortar. The leaves were ground into fine powder in liquid N with the help of pestle and they were crushed using extraction buffer (consisting of 100 mM Tris HCl of 8 pH, 2M NaCl, 25 mM EDTA, 2% C-TAB, 2 % PVP and 0.2% β- Mercaptoethanol) using pestle and mortar. Homogenate was transferred to 2 ml Eppendorf tube.
3. The tubes were incubated at 60^oC for 1 hour with occasional shaking. After incubation, the tubes were cooled to the room temperature.
4. Equal volume of chloroform: Iso-amyl alcohol (24:1) was added and the tubes were inverted gently for minimum twenty times for mixing two phases and centrifuged at 8000 rpm for 20 minutes at 10^0C for separation of DNA from rest of the materials (proteins). The upper aqueous phase (supernatant) was taken without disturbing the lower solid portion to another tube then this step is repeated once again.
5. The supernatant was transferred without disturbing the lower solid portion to fresh labeled 1.5ml tubes then 300µl isopropanol(IPA) was added and the tubes were inverted gently for mixing two phases and then centrifuged at 8000 rpm for 15 min at 10°C.
6. The pellet formed after centrifugation was washed with 70% (v/v) ethanol for 30 min. Then alcohol was decanted and pellets were dried at least for 30min till there was no alcohol smell. After drying the pellet was later dissolved in (150 µl) $T_{10}E_1$ buffer Tris-EDTA (10-1mM) and stored at $–20^oC$ until use.
7. RNase treatment: Required quantity (3 µl) of RNAse was mixed to the DNA sample and tubes were incubated on water bath at 37^o C for one hour and 50^o C for five minutes to remove the RNA present in the DNA.

3.7.4 DNA quantification using spectrophotometer

1. UV-Visible spectrophotometer was used to measure the absorbance of isolated genomic DNA at A_{260} and A_{280} nm.

2. While the purity of extracted DNA was determined based on the ratio of A260/A280, the concentration of DNA in different cultivars was measured according to the formula DNA (ng/ ml) = A260 x 50 x Dilution factor (Table 2).

3. A sample run on 0.8 per cent agarose gel was utilized to have a visible test of quantity and quality of extracted DNA

4. The gel was run in 1 x TAE (Tris-base, glacial acetic acid, 0.5 M EDTA) buffer and stained in 5 µl ethidium bromide solution. Pure DNA preparations showed a ratio between 1.80 to 2.00.

5. DNA dilution for PCR: The re-suspended DNA was then diluted in sterile distilled water to 100 ng/ µl concentration for use in amplification reactions. Again dilution was made up to 10 ng/ µl for SRAP.

3.8 Sequence Related Amplified Polymorphism (SRAP) analysis

3.8.1 PCR amplification

Reagents used in PCR reaction mixture

10 x assay buffer	:	2 µl (10 mM Tris-HCL, pH 8.8; 500 mM $(NH_4)_2SO_4$; 15 mM $MgCl_2$; 0.1 per cent Gelatin; 0.05 per cent Tween-20 and 0.05 per cent NP 40)
dNTPs 2mM	:	2.0 µl
$MgCl_2$ 25 mM	:	2.0µl
PCR primer 3µM Forward (F)	:	2.0 µl
PCR primer 3µM Reverse (R)	:	2.0 µl
Template DNA 10 ng/ µl	:	3.0 µl
Taq polymerase 5 U/ µl	:	0.2 µl
Sterile water	:	6.80 µl

1. PCR was used to amplify specific region of a DNA strand.

2. Amplification reaction was performed in a final volume 20 µl PCR reaction mixture.

Table 2. Quantity of DNA in different gladiolus varieties

Varieties	Concentration (ng/ µl)
American Beauty	1070
Candyman	740
Charms Flow	960
Delhi Local	750
Green Bay	1000
Her majesty	490
Jester	1120
Punjab morning	370
Eighth Wonder	560
Red Majesty	370
Summer Sunshine	310
Copper King	650
White Prosperity	580
Vedanapoli	420
Darshan	280

Thermo profile for PCR

The thermal cycling was done as follows (Mishra *et al.*, 2011).

Sl. No.	Step	Temperature (°C)	Duration	Number of Cycles
1.	Initial denaturation	94	5:00	1
2.	Denaturation	94	1:00	5 Cycles
3.	Annealing	35	1:15	
4.	Extension	72	2:00	
5.	Denaturation	94	1:00	30 Cycles
6.	Annealing	50	1:15	
7.	Extension	72	2:00	
8.	Final extension	72	10:00	1
9.	Hold	4	1:00	1

After completion of 35 cycles, the samples were stored at 4°C in thermocycler and all the contents were loaded on to the gel for electrophoresis. Separation of PCR amplified products by agarose gel electrophoresis.

3.8.2 Primer selection

SRAP analysis was carried out using twenty five primer combinations among which, eleven primer combination yielded maximum number of bands that were consistent, strong, intense and clear were selected for fingerprinting and diversity analysis (Table 3).

Agarose gel electrophoresis

Materials

Agarose : 2.00 per cent

Running buffer : 1x TAE (5 ml 50x TAE buffer and make the volume with distill water for preparation one liter) and 50x TAE buffer (Tris base 242g, 57.1ml Glacial acetic acid, 100 ml EDTA 0.5 M; pH 8.0 for one liter)

Ethidium bromide : 6.0 µl/ 100 ml stock

Loading buffer 4.0 µl **(**0.25% Bromophenol blue)

1. Amplified of products was resolved by electrophoresis in 2.0 per cent agarose gel containing ethidium bromide (6.0 µl/ 100 ml) using 1 x TAE buffer (Sambrook *et at.,* 1989).

Table 3. Sequences of SRAP forward and reverse primer and primer combinations used for fingerprinting and diversity analysis

Forward primer		Reverse primer		Primer combinations	
Name	**Sequence**	**Name**	**Sequence**	**Forward**	**Reverse**
ME1	TGAGTCCAAACCGGATA	EM1	GACTGCGTACGAATTAAT	ME1	EM3
ME2	TGAGTCCAAACCGGAGC	EM2	GACTGCGTACGAATTTGC	ME2	EM3/ EM5
ME3	TGAGTCCAAACCGGAAT	EM3	GACTGCGTACGAATTGAC	ME3	EM1/ EM5/ EM12
ME4	TGAGTCCAAACCGGACC	EM4	GACTGCGTACGAATTTGA	ME4	EM2 / EM3/ EM4
ME5	TGAGTCCAAACCGGAAG	EM5	GACTGCGTACGAATTAAC	ME5	EM1/ EM2
		EM12	GACTGCGTACGAATTCTC		

2. Wells were loaded with 20 µl of reaction mixture mixed with 4 µl of loading buffer.

3. Electrophoresis was conducted at a constant voltage of 70 V for 3-4 hours in 1 x TAE buffer.

4. The gels were photographed under UV light by using Alpha Digi Doc Gel Documentation System.

3.9 Data analysis, estimation of genetic distance and clustering analysis

3.9.1 Data scoring and analysis

Consistent, well resolved fragments, in the size range of 200 bp to 10000 kb were manually scored. Each band was treated as a marker. Scoring of bands was done on the basis of their presence (‘1’) or absence (‘0’) in the gel. The genetic associations were evaluated by calculating the Dice similarity coefficient for pair-wise comparisons based on the proportion of shared bands produced by the primers.

Fragments amplified by the primer used and molecular weights in base pairs (bp) were scored for their presence or absence (Echt *et al.,* 1992) and a matrix of different SRAP, phenotypes was assembled. Further, a fragment was counted only if it was intense, clear and strong. Diffuse and/or very weak fragments were not scored as such fragments have been reported to possess the greatest propensity for poor reproducibility (Heun and Helentjaris, 1993). The band sizes were estimated by using a 1000 bp ladder marker, which was run along with the amplified products.

Per cent polymorphism computed was,

$$\text{Per cent polymorphism} = \frac{\text{Total number of polymorphic bands X 100}}{\text{Total number of bands}}$$

Statistical analysis and estimating genetic distances

The scored band data was subjected to statistical analysis using the computer programme NTSYS-PC Ver. 2.2 software (Rohlf, 2000). The resultant similarity matrix was used to generate a tree by UPGMA (Unweighted Pair Group Method with Arithmetical averages).

Cluster analysis

The agglomerative method of clustering using UPGMA (Unweighted Pair Group Method with Arithmetical averages) for developing dendrogram was adopted. This calculates

the congruence between assays of values typically densitometric assays. As it compares curves as a whole, it is independent of band definitions and thus ideally suited for a quick comparison of pattern without first having to edit the bands. It is largely intensive to relative concentrations, but is sensitive to differences in background. Dice co-efficient considers only the presence of band as similarity and hence, it is more conservative in declaring genetic diversity. The subset of data involving fifteen varieties was analyzed separately.

4. EXPERIMENTAL RESULTS

The present investigations were carried out using fifteen varieties of gladiolus to assess the extent of genetic variability and diversity among them based on morphological and molecular basis and are presented in this chapter.

4.1 Assessment of genetic variability and genetic diversity based on morphological data

4.1.1 Analysis of variance

The analysis of variance indicated significantly higher amount of variability among the varieties for all the characters studied and are presented in Table 4. The variation due to replication was non-significant for all the characters studied. The mean values of different characters for the varieties under study are given in Appendix V, VI and VII.

4.1.2 Genetic variability, heritability and genetic advance

To understand the extent to which the observed variation were due to genetic factors, viz., the genotypic variance (GV), phenotypic variance (PV), genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), broad sense heritability (h^2), genetic advance (GA) and genetic advance over per cent mean (GAM). The data revealed the existence of large amount of variability with respects to all characters studied and given in the Table 5 and Table 6.

4.1.2.1 Growth Parameters

4.1.2.1.1 Plant height (cm)

The plant height at 30 days after planting varied from 30.77 cm in variety 'Jester Gold' to 53.18 cm in variety 'Punjab Morning' with a grand mean of 40.59 cm. The genotypic and phenotypic variances were 38.32 and 46.00, respectively. The estimates of genotypic and phenotypic coefficient of variations were moderate of 15.25 per cent and 16.71 per cent, respectively. The high heritability (83.31%) was coupled with moderate genetic advance as per cent of mean (28.68)

.

Table 4. Analysis of variance for different characters in gladiolus (*Gladiolus hybridus* Hort.) varieties

Sl. No	Character	Mean sum of squares		
		Replication d.f = 1	Genotypes d.f = 14	Error d.f = 14
Growth parameters				
1	Plant height 30 DAP (cm)	4.287	84.325*	7.680
2	Plant height 60 DAP (cm)	1.936	135.008*	8.038
3	Plant height 90 DAP (cm)	10.526	138.574*	8.503
4	Stem girth 30 DAP (cm)	0.217	0.047*	0.018
5	Stem girth 60 DAP (cm)	0.126	0.079*	0.030
6	Stem girth 90 DAP (cm)	0.106	0.065*	0.023
7	Number of leaves 30 DAP	0.108	0.962*	0.102
8	Number of leaves 60 DAP	0.120	0.803*	0.2318
9	Number of leaves 90 DAP	0.225	1.185*	0.123
10	Leaf length 30 DAP (cm)	1.358	51.426*	6.735
11	Leaf length 60 DAP (cm)	7.076	114.759*	9.908
12	Leaf length 90 DAP (cm)	4.008	128.996*	7.348
13	Leaf width 30 DAP (cm)	0.009	0.369*	0.016
14	Leaf width 60 DAP (cm)	0.007	0.266*	0.040
15	Leaf width 90 DAP (cm)	0.126	0.361*	0.039
16	Leaf area 90 DAP (cm^2)	97.725	1938.20*	21.465

* Significant at p = 0.05 probability

Table 4. Cont……

Sl. No	Character	Mean sum of squares		
		Replication d.f = 1	Genotypes d.f = 14	Error d.f = 14
Flowering parameter				
1	Days taken for spike initiation	103.05	150.56*	13.03
2	Days to first three florets color showing stage	77.44	158.89*	10.30
3	Duration of flowering (days)	7.50	18.43*	2.14
Quality and yield parameters				
4	Spike length (cm)	103.05	150.56*	13.03
5	Rachis length (cm)	77.44	158.89*	10.30
6	Number of florets per spike	0.22	8.42*	0.16
7	Floret diameter (cm)	0.14	3.30*	0.09
8	Weight of spike (g)	2.22	839.45*	2.47
9	Vase life (days)	0.13	2.33*	0.03
10	Number of spikes per plant	0.03	0.72*	0.04
Corm and cormels parameters				
11	Number of daughter corms per plant	0.021	0.942*	0.012
12	Diameter of daughter corm (cm)	0.02	0.890*	0.04
13	Weight of daughter corm (g/plant)	2.81	435.01*	4.11
14	Number of cormels per plant	29.07	3716.71*	30.67
15	Weight of cormels (g/plant)	6.62	234.65*	2.74
16	Weight of ten cormels (g)	0.06	25.72*	0.24

* Significant at p = 0.05 probability

At 60 days after planting, plant height varied from 52.85 cm in variety 'Delhi Local' to 82.09 cm in variety 'Red Majesty' with a grand mean plant height of 62.81 cm. The genotypic and phenotypic variances were 63.49 and 71.52, respectively. Moderate genotypic and phenotypic coefficient of variations were observed (12.69 per cent and 13.47 per cent, respectively). The high heritability (88.76%) was coupled with moderate genetic advance as per cent of mean (24.62)

The plant height at 90 days after planting ranged from 53.80 cm in variety 'Delhi Local' to 85.70 cm in variety 'Red Majesty' with a grand mean plant height of 66.51 cm. The genotypic and phenotypic variances were 65.04 and 73.54, respectively. The estimates of genotypic and phenotypic coefficient of variations were 12.13 per cent and 12.89 per cent, respectively. The high heritability (88.44%) was coupled with moderate genetic advance as per cent of mean (23.49).

4.1.2.1.2 Stem girth (cm)

Stem girth at 30 days after planting ranged from 1.84 cm (Delhi Local) to 2.38 cm (Darshan) with grand mean of 2.18cm. The genotypic and phenotypic variances were 0.02 and 0.03, respectively. The estimates of genotypic and phenotypic coefficient of variations were 5.98 per cent and 8.12 per cent, respectively. The heritability (54.15) was coupled with low genetic advance as per cent of mean (9.06)

Stem girth at 60 days after planting ranged from 1.80 cm (Delhi Local) to 2.30 cm (Darshan) with grand mean of 2.11cm. The genotypic and phenotypic variances were 0.02 and 0.03, respectively. The estimates of GCV and PCV 7.15 per cent and 10.31 per cent, respectively. The heritability (50.93) was coupled with moderate genetic advance as per cent of mean (10.32).

Stem girth at 90 days after planting ranged from 1.64cm (Delhi Local) to 2.25 cm (Darshan) with grand mean of 2.03cm. The genotypic and phenotypic variances were 0.02 and 0.04, respectively. The estimates of genotypic and phenotypic coefficient of variations were 7.15 per cent and 10.31 per cent, respectively. The heritability (48.04) coupled with moderate genetic advance as per cent of mean (10.20)

4.1.2.1.3 Number of leaves per plant

Number of leaves per plant at 30 days after planting ranged from 1.90 (Green Bay) to 4.70 (Delhi Local) with grand mean of 2.85. The genotypic and phenotypic variances were 0.43 and 0.53, respectively. The genotypic and phenotypic coefficient of variations were 23.04

Table 5. Estimates of mean, range, components of variance, heritability and genetic advance for growth parameters in gladiolus (*Gladiolus hybridus* Hort.) varieties.

Sl. No	Character	Mean ± S.Em	Range	GV	PV	GCV (%)	PCV (%)	h2 (%)	GA	GAM (% mean)
Growth parameters										
1	Plant height 30 DAP (cm)	40.59 ± 1.96	30.77 - 53.18	38.32	46.00	15.25	16.71	83.31	11.64	28.68
2	Plant height 60 DAP (cm)	62.81 ± 2.00	52.85 - 82.09	63.49	71.52	12.69	13.47	88.76	15.46	24.62
3	Plant height 90 DAP (cm)	66.51 ± 2.06	53.80 - 85.70	65.04	73.54	12.13	12.89	88.44	15.62	23.49
4	Stem girth 30 DAP (cm)	2.18 ± 0.08	1.84 -2.38	0.02	0.03	5.98	8.12	54.15	0.20	9.06
5	Stem girth 60 DAP (cm)	2.11 ± 0.09	1.80 - 2.30	0.02	0.03	6.22	8.71	50.93	0.19	9.14
6	Stem girth 90 DAP (cm)	2.03 ± 0.11	1.64 - 2.25	0.02	0.04	7.15	10.31	48.04	0.21	10.20
7	Number of leaves 30 DAP	2.85 ± 0.23	1.90 - 4.70	0.43	0.53	23.04	25.63	80.79	1.21	42.66
8	Number of leaves 60 DAP	6.42 ± 0.34	5.10 - 7.30	0.29	0.52	8.32	11.20	55.23	0.82	12.74
9	Number of leaves 90 DAP	8.41 ± 0.25	7.40 - 9.60	0.53	0.65	8.66	9.61	81.16	1.35	16.07
10	Leaf length 30 DAP (cm)	34.04 ± 1.84	25.38 - 42.40	22.35	29.08	13.89	15.84	76.84	8.54	25.08
11	Leaf length 60 DAP (cm)	53.04 ± 2.23	44.76 - 72.99	52.43	62.33	13.65	14.88	84.11	13.68	25.79
12	Leaf length 90 DAP (cm)	59.92 ± 1.92	48.90 - 80.22	60.82	68.17	13.01	13.78	89.22	15.18	25.32
13	Leaf width 30 DAP (cm)	2.70 ± 0.09	2.11 - 3.53	0.18	0.19	15.59	16.30	91.55	0.83	30.73
14	Leaf width 60 DAP (cm)	3.06 ± 0.14	2.38 - 3.70	0.11	0.15	10.97	12.78	73.70	0.59	19.40
15	Leaf width 90 DAP (cm)	3.36 ± 0.14	2.53 - 3.98	0.16	0.20	11.96	13.33	80.50	0.74	22.11
16	Leaf area 90 DAP (cm^2)	120.83 ± 3.28	76.53 -194.19	958.37	979.83	25.62	25.91	97.81	63.07	52.20

DAP – Days after planting

GV - Genotypic variance
PV - Phenotypic variance
GAM - Genetic advance over per cent mean
GCV - Genotypic coefficient of variation
PCV - Phenotypic coefficient of variation
h^2 - Heritability
GA - Genetic advance

per cent and 25.63 per cent respectively. High heritability of 80.79% coupled with high genetic advance as per cent mean of 42.66.

Number of leaves per plant at 60 days after planting varied from 5.10 (Green Bay) to 7.30 (White Prosperity) with grand mean of 6.42. The genotypic and phenotypic variances were 0.29 and 0.52, respectively were observed. The genotypic and phenotypic coefficient of variations were 8.32 per cent and 11.20per cent respectively. High heritability of 55.23% coupled with moderate genetic advance as per cent mean of 12.74.

At 90 days after planting, number of leaves per plant ranged from 7.40 (Candyman) to 9.60 (White Prosperity and Red Majesty) with grand mean of 8.41. The genotypic and phenotypic variances were 0.53 and 0.65, respectively. The genotypic and phenotypic coefficient of variations were 8.66 per cent and 9.61 per cent respectively. High heritability of 81.16% coupled with genetic advance as per cent mean of 16.07.

4.1.2.1.4 Leaf length (cm)

The leaf length at 30 days after planting varied from 25.38 cm in variety ‘American beauty’ to 42.40 cm in variety ‘Punjab Morning’ with a grand mean leaf length of 34.04 cm. The genotypic and phenotypic variances were 22.35 and 29.08, respectively. Moderate genotypic and phenotypic coefficient of variations were 13.89 per cent and 15.84 per cent, respectively. The high heritability (76.84%) was coupled with genetic advance as per cent of mean (25.08).

The leaf length at 60 days after planting varied from 44.76 cm in variety ‘Delhi Local’ to 72.99 cm in variety ‘Red Majesty’ with a grand mean of 53.04 cm. The genotypic and phenotypic variances were 52.43 and 62.33, respectively. The estimates of genotypic and phenotypic coefficient of variations were 13.65 per cent and 14.88 per cent, respectively. The high heritability (84.11%) was coupled with genetic advance as per cent of mean (25.79)..

The leaf length at 90 days after planting varied from 48.90 cm in variety ‘Delhi Local’ to 80.22 cm in variety ‘Red Majesty’ with a grand mean of 59.92 cm. The genotypic and phenotypic variances were 60.82 and 68.17, respectively. The estimates of genotypic and phenotypic coefficient of variations were 13.01 per cent and 13.78 per cent, respectively. High heritability (89.22%) was coupled with high genetic advance as per cent of mean (25.32).

4.1.2.1.5 Leaf width (cm)

Leaf width at 30 days after planting varied from 2.11cm (Delhi Local) to 3.53 cm (Summer Sunshine) with grand mean of 2.70 cm. Genetic variance of 0.18 and phenotypic variance of 0.19 were observed. The estimates of genotypic and phenotypic coefficient of variations were 15.59 per cent and 16.30 per cent, respectively. The heritability and genetic advance as per cent mean were 91.55 per cent and 30.73, respectively.

At 60 days after planting, leaf width ranged from 2.38 cm (Delhi Local) to 3.70 cm (Summer Sunshine) with grand mean of 3.06 cm. Genetic variance of 0.11 and phenotypic variance of 0.15 were observed. The estimates GCV and PCV were 10.97 per cent and 12.78 per cent, respectively. The heritability and genetic advance as per cent mean were 73.70 per cent and 19.40, respectively.

Leaf width at 90 days after planting ranged from 2.53 cm (Delhi Local) to 3.98 cm (Summer Sunshine) with grand mean of 3.36 cm. Genetic variance of 0.16 and phenotypic variance of 0.20 were observed. The estimates of genotypic and phenotypic coefficient of variations were 11.96 per cent and 13.33 per cent, respectively. The heritability and genetic advance as per cent mean were 80.50 per cent and 22.11, respectively.

4.1.2.1.6 Leaf area (cm^2)

Leaf area at 90 days after planting ranged from 76.53 cm^2 (Delhi Local) to 194.19 cm^2 (Red Majesty) with grand mean of 120.83 cm^2. Genetic variance of 958.37 and phenotypic variance of 979.83 were observed. The estimates of genotypic and phenotypic coefficient of variations were 25.62 per cent and 25.91 per cent, respectively. The high heritability (97.81%) was coupled with moderate genetic advance as per cent of mean (52.20).

4.1.2.2 Flowering parameters

4.1.2.2.1 Days taken for spike initiation (days)

The variety Punjab Morning took minimum number of days (59.90 days), while Eighth Wonder took the maximum number of days (88.20 days) for spike initiation with grand mean of 73.36 days. The genotypic and phenotypic variances were 68.76 and 81.80, respectively. Low estimates of GCV (11.30%) and PCV (12.33%) were coupled with high heritability (84.07%) and genetic advance as per cent of mean (21.35) was observed.

4.1.2.2.2 Days to first three florets colour showing stage (days)

Days taken for three florets colour showing stage was minimum in Delhi Local (70.90 days) and maximum was found in Eighth Wonder variety (98.70 days) with grand mean of 84.10 days. Estimates of genotypic and phenotypic variances were 74.30 and 84.60, respectively. Low estimates of GCV (10.25%) and PCV (10.94%) were coupled with high heritability (87.83%) and genetic advance as per cent of mean (19.79) was observed.

4.1.2.2.3 Duration of flowering (days)

Duration of flowering was minimum in Red Majesty (23.50days) and maximum was found in Delhi Local variety (33.00 days) with grand mean of 28.50 days. Estimates of

Table 6. Estimates of mean, range, components of variance, heritability and genetic advance for flowering, quality and yield parameters in gladiolus (*Gladiolus hybridus* Hort.) varieties.

Sl. No	Character	Mean ± S.Em	Range	GV	PV	GCV (%)	PCV (%)	h^2 (%)	GA	GAM (% mean)
Flowering parameters										
1	Days taken for spike initiation	73.36 ± 2.55	59.9 - 88.20	68.76	81.80	11.30	12.33	84.07	15.66	21.35
2	Days to first three florets color showing stage	84.10 ± 2.27	70.90 - 98.70	74.30	84.60	10.25	10.94	87.83	16.64	19.79
3	Duration of flowering (days)	28.50 ± 1.04	23.50 - 33.00	8.14	10.29	10.01	11.25	79.17	5.23	18.35
Quality and yield parameters										
4	Spike length (cm)	78.75 ± 2.48	60.90 - 103.7	135.39	147.68	14.77	15.43	91.67	22.95	29.14
5	Rachis length (cm)	40.02 ± 2.03	27.85 - 49.35	34.56	42.84	14.69	16.35	80.68	10.88	27.18
6	Number of florets per spike	13.52 ± 0.29	11.10 - 17.30	4.13	4.29	15.02	15.32	96.20	4.10	30.36
7	Floret diameter (cm)	9.29 ± 0.22	6.99 - 11.45	1.60	1.70	13.64	14.02	94.54	2.54	27.31
8	Weight of spike (g)	87.74 ± 1.11	65.10 - 121.3	418.49	420.96	23.32	23.38	99.41	42.02	47.89
9	Vase life (days)	8.40 ± 0.13	6.60 - 10.60	1.15	1.18	12.75	12.94	97.18	2.18	25.90
10	Number of spikes per plant	1.50 ± 0.14	1.0 - 3.0	0.34	0.38	38.82	40.99	89.67	1.14	75.72
Corm and cormels parameters										
11	Number of daughter corms per plant	1.56 ± 0.08	1.0 - 3.5	0.46	0.48	43.70	44.30	97.33	1.39	88.81
12	Diameter of daughter corm (cm)	5.49 ± 0.14	4.46 - 6.70	0.42	0.46	11.86	12.41	91.37	1.28	23.35
13	Weight of daughter corm (g/plant)	52.06 ± 1.43	33.67 - 84.01	215.45	219.56	28.20	28.46	98.13	29.95	57.54
14	Number of cormels per plant	60.61 ± 3.92	18.00 - 183.0	1843.02	1873.69	70.83	71.42	98.36	87.71	144.71
15	Weight of cormels (g/plant)	33.87 ± 1.17	21.87 - 60.94	115.95	118.69	31.79	32.16	97.69	21.92	64.73
16	Weight of ten cormels (g)	6.35 ± 0.35	3.35 - 17.75	12.74	12.98	56.22	56.75	98.14	7.28	114.74

genotypic and phenotypic variances were 8.14 and 10.29, respectively. The estimates of genotypic and phenotypic coefficient of variations (10.01 per cent and 11.25 per cent, respectively) were coupled with high heritability (79.17%) and genetic advance as per cent of mean (18.35) was observed.

4.1.2.3 Quality and Yield parameters

4.1.2.3.1 Spike length (cm)

The average spike length obtained was 78.75 cm with a range of 60.90 cm in the variety 'Darshan' to 103.70 cm in the variety Red Majesty'. Genetic variance of 135.39 and phenotypic variance of 147.68 were observed. Moderate genotypic coefficient of variability (14.77%) and phenotypic coefficient of variability (15.43%) were observed. High heritability (91.67%) with genetic advance as per cent mean (29.14) was observed.

4.1.2.3.2 Rachis length (cm)

Rachis length was minimum in the variety Her Majesty (27.85 cm) and maximum in Red Majesty (49.35 cm) with grand mean of 40.02 cm. The genotypic and phenotypic variances were 34.56 and 42.84, respectively. The genotypic coefficients of variations (14.69%) and phenotypic coefficients of variations (16.35%) were moderate. High heritability of 80.68 per cent with genetic advance as per cent mean of 27.18.

4.1.2.3.3 Number of florets per spike

Number of florets per spike ranged from 11.10 (Copper King) to 17.30 (Eight Wonder), with grand mean of 13.52 florets per spike. The low estimates of genotypic (4.13) and phenotypic (4.29) variance were observed. The GCV (15.02%) and PCV (15.32%) with high heritability (96.20%) and genetic advance as per cent of mean (30.36) was found.

4.1.2.3.4 Floret diameter (cm)

Diameter of the floret varied from 6.99cm (Delhi Local) to 11.45 cm (Red Majesty) with grand mean of 9.29cm. Estimates of genotypic and phenotypic variance were 1.60 and 1.70, respectively. The GCV (13.64%) and PCV (14.02%) were moderate. The high heritability of 94.54% coupled with genetic advance as per cent mean of 27.31.

4.1.2.3.5 Weight of spike (g)

The average weight of spike was 87.74 g with a wide range of 65.10 g (Green Bay) to 121.30 g (Candyman). The genotypic and phenotypic variances were 418.49 and 420.96, respectively. The estimates of GCV (23.32%) and PCV (23.38%) with high heritability of 99.41 per cent and genetic advance as per cent mean of 47.89.

4.1.2.3.6 Vase life (days)

Vase life varied from 6.60 days (Delhi local) to 10.60 days (Candyman) with grand mean of 8.40 days. The genotypic and phenotypic variances were 1.15 and 1.18, respectively. The estimates of GCV and PCV were 12.75 per cent and 12.94 per cent, respectively with high heritability (97.18%) and genetic advance as per cent mean (25.90).

4.1.2.3.7 Number of spikes per plant

Spike yield per plant was highest in Delhi Local (3.00) and the least (1.00) in many other varieties like Charms Flow, Jester Gold and White Prosperity. The grand mean was 1.50 spikes per plant. The genotypic and phenotypic variances were 0.34 and 0.38, respectively. The GCV (38.82%) and PCV (40.99%) were associated with high heritability (89.67%) coupled with high genetic advance as per cent of mean (75.72).

4.1.2.4 Corm and cormel parameters

4.1.2.4.1 Number of daughter corm per plant

Number of daughter corm per plant ranged from 1.00 (Charms Flow and Candymam) to 3.50 (Delhi Local) with grand mean of 1.56. Genotypic variance of 0.46 and phenotypic variance of 0.48 were observed. The estimates of genotypic coefficients of variation (43.70%) and phenotypic coefficients of variation (44.30%) were associated with high heritability of 97.33 per cent and high genetic advance as per cent mean of 88.81.

4.1.2.4.2 Diameter of daughter corm

Diameter of daughter corm varied from 4.46 cm (Delhi Local) to 6.70 cm (Summer Sunshine), with grand mean of 5.49 cm. Genotypic variance of 0.42 and phenotypic variance of 0.46 were observed. Coefficients of variations for both genotypic and phenotypic were 11.86 per cent and 12.41 per cent, respectively. High heritability of 91.37% coupled with genetic advance as per cent mean of 23.35.

4.1.2.4.3 Weight of daughter corm (g)

The average weight daughter corm was 52.06 g with a wide range of 33.67 g (Delhi Local) to 84.01 g (Summer Sunshine). The genotypic and phenotypic variance was 215.45 and 219.56, respectively. Estimates of GCV (28.20%) and PCV (28.46%) along with high heritability of 98.13 per cent and high genetic advance as per cent mean of 57.54.

4.1.2.4.4 Number of cormels per plant

Number of cormels per plant ranged from 18.00 (Punjab Morning) to 183.00 (Red Majesty), with grand mean of 60.61. High genotypic (1843.02) and phenotypic (1873.69)

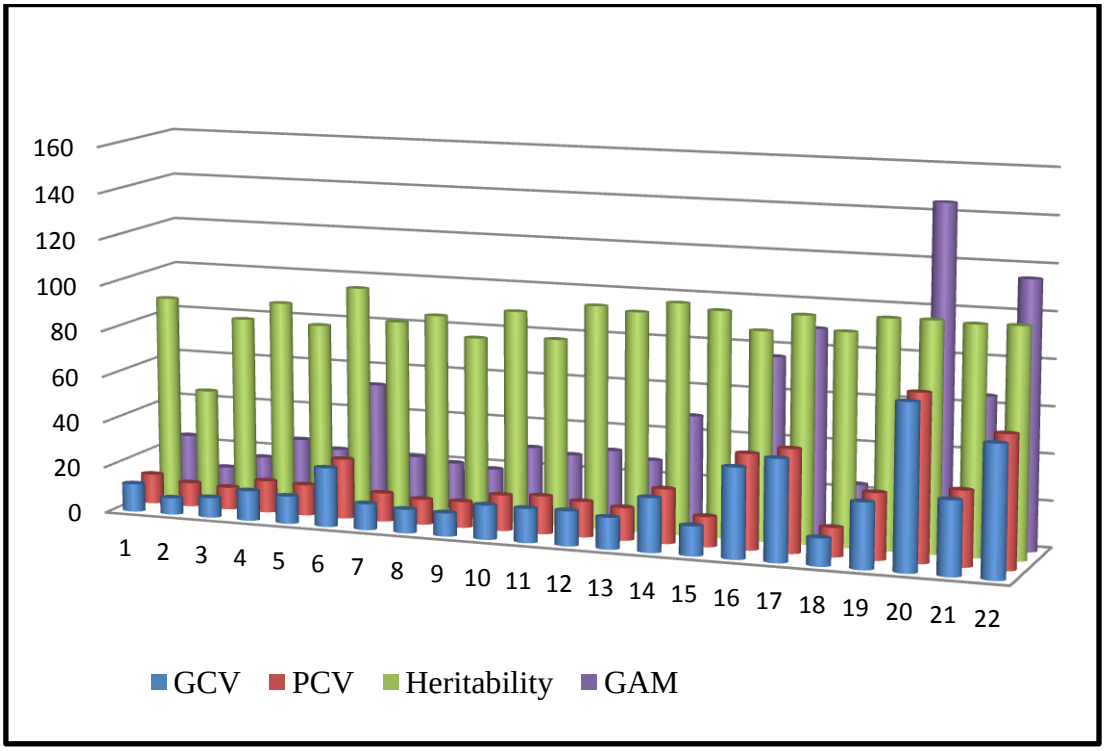


Fig 1. GCV, PCV, Heritability and GAM for different characters in gladiolus

1. Plant height 90 DAP (cm)
2. Stem girth 90 DAP (cm)
3. Number of leaves 90 DAP
4. Leaf length 90 DAP (cm)
5. Leaf width 90 DAP (cm)
6. Leaf area 90 DAP (cm^2)
7. Days taken for spike initiation
8. Days to first three florets color showing stage
9. Duration of flowering (days)
10. Spike length (cm)
11. Rachis length (cm)
12. Number of florets per spike
13. Floret diameter (cm)
14. Weight of spike (g)
15. Vase life (days)
16. Number of spike per plant
17. Number of daughter corms per plant
18. Diameter of daughter corm (cm)
19. Weight of daughter corm (g/plant)
20. Number of cormels per plant
21. Weight of cormels (g/plant)
22. Weight of ten cormels (g)

variance were found. High estimates of GCV (70.83%) and PCV (71.42%) were observed. High heritability of (98.36%) coupled with high genetic advance as per cent mean (144.71).

4.1.2.4.5 Weight of cormels per plant

Weight of cormels per plant varied from 21.87g (Charms Flow) to 60.94g (Summer Sunshine), with grand mean of 33.87g. The genotypic and phenotypic variance were 115.95 and 118.69, respectively. Estimates of genotypic coefficients of variations (31.79%) and phenotypic coefficients of (32.16%) along with high heritability of 97.69 per cent and high genetic advance as per cent mean of 64.73.

4.1.2.4.6 Weight of ten cormels per plant

Weight of ten cormels per plant varied from 3.35g (Charms Flow) to 17.75g (Summer Sunshine), with grand mean of 6.35g. The genotypic and phenotypic variance was 12.74 and 12.98, respectively. Estimates of GCV (56.22%) and PCV (56.75%) were observed. High heritability of 98.14 per cent coupled with high genetic advance as per cent mean of 114.74.

4.1.3 Correlation studies

The genotypic and phenotypic correlation studies were carried out for all the 18 characters to know the nature of relationship existing between number of spikes per plant and its other component characters (Table 7 and 8).

4.1.3.1 Genotypic and phenotypic correlations

In general, genotypic correlation coefficients were higher than the phenotypic correlation coefficients. This indicates the presence of inherent association between various characters.

Plant height had a significant and positive correlation with number of leaves (0.489 and 0.454), leaf length (0.996 and 0.976), leaf width (0.881 and 0.667), leaf area (0.886 and 0.832), spike length (0.745 and 0.666), number of florets per spike (0.479 and 0.448), floret diameter (0.640 and 0.598), weight of spike (0.372 and 0.344), weight of daughter corm (0.676 and 0.629), number of cormels per plant (0.709 and 0.658) at both genotypic and phenotypic levels and with rachis length (0.357) at genotypic level. Whereas, significant and negative correlation was observed at genotypic and phenotypic level with duration of flowering (-0.548 and -0.474), number of daughter corms (-0.597 and -0.548) and number of spikes per plant (-0.500 and -0.464). Correlations with other characters were low and non significant.

Number of leaves showed significant and positive correlation at both genotypic and phenotypic levels with plant height, leaf length (0.434 and 0.424), leaf width (0.682 and 0.486), leaf area (0.537 and 0.472), spike length (0.689 and 0.554), rachis length (0.496 and

Table 7. Genotypic correlation coefficient for growth, flowering and yield characters in gladiolus varieties.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	1.000	0.489**	0.996**	0.881**	0.291	0.886**	0.169	-0.548**	0.745**	0.357*	0.497**	0.640**	0.372*	0.250	0.676**	-0.597**	0.709**	-0.500**
2		1.000	0.434**	0.682**	0.342*	0.537**	0.316	-0.134	0.689**	0.496**	0.549**	0.560**	0.456**	0.300	0.403*	-0.421*	0.752**	-0.548**
3			1.000	0.826**	0.254	0.897**	0.019	-0.544**	0.715**	0.377*	0.528**	0.611**	0.346*	0.218	0.651**	-0.563**	0.714**	-0.462**
4				1.000	0.476**	0.740**	0.304	-0.332*	0.736**	0.226	0.378*	0.714**	0.676**	0.684**	0.699**	-0.722**	0.712**	-0.631**
5					1.000	0.298	0.258	-0.041	-0.115	-0.486**	-0.019	0.255	-0.086	0.438**	0.415*	-0.686**	0.297	-0.747**
6						1.000	0.139	-0.560**	0.708**	0.314	0.603**	0.694**	0.453**	0.385*	0.591**	-0.639**	0.659**	-0.600**
7							1.000	-0.348*	0.331*	0.277	0.130	0.405*	0.117	0.148	-0.029	-0.347*	0.141	-0.391*
8								1.000	-0.355*	-0.249	-0.035	-0.351*	-0.074	0.019	-0.127	0.397*	-0.353*	0.387*
9									1.000	0.742**	0.478**	0.742**	0.631**	0.498**	0.682**	-0.392*	0.712**	-0.418*
10										1.000	0.468**	0.700**	0.642**	0.474**	0.365*	-0.142	0.549**	-0.237
11											1.000	0.460**	0.374*	0.349*	0.314	-0.494**	0.303	-0.562**
12												1.000	0.739**	0.723**	0.697**	-0.688**	0.709**	-0.709**
13													1.000	0.851**	0.566**	-0.384*	0.496**	-0.392*
14														1.000	0.654**	-0.576**	0.323	-0.569**
15															1.000	-0.559**	0.435**	-0.511**
16																1.000	-0.342*	0.998**
17																	1.000	-0.343*
18																		1.000

* Significant at p = 0.05 probability (0.329) ** Significant at p = 0.01 probability (0.424) DAP- Days after planting

1 Plant height (90 DAP) (cm)
2 Number of leaves (90 DAP)
3 Leaf length (90 DAP) (cm)
4 Leaf width (90 DAP) (cm)
5 Stem girth (90 DAP) (cm)
6 Leaf area (90 DAP) (cm^2)
7 Days taken for spike initiation
8 Duration of flowering (days)
9 Spike length (cm)
10 Rachis length (cm)
11 Number of florets per spike
12 Floret diameter (cm)
13 Weight of spike (g)
14 Vase life (days)
15 Weight of daughter corm (g/plant)
16 Number of daughter corms per plant
17 Number of cormels per plant
18 Number of spikes per plant

Table 8. Phenotypic correlation coefficient for growth, flowering and yield characters in gladiolus varieties.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	1.000	0.454**	0.976**	0.667**	0.150	0.832**	0.107	-0.474**	0.666**	0.285	0.448**	0.598**	0.344*	0.235	0.629**	-0.548**	0.658**	-0.464**
2		1.000	0.424**	0.486**	0.244	0.472**	0.329*	-0.051	0.554**	0.336*	0.525**	0.515**	0.390*	0.294	0.386*	-0.396*	0.661**	-0.485**
3			1.000	0.595**	0.110	0.839**	-0.009	-0.443**	0.633**	0.290	0.483**	0.576**	0.324	0.214	0.598**	-0.523**	0.657**	-0.438**
4				1.000	0.394*	0.700**	0.310	-0.242	0.672**	0.266	0.343*	0.639**	0.605**	0.584**	0.623**	-0.618**	0.650**	-0.514**
5					1.000	0.216	0.110	-0.012	0.047	-0.165	0.079	0.247	-0.063	0.290	0.294	-0.506**	0.158	-0.447**
6						1.000	0.141	-0.477**	0.682**	0.301	0.585**	0.682**	0.445**	0.372*	0.575**	-0.613**	0.651**	-0.559**
7							1.000	-0.266	0.240	0.173	0.114	0.344*	0.093	0.114	-0.007	-0.301	0.132	-0.328
8								1.000	-0.257	-0.117	-0.001	-0.262	-0.077	0.049	-0.124	0.358*	-0.306	0.279
9									1.000	0.709**	0.461**	0.727**	0.605**	0.470**	0.628**	-0.378*	0.672**	-0.374*
10										1.000	0.434**	0.634**	0.580**	0.427**	0.308	-0.120	0.475**	-0.209
11											1.000	0.449**	0.361*	0.348*	0.312	-0.491**	0.290	-0.509**
12												1.000	0.717**	0.703**	0.667**	-0.662**	0.678**	-0.666**
13													1.000	0.834**	0.554**	-0.378*	0.491**	-0.374*
14														1.000	0.635**	-0.569**	0.316	-0.548**
15															1.000	-0.548**	0.428**	-0.472**
16																1.000	-0.329*	0.917**
17																	1.000	-0.319
18																		1.000

* Significant at p = 0.05 probability (0.329) ** Significant at p = 0.01 probability (0.424) DAP- Days after planting

1 Plant height (90 DAP) (cm)
2 Number of leaves (90 DAP)
3 Leaf length (90 DAP) (cm)
4 Leaf width (90 DAP) (cm)
5 Stem girth (90 DAP) (cm)
6 Leaf area (90 DAP) (cm^2)
7 Days taken for spike initiation
8 Duration of flowering (days)
9 Spike length (cm)
10 Rachis length (cm)
11 Number of florets per spike
12 Floret diameter (cm)
13 Weight of spike (g)
14 Vase life (days)
15 Weight of daughter corm (g/plant)
16 Number of daughter corms per plant
17 Number of cormels per plant
18 Number of spikes per plant

0.336), number of florets per spike (0.549 and 0.525), floret diameter (0.560 and 0.515), weight of spike (0.456 and 0.390) , weight of daughter corm (0.403 and 0.386) and number of cormels per plant (0.752 and 0.661). It had significant and positive correlation with stem girth (0.342) at genotypic level and with days taken for spike initiation (0.329) at phenotypic level. Whereas, significant and negative correlation was observed at genotypic and phenotypic level with number of daughter corms (-0.421 and -0.396) and number of spikes per plant (-0.548 and -0.485). However it showed non-significant correlation with other characters.

Leaf length was recorded to be significantly and positively correlated with plant height, number of leaves, leaf width (0.826 and 0.595), leaf area (0.897 and 0.839), spike length (0.715 and 0.633), number of florets per spike (0.528 and 0.483), floret diameter (0.611 and 0.576), weight of daughter corm (0.651 and 0.598) and number of cormels per plant (0.714 and 0.657) at both genotypic and phenotypic levels and with rachis length (0.377) and weight of spike (0.346) at genotypic level. Whereas, significant and negative correlation was observed at genotypic and phenotypic level with duration of flowering (-0.544 and -0.443), number of daughter corms (-0.563 and -0.523) and number of spikes per plant (-0.462 and -0.438). However it showed non-significant correlation with other characters.

Leaf width exhibited significant and positive correlation at both genotypic and phenotypic levels with plant height, number of leaves, leaf length, stem girth (0.476 and 0.394) leaf area (0.740 and 0.700), spike length (0.736 and 0.627), number of florets per spike (0.378 and 0.343), floret diameter (0.714 and 0.639), weight of spike (0.676 and 0.605), vase life (0.684 and 0.584), weight of daughter corm (0.699 and 0.623) and number of cormels per plant (0.712 and 0.650). Whereas, significant and negative correlation with number of daughter corms (-0.722 and -0.618) and Number of spikes per plant (-0.631 and -0.514) was observed at genotypic and phenotypic level and with duration of flowering (-0.332) at genotypic level. Correlations with other characters were low and non significant.

Stem girth had a significant and positive correlation with number of leaves, vase life (0.438) and weight of daughter corm (0.415) at genotypic levels. Whereas, significant and negative correlation was observed with number of daughter corms (-0.686 and -0.506) number of spikes per plant (-0.747 and -0.447) at both the levels and with rachis length (-0.486) at genotypic level. Correlation between stem girth with other characters was non significant.

Leaf area exhibited significant and positive correlation with at both genotypic and phenotypic levels with plant height, number of leaves, leaf length, leaf width, spike length (0.708 and 0.682), number of florets per spike (0.603 and 0.585), floret diameter (0.694 and 0.682), weight of spike (0.453 and 0.445), vase life (0.385 and 0.372), weight of daughter corm (0.591 and 0.575) and number of cormels per plant (0.656 and 0.651). It had significant and negative correlation with duration of flowering (-0.560 and -0.477), number of daughter

corms (-0.639 and -0.613) and number of spikes per plant (-0.600 and -0.559). Association with other characters were non significant.

Days taken for spike initiation showed significant and positive correlation with floret diameter (0.405 and 0.344) at both genotypic and phenotypic levels and with spike length (0.331) at genotypic level. Whereas, significant and negative correlation was observed at genotypic level with duration of flowering (-0.348), number of daughter corms (-0.347) and number of spikes per plant (-0.391). However it showed non-significant correlation with other characters.

Duration of flowering had a significant and positive correlation with number of daughter corms (0.397 and 0.358) at both genotypic and phenotypic levels and with Number of spikes per plant (0.387) at genotypic level. Whereas, significant and negative correlation with plant height, leaf length, leaf area at both levels and with leaf width, days taken for spike initiation (-0.348), spike length (-0.355), floret diameter (-0.351), number of cormels per plant (-0.353) at genotypic level. However it showed non-significant correlation with other characters.

Spike length exhibited significant and positive correlation with plant height, number of leaves, leaf length, leaf width, leaf area, rachis length (0.742 and 0.709), number of florets per spike (0.478 and 0.461), floret diameter (0.742 and 0.727), weight of spike (0.631 and 0.605), vase life (0.498 and 0.470), weight of daughter corm (0.682 and 0.628), number of cormels per plant (0.712 and 0.672) at both levels and with days taken for spike initiation (0.331) at genotypic level. Whereas, significant and negative correlation with number of daughter corms (-0.392 and -0.378), number of spikes per plant (-0.418 and -0.374) at both levels and with duration of flowering (-0.355) at genotypic level. Association with other characters were non significant.

Rachis length showed significant and positive correlation at both genotypic and phenotypic levels with number of leaves, spike length, number of florets per spike (0.468 and 0.434), floret diameter (0.700 and 0.634), weight of spike (0.642 and 0.580), vase life (0.474 and 0.427) and number of cormels per plant (0.549 and 0.475). It showed significant and positive correlations at genotypic level with plant height, leaf length and weight of daughter corm (0.365) but had significant and negative correlation at genotypic level with stem girth (-0.486). Other remaining characters showed non significant results.

Number of florets per spike had a significant and positive correlation at both genotypic and phenotypic levels with plant height, number of leaves, leaf length, leaf width, leaf area, spike length, rachis length, floret diameter (0.460 and 0.449), weight of spike (0.374 and 0.361) and vase life (0.349 and 0.348). It had significant and negative correlation with number of daughter corms (-0.494 and -0.491) and number of spikes per plant (-0.562 and -

0.509) at genotypic and phenotypic level. Association with other characters were non significant.

Floret diameter exhibited significant and positive correlation at both genotypic and phenotypic levels with plant height, number of leaves, leaf length, leaf width, leaf area, days taken for spike initiation, spike length, rachis length, number of floret per spike, weight of spike (0.739 and 0.717) vase life (0.723 and 0.703), weight of daughter corm (0.697 and 0.667) and number of cormels per plant (0.709 and 0.678). Whereas, significant and negative correlation with number of daughter corms (-0.688 and -0.662), number of spikes per plant (-0.709 and -0.666) and with duration of flowering (-0.351) at genotypic level. Correlations with other characters were low and non significant.

Weight of spike was observed to have significant and positive correlation at both genotypic and phenotypic levels with plant height, number of leaves, leaf width, leaf area, spike length, rachis length, number of florets per spike, floret diameter, vase life (0.851 and 0.834), weight of daughter corm (0.566 and 0.554) and number of cormels per plant (0.496 and 0.491). Whereas, significant and negative correlation with number of daughter corms (-0.384 and -0.378), Number of spikes per plant (-0.392 and -0.374). Association with other characters were non significant.

Significant and positive correlation at both genotypic and phenotypic levels were observed in vase life with leaf width, leaf area, spike length, rachis length, number of florets per spike, floret diameter, weight of spike and weight of daughter corm (0.654 and 0.635). Whereas, significant and negative correlation at both genotypic and phenotypic levels with number of daughter corms (-0.576 and -0.569), number of spikes per plant (-0.569 and -0.548). Association with other characters were non significant.

Weight of daughter corm showed significant and positive correlation at both genotypic and phenotypic levels with plant height, number of leaves, leaf length, leaf width, leaf area, spike length, floret diameter, vase life and number of cormels per plant (0.435 and 0.428). It showed significant and positive correlation at genotypic level with stem girth and rachis length. Whereas, significant and negative correlation at genotypic and phenotypic levels with number of daughter corms (-0.559 and -0.548) and number of spikes per plant (-0.511 and -0.472). Association with other characters were non significant.

Number of daughter corms exhibited significant and positive correlation at both genotypic and phenotypic levels with duration of flowering (0.394 and 0.358) and number of spikes per plant (0.998 and 0.917). Whereas, significant and negative correlation at both genotypic and phenotypic levels with plant height, stem girth, number of leaves, leaf length, leaf width, leaf area, spike length, number of florets per spike, floret diameter, vase life, weight of spike, number of cormels per plant (-0.342 and -0.329). Correlations with other characters were low and non significant.

Number of cormels per plant exhibited significant and positive correlation at both genotypic and phenotypic levels with plant height, number of leaves, leaf length, leaf width, leaf area, spike length, rachis length, floret diameter, vase life, weight of spike and weight of daughter corm. However, its relationship with number of daughter corms (-0.342 and -0.329) at both genotypic and phenotypic levels and with duration of flowering (-0.353), number of spikes per plant (-0.343) were significant and negatively correlated. Association with other characters were non significant.

Number of spikes per plant exhibited significant and positive correlation at both genotypic and phenotypic levels with number of daughter corm per plant (0.998 and 0.917). It showed significant and positive correlation with duration of flowering (0.387) at genotypic level. Whereas, significant and negative correlation with plant height (-0.500 and -0.464), number of leaves (-0.548 and -0.485), stem girth (-0.747 and -0.447), leaf length (-0.462 and -0.438), leaf width (-0.631 and -0.514), leaf area (-0.600 and 0.559), spike length (-0.418 and -0.374), number of florets per spike (-0.562 and -0.509), floret diameter (-0.709 and -0.666), weight of spike (-0.392 and -0.374), vase life (-0.569 and -0.548) and weight of daughter corm (-0.511 and -0.472). It showed significant and negative correlation with days taken for spike initiation (-0.391) and number of cormels per plant (-0.343) at genotypic level. Association with other characters were non significant.

4.1.4 Path Coefficient Analysis

The correlation coefficients only indicate the relationship of independent variable with the dependent variable without specifying cause and effect relationship. Using Path coefficient analysis, it is possible to resolve the correlations, which will provide and indirect contribution of different quantitative traits. The analysis was done for number of spikess per plant, which is dependent variable. Both genotypic and phenotypic paths for the dependent variable were computed.

4.1.4.1 Genotypic and phenotypic path coefficient analysis for Number of spikes per plant.

The path analysis of spike yield per plant was done with eleven independent characters involving growth, flowering, quality and corm parameters. The genotypic and phenotypic paths for various characters are given in Tables 9 and 10.

4.1.4.1.1 Number of spikes per plant as dependent variable

Number of spikes per plant was directly and positively influenced by stem girth (0.565), leaf area(1.837), days taken for spike initiation (0.410), rachis length (2.211), number of daughter corms (0.632), weight of daughter corm (0.754) and negative direct effect through plant height (-0.550), number of leaves (-0.389), spike length (-0.966), number

of florets per spike (-0.641), floret diameter (-2.351) at genotypic level. However, at phenotypic level it was directly and positively influenced by plant height (0.122), stem girth (0.032), days taken for spike initiation (0.008), spike length (0.144), number of florets per spike (0.045), number of daughter corms (0.936), weight of daughter corm (0.039) and negative direct effect through number of leaves (-0193), leaf area (-0.087), rachis length (-0.156) and floret diameter (-0.022).

4.1.4.2 Genotypic path coefficient analysis

Genotypic path coefficient for various characters studied are given in Table 9.

Plant height had high direct negative effect (-0.550) on number of spikes per plant, while indirect positive effects through stem girth (0.165), leaf area (1.628), days taken for spike initiation (0.069), rachis length (0.788) and weight of daughter corm (0.510). It had negative indirect effect through days number of leaves (-0.190), spike length (-0720), number of florets per spike (-0.319), floret diameter (-1.504) and number of daughter corms (-0.377).

High positive direct effect (0.565) was found for stem girth on number of spikes per plant, while indirect positive effects through leaf area (0.547), days taken for spike initiation (0.106), spike length (0.111), number of florets per spike (0.012) and weight of daughter corm (0.313). It had negative indirect effect through plant height (-0.160), number of leaves (-0.133), rachis length (-1.074), floret diameter (-0.600) and number of daughter corms (-0.433).

Negative direct effect (-0.389) was observed for number of leaves per plant on number of spikes per plant, while indirect positive effects through stem girth (0.193), leaf area (0.987), days taken for spike initiation (0.129), rachis length (1.098), weight of daughter corm (0.304) and remaining characters had negative indirect effects.

Leaf area had high direct positive effect (1.837) on number of spikes per plant. Stem girth (0.168), days taken for spike initiation (0.057), rachis length (0.695), weight of daughter corm (0.446) had positive indirect effects and remaining characters had negative indirect effects.

Days taken for spike initiation had direct positive effect (0.410) on number of spikes per plant. Though, indirect positive effects were observed through stem girth (0.146), leaf area (0.255), rachis length (0.612) and remaining characters had negative indirect effects.

Spike length exhibited high direct negative effect (-0.966) on number of spikes per plant, while indirect positive effects through leaf area (1.300), days taken for spike initiation (0.136), rachis length (1.640), weight of daughter corm (0.515) and remaining characters had negative indirect effects.

Table 9. Genotypic path coefficient analysis of eleven different quantitative characters on Number of spikes per plant of gladiolus varieties through direct and indirect effects.

	1	2	3	4	5	6	7	8	9	10	11	rG
1	**-0.550**	0.165	-0.190	1.628	0.069	-0.720	0.788	-0.319	-1.504	-0.377	0.510	-0.500*
2	-0.160	**0.565**	-0.133	0.547	0.106	0.111	-1.074	0.012	-0.600	-0.433	0.313	-0.747**
3	-0.269	0.193	**-0.389**	0.987	0.129	-0.665	1.098	-0.352	-1.317	-0.266	0.304	-0.548**
4	-0.488	0.168	-0.209	**1.837**	0.057	-0.684	0.695	-0.386	-1.633	-0.404	0.446	-0.600**
5	-0.093	0.146	-0.123	0.255	**0.410**	-0.320	0.612	-0.084	-0.952	-0.219	-0.022	-0.391
6	-0.410	-0.065	-0.268	1.300	0.136	**-0.966**	1.640	-0.306	-1.745	-0.247	0.515	-0.418*
7	-0.196	-0.274	-0.193	0.577	0.113	-0.717	**2.211**	-0.300	-1.645	-0.089	0.275	-0.237
8	-0.273	-0.011	-0.214	1.107	0.053	-0.461	1.034	**-0.641**	-1.081	-0.312	0.237	-0.562**
9	-0.352	0.144	-0.218	1.276	0.166	-0.717	1.547	-0.295	**-2.351**	-0.435	0.526	-0.709**
10	0.328	-0.388	0.164	-1.174	-0.142	0.379	-0.313	0.317	1.618	**0.632**	-0.422	0.998**
11	-0.372	0.234	-0.157	1.087	-0.012	-0.659	0.807	-0.201	-1.639	-0.353	**0.754**	-0.511*

Residual Effect= 0.0458

Bold : Direct effect Above and below diagonal : Indirect effect

* Significant at 0.05 probability level (0.404) ** Significant at 0.01 probability level (0.515)

1 Plant height (90 DAP) (cm)
2 Stem girth (90 DAP) (cm)
3 Number of leaves (90 DAP)
4 Leaf area (90 DAP) (cm)
5 Days taken for spike initiation
6 Spike length (cm)
7 Rachis length (cm)
8 Number of florets per spike
9 Floret diameter (cm)
10 Number of daughter corms per plant
11 Weight of daughter corm(g/plant)
rG Correlation with Number of spikes per plant

Rachis length had high direct positive effect (2.211) on number of spikes per plant. Though, indirect positive effects were observed through leaf area (0.577), days taken for spike initiation (0.113), weight of daughter corm (0.275) and remaining characters had negative indirect effects.

Number of florets per spike exhibited high direct negative effect (-0.641) on number of spikes per plant, while indirect positive effects through leaf area (1.107), days taken for spike initiation (0.053), rachis length (1.034), weight of daughter corm (0.237) and remaining characters had negative indirect effects.

High direct negative effect (-2.351) were observed for floret diameter on number of spikes per plant. Whereas, indirect positive effects were observed through stem girth (0.144), leaf area (1.276), days taken for spike initiation (0.166), rachis length (1.547) and weight of daughter corm (0.526).

Number of daughter corms had high direct positive effect (0.632) on number of spikes per plant. Plant height (0.328), number of leaves (0.164), spike length (0.379), number of florets per spike (0.317), floret diameter (1.618) exhibited indirect positive effects and remaining characters had negative indirect effects.

Weight of daughter corm showed high direct positive effect (0.754) on number of spikes per plant. Indirect positive effects were observed through stem girth (0.234), leaf area (1.087), rachis length (0.807) and remaining characters had negative indirect effects.

4.1.4.3 Phenotypic path coefficient analysis

Phenotypic path coefficient for various characters studied are given in Table 10.

Plant height had direct positive effect (0.122) on number of spikes per plant. Though, indirect positive effects were observed through stem girth (0.005), days taken for spike initiation (0.001), spike length (0.096), number of florets per spike (0.020), weight of daughter corm (0.024) and remaining characters had negative indirect effects.

Stem girth exhibited direct positive effect (0.032) on number of spikes per plant. Whereas, indirect positive effects were observed through plant height (0.018), days taken for spike initiation (0.001), spike length (0.007), rachis length (0.026), number of florets per spike (0.004), weight of daughter corm (0.011) and remaining characters had negative indirect effects.

Number of leaves per plant had direct negative effect (-0.193) on number of spikes per plant, while indirect positive effects through plant height (0.055), stem girth (0.008), days taken for spike initiation (0.003), spike length (0.080), number of florets per spike (0.023), weight of daughter corm (0.015) and remaining characters had negative indirect effects.

Table 10. Phenotypic path coefficient analysis of eleven different quantitative characters on Number of spikes per plant of gladiolus varieties through direct and indirect effects.

	1	2	3	4	5	6	7	8	9	10	11	rP
1	**0.122**	0.005	-0.088	-0.072	0.001	0.096	-0.045	0.020	-0.013	-0.513	0.024	-0.464*
2	0.018	**0.032**	-0.047	-0.019	0.001	0.007	0.026	0.004	-0.005	-0.474	0.011	-0.447*
3	0.055	0.008	**-0.193**	-0.041	0.003	0.080	-0.053	0.023	-0.011	-0.371	0.015	-0.485*
4	0.101	0.007	-0.091	**-0.087**	0.001	0.098	-0.047	0.026	-0.015	-0.574	0.022	-0.559**
5	0.013	0.003	-0.064	-0.012	**0.008**	0.035	-0.027	0.005	-0.008	-0.282	0.001	-0.328
6	0.081	0.001	-0.107	-0.059	0.002	**0.144**	-0.111	0.021	-0.016	-0.354	0.024	-0.374
7	0.035	-0.005	-0.065	-0.026	0.001	0.102	**-0.156**	0.019	-0.014	-0.112	0.012	-0.209
8	0.054	0.002	-0.101	-0.051	0.001	0.066	-0.068	**0.045**	-0.010	-0.459	0.012	-0.509**
9	0.073	0.008	-0.100	-0.059	0.003	0.105	-0.099	0.020	**-0.022**	-0.62	0.026	-0.666**
10	-0.067	-0.016	0.076	0.053	-0.002	-0.054	0.019	-0.022	0.015	**0.936**	-0.021	0.917**
11	0.077	0.009	-0.075	-0.050	0.001	0.090	-0.048	0.014	-0.015	-0.513	**0.039**	-0.472*

Residual Effect= 0.1200

Bold : Direct effect Above and below diagonal : Indirect effect

* Significant at 0.05 probability level (0.404) ** Significant at 0.01 probability level (0.515)

1 Plant height (90 DAP) (cm)
2 Stem girth (90 DAP) (cm)
3 Number of leaves (90 DAP)
4 Leaf area (90 DAP) (cm)
5 Days taken for spike initiation
6 Spike length (cm)
7 Rachis length (cm)
8 Number of florets per spike
9 Floret diameter (cm)
10 Number of daughter corms per plant
11 Weight of daughter corm(g/plant)
rP Correlation with Number of spikes per plant

Leaf area exhibited direct negative effect (-0.087) on number of spikes per plant. Plant height (0.101), stem girth (0.007), days taken for spike initiation (0.001), spike length (0.098), number of florets per spike (0.026), weight of daughter corm (0.022) had indirect positive effects.

Days taken for spike initiation had positive direct effect (0.008) on number of spikes per plant. Indirect positive effects were observed through plant height (0.013), stem girth (0.003), spike length (0.035), number of florets per spike (0.005), weight of daughter corm (0.001) and remaining characters had negative indirect effects.

Positive direct effect (0.144) was observed for spike length on number of spikes per plant. Plant height (0.081), stem girth (0.001), days taken for spike initiation(0.002), number of florets per spike (0.021), weight of daughter corm (0.024) had positive indirect effects and remaining characters had negative indirect effects.

Rachis length had direct negative effect (-0.156) on number of spikes per plant. Though, indirect positive effects were observed through plant height (0.035), days taken for spike initiation (0.001), spike length (0.102), number of florets per spike (0.019), weight of daughter corm (0.012) and remaining characters had negative indirect effects.

Number of florets per spike exhibited direct positive effect (0.045) on number of spikes per plant, while indirect positive effects through plant height (0.054), stem girth (0.002), days taken for spike initiation (0.001), spike length (0.066), weight of daughter corm (0.012) and remaining characters had negative indirect effects.

Floret diameter had direct negative effect (-0.022) on number of spikes per plant. Indirect positive effects were observed through plant height (0.073), stem girth (0.008), days taken for spike initiation (0.003), spike length (0.105), number of florets per spike (0.020), weight of daughter corm (0.026) and remaining characters had negative indirect effects.

Number of daughter corms had high direct positive effect (0.936) on number of spikes per plant, while indirect positive effects through number of leaves (0.076), leaf area (0.053), rachis length (0.019), floret diameter (0.015) and remaining characters had negative indirect effects.

Weight of daughter corm had direct positive effect (0.039) on number of spikes per plant. Whereas, indirect positive effects were observed through plant height (0.077), stem girth (0.009), days taken for spike initiation (0.001), spike length (0.090), number of florets per spike (0.014) and remaining characters had negative indirect effects.

4.1.5 Disease incidence

Fusarium oxysporum incidence in terms of disease score was recorded by using 0 – 3 grade scale and data are presented in Table 11.

Among the varieties the minimum score was obtained for Eighth Wonder (0.0) and Charms Flow(0.0) followed by American Beauty, Candyman, Jester Gold, Red Majesty, Summer Sunshine, Copper King, White Prosperity, Vedanapoli (1.0 each). The highest score was obtained by Her Majesty (3.0). Among the all varieties two were found resistant (R) to *Fusarium* disease (Eighth Wonder and Charms Flow).

Table 11. Screening of gladiolus varieties against *Fusarium oxysporum* disease under natural disease pressure conditions

Sl. No	Varieties	Disease score (0 – 3 grade scale)	Disease reaction
1	American Beauty	1.0	Moderately resistance
2	Candyman	1.0	Moderately resistance
3	Charms Flow	0.0	Resistance
4	Delhi Local	2.0	Moderately susceptible
5	Green Bay	2.0	Moderately susceptible
6	Her majesty	3.0	Susceptible
7	Jester Gold	1.0	Moderately resistance
8	Punjab Morning	2.0	Moderately susceptible
9	Eighth Wonder	0.0	Resistance
10	Red Majesty	1.0	Moderately resistance
11	Summer Sunshine	1.0	Moderately resistance
12	Copper King	1.0	Moderately resistance
13	White Prosperity	1.0	Moderately resistance
14	Vedanapoli	1.0	Moderately resistance
15	Darshan	2.0	Moderately susceptible

4.1.6 Similarity matrix and dendrogram generated by morphological characters

Relationships between the 18 morphological parameters of 15 gladiolus varieties revealed by similarity matrix and cluster analyses are presented in Table 12 and Figure 1 respectively. Morphological values (qualitative and quantitative) were transformed to 0-1 scale using the formula as mentioned in material and methods. Transformed data was computed in NTSYS -PC ver.2.1 software for similarity matrix using Jaccard's Co-efficient (J) and the similarity matrix was subjected to unweighed pairgroup method using arithmetic average (UPGMA) analysis to construct a dendrogram.

The similarity matrix coefficient ranged from 00 to 90 percent (Table 12), suggesting a low to higher genetic variation within gladiolus varieties. The highest genetic similarity between the Delhi local and Jester Gold, Punjab Morning and Vedanapoli of each 90 per cent was observed, while least (00 %) was noticed between Charms Flow and Delhi local, Charms Flow and Copper King, Charms Flow and Vedanapoli, Her Majesty and Eighth Wonder, Her Majesty and Vedanapoli, Her Majesty and Copper King, Eighth Wonder and Darshan, Copper King and Darshan.

A dendrogram based on morphological data showed that 15 gladiolus varieties (Fig. 2) formed two main clusters; major cluster I and major cluster II. The major cluster I in dendrogram is further divided into two sub clusters. Sub cluster I again divided into two groups; group I and group II. Group I consists two sub groups; sub group I consists of varieties American Beauty, Green Bay, Copper King and Vedanapoli having the range of colours from reddish to greenish, and sub group II consists of varieties Candyman, Summer Sunshine, Eighth Wonder, Red Majesty White Prosperity, Jester Gold and Punjab Morning having good commercial characters like more plant height, spike length, number of florets per spike, floret diameter, vase life. Sub cluster II includes varieties Delhi Local and Darshan having short stature and more spike per plant, with orange to pinkish colour. Major cluster II consists of two varieties Charms Flow and Her Majesty. All the varieties with superior commercial characters clustered together in dendrogram, but some varieties clustered together in the dendrogram were haphazardly.

Table 12. Similarity co-efficient of gladiolus varieties based on morphological data

	American Beauty	Candyman	Charms Flow	Delhi Local	Green Bay	Her Majesty	Jester Gold	Punjab Morning	Eighth Wonder	Red Majesty	Summer Sunshine	Copper King	White Prosperity	Vedanapoli	Darshan
American Beauty	1.00														
Candyman	0.14	1.00													
Charms Flow	0.12	0.30	1.00												
Delhi Local	0.11	0.76	0.00	1.00											
Green Bay	0.57	0.71	0.14	0.12	1.00										
Her Majesty	0.16	0.10	0.33	0.00	0.20	1.00									
Jester Gold	0.55	0.38	0.10	0.90	0.30	0.12	1.00								
Punjab Morning	0.20	0.45	0.28	0.25	0.22	0.16	0.27	1.00							
Eighth Wonder	0.28	0.57	0.71	0.66	0.21	0.00	0.53	0.28	1.00						
Red Majesty	0.20	0.69	0.15	0.66	0.13	0.83	0.42	0.38	0.71	1.00					
Summer Sunshine	0.33	0.66	0.30	0.16	0.15	0.10	0.38	0.45	0.46	0.57	1.00				
Copper King	0.25	0.76	0.00	0.33	0.28	0.00	0.33	0.11	0.23	0.66	0.76	1.00			
White Prosperity	0.36	0.46	0.20	0.18	0.16	0.11	0.41	0.36	0.31	0.50	0.58	0.83	1.00		
Vedanapoli	0.33	0.23	0.00	0.25	0.22	0.00	0.40	0.90	0.38	0.20	0.23	0.42	0.25	1.00	
Darshan	0.28	0.83	0.20	0.40	0.33	0.33	0.10	0.28	0.00	0.71	0.18	0.00	0.20	0.12	1.00

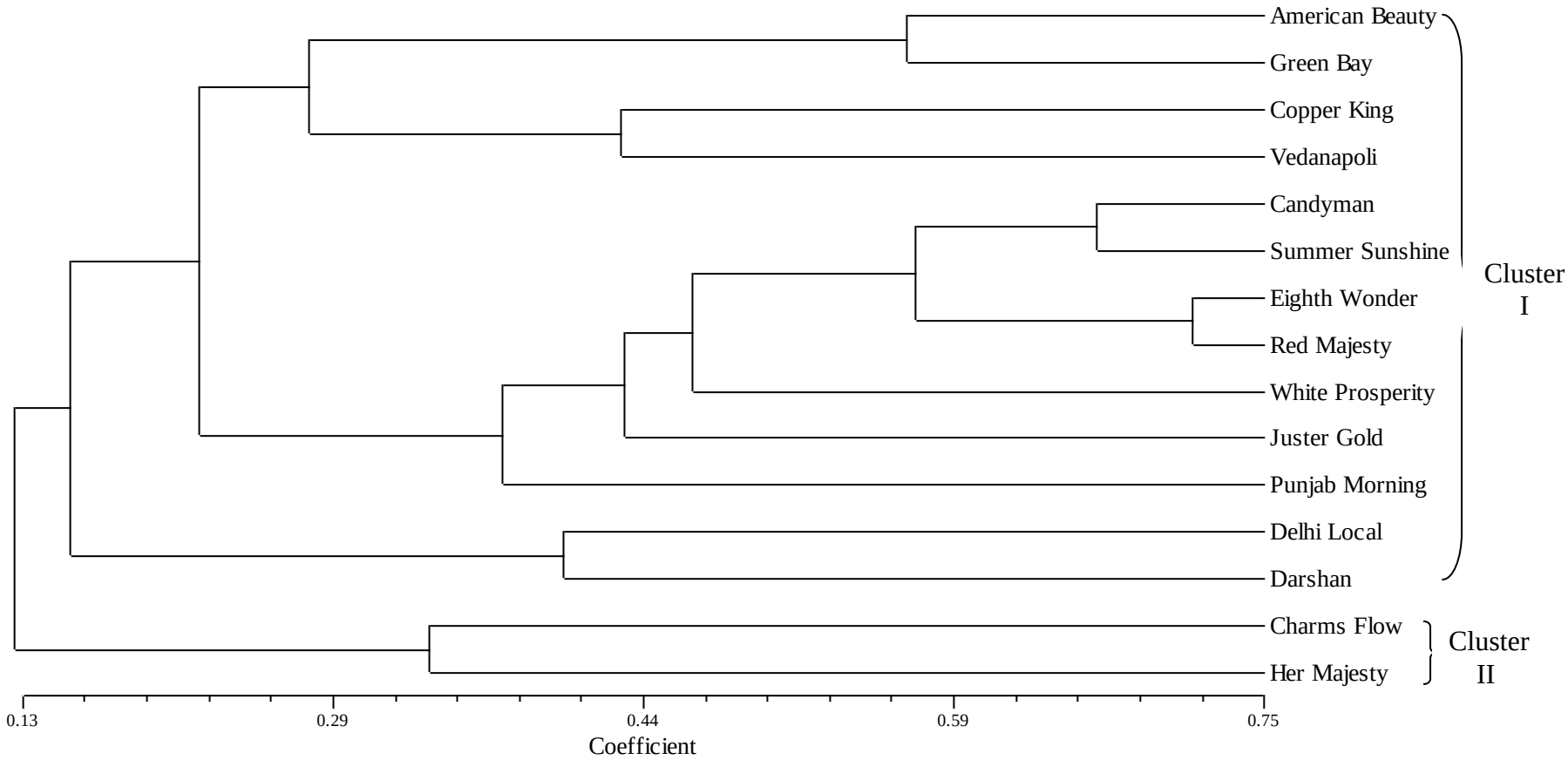


Fig. 2. Dendrogram showing genetic relationship among 15 gladiolus varieties based on morphological data using unweighted pair group method with arithmetic average (UPGMA) analysis.

4.2 Assessment of genetic diversity based on molecular data

4.2.1 DNA isolation protocol

Genomic DNA was extracted from 15 gladiolus varieties from fresh young leaves by modified CTAB method as described in Material and Methods chapter. The extraction method yielded good amount of DNA. The quality and quantity of isolated DNA samples were tested and found good by agarose gel electrophoresis (Plate 3).

4.2.2 DNA Quantification

The isolated DNA was analyzed quantitatively by using UV Spectrophotometer. DNA had an A_{260}/A_{280} ratio of 1.80 to 2.00 showing that it was relatively pure and free from impurities. The quality of DNA isolated was very good. The DNA yield for all the varieties are presented in the Table 2. Varieties Jester Gold, American Beauty and Green Bay yielded highest quantity of DNA (1120, 1070 and 1000 ng/ µl respectively) and Darshan had the lowest amount of DNA (280 ng/ µl).

4.2.3 SRAP analysis of gladiolus varieties

4.2.3.1 PCR protocol

Reaction parameters and amplification conditions

It is important to optimize the concentration of PCR mixture, in order to produce informative and reproducible SRAP fingerprints. For fingerprinting and diversity analysis, PCR reaction mixture and amplification conditions were optimized based on the protocol outlined by Mishra *et al.* (2011) with minor modifications as mentioned in Material and Methods chapter.

Primer selection for SRAP analysis

SRAP analysis was carried out using twenty five primer combinations among which, eleven primer combination (Me 1 + Em 3, Me 2 + Em 3, Me 2 + Em 5, Me 3 + Em 1, Me 3 + Em 5, Me 3 + Em 12, Me 4 + Em 2, Me 4 + Em 3, Me 4 + Em 4, Me 5 + Em 1 and Me 5 + Em 2) yielded maximum number of bands that were consistent and clear were selected for fingerprinting and diversity analysis (Table 13).

4.2.3.2 SRAP profile analysis

The SRAP fingerprint for 15 selected varieties of gladiolus using eleven primer combinations revealed a total of 80 scorable bands that were well defined, consistent, unambiguous, readable and reproducible polymorphic bands which were used to estimate genetic diversity. The number of bands scored for each primer combination varied from 4-10

Table 13. Polymorphism rates for the 15 gladiolus varieties related to the eleven SRAP primer combinations

SL. No.	Primer combination	No. of polymorphic bands	No. of bands produced	Unique bands	Percentage polymorphism
1.	Me 1 + Em 3	9	10	1	100.00
2.	Me 2 + Em 3	6	7	0	85.71
3.	Me 2 + Em 5	5	7	1	85.71
4.	Me 3 + Em 1	6	7	1	100.00
5.	Me 3 + Em 5	1	4	2	75.00
6.	Me 3 + Em 12	5	7	0	71.43
7.	Me 4 + Em 2	4	9	2	66.67
8.	Me 4 + Em 3	8	8	0	100.00
9.	Me 4 + Em 4	8	8	0	100.00
10.	Me 5 + Em 1	1	4	0	25.00
11.	Me 5 + Em 2	7	9	1	88.89
Total		60	80	8	
Mean		5.45	7.27	-	81.67

bands (Table 13) with an average of 7.27 bands per primer combination and the size ranged from 200 to 1500 bp. The bands which are more than 200 bp were selected for scoring.

A total of 80 bands were observed, among which 68 were polymorphic with an average of 6.18 polymorphic bands per pair of primers. Among eleven primer combinations screened, Me1+Em3 scored maximum number of polymorphic bands (10) followed by Me 4 + Em 2 and Me 5+ Em 1 (9 bands each), while Me 3 + Em 5 and Me 5 + Em 2 produced minimum number of bands (04 each). The combinations of primer Me 1 + Em 3, Me 3 + Em 1, Me 4 + Em 3 and Me 4 + Em 4 produced highest polymorphisim of 100 per cent followed by Me 5 + Em 1 (88.89%), where as primer combination Me 5 + Em 2 produced least polymorhisim of 25 per cent .

The bands that were unique could be used as the special marker to distinguish the varieties. For instance, Delhi Local and Darshan variety each had two special amplified unique bands generated by Me3/Em5 and Me4/Em2 primer combination respectively. Vedanapoli, Summer Sunshine, Punjab Morning, Red Majesty each had one special amplified unique bands generated by Me1/Em3, Me2/Em5, Me3/Em1 and Me5/Em2 primer combination respectively.

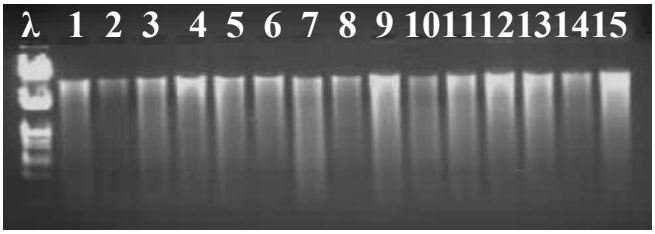
The SRAP profiles generated for fifteen gladiolus varieties obtained by using the eleven primer combination varied from one another. For the purpose of illustration, the SRAP fingerprints/gel profiles generated are illustrated through Plate 4, 5 and 6.

4.2.4 Genetic diversity analysis of 15 gladiolus varieties

4.2.4.1 Cluster analysis and genetic similarity matrix

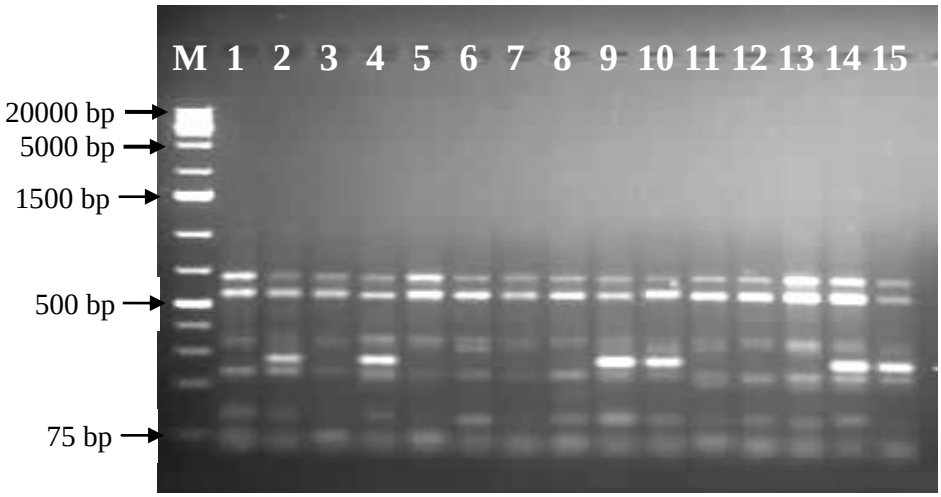
SRAP bands were scored from the gel profile by assigning binary values ‘1’ for the presence of band and 0’ for the absence of band. Such binary data generated from all the gel profiles were used for statistical analysis. The similarity matrix was computed using Jaccard’s Co-efficient (J). The pooled SRAP binary data was utilized for cluster analysis using the NTSYS-PC ver.2.1 software for the 15 gladiolus varieties.

The similarity matrix coefficient ranged from 31 to 73 percent (Table 14), suggesting a low to higher genetic variation within gladiolus varieties. The highest genetic similarity of 73 per cent was observed between the Charms Flow and Green Bay, followed by Punjab Morning and Eighth Wonder of per cent 68, while least (31 %) was between Jester Gold and Vedanapoli.



λ – EcoRI + HindIII double

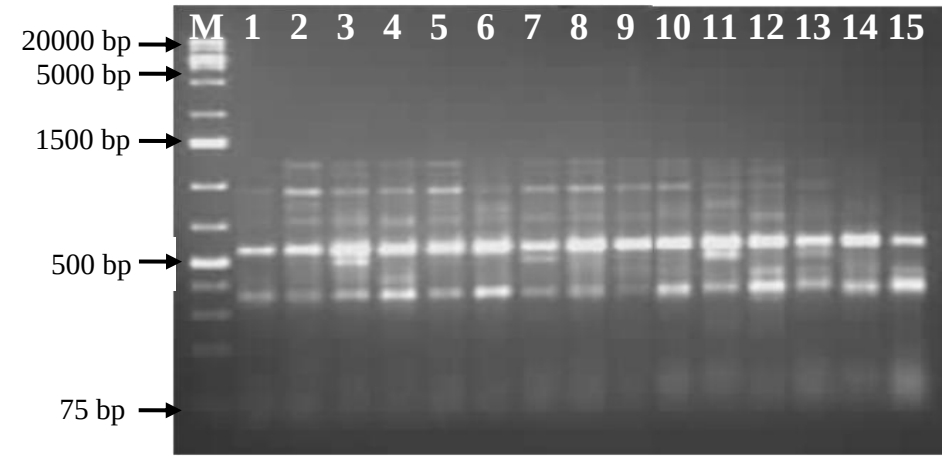
Plate 3. Quality DNA of 15 gladiolus varieties digest (Lane 1-15 represents the varieties in the same order as listed in Table 1)



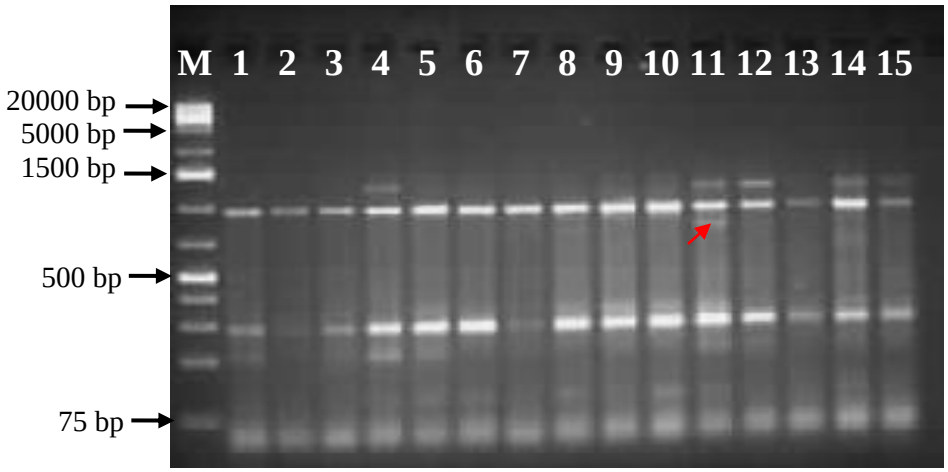
ME 3 + EM 12

ME 1 + EM 3

Plate 4. Gel profile of 15 gladiolus varieties using different SRAP primer combination. (Lane 1-15 represents the varieties in the same order as listed in Table 1). M – Molecular marker (1 kb plus ladder)



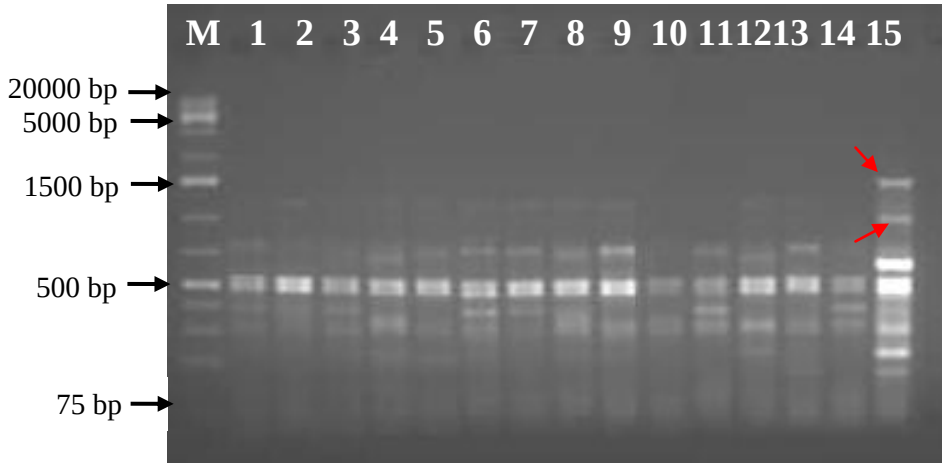
ME 2 + EM 3



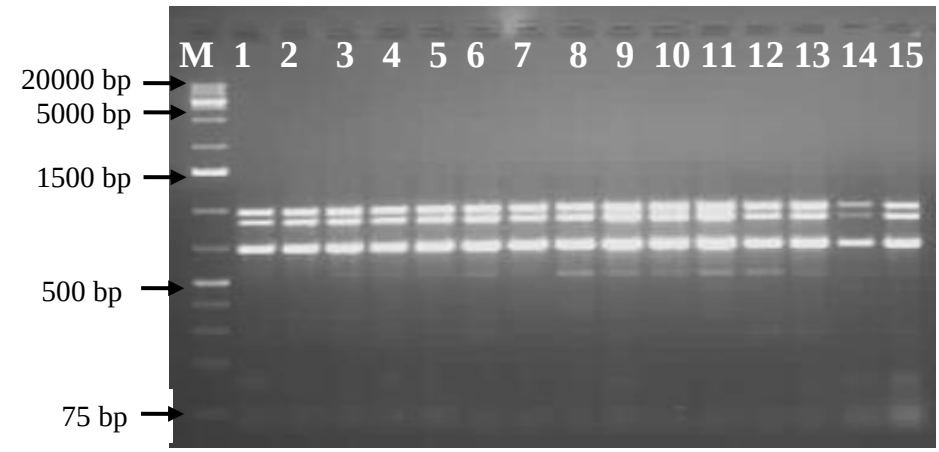
ME 2 + EM 5

ME 3 + EM 5

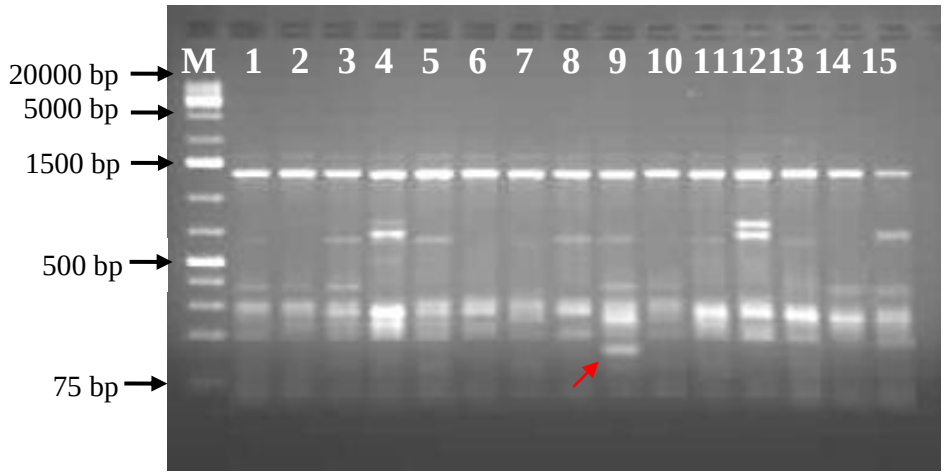
Plate 5. Gel profile of 15 gladiolus varieties using different SRAP primer combination. (Lane 1-15 represents the varieties in the same order as listed in Table 1). M – Molecular marker (1 kb plus ladder)



ME 4 + EM 2



ME 5 + EM 1



ME 5 + EM 2

Plate 6. Gel profile of 15 gladiolus varieties using different SRAP primer combination.(Lane 1-15 represents the varieties in the same order as listed in Table 1). M – Molecular marker (1 kb plus ladder)

4.2.4.2 Dendrogram

The dendrogram was constructed by UPGMA (Unweighted Pair Group Method with Arithmetical averages) method of clustering using Jaccard's Co-efficient (J). Cluster analysis based on a total of 80 bands with 68 polymorphic bands using eleven primer combinations, revealed that the 15 gladiolus varieties clustered in the dendrogram. All varieties were grouped into two major clusters.

Within major group, there were further sub-clusters (Fig. 3). The first major constituted fourteen varieties from American Beauty to Darshan. The second major constituted a single variety Jester Gold.

The major cluster I in dendrogram is further divided into five sub clusters. Sub cluster I again divided into two groups; group I and group II. Group I consists of two sub groups; sub group I consists of American Beauty, Charms Flow and Green Bay verities and sub group II consists of Her Majesty, Punjab Morning and Eighth Wonder varieties. Sub cluster II having two groups; group I includes two varieties (Red Majesty and White Prosperity) and group II includes only one variety Summer Sunshine. Sub cluster III consists of two varieties Delhi Local and Copper King. Sub cluster IV had only one variety Candyman. Sub cluster V includes Vedanapoli and Darshan. Major cluster II consists a single variety Jester Gold. All the varieties share more than 30 per cent similarity among themselves.

4.2.4.3 Similarity matrix among gladiolus varieties

In the dendrogam the major cluster I in dendrogram is further divided into five sub clusters. Sub cluster I again divided into two groups. Group I consists of two sub groups; sub group I had three varieties and similarity index among the members of this group ranged from 0.61 (between American Beauty and Green Bay) and 0.73 (between Charms Flow and Green Bay), whereas, sub group II also consists of three varieties and similarity index among the members of this group ranged from 0.62 (between Her Majesty and Eighth Wonder) and 0.68 (between Punjab Morning and Eighth Wonder). Sub cluster II having two groups; group I consists of Red Majesty and White Prosperity were similar at a similarity coefficient of 0.63 and group II includes only one variety Summer Sunshine had a similarity matrix coefficient of 0.55. Sub cluster III consists of two varieties at a similarity coefficient of 0.62 (Delhi Local and Copper King). Sub cluster IV had only one variety Candyman had a similarity matrix coefficient of 0.47. Sub cluster V includes Vedanapoli and Darshan were similar at a similarity coefficient of 0.52. Major cluster II consists a single variety Jester Gold and had least similarity index of 0.31.

4.2.4.4 Association of some phenotypic characters with SRAP cluster

A dendrogram based on clustering results of fifteen varieties with their respect to their origin revealed that, the geographical distribution of most varieties in each of the sub clusters

was not so well defined. Majority of the verities in major cluster I were originated from USA, Holland, Australia and India. American Beauty, Charms Flow and Green Bay (USA), Red

Table 14. Similarity co-efficient of gladiolus varieties using SRAP marker.

	American Beauty	Candyman	Charms Flow	Delhi Local	Green Bay	Her Majesty	Jester Gold	Punjab Morning	Eighth Wonder	Red Majesty	Summer Sunshine	Copper King	White Prosperity	Vedanapoli	Darshan
American Beauty	1.00														
Candyman	0.56	1.00													
Charms Flow	0.67	0.54	1.00												
Delhi Local	0.55	0.49	0.47	1.00											
Green Bay	0.62	0.52	0.73	0.57	1.00										
Her Majesty	0.64	0.54	0.55	0.56	0.63	1.00									
Jester Gold	0.43	0.50	0.48	0.41	0.47	0.46	1.00								
Punjab Morning	0.50	0.44	0.54	0.53	0.59	0.64	0.48	1.00							
Eighth Wonder	0.58	0.56	0.56	0.57	0.64	0.63	0.43	0.69	1.00						
Red Majesty	0.57	0.54	0.54	0.53	0.53	0.52	0.44	0.51	0.66	1.00					
Summer Sunshine	0.52	0.49	0.55	0.49	0.60	0.59	0.39	0.55	0.57	0.55	1.00				
Copper King	0.51	0.48	0.55	0.62	0.60	0.56	0.49	0.61	0.57	0.49	0.53	1.00			
White Prosperity	0.62	0.52	0.60	0.57	0.55	0.57	0.50	0.53	0.58	0.63	0.60	0.60	1.00		
Vedanapoli	0.50	0.39	0.48	0.45	0.50	0.60	0.31	0.46	0.53	0.60	0.57	0.44	0.50	1.00	
Darshan	0.46	0.40	0.42	0.54	0.44	0.48	0.38	0.47	0.55	0.47	0.52	0.51	0.52	0.53	1.00

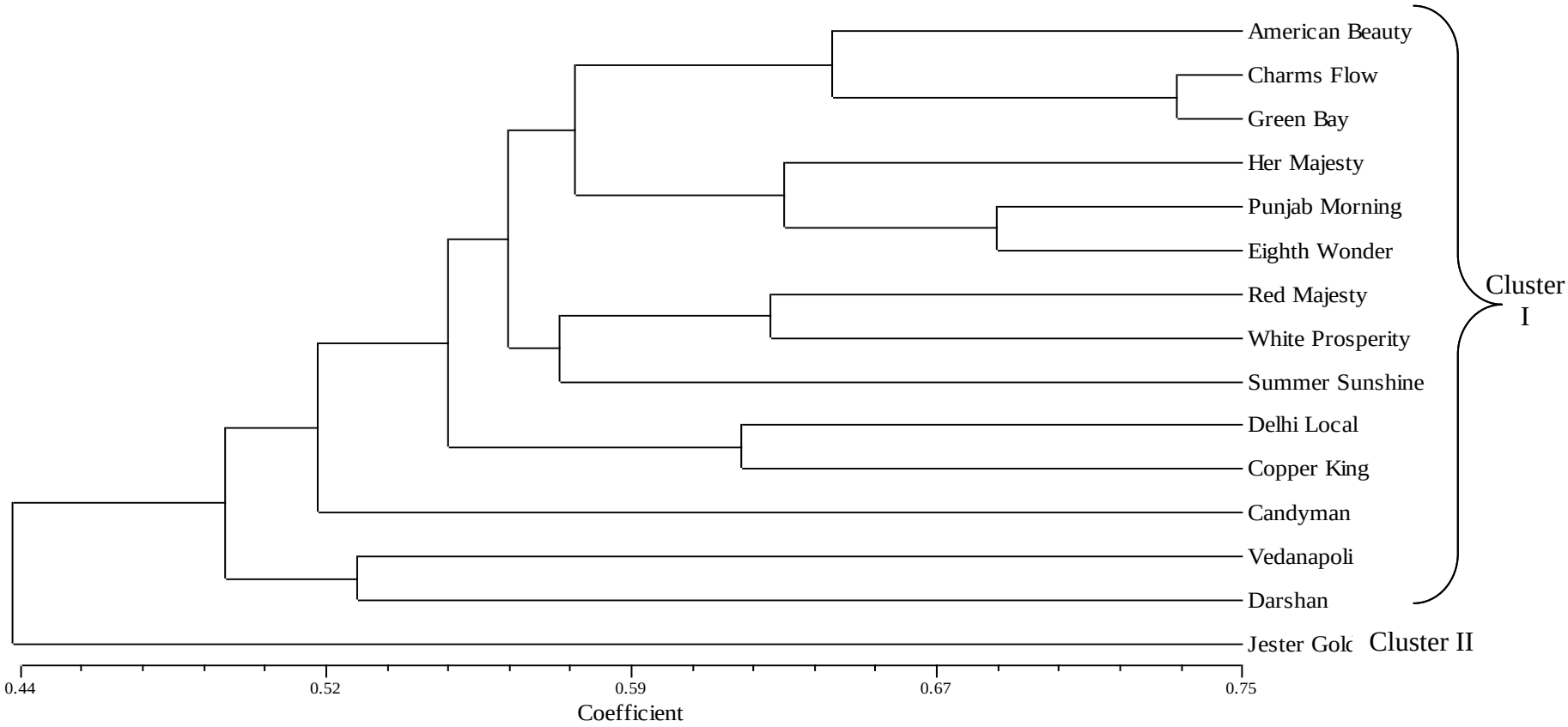


Fig. 3. Dendrogram showing genetic relationship among 15 gladiolus varieties based on SRAP markers according to unweighted pair group method with arithmetic average (UPGMA) analysis.

Majesty and White Prosperity (USA), Vedanapoli and Darshan (India) grouped together. Major cluster II consists a single variety Jester Gold from Holland. Majority of sub groups were grouped according to origin and the rest of them grouped incongruently. However, in the dendrogram varieties did not grouped based upon the parentages.

Verities are distributed based on floret shape, flower color and commercial characters like spike length, floret diameter, number of florets per spike. All varieties were grouped into two major cluster. The major cluster I in dendrogram is further divided into five sub clusters. Sub cluster I again divided into two groups; group I and group II. Group I consists two sub groups; sub group I consists of varieties having round shape florets (American Beauty, Charms Flow and Green Bay) and sub group II consists of varieties having star shape florets (Her Majesty, Punjab Morning and Eighth Wonder). Sub cluster II includes varieties Red Majesty, White Prosperity and Summer Sunshine were having good spike length, more number of florets per spike and commercially cultivated varieties. Sub cluster III consists of two varieties Delhi Local and Copper King were butterfly type of florets, short stature, less floret diameter and yield more no of spike per plant. Sub cluster IV had only one variety Candyman had good quality parameters such as more spike length, floret diameter and vase life. Sub cluster V includes Vedanapoli and Darshan were pink in floret color. Major cluster II consists a single variety Jester Gold had good spike length and yellow color florets with reddish blotch.

6. DISCUSSION

The present investigation on gladiolus varieties was conducted mainly to evaluate the extent of genetic variability for crop improvement programme on fifteen different varieties based on morphological and molecular markers. Success of crop improvement programmes depends largely on the extent of variability present in the germplasm stock for the traits for which the improvement is aimed at. The knowledge of genetic variability and association of various characters are essential in planning the breeding programmes.

The studies on the genetic polymorphisms and phenotypic relationships can provide a scientific basis for the utilization of these phenotypes for the efficient crop improvement. Hence, characterization of varieties at phenotypic levels based on morphological characters supplemented with molecular characterization at genetic level is first step towards efficient utilization, conservation and maintenance of the existing genetic diversity. Considering these facts, an investigation on "Morphological and molecular characterization of gladiolus" was carried out. The results of the present studies are discussed in this chapter.

5.1 Assessment of genetic diversity based on morphological data

5.1.1 Variability studies

Assessment of genetic variability is necessary to evaluate the performance of individual cultivars. The analysis of variance permits estimation of phenotypic and genotypic coefficients of variability of various polygenic traits. The genotypic coefficient of variation measures the extent of variability among the different traits caused due to the inherent capacity of the genotype. The genotypic and phenotypic coefficients of variation are needed to understand the effect of environment on various polygenic traits (Allard, 1960).

Estimates of genotypic coefficient of variation was less than the estimates of phenotypic coefficient of variation indicating that the apparent variation is not only due to genotype but also due to the influence of environment. Similar results were obtained by Patil *et al*. (2004), Nimbalkar *et al.* (2007) and Kumar *et al*. (2011a) in gladiolus.

Narrow differences between genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) were observed for the characters like plant height (30, 60 and 90 DAP), leaf length (30, 60 and 90 DAP), leaf width (30, 60 and 90 DAP), leaf area, days taken for spike initiation, days to first three florets colour showing stage, duration of flowering, spike length, rachis length, number of florets per spike, floret diameter, weight of spike, vase life, number of spikes per plant, number of daughter corms per plants, diameter of daughter corm, weight of daughter corm, number of cormels per plant, weight of cormels, weight of ten cormels except stem girth (30, 60 and 90 DAP) and number of leaves (30, 60 and 90 DAP).

Narrow difference between GCV and PCV values indicate the least influence of environment on these characters. Similar results were obtained by Balamurugan *et al.* (2002), Katewate *et al.* (2002) and Kumar *et al*. (2011a) in gladiolus.

5.1.2 Heritability and genetic advance as per cent mean

Heritability values were found to be moderate to high for growth and high for flowering, quantity, yield, corm and cormel parameters. However, high heritability coupled with high genetic advance were noticed for the traits, plant height (30, 60 and 90 DAP), number of leaves (30 DAP), leaf length (30, 60 and 90 DAP), leaf width (30 and 90 DAP), leaf area, days taken for spike initiation, spike length, rachis length, number of florets per spike, floret diameter, weight of spike, vase life, number of spikes per plant, number of daughter corms per plants, diameter of daughter corm, weight of daughter corm, number of cormels per plant, weight of cormels, weight of ten cormels. This indicates the lesser influence of environment in expression of these characters and prevalence of additive gene action in their inheritance hence, these traits are suitable for selection. Similar results observed by Bichoo *et al.* (2002), Katwate *et al*. (2002), Neeraj *et al*. (2005), Pratap and Rao (2006), and Balaram and Janakiram (2009) in gladiolus.

Whereas, high heritability with moderate genetic advance over percent mean were observed for Stem girth 90 DAP, number of leaves (60 and 90 DAP), leaf width (60 DAP) days to first three florets colour showing stage, duration of flowering can be exploited through hybridization. However, high heritability with low GA was observed for stem girth (30 and 60 DAP) thereby indicating non-additive gene action *i.e*. the character is highly influenced by environmental effects and selection would be ineffective and which can be exploited through hybridization or heterosis breeding as reported by Neeraj *et al*. (2005), Nimbalkar *et al.* (2007) in gladiolus.

5.1.3 Correlation

Variability studies provide information on the extent of improvement in different characters, but they do not throw light on the extent and nature of relationship existing between various characters. Therefore, for rational approach towards the improvement of yield, selection has to be made for the components of yield, since there may not be genes for yield *perse*, but only for various yield components (Grafius, 1959). Genetic correlations between two characters arise because of linkage, pleiotrophy or development induced functional relationship (Harland, 1939). Hence, correlation study has greater significance and could be effectively utilized in formulating an effective selection scheme. Many of these yield contributing characters are interact in desirable and undesirable direction. Therefore, knowledge of association between the traits can greatly help in avoiding inversely related compensation effects during selection. A narrow difference between the genotypic and phenotypic correlation coefficients was observed (Table 7 and Table 8) for various traits in the

present finding and this indicates the lesser influence of environment on the expression of these traits and presence of strong inherent association among the traits. Similar results have been observed by Misra and Saini (1990), Balaram and Janakiram (2009) and Choudhary *et al.* (2011) in gladiolus. Therefore, in the present study, though the genotypic and phenotypic correlation coefficients were worked out for growth, flowering, yield and quality components in gladiolus.

Plant height had significant and positive correlation with number of leaves, leaf length, leaf width, leaf area, spike length, rachis length, number of florets per spike, floret diameter, weight of spike, weight of daughter corm, number of cormels per plant. This indicate that the plant height, which is an important trait for healthy spike production, could be increased by making the selection to improve the associated characters. Similar results were observed by Misra and Saini (1990), Rajeevan *et al.* (1994), Anuradha (1990) and Kumar *et al.* (2011b) in gladiolus. Whereas, significant and negative correlation was observed with duration of flowering, number of daughter corms and number of spikes per plant.

Spike length showed significant and positive correlation with plant height, number of leaves, leaf length, leaf width, leaf area, rachis length, number of florets per spike, floret diameter, weight of spike, vase life, weight of daughter corm, number of cormels per plant, days taken for spike initiation. Similar findings were made by Jhon *et al.* (2002), Katwate *et al.* (2002), Misra and Saini (1990) and Choudhary *et al.* (2011) in gladiolus. It shows that spike length, which is an important attribute of cut flower quality, can be increased with increase in any one of these characters.

Number of spikes per plant showed significant and positive correlation with duration of flowering and number of daughter corm per plant. Similar results were observed by Sahana (2010) and Choudhary *et al*. (2011) in gladiolus. Whereas, significant and negative correlation with plant height, number of leaves, stem girth, leaf length, leaf width, leaf area, days taken for spike initiation, spike length, number of florets per spike, floret diameter, weight of spike, vase life, weight of daughter corm and number of cormels per plant. Association with other characters were non significant.

Significant and positive correlation was found between other important characters i.e. number of leaves with plant height, stem girth, leaf length, leaf width, leaf area, days taken for spike initiation, spike length, rachis length, number of florets per spike, floret diameter, weight of spike, weight of daughter corm and number of cormels per plant, leaf area with leaf length, leaf width, spike length, number of florets per spike, floret diameter, weight of spike, vase life, weight of daughter corm and number of cormels per plant, days taken for spike initiation with floret diameter and spike length, rachis length with number of florets per spike, floret diameter, weight of spike, vase life and number of cormels per plant, number of florets per spike with floret diameter, weight of spike and vase life, floret diameter with weight of spike, vase life, weight of daughter corm and number of cormels per plant, number of daughter

corms with duration of flowering and number of spikes per plant. Similar findings were made by De and Misra (1994), Lone *et al.* (1999), Hussain *et al.* (2001), and Kumar *et al.* (2011b) in gladiolus.

Whereas, traits like number of leaves with number of daughter corms and number of spikes per plant, leaf area with duration of flowering, number of daughter corms and number of spikes per plant, days taken for spike initiation with duration of flowering, number of daughter corms and number of spikes per plant, rachis length with leaf length, stem girth and weight of daughter corm, number of florets per spike with number of daughter corms and number of spikes per plant, floret diameter with number of daughter corms, number of spikes per plant and duration of flowering, number of daughter corms with vase life, weight of spike and number of cormels per plant showed significant and negative correlation. In general significant negative correlation between these traits implies these traits are independent in nature and could be improved independently through selection programme. The results are in conformity with the findings by Misra and Saini (1990), Lone *et al.* (1999), Kumar *et al.* (2011c) in gladiolus.

5.1.4 Path analysis

The implication of studies becomes more evident when correlations are partitioned into components through path analysis in order to determine the relative magnitude of various attributes contributing to correlation. Partitioning of total correlation into direct and indirect effects would be worthwhile for an effective selection programme.

Path coefficient analysis was carried out using genotypic correlation coefficient for number of spikes per plant. The number of spikes per plant was directly and positively influenced by stem girth, leaf area, days taken for spike initiation, rachis length, number of daughter corms per plant and weight of daughter corm. Plant height, number of leaves, spike length, number of florets per spike and floret diameter had direct negative effect on number of spikes per plant. Similar results were observed by Anuradha *et al.* (2002) and Sahana (2010) in gladiolus. However, indirect positive contribution through component characters like stem girth, leaf area, days taken for spike initiation, rachis length, number of daughter corms and weight of daughter corm. It can be concluded that association as well as indirect effects were appreciable which can be efficiently used for selecting superior genotypes in future breeding programme. Positive and significant genotypic correlation of number of spikes per plant with number of daughter corms per plant was mainly due to their high direct effects. The residual effect of the genotypic path analysis was 4.58 percent indicates that the studied characters contributed 95.42 percent variation on number of spikes per plant. For the study, number of spikes per plant is taken as a dependent variable.

At phenotypic level, number of spikes per plant was directly and positively influenced by plant height, stem girth, days taken for spike initiation, spike length, number of florets per spike, number of daughter corms, weight of daughter corm and their indirect effect were higher. Direct effect of number of leaves, leaf area, rachis length and floret diameter were recorded to be negative. The indirect positive effects suggested that selection for any of these characters would improve the yield through the associated characters. However, indirect positive contribution through component characters like stem girth, leaf area, days taken for spike initiation, rachis length, number of daughter corms per plant, weight of daughter corm. The number of daughter corms per plant was significant and positively

correlated with the number of spike per plant though it had a positive direct effects and its indirect effects were positive with some of the characters thus, improvement of number of daughter corm per plant can improve yield. It can be concluded that association as well as indirect effects were appreciable which can be efficiently used for selecting superior genotypes in future breeding programme. The residual effect of the phenotypic path analysis was 12 percent indicates that the studied characters contributed 88 percent variation on number of spikes per plant.

5.1.5 Disease incidence

With regard to *Fusarium* disease, minimum score was obtained in varieties Eighth Wonder and Charms Flow. Hence, these varieties were resistant to *Fusarium* disease. Whereas, the high score obtained varieties were highly susceptible (Her Majesty). The degree of variation occurred with respect to the response of varieties to *Fusarium* leaf spot disease was expected since, any resistance or susceptibility of the cultivars to the disease is controlled by the genetic constitution of varieties.

5.2 Genetic diversity through SRAP

SRAP marker have various applications for genetic studies and practical breeding programs. The dominant SRAP markers could provide more accurate information on genetic diversity than traditional methods due to it is simple, reproducible and targets open reading frames (ORFs). Exons are typically GC-rich and thus, the 'CCGG' sequence in the core of the forward SRAP primers is designed to target such coding regions (Li and Quiros, 2001). The results of SRAP in this study show good amplification, stability and reproducibility with easily found polymorphism.

Genetic diversity of 15 gladiolus varieties using eleven primer combinations revealed a total of 80 scorable bands, among which 68 were polymorphic with an average of 6.18 polymorphic bands per pair of primers. The number of bands scored for each primer combination varied from 4-10 with an average of 7.27 bands per primer. This was comparable with Cai *et al.* (2011) in *Dendrobium loddigesii* (231 bands from 17 primer combinations with an average of 13.5 bands per primer combinations), Xue *et al*. (2010) in *Dendrobium* spp. (98 bands from 10 primer combinations with an average of 9.8 markers per primer pair).

Among eleven primer combinations screened, Me1+Em3 scored maximum number of polymorphic bands (10) followed by Me 4 + Em 2 and Me 5+ Em 1 (9 bands each), while Me 3 + Em 5 and Me 5 + Em 2 produced minimum number of bands (04 each). The combinations of primer Me 1 + Em 3, Me 3 + Em 1, Me 4 + Em 3 and Me 4 + Em 4 produced highest polymorphism of 100 per cent respectively, followed by Me 5 + Em 1 (88.89%), where as primer combination Me 5 + Em 2 produced least polymorphism of 25 per cent. Similarly Xue

et al. (2010) analyzed 10 polymorphic primer combinations out of 11 primer combinations in *Dendrobium* spp.

The bands that were unique could be used as the special marker to distinguish the varieties. For instance, Delhi Local and Darshan variety each had two special amplified unique bands generated by Me3/Em5 and Me4/Em2 primer combination respectively. Vedanapoli, Summer Sunshine, Punjab Morning, Red Majesty each had one special amplified unique bands generated by Me1/Em3, Me2/Em5, Me3/Em1 and Me5/Em2 primer combination respectively.

The similarity matrix coefficient ranged from 31 to 73 percent (Table 14), suggesting a higher genetic variation within gladiolus varieties. The highest genetic similarity of 73 per cent was observed between the Charms Flow and Green Bay, while least was between Jester Gold and Vedanapoli of 31 per cent. Similar conclusion was drawn by Pragya *et al.* (2010b) reported greatest similarity was found between Pusa Lohit and Pusa Swarnima (Jaccard coefficient = 0.788), while Pusa Gunjan was found to be the most distinct genotype by AFLP marker in gladiolus.

The dendrogram based on UPGAM clustering (Fig 2) resulted in the identification of two major clusters. Majority of the verities in major cluster I were originated from USA, Holland, Australia and India. In the same sub cluster were clustered together based on origin of varieties with some of exceptions. American Beauty, Charms Flow and Green Bay (USA), Red Majesty and White Prosperity (USA), Vedanapoli and Darshan (India) grouped together as sub cluster in major cluster I. Jester Gold originated from Holland was branched out singly from the cluster showing quite different from other varieties having lowest genetic similarity between Jester Gold and Vedanapoli (31 %) this could have happened due to highly heterozygous nature of the crop. Similar results were reported by Pragya *et al.* (2010a) in gladiolus reveled that in cluster analysis by RAPD marker, the cultivar ‘Pusa Lohit’ branched out from the dendrograms, confirming that it is quite different from all other genotypes.

5.3 Comparison of morphological and molecular data

The genetic relationships between the selected varieties on the basis of morphological and molecular tools revealed some controversy. Traditionally, genetic diversity is assessed based on morphological features such as growth habit and flower colour. The analysis of genetic variability on the basis of morphological traits listed in the appendices V to VII, but these morphological characters may not be good enough to classify varieties and may not provide an accurate indication of the genetic divergence as these are few in number and highly influenced by environmental conditions.

Based on morphological traits, it is revealed that the varieties were not clearly grouped as separate cluster by morphological dendrogram. This may be due to less number

of morphological traits considered for the study**.** Hence there is a need for use of DNA based markers to study the genetic diversity.

As morphological characters were considered, it was found as a distinct cultivars, but when observed with different characters separately, they showed similarity in one of the most important morphological features for discrimination of varieties. Compared with other varieties, Candyman, Summer Sunshine, Eighth Wonder, Red Majesty White Prosperity, Jester Gold and Punjab Morning showed the superior commercial characters, such as spike length, rachis length, floret diameter etc. were clustered together. Delhi Local and Darshan were grouped together which had short stature and more spike per plant. This could be because of the reason that morphological characters like plant height and spike length are highly affected by environment while flower colour is least affected. However, SRAP analysis cluster containing Red Majesty, White Prosperity and Summer Sunshine showed the superior commercial characters. Delhi Local and Copper King were short stature, less floret diameter and yield more no of spike per plant were clustered together. On the basis of floret color (pink) Vedanapoli and Darshan were grouped together. The results were similar to Pragya *et al.* (2010a) in gladiolus using RAPD marker, that the the reliability of morphological traits could also be illustrated as phenotypes are the expressions of genotypes nevertheless it is affected by many factors.

The dendrogram based on morphological and SRAP analysis, difference have arisen due to several reasons, but the most important is that the genetic, while morphological expression (phenotype) are subjected to the physiological state of the individual plant and ambient environmental conditions.

Future line of work

1. Diverse varieties can be used for further crop improvement programme
2. Studies on identification of markers linked to traits of interest may be carried out.
3. Molecular markers could be used in classical breeding programmes to enhance speed and efficiency of gladiolus breeding programme.

6. SUMMARY AND CONCLUSIONS

A study was conducted to understand the magnitude of variability and genetic diversity based on morphological traits and molecular markers in gladiolus. Fifteen varieties of gladiolus were evaluated for their morphological traits and molecular studies at Kittur Rani Channamma College of Horticulture, Arabhavi. The experimental results are summarized below.

6.1 Genetic diversity according to morphological data

Analysis of variance revealed highly significant difference among the 15 varieties for all the characters studied. Narrow differences between genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) were observed for all the characters except for stem girth (30, 60 and 90 DAP) and number of leaves (30, 60 and 90 DAP). Narrow difference between GCV and PCV values indicate the least influence of environment on these characters.

High heritability coupled with high genetic advance were noticed for the traits, plant height, number of leaves, leaf length , leaf width, leaf area, days taken for spike initiation, spike length, rachis length, number of florets per spike, floret diameter, weight of spike, vase life, number of spikes per plant, number of daughter corms per plant, diameter of daughter corm, weight of daughter corm, number of cormels per plant, weight of cormels and weight of ten cormels. This indicating the prevalence of additive gene action in their inheritance hence, suitable for selection. Whereas, high heritability coupled with moderate genetic advance were observed for other traits which can be exploited through hybridization.

Spike length showed significant and positive correlation with plant height, number of leaves, leaf length, leaf width, leaf area, rachis length, number of florets per spike, floret diameter, weight of spike, vase life, weight of daughter corm, number of cormels per plant and days taken for spike initiation. This suggests that, spike length which is an important attribute of cut flower quality, can be increased with increase in any one of these characters. Duration of flowering and number of daughter corms per plant were highly significant and positively correlated with number of spikes per plant. Strong association of these traits revealed that selection based on these traits would ultimately improve the quality and yield.

The path coefficient analysis method splits the correlation coefficients into direct and indirect effects which help in assessing the relative influence of each important character on the ultimate yield and flower quality. Path analysis for number of spikes per plant revealed that plant height, leaf area, days taken for spike initiation, rachis length, spike length, number of florets per spike, number of daughter corms per plant, weight of daughter corm had high direct effects which indicated the possibility of increasing spike yield by selecting the varieties for these characters directly.

Based on morphological traits, it is revealed that the varieties were not clearly grouped as separate cluster by morphological dendrogram. This may be due to less number of morphological traits considered for the study**.** Hence there is a need for use of DNA based markers to study the genetic diversity at DNA level.

6.2 Genetic diversity according to molecular data

In gladiolus, identification of the phenotypes is still based on morphological characters. However, many of them cannot be readily distinguished by morphological indices, particularly if they are closely related. Further more, phenotype identification based on morphological traits is subject to environmental variation.

SRAP analysis

The SRAP fingerprinting of 15 selected varieties of gladiolus using eleven primer combinations revealed a total of 80 scorable bands, among which 68 were polymorphic with an average of 6.18 polymorphic bands per pair of primers. The number of bands scored for each primer combination varied from 4-10 bands (200 to 1500 bp) with an average of 7.27 bands per primer combination.

Among eleven primer combinations screened, Me1+Em3 scored maximum number of polymorphic bands (10) followed by Me 4 + Em 2 and Me 5+ Em 1 (9 bands each), while Me 3 + Em 5 and Me 5 + Em 2 produced minimum number of bands (04 each). The bands that were unique could be used as the special marker to distinguish the varieties.

The similarity matrix coefficient ranged from 31 to 73 percent, suggesting a higher genetic variation within gladiolus varieties. Cluster analysis of the gladiolus varieties employing Jaccard's Co-efficient method led to the grouping of the varieties into two distinct major clusters. Cluster analysis showed that groupings of the varieties with their geographic region of origin. Fourteen varieties were grouped together form a major cluster with a single variety Jester Gold form another major cluster.

On comparing the genetic diversity as revealed by the dendrogram, it was evident that Jester Gold was identified quite distinct variety. In conclusion, SRAP marker provide more accurate information on genetic diversity than traditional methods due to it is simple, reproducible and targets open reading frames (ORFs). Thus, SRAP markers have successfully been used for fingerprint and assess the extent of genetic variation among gladiolus varieties.

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MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION IN GLADIOLUS (*Gladiolus hybridus* Hort.) VARIETIES

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ABSTRACT

An investigation was carried out on morphological and molecular characterization in gladiolus (*Gladiolus hybridus* Hort.) varieties during 2011-2013 at department of Floriculture and Landscape Architecture, Kittur Rani Channamma College of Horticulture, Arabhavi.

Analysis of variance revealed highly significant difference among the 15 varieties for all the characters studied. Narrow differences between genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) were observed for all the characters except for stem girth and number of leaves. Duration of flowering and number of daughter corms per plant were highly significant and positively correlated with number of spikes per plant, suggesting the possibility of simultaneous selection for these characters. Path analysis for number of spikes per plant revealed that plant height, leaf area, days taken for spike initiation, spike length, number of florets per spike, number of daughter corms per plant, had high direct effects which indicated the possibility of increasing spike yield by selecting the varieties for these characters directly. Based on morphological traits, the varieties were grouped into two clusters and the variety which are distinct can be used for further breeding programme.

Molecular characterization was carried out to know the genetic diversity using PCR based Sequence-Related Amplified Polymorphism (SRAP) markers. Among twenty five primer combinations screened, eleven primer combinations gave consistent banding patterns and were used to generate the SRAP fingerprinting. The combinations of primer Me 1 + Em 3, Me 3 + Em 1, Me 4 + Em 3 and Me 4 + Em 4 produced highest polymorphism. The similarity matrix coefficient ranged from 31 to 73 per cent suggesting a moderate to higher genetic variation within gladiolus varieties. Cluster analysis showed groupings of the varieties with their geographic region of origin. On comparing the genetic diversity as revealed by the dendrogram, it was evident that Jester Gold was identified quite distinct variety. SRAP marker provided more accurate information on genetic diversity than traditional methods due to its reproducible and targets open reading frames (ORFs).

"ಬಾಹ್ಯ ಲಕ್ಷಣಗಳು ಮತ್ತು ಜೀವಾಣು ಮುದ್ರೆಗಳನ್ನು ಬಳಸಿ ಗ್ಲಾ್ಯಡಿಯೋಲಸ್‌ನ (*ಗ್ಲಾ್ಯಡಿಯೋಲಸ್ ಹೈಬ್ರಿಡಸ ಹಾರ್ಟ.*) ವಿವಿಧ ತಳಿಗಳಲ್ಲಿ ಅನುವಂಶಿಕ ವೈವಿಧ್ಯತೆಯ ತುಲನೆ"

ಗೀತಾ ವೆ0ಕಟಾಪೂರ 2013 ಎ. ಎಮ್. ಶಿರೊಳ

ಮುಖ್ಯ ಸಲಹೆಗಾರರು

ಸಾರಾಂಶ

ಗ್ಲಾ್ಯಡಿಯೋಲಸ್‌ನ (*ಗ್ಲಾ್ಯಡಿಯೋಲಸ್ ಹೈಬ್ರಿಡಸ ಹಾರ್ಟ.*) ವಿವಿಧ ತಳಿಗಳಲ್ಲಿ ಬಾಹ್ಯ ಲಕ್ಷಣಗಳು ಮತ್ತು ಜೀವಾಣು ಮುದ್ರೆಗಳನ್ನು ಬಳಸಿ ಅನುವಂಶಿಕ ವೈವಿಧ್ಯತೆಯ ತುಲನಯ ಬಗ್ಗೆ ಕ್ಷೇತ್ರ ಸಂಶೋಧನೆಯನ್ನು ಕೆ. ರಾ. ಚ. ತೋಟಗಾರಿಕಾ ಮಹಾವಿದ್ಯಾಲಯದ ಪುಷ್ಪ ಕೃಷಿ ಮತ್ತು ಉದ್ಯಾನ ವಿನ್ಯಾಸ ಶಾಸ್ತ್ರ ವಿಭಾಗದಲ್ಲಿ 2012–2013 ನೇ ಸಾಲಿನಲ್ಲಿ ಹಮ್ಮಿಕೊಳ್ಳಲಾಗಿತ್ತು.

ವಿವಿಧ ತಳಿಗಳ ಗುಣಲಕ್ಷಣಗಳಲ್ಲಿ ಗಣನೀಯ ವೈವಿಧ್ಯತೆಇದೆ ಎಂಬುದು ತುಲನಾತ್ಮಕವಾಗಿ ಧೃಢಪಟ್ಟಿದೆ. ಕಾಂಡದ ಸುತ್ತಳತೆ ಮತ್ತು ಎಳೆಗಳ ಸಂಖ್ಯೆ ಹೊರತು ಪಡಿಸಿ ಉಳಿದೆಲ್ಲಾ ಗುಣಲಕ್ಷಣಗಳಲ್ಲಿ ಜಿಸಿವಿ ಮತ್ತು ಪಿಸಿವಿ ನಡುವೆ ಅಲ್ಪ ವ್ಯತ್ಯಾಸ ಕಂಡುಬಂದಿದೆ. ಹೂಬಿಡಲು ತೆಗೆದುಕೊಂಡ ಸಮಯ ಮತ್ತು ಮರಿಗಡ್ಡೆಗಳ ಸಂಖ್ಯೆಯು ಹೂವಿನ ಗೊಂಚಲಿನ ಇಳುವರಿಗೆ ನೇರ ಸಂಭಂಧ ಹೊಂದಿದೆ. ಮಾರ್ಗ ವಿಶ್ಲೇಷಣೆಯಲ್ಲಿ ಹೂವಿನ ಗೊಂಚಲಿನ ಇಳುವರಿಗೆ ಎಲೆಗಳ ವಿಸ್ತೀರ್ಣ, ಹೂಗೊಂಚಲು ಬಿಡಲು ತೆಗೆದುಕೊಂಡ ಸಮಯ, ಹೂದೇಟಿನ ಉದ್ದ, ಹೂದೇಟಿನಲ್ಲಿ ಇರುವ ಹೂಗಳಸಂಖ್ಯೆ ಮತ್ತು ಮರಿ ಗಡ್ಡೆಗಳ ಸಂಖ್ಯೆವಗಳು ನೇರ ಪರಿಣಾಮ ಹೊಂದಿದೆ. ಈ ಎಲ್ಲಾ ಗುಣಲಕ್ಷಣಗಳ ಆಧಾರದ ಮೇಲೆ ತಳಿಗಳನ್ನು ಆಯ್ಕೆ ಮಾಡಬಹುದೆಂದು ತಿಳಿದು ಬಂದಿದೆ. ಗ್ಲಾ್ಯಡಿಯೋಲಸನ ವಿವಿದ ತಳಿಗಳನ್ನು ಬಾಹ್ಯ ಲಕ್ಷಣಗಳ ಆಧರದ ಮೇಲೆ ಎರಡು ಗು0ಪುಗಳಾಗಿ ವಿ0ಗಡಿಸಲಾಯಿತು. ವಿಭ್ನಿನ ತಳಿಗಳನ್ನು ಹೊಸ ತಳಿಸಂವರ್ಧನೆಗಳಸುವ ಯೋಜನೆಯಲ್ಲಿ ಬಳಸಬಹುದು.

ಪ್ರಸ್ತುತ ಅಧ್ಯಯನದಲ್ಲಿ ಗ್ಲಾ್ಯಡಿಯೋಲಸ್ ತಳಿಗಳನ್ನು ಆನುವಂಶಿಕ ವೈವಿದ್ಯತೆಯನ್ನು ಎಸ್‌ಆರ್‌ಎಪಿ ಜೀವಾಣು ಮುದ್ರೆ ಬಳಿಸಿ ಪರೀಕ್ಷಿಸಲಾಯಿತು. 25 ಪ್ರೈಮರ ಕಾಂಬಿನೇಷನಲ್ಲಿ 11 ಪ್ರೈಮರ ಕಾಂಬಿನೇಷನ ಮಾದರಿ ಬ್ಯಾಂಡಿಂಗಳು ಬಂದಿವೆ. ಪ್ರೈಮರ ಕಾಂಬಿನೇಷನ ಎಮ್‌ಈ 1 + ಈಎಮ್ 3, ಎಮ್‌ಈ 1 + ಈಎಲಮ್ 3, ಎಮ್‌ಈ 1 + ಈಎಮ್ 3 ಹಾಗೂ ಎಮ್‌ಈ 1 + ಈಎಮ್ 3 ಹೆಚ್ಚಿನ ಬಹುರೂಪತೆಯನ್ನು ಪ್ರದರ್ಶಿಸಿದವು. ಗ್ಲಾ್ಯಡಿಯೋಲಸ್ ತಳಿಗಳಲ್ಲಿ ಸಿಮಿಲ್ಯಾರಿಟಿ ಮ್ಯಾಟ್ರಿಕ್ ಕೊಎಪಿಸಿಎಂಟ್ ರೇಂಜವು ಸಾಧಾರಣದಿಂದ ಹೆಚ್ಚಿಗೆ ಅಂದರೆ ಶೇಕಡಾ 31 ರಿ0ದ 73 ಜೀವಾಣು ವೈವಿದ್ಯತೆ ಕಂಡು ಬಂದಿದೆ. ಪ್ರಧಾನ ಘಟಕ ವಿಶ್ಲೇಷಣೆಯ ಚಿತ್ರವು 15 ಗ್ಲಾ್ಯಡಿಯೋಲಸ್ ತಳಿಗಳನ್ನು ಎರಡು ಗುಂಪುಗಳಾಗಿ ವಿಂಗಡಿಸಲಾಯಿತು. ಕ್ಲಸ್ಟರ ವಿಶ್ಲೆಷಣೆಯಲ್ಲಿ ಗ್ಲಾ್ಯಡಿಯೋಲಸ ತಳಿಗಳು ಮೂಲ ಭೌಗೋಳಿಕ ಪ್ರದೇಶಗಳ ಅನುಗುಣವಾಗಿ ಗುಂಪುಗಳು ಕ0ಡು ಬ0ದವು. ಅದರಲ್ಲಿ ಜೆಸ್ಟರ ಗೋಲ್ಡ ತಳಿಯು ಸಾಕಷ್ಟು ವಿಭಿನ್ನತೆ ಕ0ಡು ಬಂದಿತು. ಸಾಂಪ್ರದಾಯಕ ವಿಧಾನ ಕ್ಕಿ0ತಲು ಎಸ್‌ಆರ್‌ಎಪಿ ಜೀವಾಣು ಮುದ್ರೆಯು ಆನುವ0ಶಿಕ ವೈವಿದ್ಯತೆಯ ಮೇಲೆ ಹೆಚ್ಚು ನಿಖರವಾದ ಮಾಹಿತಿಯನ್ನು ಒದಗಿಸುತ್ತದೆ ಏಕೆಂದರೆ ಅದು ಪುನರ–ಉತ್ಪಾದಕ ಮತ್ತು ಓಪನ ರಿಡಿ0ಗ ಪ್ರೈಮಗಳನ್ನು ಗುರುತಿಸುತ್ತದೆ.