GENETIC DIVERSITY ANALYSIS OF GLADIOLUS GENOTYPES (*Gladiolus grandiflorus* L.) USING MOLECULAR MARKERS

By

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THESIS

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DECLARATION

I hereby declare that this thesis entitled "**Genetic diversity analysis of Gladiolus genotypes (*Gladiolus grandiflorus* L.) using molecular markers**" is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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DEDICATED TO MY FAMILY

MOTHER

Mrs. ANITHAKUMARI R.

FATHER

Mr. SUKUMARAN NAIR K.

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LIST OF ABBREVIATIONS

%	Percentage
(L.)	Linnaeus
°C	Degree Celsius
µl	Microliter
µM	Micromolar
A	alpha
A_{260}	Absorbance at 260 nm
A_{280}	Absorbance at 280 nm
AGE	Agarose Gel Electrophoresis
Bp	Basepair
Cm	Centimetre
CTAB	Cetyl trimethyl ammonium bromide
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleosidetriphosphate
EDTA	Ethylenediaminetetraaceticacid
et al.	*et alia*
g	Gram
hr	Hour
IIHR	Indian Institute of Horticultural Research
L	Litre
M	Molarity
mg	milligram
Min	minute(s)
Ml	Millilitre
mM	Millimolar
NaCl	Sodium chloride
NaOH	Sodium hydroxide
NBRI	National Botanical Research Institute

ng	Nanogram
nm	Nanometer
PCR	Polymerase Chain Reaction
pM	Picomolar
PVP	Polyvinylpyrrolidone
RNA	Ribonucleic acid
RNase	Ribonuclease
rpm	revolutions per minute
rRNA	ribosomal RNA
RT	Room temperature
s	second(s)
S	Sydberg unit
ß	Beta
Ta	Annealing Temperature
TAE buffer	Tris Acetate EDTA buffer
Taq	Thermus aquaticus
Tm	Melting Temperature
Tris HCl	Tris (hydroxymethyl) aminomethane hydrochloric acid
UV	Ultra violet
V	Volt

INTRODUCTION

1. INTRODUCTION

Flowers are associated with humankind from the dawn of civilisation. They symbolises love, joy, sorrow, beauty, respect and homage. In India flowers are strongly associated with almost all social occasions in one way or the other way. The cultivation of flower crops, floriculture is gaining attention as a good source of earning apart from giving pleasure. *Gladiolus* is an important commercial flower crop used widely both as cut flower and as a garden plant.

Gladiolus (*Gladiolus grandiflorus* L.), a tetraploid (2n=4x=60) species belonging to the family Iridaceae, is praised for its beautiful and majestic spikes having flowers of different shapes, attractive colours and long keeping quality. Native to Sub Saharan Africa, gladiolus is one of the most popular perennial herbaceous flowering plant and is normally propagated using asexual means. It is commonly used for floral arrangements, interior decoration and making high quality bouquets.

The genus *Gladiolus* includes about 300 species, Southern Africa has 260 endemic species, tropical Asia has 76 and Eurasia has about 10 species (World Checklist of Selected Plant Families, 2017). Following carnation, rose and chrysanthemum, gladiolus stands fourth in international cut flower trade (Singh *et al.*, 2017). USA, Holland, Italy, France, Poland and Bulgaria are some of the major countries producing gladiolus cut flowers. In India, *Gladiolus* is of major demand in cities like Delhi, Chennai, Kolkata, Bangalore, Mumbai and Pune. *Gladiolus* is commercially cultivated in Himachal Pradesh, West Bengal, Sikkim, Karnataka, Tamil Nadu, Uttar Pradesh, Punjab and Delhi. In India, although the cultivation of gladiolus is seen in all four directions, the area under cultivation is varied in each state depending on various conditions like market demand and climate.

The latest gladiolus cultivars offer a large variety of colours, sizes and shapes. *Gladiolus* is very rich in its varietal wealth and each year through organised breeding programme there is addition of new cultivars with improved qualities like extended flowering periods, floral uniqueness, or extended vase life (Sinha and Roy, 2002; Kumar *et al.*, 1999). However, the huge diversity has been exploited very meagrely.

Improvement of some traits like resistance to pathogens and flower fragrance is desirable (Cohat, 1993). Like any other crop, there is scope to improve existing varieties through continuous crop improvement programme in gladiolus. The performance of gladiolus genotypes depends on the climatic conditions of the region where they are grown (Swaroop and Janakiram, 2010). This shows the need for evaluation of genotypes in particular agro-climatic region. To generate new gene complexes for improvement in various traits and to implement effective breeding programmes, the genetic variability present in both wild and cultivated species should be exploited. Therefore, good knowledge of the genetic parameters existing in different characters and the relative ratio of this information in different quantitative traits is a pre requisite for successful crop improvement. Studies on genetic variability based on morphological traits in some gladiolus varieties were conducted by some workers (Patil and Bhajantri, 2013; Choudhary *et al.*, 2012 and Kumar *et al.*, 2012).

Response to change in environmental factors is a major limitation to morphological markers in assessing genetic divergence (Asare *et al.*, 2011). Thus molecular markers are more reliable for genetic variability analysis because they offer numerous advantages over conventional morphology based method. Molecular markers are stable, detectable in all tissues and are not confounded by environmental effects (Mondini *et al.*, 2009). In gladiolus the most widely used markers are Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Simple Sequence Repeat (SSR) and Inter Simple Sequence Repeat (ISSR). However, ISSR (Inter Simple Sequence Repeat) possess high repeatability as they do not require prior information on DNA sequence, reveal high degree of polymorphism, are economic, identify genetically identical individuals and generates reliable information for DNA analysis (Joshi *et al.*, 2000). ISSR based cultivar identification is reported in various bulbous crops (Kiani *et al.*, 2012; Jingang, 2008). Molecular markers sometimes show similarity to morphological expression of a genomic trait (Mondini *et al.*, 2009). In this background the present study was undertaken with the following objectives.

1. To analyse the genetic diversity of gladiolus using morphological and ISSR markers.
2. To identify gladiolus varieties that is suitable for Kerala climate condition.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

In the current global genetic scenario, characterization has become the major requisite for the proper handling of available germplasm in all commercial crops. Organized characterization is the basics for genetic diversity analysis and germplasm conservation. This is done by analysis of markers that vary between the analysed entities. These markers can be either morphological, biochemical or molecular. An ideal marker should be easy to measure and evaluate, highly heritable, either unlinked or neutral and produce comparable results discriminating the entities (Hilliz and Moritz, 1990).

In this chapter literature regarding morphological characterization in gladiolus (*Gladiolus grandiflorus* L.), and molecular characterization associated with the work undertaken have been reviewed.

2.1 GLADIOLUS

Gladiolus (*Gladiolus grandiflorus* L.), is a bulbous flowering plant belonging to the family Iridaceae. It is a herbaceous perennial and one of the most widely cultivated commercial flowering plant worldwide. *Gladiolus grandiflorus*, generally known as 'Sword lily' is also called as the 'queen of bulbous flowers'. *Gladiolus* flowers have high value both in national and international markets due to its beautiful, glamorous and attractive flowers.

Gladiolus is generally propagated from corms, which have one or more buds. The buds develop into leaves and flowering spikes. On an average 1, 50,000 to 1, 60,000 corms per hectare are obtained (Singh *et al.*, 2017). *Gladiolus* spikes are most suitable for interior decoration, floral arrangements, display and making high quality bouquet (Lepcha *et al.*, 2007). *Gladiolus* is ranked fifth among bulbous flowering plants following tulip (*Tulip* sp.), lily (*Lilium* sp.), fressia (*Fressia* sp.) and hippestrum (*Hippestrum* sp.) (Flower council of Holland, 2008). *Gladiolus* ranked fourth in the cut flower trade following rose, carnation and chrysanthemum (Rathod *et al.*, 2011). Plate 1.1 and 1.2 shows the plant profile of Gladiolus.

2.1.1 Taxonomic profile of *Gladiolus grandiflorus* L.

Domain : Eukaryota

Kingdom : Plantae

Division : Magnoliphyta

Class : Liliopsida

Order : Asparagales

Family : Iridaceae

Genus : Gladiolus

Species : *Gladiolus grandiflorus* L.

2.1.2 Origin and Distribution

Gladiolus is domestic to sub-Saharan Africa, mainly South Africa. Cape floristic region is its major centre of diversity and it also occurs in Mediterranean Europe, Asia and tropical Asia. The genus *Gladiolus* contains around 260 species (Goldblatt and Manning, 1998). From these, 180 species are from Africa (Duncan, 2000; Manning *et al.,* 2002). Ploidy in the gladiolus genus ranges from 2n=30 (diploid) to 2n=180 (dodecaploid), with basic chromosome number n=15. It is believed to be in cultivation since 1578, which was first introduced into France and later spread to England, Holland, Germany and North America. It was introduced in India during British period and no species is found to be native of India.

2.1.3 General plant morphology

In botanical description the plant type is generally referred to as herbaceous plant that bears underground storage stems called corms. *Gladiolus* is generally propagated through corms and cormels, where in propagation by corms is most suited for cut flower production. The corms should be healthy and disease free. Corms of diameter ranging from 4-7 cm produces best results and conical shaped is preferred over flat shaped corms for planting.

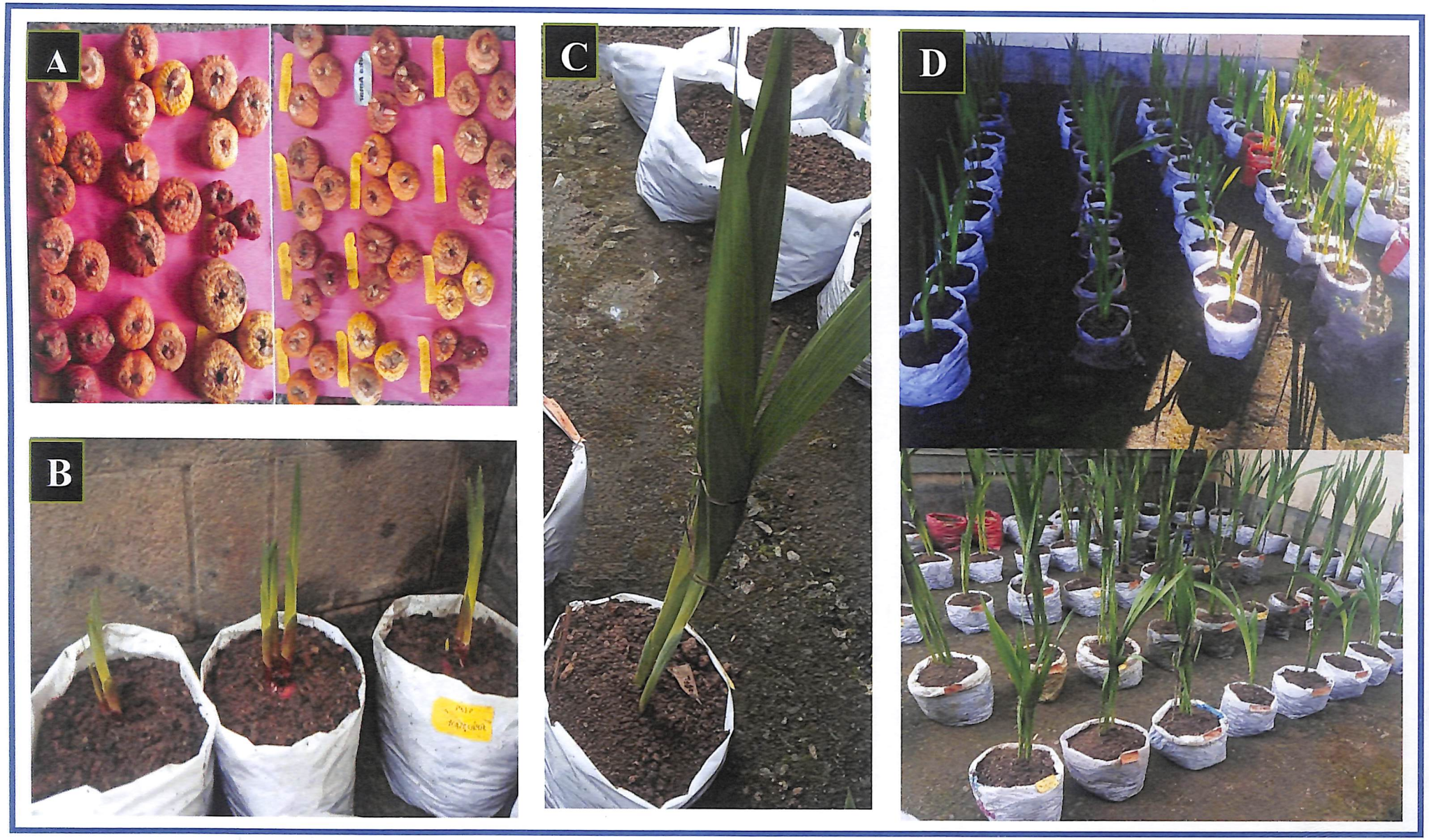


***Plate 1.1 Plant profile: Gladiolus grandiflorus* L.** *(A) Corms (B) Sprouting gladiolus (C) Habitat (D) Field view*

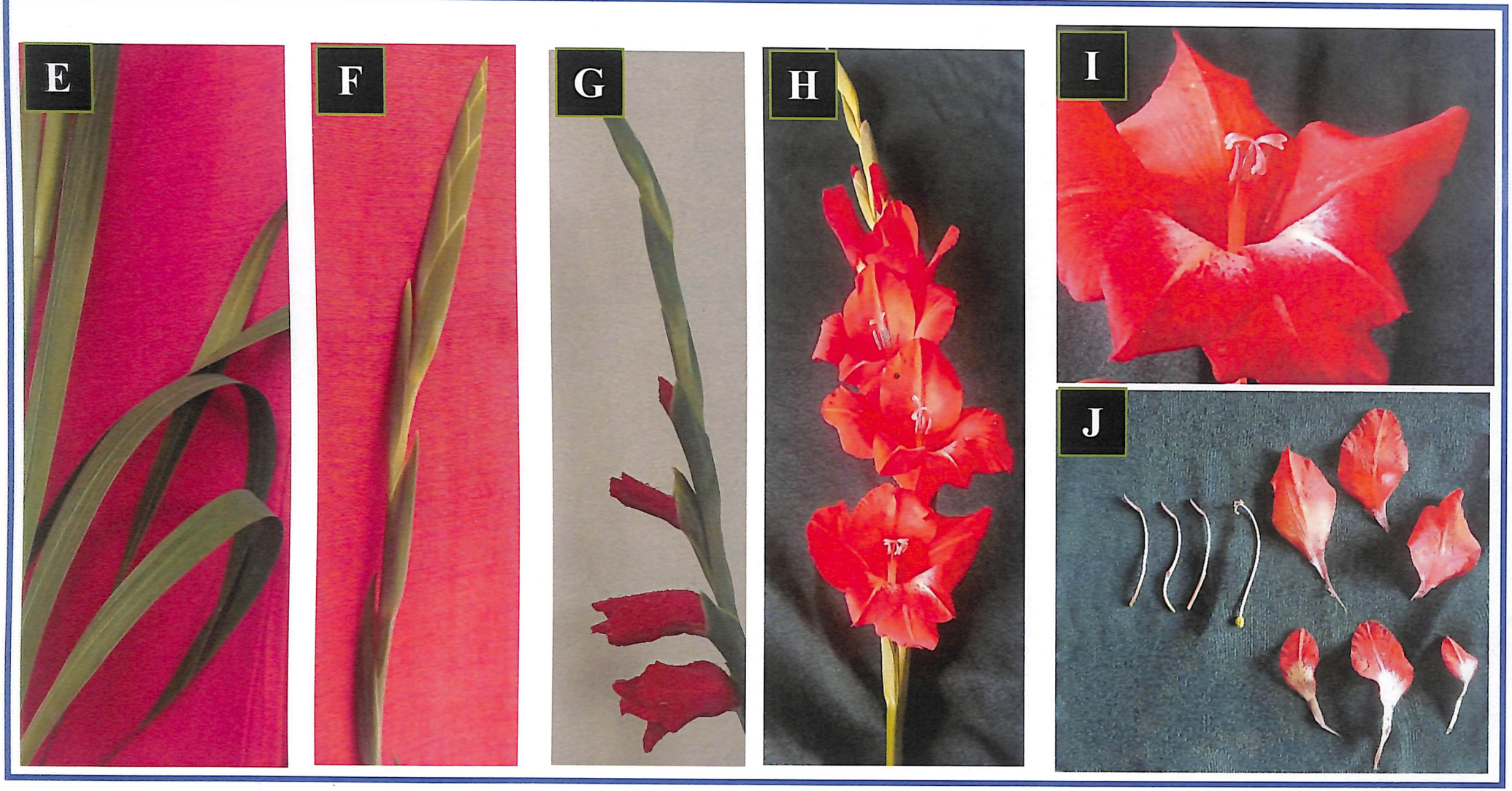


***Plate 1.2 Plant profile: Gladiolus grandiflorus* L.** *(E) Leaf (F) Bud (G) Bud at colour showing stage (H) Single flower/ floret (I) Gladiolus flower (J) Stamen, Pistil and Petals*

The stems are unbranched with 1to 9 sword shaped, narrow, longitudinal grooved leaves enclosed in a sheath. The shape of the leaf blade is plane or cruciform.

Gladiolus flowers are bisexual and the inflorescence is called as spikes. Each flower consists of two leathery bracts known as tepals, which are united at the base to a tube shaped structure. Stamens are attached with its base. *Gladiolus* produces trilocular and oblong capsules that contain many winged, longitudinally arranged, brown seeds. They have axile type placentation and fruit type is referred to as capsule (Singh *et al.,* 2017). Red, green, yellow, white, salmon, brown, smokes, tan, orange, pink, violet and many other flower colours of gladiolus are available and in each colour pale, medium, and deep shades are also available. Mode of pollination is cross pollination usually by flies, bees, sunbirds, and moths. The florets in gladiolus open in sequence over long duration and thus, has high keeping quality among cut flowers. Inflorescence is generally simple and consists of 10-25 florets arranged in rows, all facing one side. The nature of florets are protandrous.

2.1.4 Agro climatic conditions

Gladiolus, the bulbous flowering plant grows well in moderate climatic conditions. It could be grown round the year except when sun burn will be severe. Under Indian condition, it is cultivated during winter in plains and in summer in hills. However, gladiolus is sensitive to frost condition. The temperature needed for flowering ranges from 25- 30°C, whereas optimum temperature for better growth is 15-20°C. *Gladiolus* usually grows better in well drained loamy to sandy soil with optimum pH ranging from 6.5 to 7, which is not too acidic nor too basic. *Gladiolus* flourishes well in moderate humidity and water logging should be prevented.

Gladiolus is usually planted in September to October in plains and in March to April in hilly areas. Raised beds or ridges can be used for planting corms. Depth of planting is an important criteria. Cormel production is enhanced in shallow planting but it can leads to lodging during severe winds. Deep planting can leads to decaying of corms in soil. Most suitable depth for planting ranges from 6-10cm. The number of irrigation depends upon the soil type and the existing weather conditions. Irrigation is usually done twice a week during warm condition whereas in winter season it is

reduced to once in a week. However, after harvesting of flowers watering is reduced for the easy lifting of corms from the soil. Judicious usage of organic manures and fertilisers also favours the growth of gladiolus plant. Fertlisers such as zinc, FYM and NPK is applied time to time for better development of corms and flower production. Earthing up is done to prevent lodging due to wind and staking is done to provide better support or anchorage to plant when needed. Weeding is also done as weeds can result in reduction in gladiolus production. Hand weeding, barriers, hoeing or herbicides are used to control weeds. Mulching is usually done with hay or dry matter. Mulching helps to control weeds, create a cooler growing environment and prevent water loss.

Priyakumari (2001) reported the standardised protocol for *in vitro* clonal propagation of *Gladiolus grandiflorus* L. varieties, conducted in the Department of Pomology and Floriculture, College of Agriculture, Vellayani. Two varieties Peach Blossom and Tropic Seas were selected for the study. Cormels were used as explant.

Studies were conducted to standardize *ex vitro* establishment techniques in *Gladiolus grandiflorus* L. variety 'Vink's Glory' in the Department of Pomology and Floriculture, College of Agriculture, Vellayani. The effects of various potting media, triazole, mycorrhizae and height of potting media in the container on *ex vitro* establishment of the micro propagated plantlets were studied (Sheena, 2004). Harvesting is usually done when bottom flowers in the spike has started opening and it takes 3 to 5 months for flower production depending on the variety. The general yield of spikes is two to three lakhs per hectare of land. The corm yield per hectare of land on an average is 18,000 to 20,000.

Studies were conducted on enhancement of spike qualities of Gladiolus (*Gladiolus grandiflorus* L.) in the Department of Pomology and Floriculture, College of Horticulture, Vellanikkara, in two seasons. The performance of *Gladiolus* varieties under open and rain shelter conditions and standardization of the post-harvest treatments to improve the spike qualities were studied (Simmy, 2015). Elansery (2020) reported in a work that the morphological, physiological, biochemical, and genetic responses of *Gladiolus grandiflorus* cut spikes to *Magnolia acuminata* and *Taxus cuspidata* bark extracts, is used as additives in holding solutions.

2.2 MORPHOLOGICAL CHARACTERIZATION

Morphological markers are the earliest markers used to describe the phenotypic characters of any organism. The usage of these markers is irreplaceable because of their omnipresence which makes them suitable indicators (Patterson, 1996). The major advantages in using morphological markers are that they are simple, inexpensive and fast. But these are not much suitable to be incorporated in genetic improvement studies because of the environmental influence that alter the phenotypic characters either in undesirable or desirable manner. However these markers are still used for initial genetic evaluation studies. Phenotypic traits are the ancient and widely used markers and still they are considered useful for identification of certain cultivars and germplasm on the basis of shape, height, colour, fruit size, leaf and other physical features.

2.2.1 Studies on various growth and flowering performance on gladiolus geneotypes

The status of performance of gladiolus varieties differs based on corm and cormel production, growth and flowering traits. This is especially evident when gladiolus is cultivated under different agro-climatic conditions.

Different gladiolus varieties evaluated in Punjab climatic conditions recorded that the longest spike of 102.27 cm was produced by 'White Prosperity' variety whereas big size florets were produced by 'Rose Supreme' (Sidhu and Arora, 2000).

In another study, Rai *et al.* (2000) 'White Goddess', 'White Prosperity', 'Red Beauty', 'Aldebran', 'Friendship', 'First Lady' were reported as superior to various traits such as spike length, plant height and number of florets per spike.

Gupta *et al.* (2001) noticed maximum spike length in 'Pacific White'. The number of spikes formed per plant ranged from 1.00 in Blue Sky to 2.80 in 'Aldebran'. The number of florets per spike were also recorded highest in the cultivar Pacific White. The cultivar Red Sparkle was recorded with minimum spike length.

Kamble (2001) studied the different gladiolus varieties and found that the variety 'Summer Sunshine' was superior to other varieties in many economically

important phenotypic traits. The values were high for different traits such as weight of corm (143.87 g), diameter of daughter corm (6.83 cm), diameter of florets (11.91 cm) and spike length (93.90 cm).

Under Malwa region of Madhya Pradesh, Gupta *et al.* (2002) evaluated different gladiolus cultivars and concluded was that the maximum spikes per corm was recorded by 'American Beauty' and 'Spring Green' while 'White Prosperity' recorded highest spike length of 83.20 cm.

In another similar study, under valley conditions of Uttaranchal, the maximum number of florets per spike and spike length were observed in 'Oscar' (Jagdish *et al.*, 2003).

The cultivar 'Sancerre' showed high number of florets per spike and larger spikes. 'Yellow Stone' and 'Tropic Sea' also showed good performance in these traits. This evaluation study was conducted in Mahabaleshwar (Patil, 2003). While another study conducted by Seetharamu *et al.* (2003) under polyhouse conditions reported that maximum stalk length, uniform distribution of florets on spike, number of florets/spike and corm production were high for 'American Beauty', followed by 'Her Majesty' and 'Cheaper White'.

In similar evaluation studies, 'Pusa Suhagin' was reported to have the longest vase life of 9.20 days, 'Darshan' produced three spikes per plant (highest) and 'Dhiraj' showed the maximum number of florets in a spike (Nair and Shiva, 2003).

Kamble *et al.* (2004) reported that 'Melody', 'Summer Sunshine', 'Yellow Cup' and 'Trust' exhibited highest values for yield and spike length. On the other hand Kumar and Yadav (2005) showed that 'Pusa Dhanvantari' was preferred for recording highest values for plant height, leaves per plant, length and breadth of leaves, length of spike, diameter of corm and coefficient of propagation but showed late in flowering. Variety 'Smoke Lady' was earliest in flowering. The findings were also confirmed by Swaroop (2010).

Under subtropical conditions of Jammu, 'Jyotsana', 'American Beauty', 'Eurovision' and 'White Prosperity' were the best for commercial production of flowers because of their superiority in corm and cormel production, vegetative growth

and flowering production (Pandey *et al.,* 2009). Similarly under Uttarakhand conditions, five cultivars were found to be superior i.e., 'White Prosperity', 'Candy Man', 'Summer Pearl', 'Charm Flow' and 'Red Majesty' (Lepcha *et al.,* 2007).

Riaz *et al.* (2010) analysed twenty three gladiolus genotypes and found that 'Glad Red' showed the highest length of spike (55 cm), the second highest position was occupied by 'Advanced Red' (50.2 cm) followed by 'White Prosperity' (49.5 cm). The highest field life was noticed in 'Glad Red' (42.5 days), 'Friendship' (42.5 days), 'Peter Pears' (38.7 days) and 'Chinon' (37.3 days).

Saleem *et al.* (2013) revealed earlier sprouting and the highest number of cormel production in 'Fado', highest stem length and leaf area in 'Cantate', the maximum time for spike emergence in 'Eminence' and the maximum leaf chlorophyll content in 'Cantate'.

Sarkar and Chakraborty (2014) tested and found out that 'Jester' reported maximum number of florets remaining open at a time, stalk length, length of rachis, weight of harvested spike and width of floret. Highest vase life was reported for 'Darshan' while 'Kum Kum' and 'Swarnima' showed the maximum number of florets per spike. Thus, 'Kum Kum', 'Jester' and 'Swarnima' were suggested for cultivation to gain good profit.

2.2.2 Variability, heritability and genetic advance studies for morphological traits

In the past several workers have carried out studies on evaluation of genetic variability using yield parameters and morphological markers in gladiolus. It is also important to evaluate the heritability of the phenotypic variation to get an estimate of information on the transfer of characters from parent to progeny. However variability and heritability estimation alone may not give clear knowledge on efficient breeding programme. Estimation of genetic advance along with the other two, is necessary for predicting the overall effect of selecting good individuals (Johnson *et al.,* 1955). The resultant improvement achieved over the base population is analysed using genetic advance. So, in this section a brief review on the assessment of variability, heritability and genetic advance for various quality and quantitative traits in gladiolus is done.

Twenty five gladiolus genotypes studied have proved that some characters such as length of rachis, dormancy period and number of shoots could contribute for crop improvement and further selection. This is because of the high heritability and genetic advance revealed by these characters (Hedge and Patil, 1995).

Sheikh *et al.* (1995) assessed thirty varieties of gladiolus for variability, genetic heritability and genetic advance and found out that highest heritability of 99% was observed for cormels produced per plant and lowest heritability of 13.33% was observed for weight of corm. These characters showed high heritability and high genetic advance.

Balamurugan *et al.* (2002) in his study reported that the highest genotypic coefficient of variation and phenotypic coefficient of variation were observed for the number of cormels formed in per plant of gladiolus. The highest GCV in gladiolus is observed for the number of cormels produced per gladiolus (105.34%) which is followed by corms (103.94%) and side shoots (54.67%). Similar pattern was also reported for PCV. Lifespan of individual florets (6.10%), time taken for first floret opening (10.46%) and length of leaf (10.98%), respectively follows the order for lowest GCV.

Forty one gladiolus genotypes were assessed for genetic variability for seventeen characters. Significant differences among all the characters were observed in the study by estimating the variability of mean and range. This indicated the existence of phenotypic variability in gladiolus genotypes in a wide range. Highest GCV were observed for the number of cormels produced per gladiolus plant followed by the weight obtained for ten cormels (Bichoo *et al.*, 2002).

Katwate *et al.* (2002) observed low genetic advance in characters such as number of leaves, number of corms and days taken for sprouting in gladiolus. This indicated non additive gene effect and wide environment interaction in gladiolus. Characters such as number of cormels and weight of corm showed maximum genetic advance.

Nazir *et al.* (2004) studied the genetic variability in twenty two gladiolus genotypes for twenty characters. In this study the highest genotypic coefficient of

variation and phenotypic coefficient of variation was obtained for the number of cormels produced per gladiolus plant and mean weight of cormel per plant. The range of phenotypic coefficient of variance obtained is from 7.56% to 71.27% for diameter of first floret and average weight of cormels produced per plant respectively. The corresponding genotypic coefficient of variance ranges from 4.77% for diameter of first floret and 58.61% for average cormel weight per plant. High genetic advance indicated by additive gene effects was shown in most of the characters such as days taken for fifty percent heading and opening of first floret.

Genetic divergence was analysed for forty different gladiolus lines during *rabi* season. In this study the values obtained for genotypic coefficient of variation and phenotypic coefficient of variation were almost same, which shows the limited role of environment on various character expression. Both values were lowest for number of spikes and highest for number of cormels. Characters such as weight of spike, number of cormels, dormancy period of corm and weight of corm showed high heritability and genetic advance (Patil *et al.*, 2004).

Ten gladiolus varieties for six characters were evaluated and estimate of heritability and genetic advance were recorded. Highest heritability was recorded for days taken for flowering (94%) and lowest for length of spike. High estimate of heritability is associated with high genetic advance (Pratap and Rao, 2006).

Verma *et al.* (2006) in his study on genetic variability of thirty one gladiolus varieties for nineteen characters showed the little influence of environment on certain parameters such as length of rachis, days taken for germination, width of floret, diameter of corm and weight of five cormel per plant. There was a narrow change in magnitude between genotypic and phenotypic coefficient of variation. Highest values for coefficients of variation were obtained for cormels produced per plant, weight of five cormel per plant, weight of corms, length of rachis and diameter of corm. The range of coefficient of variation for weight of five cormel per plant was 4.00 to 23.3g, for number of cormels per plant was 4.00 to 13.00, for corm weight was 32.33 to 73.43 g, for height of plant, it was 73.48 cm to 146.51 cm and for number of florets per spike 8.09 to 20.40. Lowest GCV and PCV values were reported for the days taken for the

last floret bud opening. Compared to other traits in gladiolus higher heritability was recorded for weight of five cormel/plant, length of spike and flower number per spike.

The genetic variation in thirty six genotypes of gladiolus were reported by Nimbalkar *et al.* (2007). The results obtained was that the genotypic coefficient of variation was lower than the phenotypic coefficient of variation for all characters. Number of corms and cormels per plant and weight of corms and cormels per plant showed highest values for PCV and GCV. Number of cormels per plant (142.01%) and number of corms per plant (0.60%) showed highest and lowest genetic advance respectively.

Studies on performance of thirteen gladiolus genotypes under rainfed climatic condition of Uttarakhand hills have shown that the variability obtained for the number of cormels per plant was 9.75 to 56.50, which was large. Similar trends were also gained for cormel size (0.82 – 3.32 cm), cormel weight (4.61-21.33g), plant height (31.0-62.25 cm), length of spike (48.0- 87.75cm) and florets number (10.25 – 19.50/spike) (Lepcha *et al.*, 2007).

Archana *et al.* (2008) analysed the variation in fifteen characters among thirty two gladiolus varieties. GCV and PCV were obtained high for weight of corm (29.59 and 29.58 g) and height of plant after 30 day of planting (16.10 and 15.34 cm). The values obtained for high heritability and genetic advance as mean per cent for weight of corm were 93.6% and 60.92% respectively.

Studies conducted on variability analysis for seven corm characters in thirty five gladiolus varieties revealed that the number of cormels produced per corm varies in different genotypes. The GCV and PCV difference was narrow, reflecting less environmental influence on expression of characters. The number of corms, number of cormels and 25 cormel weight average showed highest genetic advance, heritability, GCV and PCV values. Additive gene effects is observed for characters such as weight of corm and number of cormels, indicated by the high heritability and high genetic advance. Low genetic advance value was indicated by non- additive genetic control balance characters (Balaram and Janakiram, 2009).

Genetic divergence, genetic advance and heritability of fifteen characters in gladiolus cultivars (forty four) were estimated by Kumar *et al.* (2011a). In this study the estimated PCV was higher than that of GCV. Diameter of floret showed a PCV value of 8.41 and GCV value of 6.83 whereas the number of florets/ spike showed a PCV value of 127.33 and GCV value of 126.33.

Kumar *et al.* (2011b) evaluated different gladiolus genotypes (twenty nine genotypes) for fifteen characters and found that there is difference between the values of genotypic coefficient of variation and phenotypic coefficient of variation. The number of daughter corm produced and cormel production per plant showed highest GCV and PCV values. Plant height, days taken for first flower opening and corm weight showed high heritability with high genetic advance.

Venkatapur (2013) characterised fifteen gladiolus varieties and found that there existed narrow variation between GCV and PCV values for all the traits studied under the work, except for the number of leaves and stem girth. A similar study held in Punjab among gladiolus varieties showed a PCV value of 40.21 and a GCV value of 39.66 for the number of cormels per plant. Heritability of 97.98% was observed for the weight of the cormels per plant (Zahor *et al.*, 2019).

2.2.3 Path coefficient and correlation analysis

Correlation coefficient along with its three types, genotypic, phenotypic and environmental correlations are used to analyse the mutual relationship between various plant characters. Galton (1889) presented the idea of correlation and later developed by Fisher (1918) and Wright (1921). Thus using correlation coefficient nature of association of characters in different varieties of gladiolus was investigated. The inherent association using two variables was estimated through genetic correlation whereas the observable correlation between two variables was estimated through phenotypic correlation. Wright (1921) developed the method of path coefficient analysis, in which it helps in the separation of correlation coefficient values into components of indirect and direct effects. It requires the use of a cause and effect relationship among the variables.

Lone *et al.* (1999) reported that number of florets per spike and length of spike exhibited positive phenotypic and genotypic correlation effects with various traits such as duration of floret, size of floret, distance between two florets. Negative correlation effects existed between weight of corms and number of corms per plant.

The correlation study in gladiolus reported that there was a positive significant correlation in number of florets in a spike with number of leaves per shoot, plant height, durability of spike, number of capsules in a spike. On the other hand number of shoots per plant to days for flowering had negative correlation (Misra and Saini, 1990).

Twenty five gladiolus genotypes were studied and reported that traits such as number of flowers remaining open at a time, durability of spike, length of rachis, number of cormels and plant height expressed positive correlation with number of florets in a spike. Path analysis evaluation revealed that positive direct effect was shown by the days for 50% heading with number of florets in a spike, whereas indirect positive effect was shown by days taken for first floret opening (Hussain *et al.*, 2001).

Jhon *et al.* (2002) reported that important traits utilised for gladiolus breeding programmes included number of florets, length of spike and size of floret. The investigation revealed that spike length showed strong positive correlation with floret size, corm weight, number of florets/spike, plant height and corm size, whereas strong negative correlation was expressed with ten cormel weight, days taken for sprouting and days taken for basal floret opening.

Nazir *et al.* (2005) evaluated twenty characters in twenty two genotypes of gladiolus and reported that days taken for first floret opening and mean weight of cormels expressed positive effects and also showed positive correlation in number of florets per spike.

Path analysis studies were carried out in gladiolus genotypes by Nimbalker *et al.* (2007). He found out that number of days taken for sprouting showed positive correlation with number of corms and cormels per plant, days taken for flowering, weight of corms and cormels, and intermodal length. Highest direct effect of

magnitude 0.819 were exhibited by rachis length, in which the residual effect was 0.349.

Positive and significant correlation were showed by weight of corm, diameter of corm, number of days taken for full emergence of spike, number of days taken for first flower initiation, plant height and number of leaves after sixty days of planting (Archana *et al.,* 2008).

Balaram and Janakiram (2009) reported that length of spike had direct effect with positive correlation with length of rachis and height of plant. The residual effect was 1.5% indicating that 98.5% of the variability of yield are explained by the contributing characters.

The correlation study in gladiolus revealed that there exists a positive significant correlation between different sets of characters considered in the study. They are plant height with corm diameter, corm weight, number of leaf per plant and rachis length, number of leaves/plant with corm diameter, weight of corm and length of rachis, floret diameter with marketable spike per corm and daughter corm formed/ plant (Kumar *et al.,* 2011c).

In an experiment on the contribution of different morphological traits in gladiolus by correlation analysis Choudhary *et al.* (2011) showed that length of spike had negative correlation with floret size, number of shoots per plant and number of corms, whereas positive correlation with rachis length, plant height, number of florets in spike and flowering duration. Thus the economically important spike quality characters according to the study are floret size, spike length and number of florets/spike.

The association between different characters towards yield in twenty two gladiolus genotypes were studied by Pal and Singh (2012). Character such as spike length, weight of corm, rachis length, number of corms and days taken to sprouting showed positive correlation with number of florets formed per spike.

2.3 MOLECULAR MARKERS

Molecular markers are more reliable for assessing the population structure and genetic diversity. These markers now generally points to DNA based markers, which are mainly of two types a) hybridization based marker and b) PCR based marker. Restriction Fragment Length Polymorphism (RFLP) is an example for hybridization based marker which use radiolabelled probes. Amplification using thermal cycler is involved in PCR based marker and is also a very widely used simple method such as Random Amplified Polymorphic DNA (RAPD), Simple Sequence Repeat (SSR), and Inter Simple Sequence Repeat (ISSR).

2.3.1 Studies on genetic variability based on molecular markers

Genetic relationship of gladiolus genotypes was analysed using RAPD markers by Takatsu *et al.* (2002). From a total of 140 tested RAPD primers, 32 primers produced a total of 133 amplified bands in 33 gladiolus varieties. The genetic distance calculated among the species produced a result of 0.842 among the most related species to 0.564 among the more distant ones. Dendrogram was constructed using these data, which separated into 4 major cluster.

Reddy *et al.* (2002) reported that ISSR- PCR technique is a simple and quick method that combines most of the advantages of microsatellites (SSRs) and AFLP to the universality of RAPD. They also identified that ISSR markers are highly polymorphic and are useful in studies on genetic diversity, phylogeny, gene tagging, genome mapping and evolutionary biology.

Gang *et al.* (2008) revealed the information on genetic relationship in 26 cultivars of *Gladiolus hybridus* Hort. using 33 RAPD primers selected as a result of screening among 520 primers. A total of 206 amplified fragments were produced from which 185 amplified fragments were detected as polymorphic. Based on this information different cultivars could be identified and as a result it showed that the germplasm resource of *Gladiolus hybridus* Hort. had a narrow genetic base on molecular level.

Wang *et al.* (2008) analysed genetic relationship using 19 arbitrary ISSR molecular markers in 26 cultivars of gladiolus. Based on further PCR and diversity analysis, a sum total of 110 ISSR fragments were reported of which 103 were

identified showing a high level (93.6%) of polymorphism. The cluster analysis showed that cut flower cultivar of *Gladiolus hybridus* Hort. had a narrow genetic base on molecular level. Also some genetic relationship existed in *Gladiolus hybridus* Hort. summer large flower cultivar.

Characterization and analysis of diversity in 54 gladiolus varieties using morphological and RAPD markers were reported. To discriminate between the cultivars nine morphological traits and 225 RAPD markers amplified using 25 arbitrary primers were utilized. Polymorphism of 93.78% was reported from 211 polymorphic bands. From the cluster analysis using both morphological and RAPD data, variety 'Pusa Lohit' branched out from the dendrogram showing that it is different from all other genotypes (Pragya *et al.*, 2010a).

Pragya *et al.* (2010b) studied on diversity analysis in gladiolus cultivars using fluorescent based AFLP markers. From screening 24 AFLP primers with three gladiolus DNA samples, nine AFLP primers which showed high polymorphic bands were selected for AFLP reactions. Polymorphic bands of 658were reported from a total of 660 AFLP fragments detected, providing a high polymorphic percentage of 99.70%. Cultivar 'Pusa Lohit' and 'Pusa Swarnima' showed greatest similarity, whereas 'Pusa Gunjan' showed the least.

Malik *et al.* (2014) studied the genetic variability diversity involving twenty two diverse genotypes of *Gladiolus* using RAPD analysis. Kumari *et al.* (2014) done the optimization of PCR conditions for ISSR analysis of *Gladiolus* genotypes was done. This optimized protocol for ISSR-PCR is suitable for molecular diversity analysis of *Gladiolus* genotypes.

Kumar *et al.* (2016) used ISSR molecular marker for the study on a total of 15 *Gladiolus* cultivars, which were also analysed using morphological traits. In this study, ISSR provided good insist of genetic diversity available in *Gladiolus* germplasm.

Chaudhary *et al.* (2018) used ISSR (Inter simple sequence repeat) markers to assess the genetic diversity and population structure in 53 indigenous and exotic genotypes of Gladiolus (*Gladiolus hybridus* Hort.). Molecular markers analysis

showed PIC ranges from 0.42 to 0.99 with an average 0.812. Singh *et al.* (2018) analysed genetic variability and relatedness in 62 *Gladiolus* cultivars using Directed Amplification of Minisatellite DNA (DAMD) and Inter Simple Sequence Repeats (ISSR). The result generated with DAMD and ISSR markers showed 83.32 % polymorphism across the *Gladiolus* cultivars.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The present study was carried out at the Department of Plant Biotechnology and at the Department of Plant Breeding and Genetics, College of Agriculture, Vellayani, Thiruvanathapuram during 2020-2021. This section consists of details regarding experimental materials and procedures used in the study.

3.1 PLANT MATERIALS

Gladiolus (*Gladiolus grandiflorus* L.) planting material were collected from different locations in India. Twelve varieties were obtained from Indian Institute of Horticultural Research (IIHR), Bangalore, Karnataka and three varieties were received from National Botanical Research Institute (NBRI), Lucknow, Uttar Pradesh making a total of fifteen commercially important varieties of gladiolus. The corms of the plant are generally used for regeneration. The plants were raised in the field according to the following experimental design:

Design	: Completely Randomised Design (CRD)
Number of treatment	: 15
Replication	: 2

Plants were planted in grow bags. The intercultural operations and weeding were carried out according to the Package of Practices Recommendations of Kerala Agricultural University (KAU, 2021). The details of the varieties and their place of collection are given in Table 1.

Table 1. Details of the gladiolus varieties used for the study

Sl NO.	Variety name	Location of collection
G1	Arka Darshan	IIHR, Bangalore
G2	Arka Pratham	IIHR, Bangalore
G3	Arka Sapna	IIHR, Bangalore
G4	Arka Naveen	IIHR, Bangalore
G5	Manohar	NBRI, Lucknow, Uttar Pradesh
G6	Arka Amar	IIHR, Bangalore
G7	Arka Aayush	IIHR, Bangalore
G8	Arka Kesar	IIHR, Bangalore
G9	Archana	NBRI, Lucknow, Uttar Pradesh
G10	Arka Kumkum	IIHR, Bangalore
G11	Arka Poonam	IIHR, Bangalore
G12	Arka Shobha	IIHR, Bangalore
G13	Arka Gold	IIHR, Bangalore
G14	Gazel	NBRI, Lucknow, Uttar Pradesh
G15	Arka Nazrana	IIHR, Bangalore

3.2 MORPHOLOGICAL OBSERVATIONS

The selected gladiolus genotypes were evaluated by examining and recording their vegetative as well as floral characters. Cultural practices such as nutrition, irrigation, and weeding were uniformly done for all varieties and were maintained the same.

3.2.1 Vegetative Characters

Observations were recorded for the following characters after planting the corms.

3.2.1.1 Plant height (cm)

Height was measured from the base of the plant to tip of the longest leaf in centimetre at 80th day after planting and then the average plant height was found out.

3.2.1.2 Number of leaves per shoot

Done by counting the number of leaves at 80th day after planting and then the average number was found out.

3.2.1.3 Length of the leaf (cm)

The length from base to tip of three leaves was measured at 80th day after planting and average value was found out. It was measured in centimeters.

3.2.1.4 Width of the leaf (cm)

The width from the middle was measured in centimeters from three leaves and the average found out. The measurement was done at 80th day of planting. Second or third leaf was chosen for this purpose.

3.2.1.5 Internode length (cm)

Measured with the help of a scale from one node to the other of the shoot in centimeters at 80th day of planting.

3.2.2 Floral Characters

3.2.2.1 Flowering nature – free flowering/ seasonal

To check whether the gladiolus flowering is free flowering or seasonal in nature.

3.2.2.2 Number of days taken for initiation of flower spike

The number of days for spike emergence from the date of planting of corm was recorded and the average was calculated.

3.2.2.3 Number of days taken for full emergence of spike

The number of days taken for full emergence i.e., to the last flower opening in a spike is calculated and the average found out.

3.2.2.4 Length of the floret (cm)

The length of the individual flower was found out by measuring the axis of the tip of the middle petal to the point of attachment of the flower. The average was found out. The measurement was in centimetre range.

3.2.2.5 Diameter of the floret (cm)

The width of the broadest part was observed at the longest part of the floret. The width of the second floret was measured, by fully opening it with the help of scale and the average found out.

3.2.2.6 Inflorescence orientation

The arrangement of flowers in the inflorescence was observed. Most popular arrangements in gladiolus inflorescence include one row, two rows, zig-zag or irregular arrangement.

3.2.2.7 Number of florets open at one time

Number of flowers which were fully open at the same time in each spike was counted in cut flower and the average worked out.

3.2.2.8 Flower length (cm)

Length of the cut flower was measured from base of the lower flower to top of the upper flower using a measuring scale and average flower length was expressed in centimeters.

3.2.2.9 Flower fragrance

It was determined by checking whether any fragrance is present or absent in flowers.

3.2.2.10 Flower width in front view

The diameter of the cut flower was measured using measuring scale and the average found out. It was expressed in centimeters.

3.2.2.11 Predominant colour in the flower

The predominant colour is the colour present in the largest surface area.

3.2.2.12 Shape of flower

The shape of the flower is determined from the shape of the front view of the flower which can be triangular, star shaped or round in shape.

3.2.2.13 Number of florets per spike

The number of all flowers including the closed buds is noted here.

3.2.2.14 Field life

The period from the first flower opening to the last flower withering on flower spike calculated in days when the spike was attached to the plant in the field.

3.2.3 Statistical Analysis

The collected values were then subjected to analysis of variance among the fifteen *Gladiolus* varieties to test for significant differences among the data, by using Panse and Sukhatme (1985). Variability, heritability, genetic advance and correlation were the other genetic parameters that were estimated.

3.2.3.1 *Analysis of Variance*

Analysis of variance with two variables X and Y was measured in genotypes 'g' developed in completely randomised design with 'r' replication and is given as below

Source	Degrees of freedom	Mean square		
		X	Y	XY
Between genotypes	(g-1)	G_{XX}	G_{YY}	G_{XY}

Error	(r-1)(g-1)	E_{XX}	E_{YY}	E_{XY}

3.2.3.2 *Coefficient of Variation*

The formula proposed by Singh and Chowdhury (1977) was used for estimating genotypic and phenotypic coefficients of variation. The formulas used for calculating the genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) are as follows:

$$GCV = (\sigma_{gx}/M)*100$$

$$PCV = (\sigma_{px}/M)*100$$

Where,

σ_{gx} = Genotypic standard deviation

σ_{px} = Phenotypic standard deviation

M = mean of the character under study

3.2.3.3 *Heritability and Genetic Advance*

Heritability (in broad sense) was estimated as the proportion of heritable components in variation. This was calculated in percentage according to the formula given by Jain (1982).

Heritability coefficient (in broad sense), $H^2 = (\sigma_{gx}^2/\sigma_{px}^2)*100$

Where,

σ_{gx}^2 = genotypic variance of the character X

σ_{px}^2 = phenotypic variance of the character X

As per Allard (1960) classification of heritability

<30% - low heritability

30-60% - high heritability

>60% - very high heritability

Genetic advance in percentage of mean (GA) = $KH^2 \sigma_{px}/M*100$

Where,

K =2.06 if 5% selection is done (selection differential value) (Miller *et al.*, 1958).

H^2 = Heritability in broad sense

σ_{px} = phenotypic standard deviation

M = mean of the characters over all varieties

Classification of genetic advance as percentage of mean by Robinson *et al.* (1949) is,

<20% - low genetic advance

>20% - high genetic advance

3.2.3.4 *Correlation Analysis*

The different correlation coefficients namely genotypic correlation coefficient, phenotypic correlation coefficient and environmental correlation coefficient between two characters 'x' and 'y' is as follows:

Genotypic correlation (r_{gxy}) = $\sigma_{gxy} / (\sigma_{gx} * \sigma_{gy})$

Phenotypic correlation (r_{pxy}) = $\sigma_{pxy} / (\sigma_{px} * \sigma_{py})$

Environmental coefficient (r_{exy}) = $\sigma_{exy} / (\sigma_{ex} * \sigma_{ey})$

Where,

σ_{gxy}, σ_{pxy} and σ_{exy} are the genotypic, phenotypic and environmental covariances between the characters 'X' and 'Y'.

σ_{gx}, σ_{px} and σ_{ex} are the genotypic, phenotypic and environmental standard deviations for the character 'X'.

σ_{gy}, σ_{py} and σ_{ey} are the genotypic, phenotypic and environmental standard deviations for the character 'Y'.

3.2.4 Principal Component Analysis

The obtained data was subjected to multivariate analysis using numeric taxonomic techniques. PCA was done to analyze the contribution of various traits in separating

accessions into various groups and to compare the clustering of accessions with each cluster in dendrogram. The principal component analysis reduces the dimensions of the multivariate data to a few principal axes, and helps to generate an Eigen vector for each axis and gives component scores for the characters (Ariyo and Odulaja, 1991; Sneath and Sokal, 1973).

3.2.5 Transformation of morphological data to 0-1 scale

To overcome the differences in the range of values contributing to the variation in qualitative and quantitative characters which might make the observations insignificant and to give equal weightage for all the characters, morphological values (qualitative and quantitative) was transformed to 0-1 scale using the following formula (Venkatapur, 2013).

$$Vt = \frac{(V - Vmin)}{(Vmax - Vmin)}$$

V – value for a particular character of any genotype

Vt – value which is transformed

Vmin – minimum value in that character among all genotypes

Vmax – maximum value in that character among all genotypes

The values less than 0.5 are taken as 0 and values greater than 0.5 are taken as 1.

3.2.6 Cluster Analysis

To analyse the trends of similarity and dissimilarity, data was subjected to UPGMA method of clustering based on Jacquards similarity coefficient. UPGMA (Unweighted Pair Group Method with Arithmetic average) (Sneath and Sokal, 1973) with NTSYS-PC (Numerical Taxonomy and Multivariate Analysis System, Biostatistic, New York, U. S. A., Software version 2.02 package) (Rohlf, 1998) is used to group morphologically similar genotypes under one group and dissimilar genotypes in distant groups. The clustering of accessions was represented using a dendrogram after analysis.

3.3 MOLECULAR CHARACTERIZATION

3.3.1 DNA extraction

3.3.1.1 Sample collection

Fresh and young tender leaves of gladiolus were collected from the varieties planted in the Department of Plant Breeding and Genetics, College of Agriculture, Vellayani, Thiruvananthapuram. DNA was extracted from the young tender leaves using the C TAB method of DNA isolation with slight modifications.

Leaves collected were weighed out (2g) and ground in pre cooled mortar and pestle to a fine powder using liquid nitrogen. Pre warmed C TAB extraction buffer (10ml) consisting of β- mercaptoethanol (1%) and Poly Vinyl Pyrolidone (2%) was added to the mixture. The homogenised lysate was kept in a waterbath for incubation at 60 °C for one hour with intermittent shaking. The mixture was then centrifuged at 10000 rpm for 10 minutes and the resulting supernatant was transferred to fresh tubes and discarded the lower phase. An equal volume of freshly prepared chloroform: isoamyl alcohol (24:1) was added to the transferred supernatant and mixed by repeated inversion for a minimum of twenty times. This mixture was then centrifuged at 10000 rpm for 10 minutes and thereafter the supernatant was transferred to fresh tubes and the process is repeated twice. The supernatant was then transferred into fresh microfuge tubes. Two volumes of ice cold isopropanol was added and mixed by gentle inversion. The mixture was then incubated at -20 °C for 1 hour or at 4 °C overnight. The precipitated DNA was pelletized at 10000 rpm for 15 minutes and without disturbing the pellet the supernatant was discarded. The pellet formed was washed with 500 µl of 70% ethanol at 10000 rpm for 5 minutes. The ethanol was then discarded and the pellet was air dried completely. The pellet thus obtained was preserved by dissolving in 100 µl TE buffer. Isolated DNA samples were stored at – 20 ° C until use. The quality and presence of impurities in the obtained DNA was analysed by subjecting to electrophoresis in 0.8 % agarose gel.

3.3.2 Analysis of the DNA extracted

3.3.2.1 Quality checking of the DNA using Agarose Gel Electrophoresis

The quality of the extracted DNA was checked using Agarose Gel Electrophoresis in 0.8 % agarose gel. After cleaning and drying the gel casting tray, it was placed on a horizontal flat surface for uniform size and was set up with combs having the required number of wells to make a mould. Agarose was weighed and dissolved in freshly

prepared 1 X TBE buffer in a conical flask. It was melted for 2-3 minutes to make 0.8% gel. When the flask attained a hand bearable temperature, about 2µl of ethidium bromide (EtBr) was added to the molten gel. Then the molten gel was immediately poured into the prepared gel casting tray and allowed to solidify. After solidification, the gel along with the tray was placed in the gel electrophoresis system and the comb was removed from the gel slowly. 1X TBE buffer was poured into the tray to a level in which the gel got fully submerged. 5 µl of extracted DNA sample was mixed with 2 µl of loading dye in a vial and was loaded into the corresponding well. The gel electrophoresis unit with the loaded gel was allowed to run for one hour at 80V. After that period the bands in the gel were visualised under the ultra violet light using the gel documentation system (G: Box, M/s Syngene) and the image was captured.

3.3.2.2. Quantification of DNA

The extracted DNA was quantified using ELICO SL 218 double beam UV-VIS spectrophotometer. This instrument is used for the assessment of both the quantity and quality of the extracted DNA with high accuracy. 5µl of the sample was used for the assessment of different measurements like absorbance at 260nm, absorbance at 280nm, the ratio of absorbance at 260nm to 280nm and concentration of DNA (ng/µl). These values were noted down and using the absorbance/OD value, suitable samples were selected.

3.3.2.3 Dilution of DNA samples

DNA samples were diluted with autoclaved distilled water to a working stock concentration of 40 ng/µl, based on the concentration obtained from DNA quantification.

3.4 PRIMER SCREENING

The prepared diluted DNA samples of two gladiolus varieties were taken for primer screening. Primer screening was done with 12 ISSR primers, initially. The following primers were used for initial screening: UBC 866, UBC 857, UBC 807, UBC 812, UBC 842, UBC 827, UBC 810, UBC 825, UBC 830, UBC 834, 22218 and UBC 890.

3.4.1 Primer dilution

The ordered primers obtained in lyophilised form were centrifuged. Nuclease free water was added to the primers. The main primer stock of 100µM concentration was made by adding nuclease free water in such a volume to make it as much as ten times DNA in nanomoles available in the tube. 10 µM concentrations of working stock was prepared from the main stock by diluting 5 µl of main stock with 45 µl of nuclease free water and kept at -20°C in the refrigerator.

3.4.2 PCR amplification

At standard conditions of temperature and time, the above mentioned 12 ISSR primers were used for amplifying the diluted DNA samples for the purpose of screening. The PCR components is given below in table 2.

Table 2. PCR master mix preparation

Components	Stock concentration	Working concentration	Volume for one reaction (20µl)
Taq buffer	10X	1X	2.5µl
dNTP mix	10Mm	1mM	2µl
Primer (ISSR)	10µM	1µM	2µl
Taq DNA polymerase	5U/µl	1U	0.33µl
DNA template	40ng/µl	80ng	2 µl
SDW	(for making the final volume to 20µl)		
	Total		20µl

3.4.3 PCR conditions

Thermofisher thermalcycler were programmed to perform the PCR reaction with an initial denaturation of 94°C for 5 minutes. It is then followed by denaturation at 94 °C for 1 minute, primer annealing at 45 - 56°C for 1 minute, extension for 1 minute at 72°C, final extension at 72°C for 8 minutes and finally hold at 4°C.

To screen the primers for selecting efficient and suitable ones, the amplicons obtained as PCR products were subjected to agarose gel electrophoresis. For that the amplicons were resolved in 2% agarose gel with 100bp ladders. UV light of G: Box gel

documentation system using GeneSyS software (Syngene) was used for visualising the bands on the gel and primers with scorable bands of high quality were selected for the characterisation of the 15 gladiolus genotypes. The list of the selected primers are given in table 3.

Table 3: List of selected ISSR primers for gladiolus characterization

Sl No.	Primer Name	Sequence	Annealing temperature (°C)
1.	UBC 866	AGCAGCAGCAGCAGCAGC	55.52
2.	UBC 857	ACACACACACACACACYG	49.83
3.	UBC 807	AGAGAGAGAGAGAGAGT	45.63
4.	UBC 812	GAGAGAGAGAGAGAGAA	45.36
5.	UBC 842	GAGAGAGAGAGAGAGAYG	49.83
6.	UBC 827	ACACACACACACACACG	47.77
7.	UBC 810	GAGAGAGAGAGAGAGAT	45.36
8.	UBC 825	ACACACACACACACACT	45.36
9.	UBC 834	AGAGAGAGAGAGAGAGYT	47.55
10.	UBC 890	VHVGTGTGTGTGTGTGT	46.96

After these initial screening steps, the selected primers were used to amplify the DNA samples and then the amplicons were analysed using agarose gel electrophoresis.

3.5 ISSR ANALYSIS OF *GLADIOLUS* GENOTYPES

The selected ISSR primers were used for the molecular characterization of the DNA of all the 15 gladiolus genotypes. 2% agarose gel were used for resolving the PCR products. For polymorphism analysis and to determine the molecular weight of the obtained bands 100bp DNA ladder was also loaded along with PCR products in the gel.

3.5.1 Agarose gel electrophoresis

For resolving the amplified PCR product, agarose gel electrophoresis was being used, which is a very important in marker analysis. Due to the sieving effect of agarose gel,

DNA molecules will get separated based on size. That is, when the agarose concentration increases sieving effect will also increases.

3.5.2 Gel preparation

Two gram of agarose was measured out and added to 100 ml 1X TBE buffer into a 250ml conical flask. The solution was then heated in a microwave oven for dissolving agarose in the buffer. It was then kept for cooling and when it attained bearable heat, 1µl/ml of ethidium bromide was added to it. The warm solution was then poured into the casting tray which was already set with combs placed in position. This was allowed to solidify for 20 minutes.

3.5.3 Gel loading and running

The prepared solidified gel was transferred to electrophoretic apparatus, filled with 1X TBE buffer, so that the gel gets submerged in the buffer. Without disturbing the wells, the comb was removed from the gel. 5 µl of each sample was loaded to the wells, along with 2µl 100bp ladder at one end for reference. It was then allowed to run at 85V for about 2 hours. UV transilluminator was used for band visualization and documentation was done using gel documentation system.

3.5.4 Visualization of gel profile

UV light of G: Box gel documentation system (Syngene) using GeneSyS software was used for visualizing the bands. The gel along with the tray was placed in the instrument and the image was captured at appropriate exposure, which was saved as .jpeg format for scoring.

3.6 MOLECULAR DATA ANALYSIS

3.6.1 Scoring of bands

Scoring was done in binary scoring format by assigning "0" for the absence of bands and "1" for the presence of required bands. For this the gel image of resolved PCR products with reproducible and clear bands was selected.

3.6.2 Polymorphic marker ratio

Based on the scoring of obtained data, polymorphic marker ratio can be calculated. Accordingly, a band is called polymorphic, when a particular band is present in one genotype and absent in any of the other genotypes.

Percentage of polymorphism is calculated based on the equation given below:

% of polymorphism = (number of polymorphic bands/ total number of bands)*100

3.6.3 Cluster analysis

Based on the above obtained information, a dendrogram was generated which helped in grouping the 15 genotypes based on jacquards similarity coefficient using UPGMA (Unweighted Pair Group Method with Arithmetic average) (Sneath and Sokal, 1973) with NTSYS-PC (Numerical Taxonomy and Multivariate Analysis System, Biostatistic, New York, U. S. A., Software version 2.02 package) (Rohlf, 1998). Pairwise distance (similarity) matrices were generated using sequential, agglomerative, hierarchical and nested (SAHN) clustering option of the NTSYS-PC, which is used to calculate the diversity and similarity between every two genotypes.

RESULTS

4 RESULTS

The results obtained on the study entitled "Genetic diversity analysis of gladiolus genotypes (*Gladiolus grandiflorus* l.) using molecular markers" carried out at the Department of Plant Biotechnology and Department of Plant Breeding and Genetics, College of Agriculture, Vellayani, Thiruvananthapuram during 2020- 2021 are described in this chapter.

4.1 MORPHOLOGICAL CHARACTERIZATION

Fifteen genotypes of gladiolus were selected based on their commercial importance. The selected genotypes included 12 genotypes from IIHR, Bangalore, Karnataka and 3 genotypes from NBRI, Lucknow, Uttar Pradesh. These varieties were evaluated in completely randomized design, with two replication each. The vegetative and floral characters recorded among the genotypes were subjected to analysis of variance (Table 6) and significant variations were observed. The mean performances of the 15 genotypes for vegetative and floral characters studied are represented in Tables 4, 5a, 5b and 5c. Graphs showing mean performance of gladiolus genotypes shown in Figure 1. Single flower/floret and inflorescence of the Gladiolus genotypes used in the study are shown in Plates 2.1, 2.2, 3.1, 3.2 and 3.3.

4.1.1 Vegetative characters

4.1.1.1 Plant height (cm)

Plant height was significantly high in G15 (72.52 cm) followed by G8 (72.05 cm) and G1 (71.59 cm). The mean height was recorded the lowest in G6 (53.815 cm).

4.1.1.2 Number of leaves per shoot

The maximum number of leaves per plant was recorded for G8 (9.39 cm). For G9 (7.38), also comparatively higher number of leaves were recorded. This was on par with G15 (6.725) and G2 (6.5). The lowest value for this character was for G14 (5.245).

4.1.1.3 Length of the leaf (cm)

The length of leaves was found to be the highest for G5 (70.64 cm) and the minimum value was recorded for G10 (50.845 cm) which was statistically on par with G9 (50.85 cm) and G15 (50.8 cm).

4.1.1.4 Width of the leaf (cm)

The highest values for the width of leaves were shown by G15 (4.665 cm) which was on par with G14 (4.46 cm) and G13 (4.44 cm). The minimum value for this trait was for genotype G2 (1,995 cm).

4.1.1.5 Internode length (cm)

Internode length showed significant variations among the 15 genotypes. The highest values were observed for G1 (14.05 cm). The lowest values were recorded for G13 (6.64 cm).

4.1.2 Floral characters

4.1.2.1 Flowering nature- free flowering/seasonal

Seasonal flowering was recorded among all 15 genotypes used in the study.

4.1.2.2 Number of days taken for initiation of flower spike

Genotype G3 took 53.515 days for the initiation of the flower spike. Maximum days for initiation of flower spike were for G13 (83.475), followed by G1 (75.08) and G10 (73.085).

4.1.2.3 Number of days taken for full emergence of spike

The number of days taken for full emergence of the spike was highest for G13 (90.78) and lowest value was recorded for G3 (60.515).

4.1.2.4 Length of the floret (cm)

Length of the floret was recorded highest for G1 (10.005 cm), followed by G15 (9.125 cm). Genotype G3 (7.45 cm) was recorded with the lowest value for the length of floret which was at par with G10 (7.77 cm) and G13 (7.78 cm).

ARKA DARSHAN

ARKA SAPNA

ARKA NAVEEN

ARKA AMAR

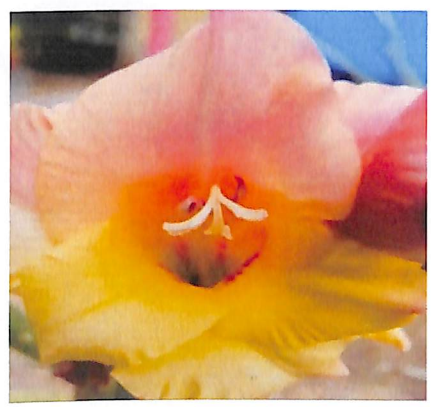
ARKA KESAR

Plate 2.1: Single flower/floret of different genotypes of Gladiolus used in the study

ARKA KESAR

ARKA KUMKUM

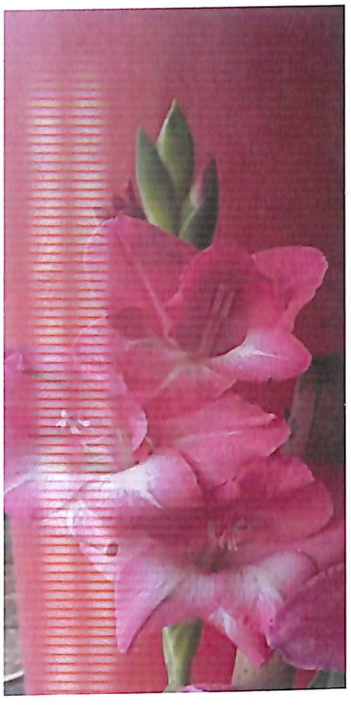
ARKA POONAM



ARKA GOLD

ARKA NAZRANA

Plate 2.2: Single flower/floret of different genotypes of Gladiolus used in the study



ARKA DARSHAN

ARKA SAPNA

ARKA NAVEEN

ARKA AMAR

Plate 3.1: Inflorescence of the Gladiolus genotypes used in the study

ARKA AAYUSH

ARKA KESAR

ARKA KUMKUM

ARKA POONAM

Plate 3.2: Inflorescence of the Gladiolus genotypes used in the study

ARKA GOLD

ARKA NAZRANA

Plate 3.3: Inflorescence of the Gladiolus genotypes used in the study

4.1.2.5 Diameter of the floret (cm)

The highest value for diameter of the floret was for G4 (7.695 cm) which was on par with G15 (7.615 cm). The minimum value for this trait was for G13 (5.215 cm) followed by G3 (5.245 cm), G8 (5.27 cm) and G10 (5.63 cm).

4.1.2.6 Inflorescence orientation

The inflorescence orientation was recorded either as one row, two row, zig zag or irregular.

4.1.2.7 Number of florets open at one time

The number of florets open at one time was recorded maximum for G3 (5.16) followed by G1 (4.285). The minimum value was noted for G8 (2.84).

4.1.2.8 Flower length (cm)

Flower length in centimeters was the highest for G11 (58.875 cm) which was statistically on par with G15 (58.4 cm). The lowest value was recorded for G8 (44.415 cm).

4.1.2.9 Flower fragrance

All the fifteen genotypes used in this study had no fragrance.

4.1.2.10 Flower width in front view

Flower width was recorded the highest for G1 (11.07 cm). The lowest value was observed in G8 (7.15 cm) which was on par with G3 (7.25 cm) and G10 (7.955 cm).

4.1.2.11 Predominant colour in the flower

All genotypes could be easily discriminated on the basis of flower colour. Flower colour as per the visual perception is presented in Table 5a.

4.1.2.12 Shape of flower

The shape of flower was recorded either as star shaped, round shaped or triangular shaped.

4.1.2.13 Number of florets per spike

The number of florets per spike was counted the maximum for G6 (9.775) which was on par with G15 (9.77) and G3 (9.2). The minimum number of florets per spike was for G8 (4.46).

4.1.2.14 Field life

Field life was recorded the maximum for G15 and G4, ie. , 15.435 and 15.06 days respectively. The lowest field life was noted for G3 (9.7) which was on par with G8 (9.94).

Being a commercial cut flower crop and based on floral parameters, 'Arka Sapna' exhibited early initiation of flower spike and maximum number of florets open at one time, 'Arka Nazrana' recorded the highest filed life, 'Arka Darshan' exhibited the maximum length of floret and flower width in front view, 'Arka Amar' recorded the maximum value for number of florets per spike and 'Arka Poonam' recorded the highest flower length.

Table 4a. Mean performance of 15 gladiolus genotypes for vegetative characters

Genotypes	Plant height (cm)	Number of leaves per shoot	Length of the leaf (cm)	Width of the leaf (cm)	Internode length (cm)
G1	71.59	5.775	66.85	2.365	**14.05**
G2	62.775	6.5	56.875	**1.995**	9.5
G3	57.085	6.35	58.675	2.195	9.25
G4	60.3	6.365	56.135	2.06	8.25
G5	**72.52**	6.235	**70.64**	2.125	9.1
G6	**53.815**	5.94	53.5	2.21	10.555
G7	64.575	5.675	58.55	2.39	11.89
G8	72.05	**9.39**	68.78	3.38	7.75
G9	55.435	7.38	50.85	3.43	13.25
G10	55.46	6.245	50.845	2.09	8.34
G11	63.15	5.64	63.5	2.245	7.285
G12	57.235	5.475	53.4	3.65	9.15
G13	62.79	6.275	60.25	4.44	**6.64**
G14	56.52	**5.245**	56.6	4.46	9.05
G15	55.8	6.725	**50.8**	**4.665**	9.31
CD (0.05)	3.311	1.178	3.077	0.936	1.072
SE_M	1.091	0.388	1.014	0.308	0.353

Table 5a. Mean performance of gladiolus genotypes for floral characters

Genotypes	Flowering nature – free flowering /seasonal	Inflorescence orientation	Flower fragrance	Predominant colour in the flower	Shape of flower
G1	Seasonal	Zig zag	Nil	Pink colour with white central linings	Star
G3	Seasonal	Two row	Nil	Whitish pink colour with light green color in the inner tip	Round
G4	Seasonal	One row	Nil	Light violet colour with whitish central shade	Star
G6	Seasonal	Two row	Nil	Red colour flower with golden yellow colour shade in central portion	Triangular
G7	Seasonal	One row	Nil	Orange colour with yellow shade and red markings	Star
G8	Seasonal	Irregular	Nil	Yellow colour	Triangular
G10	Seasonal	Zig zag	Nil	Red colour with light whitish central shade	Round
G11	Seasonal	One row	Nil	White colour with light green shades	Round
G13	Seasonal	Irregular	Nil	Yellow colour in bottom three petals and pinkish red color on other petals	Star
G15	Seasonal	Zig zag	Nil	Purple colour with golden yellow and white shades	Triangular

Table 5b. Mean performance of gladiolus genotypes for floral characters

Genotypes	Number of days taken for initiation of flower spike	Number of days taken for full emergence of spike	Length of the floret (cm)	Diameter of the floret (cm)	Number of florets open at one time
G1	75.08	81.225	**10.005**	6.38	4.285
G3	**53.515**	**60.515**	**7.45**	5.245	**5.16**
G4	62.8	68.715	8.77	**7.695**	3.785
G6	66.115	76.04	8.525	6.365	4.105
G7	65.565	70.12	8.555	6.05	3.505
G8	59.025	65.73	8.35	5.27	**2.84**
G10	73.085	79.615	7.77	5.63	3.165
G11	66.425	72.13	8.675	6.765	4.09
G13	**83.475**	**90.78**	7.78	**5.215**	3.165
G15	63.17	71.205	9.125	7.615	4.235
CD (0.05)	2.150	2.593	0.442	0.332	0.370
SE_M	0.709	0.855	0.145	0.109	0.122

Table 5c. Mean performance of gladiolus genotypes for floral characters

Genotypes	Flower length (cm)	Flower width in front view	Number of florets per spike	Field life
G1	54.275	11.07	8.79	10.065
G3	53.06	7.25	9.2	9.7
G4	56.625	9.685	6.09	15.06
G6	51.035	8.285	9.775	12.335
G7	56.625	8.45	7.09	12.35
G8	44.415	7.15	4.46	9.94
G10	54.3	7.955	5.745	10.435
G11	58.875	9.475	8.08	14.665
G13	54	8.205	5.165	10.885
G15	58.4	9.875	9.77	15.435
CD (0.05)	1.056	0.859	0.419	0.822
SE_M	0.348	0.283	0.138	0.271

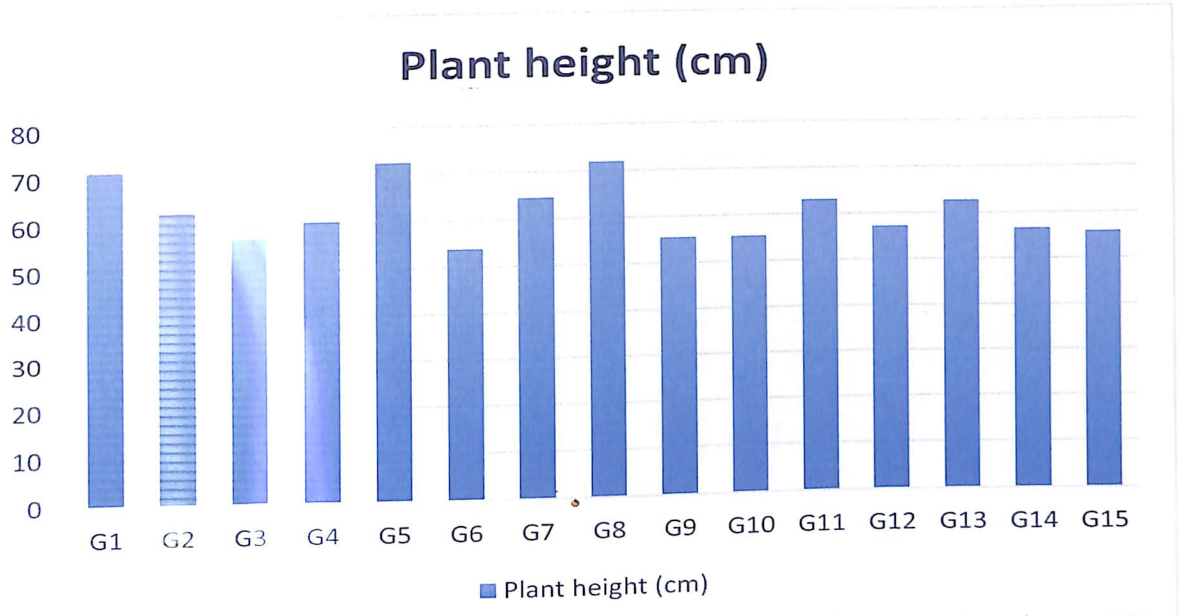


Figure 1 a. Mean performance of Gladiolus genotypes

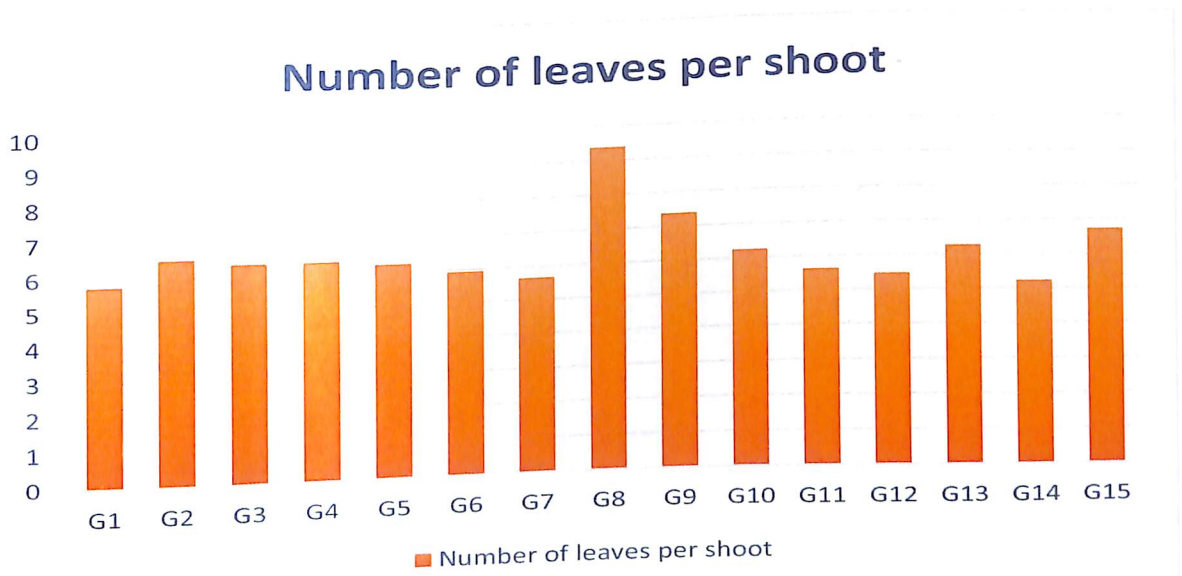


Figure 1 b. Mean performance of Gladiolus genotypes

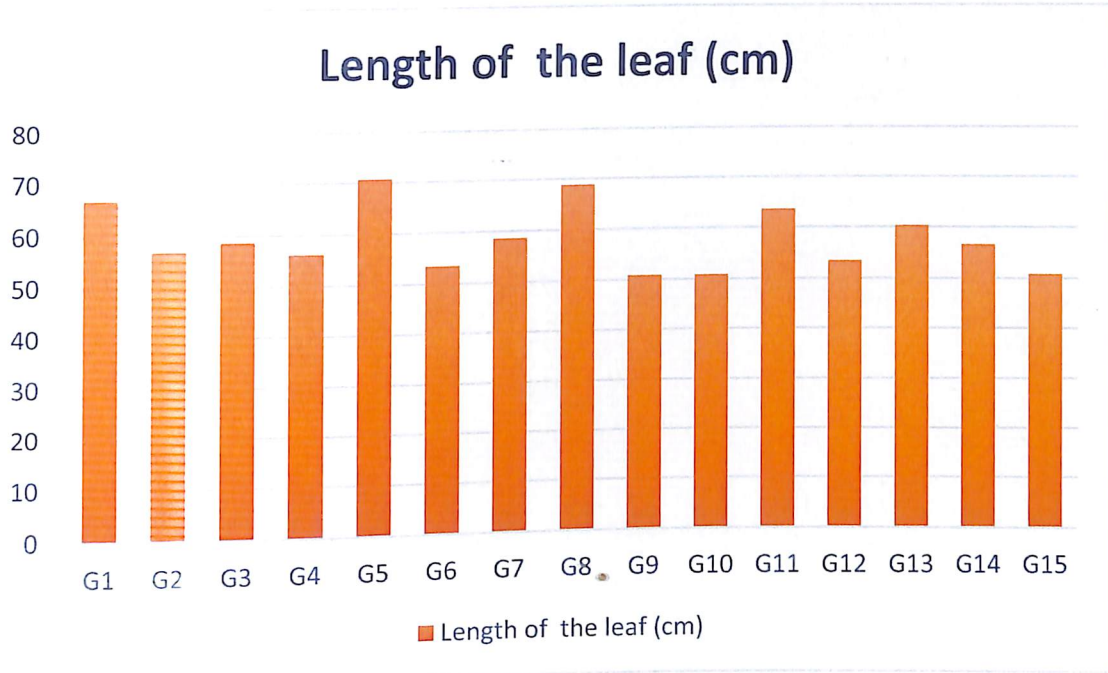


Figure 1 c. Mean performance of Gladiolus genotypes

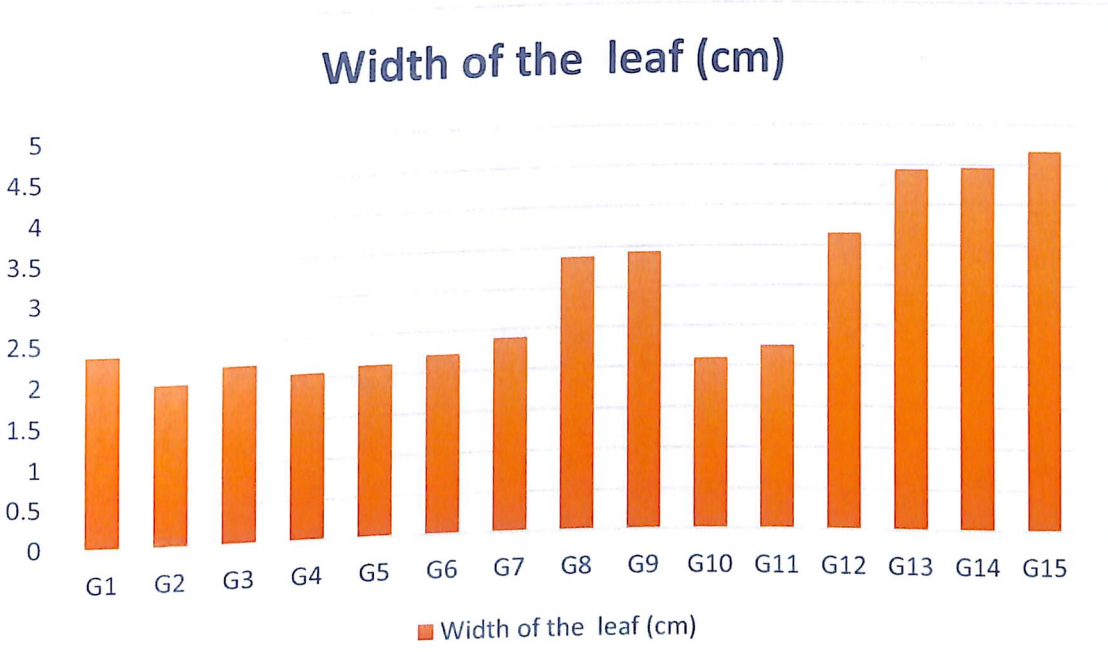


Figure 1 d. Mean performance of Gladiolus genotypes

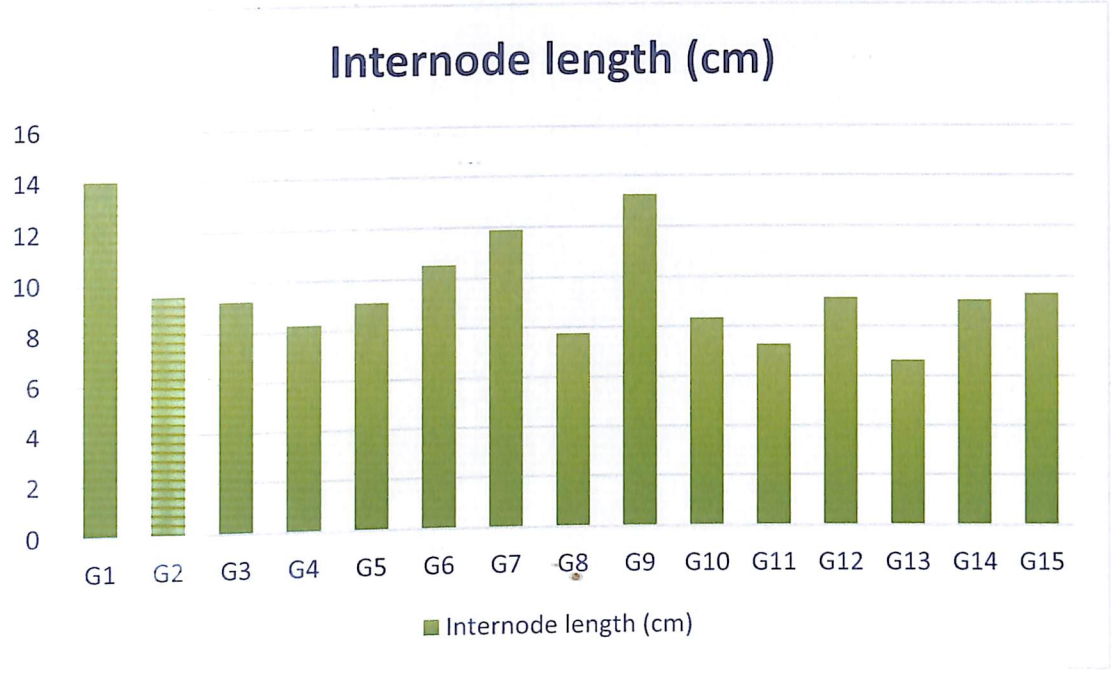


Figure 1 e. Mean performance of Gladiolus genotypes

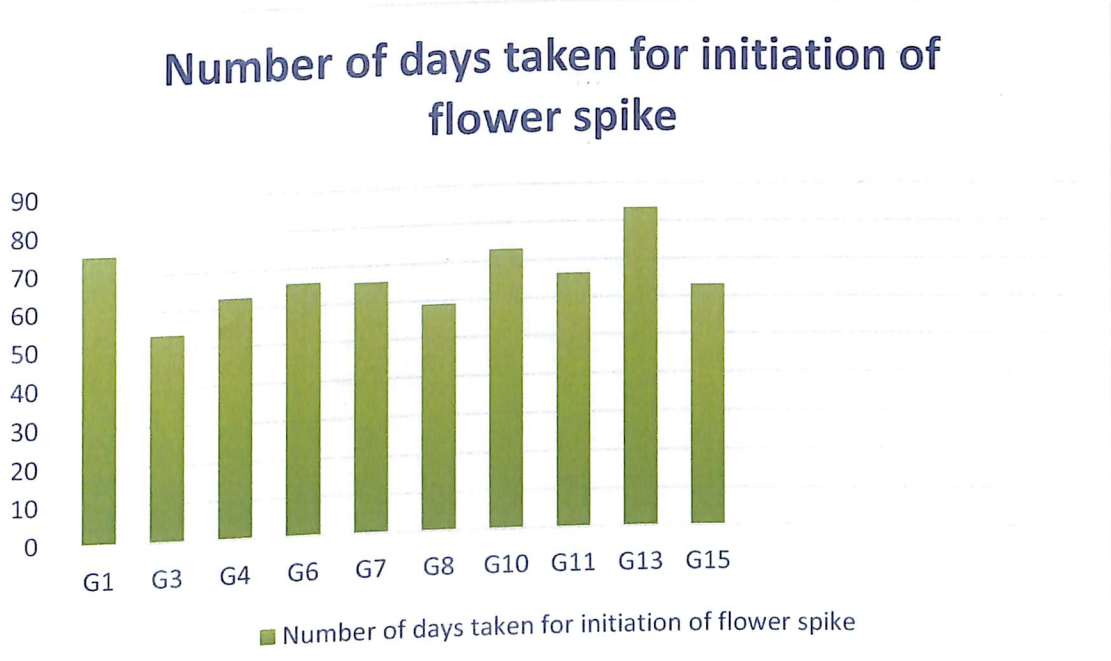


Figure 1 f. Mean performance of Gladiolus genotypes

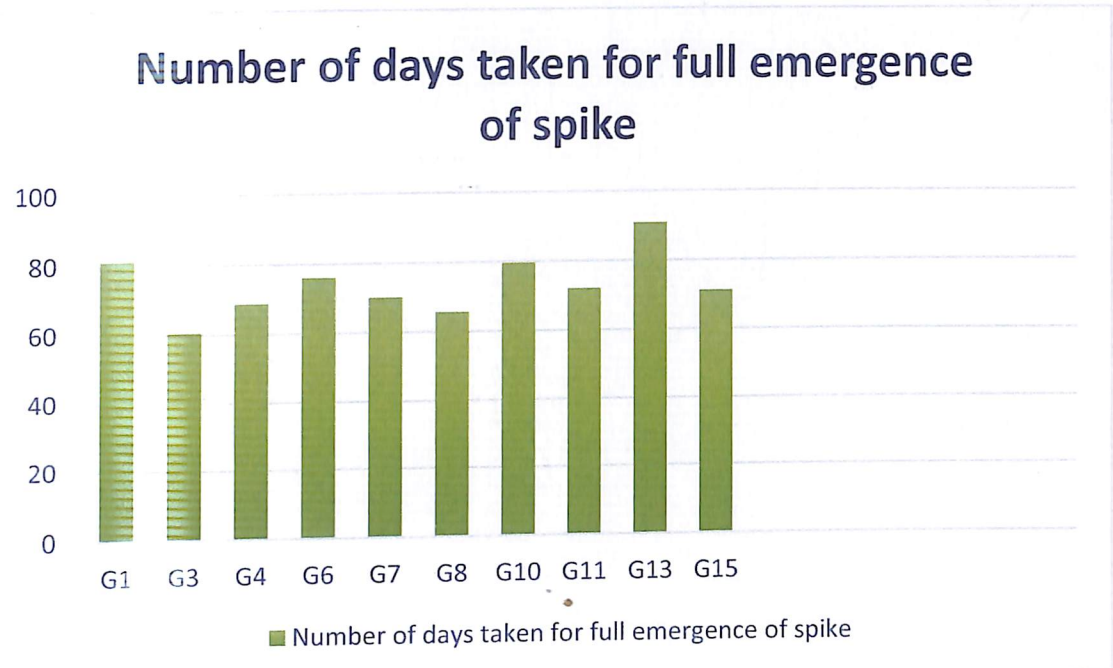


Figure 1 g. Mean performance of Gladiolus genotypes

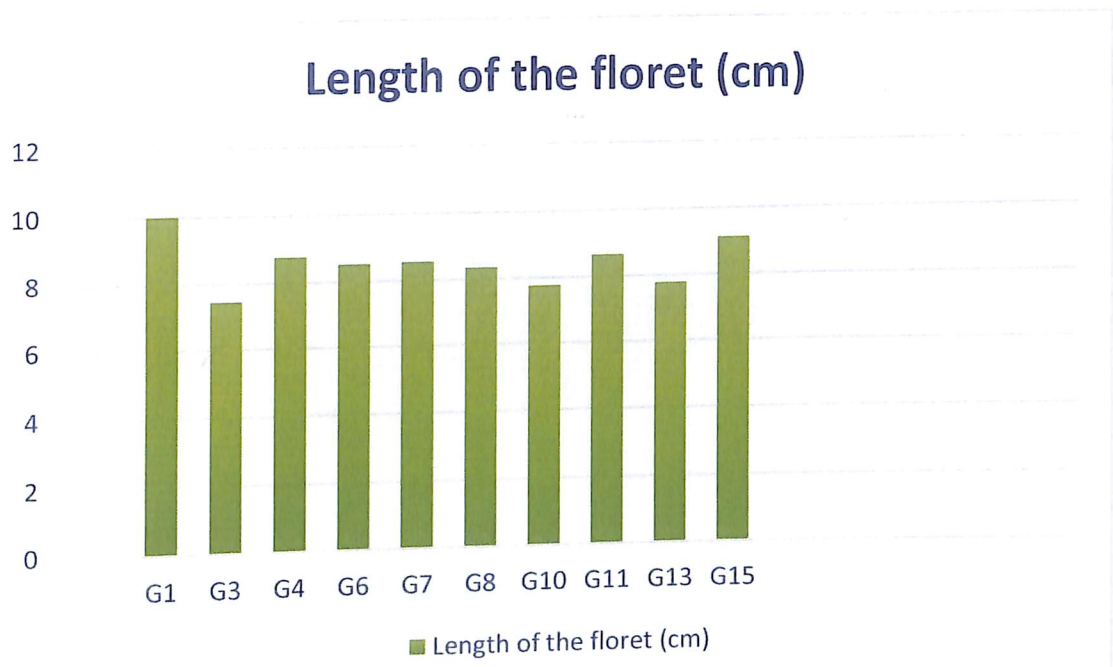


Figure 1 h. Mean performance of Gladiolus genotypes

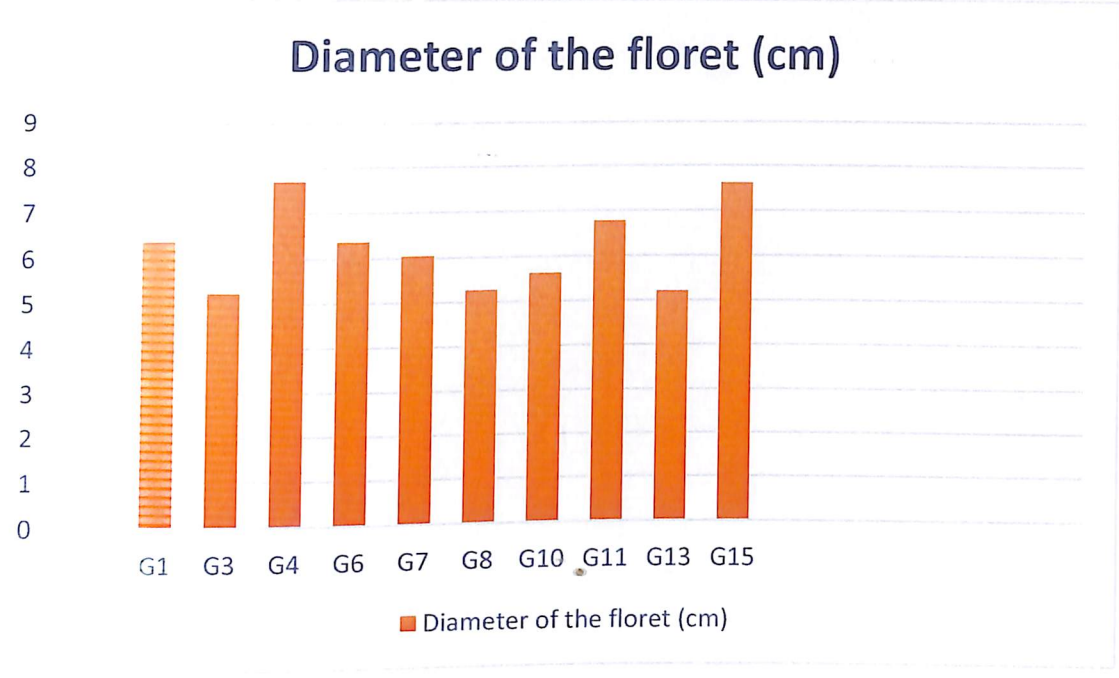


Figure 1 i. Mean performance of Gladiolus genotypes

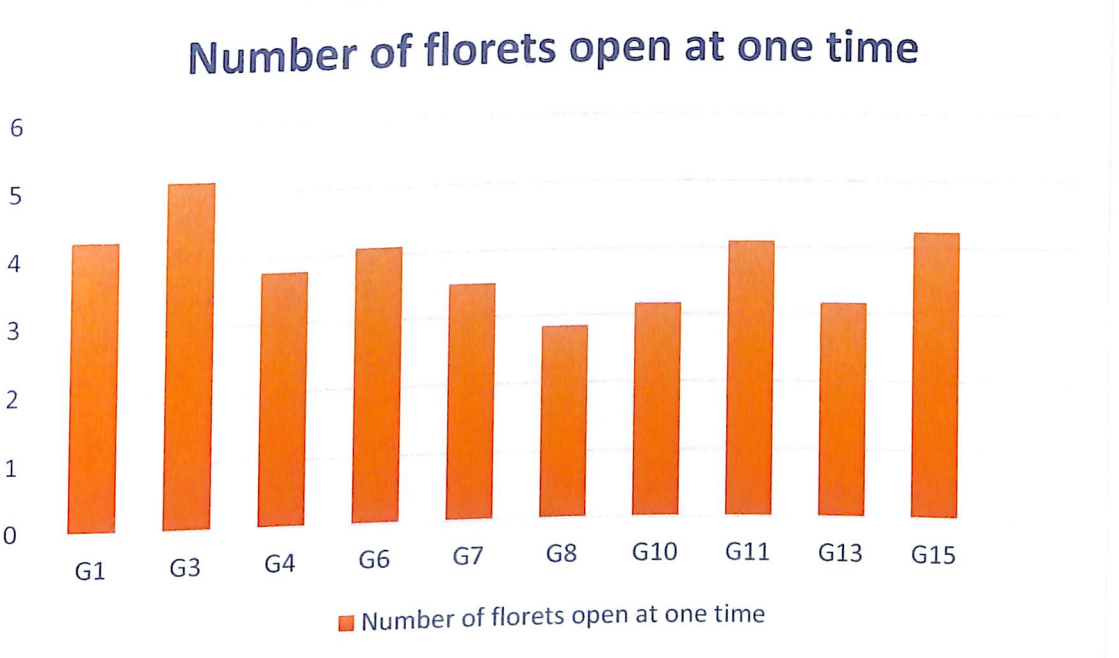


Figure 1 j. Mean performance of Gladiolus genotypes

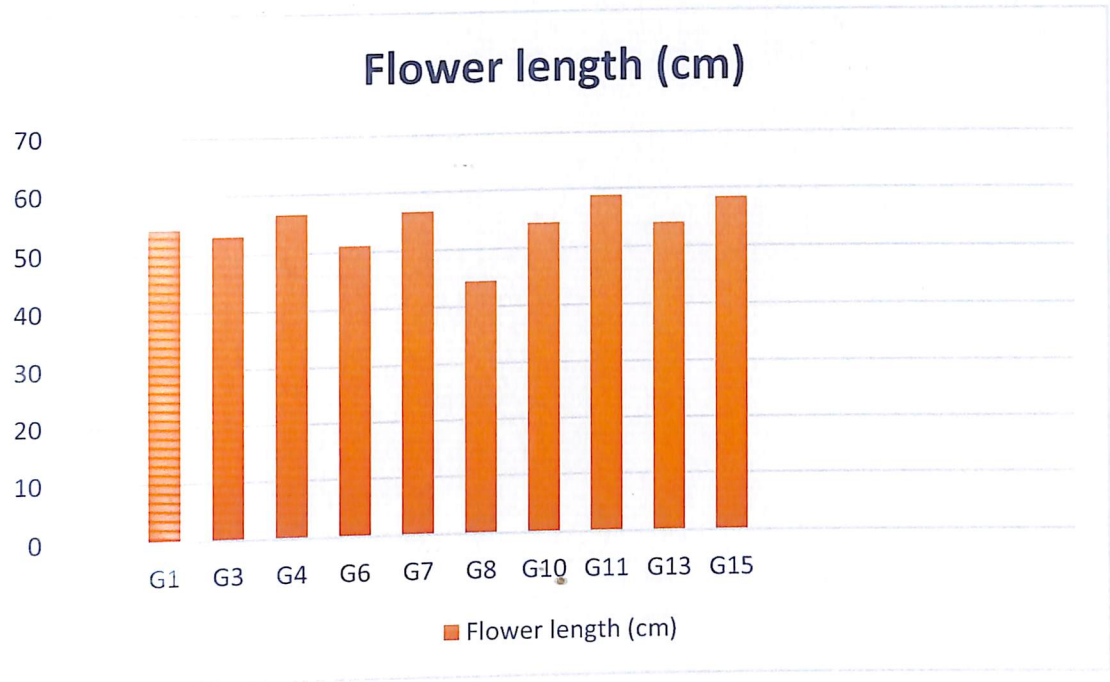


Figure 1 k. Mean performance of Gladiolus genotypes

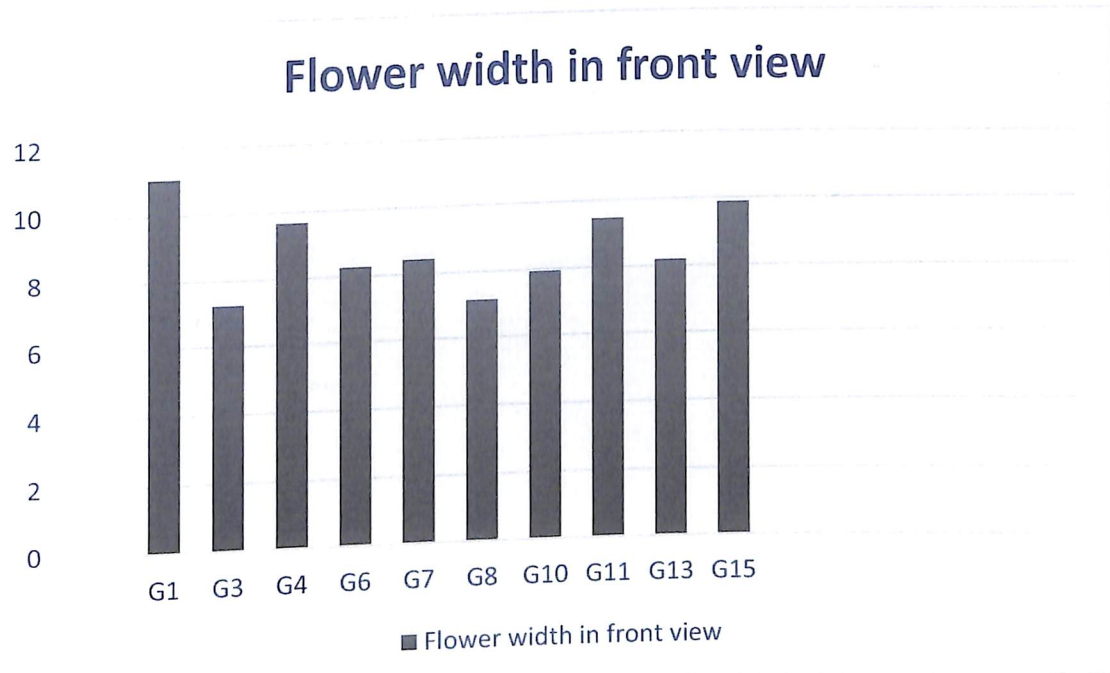


Figure 1 l. Mean performance of Gladiolus genotypes

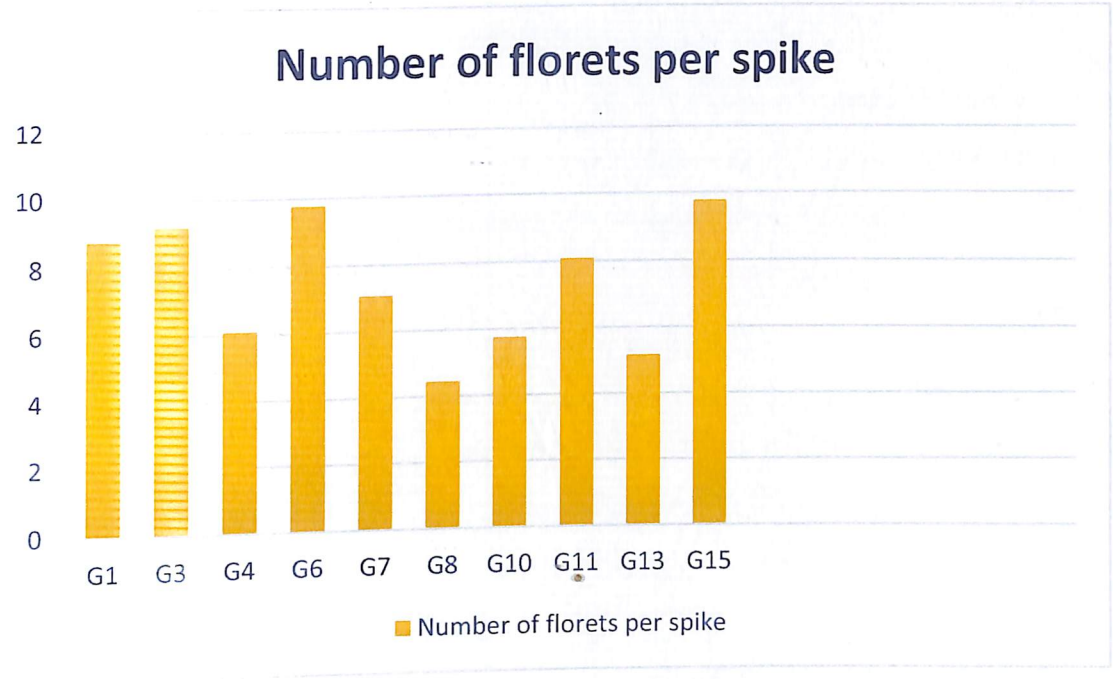


Figure 1 m. Mean performance of Gladiolus genotypes

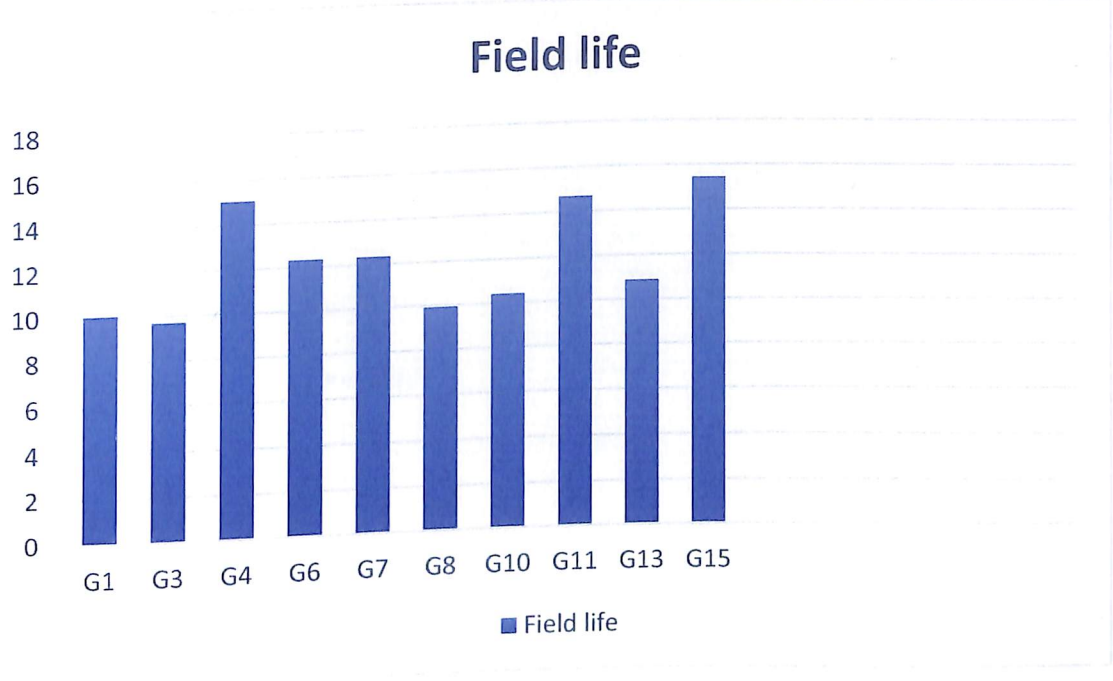


Figure 1 n. Mean performance of Gladiolus genotypes

4.1.3 Estimation of variability components *i.e.*, PCV and GCV

For analysing the variability among the 15 gladiolus genotypes, the three types of variances i.e., genotypic, phenotypic and environmental variances were estimated along with the coefficient of variation in genotypic and phenotypic levels (Table 6 and 7; Figure 2.).

The vegetative characters internode length (GCV= 21.55, PCV= 22.18%), number of leaves per shoot (GCV= 14.48, PCV= 16.87), length of the leaf (GCV= 10.83, PCV= 11.10), plant height (GCV= 10.32, PCV= 10.62) and width of the leaf (GCV= 9.27, PCV= 14.32) exhibited the highest values for genotypic and phenotypic coefficient of variation in the decreasing order.

Considering the floral characters, number of florets per spike (GCV= 79.84, PCV= 79.94), field life (GCV=76.45, PCV= 76.60), Number of florets open at one time (GCV= 76.15, PCV= 76.45), Diameter of the floret (GCV= 75.30, PCV= 75.39), Flower width in front view (GCV= 75.03, PCV= 75.34), Number of days taken for initiation of flower spike (GCV= 74.77, PCV= 74.80), Number of days taken for full emergence of spike (GCV= 74.52, PCV= 74.56), Length of the floret (GCV= 73.90, PCV= 73.98) and flower length (GCV= 73.78, PCV=73.79 %) in the decreasing order showed the highest estimates for genotypic and phenotypic coefficient of variation in the decreasing order.

4.1.4. Estimation of heritability and genetic advance

Values of heritability in broad sense and genetic advance (% mean) in the gladiolus genotypes are calculated (Table 7; Figure 3).

Allard (1960) categorized heritability per cent as low - <30%, moderate – 30 to 70 % and high - >70%. On the basis of that all the vegetative characters and floral characters considered in this study, except width of the leaf (heritability= 41.97) exhibited high heritability (>70%).

Based on the study conducted by Robinson *et al.* (1949), traits with values greater than 20% showed to have high genetic advances. The vegetative and floral characters considered under this study showed a wide range of values in genetic

advances. Most of the characters exhibited high genetic advance. Width of the leaf (12.38%) followed by plant height (20.65%) and length of the leaf (21.75%) recorded the lowest values for genetic advance among the traits studied.

The characters under study like plant height, number of leaves per shoot, length of the leaf, internode length, number of days taken for initiation of flower spike, number of days taken for full emergence of spike, length of the floret, diameter of the floret, number of florets open at one time, flower length, flower width in front view, number of florets per spike and field life exhibited high heritability (>70%) together with high genetic advance (>20%).

Table 6. GCV and PCV of the characters under study.

Sl No.	Characters	Genotypic Coefficient of Variation (GCV)	Phenotypic Coefficient of Variation (PCV)
1.	Plant height (cm)	10.32	10.62
2.	Number of leaves per shoot	14.48	16.87
3.	Length of the leaf (cm)	10.83	11.10
4.	Width of the leaf (cm)	9.27	14.32
5.	Internode length (cm)	**21.55**	**22.18**
6.	Number of days taken for initiation of flower spike	74.77	74.80
7.	Number of days taken for full emergence of spike	74.52	74.56
8.	Length of the floret (cm)	73.90	73.98
9.	Diameter of the floret (cm)	75.30	75.39
10.	Number of florets open at one time	76.15	76.45
11.	Flower length (cm)	73.78	73.79
12.	Flower width in front view	75.03	75.34
13.	Number of florets per spike	**79.84**	**79.94**
14.	Field life	76.45	76.60

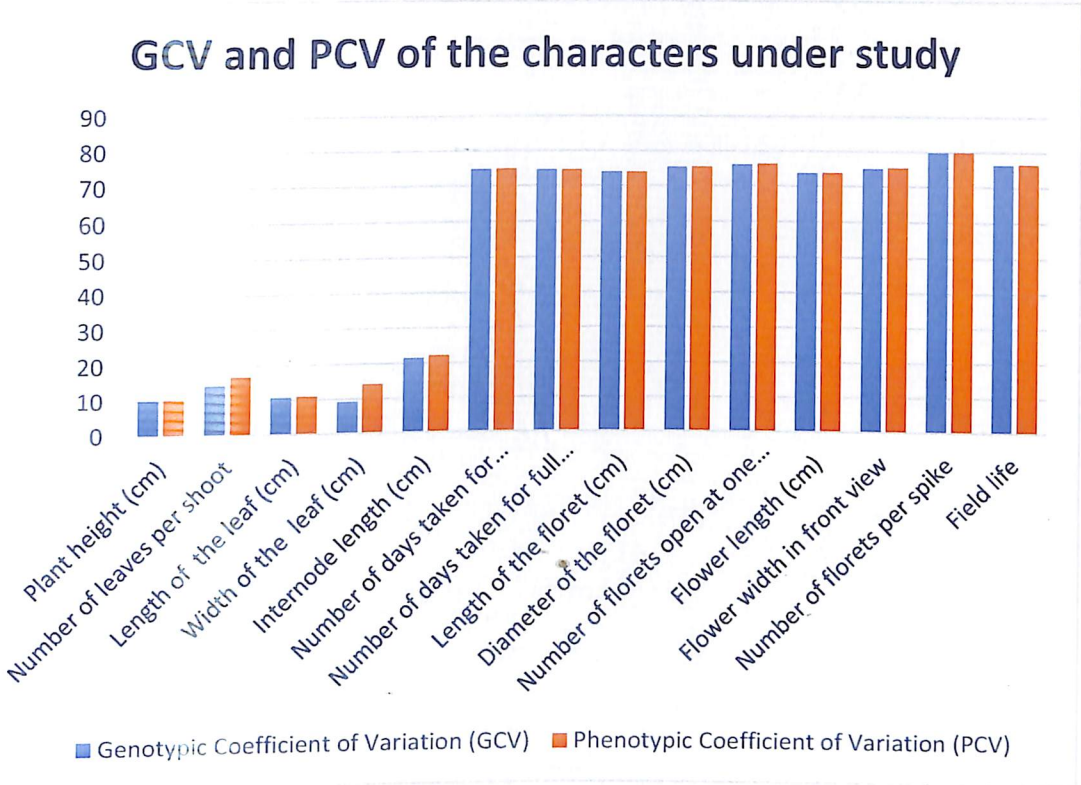


Figure 2. GCV and PCV of the characters under study

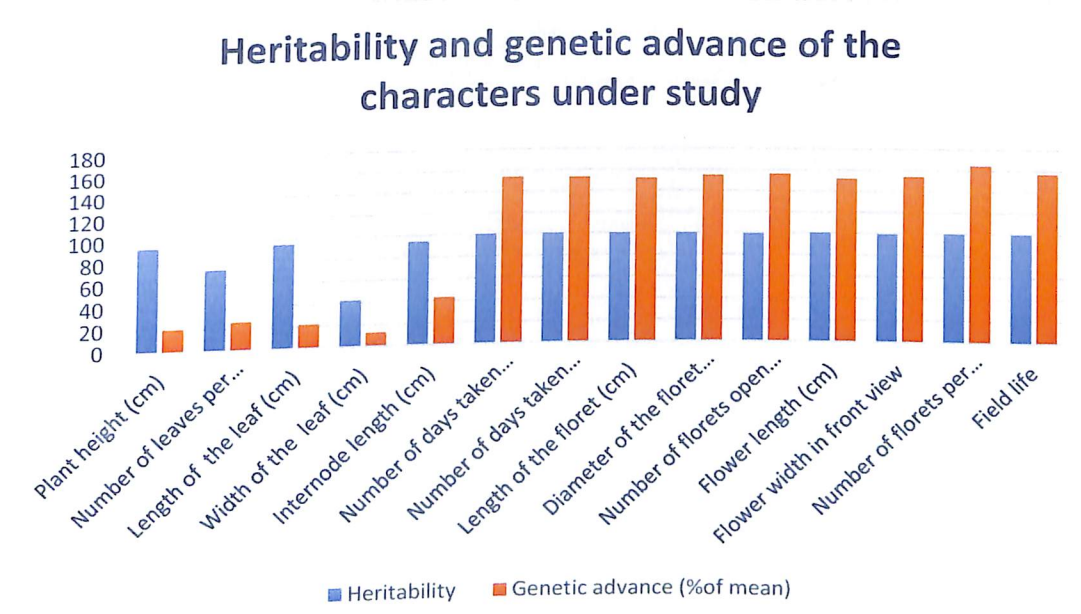


Figure 3. Heritability and genetic advance of the characters under study

Table 7. Variances, heritability and genetic advance of the characters under study.

Sl No.	Characters	Genotypic variance	Phenotypic variance	Environmental variance	Heritability	Genetic advance (%of mean)
1.	Plant height (cm)	40.15	42.54	2.38	94.40	20.65
2.	Number of leaves per shoot	0.84	1.15	0.30	73.68	25.60
3.	Length of the leaf (cm)	40.09	42.14	2.06	95.11	21.75
4.	Width of the leaf (cm)	0.138	0.33	0.2	41.97	12.38
5.	Internode length (cm)	4.25	4.50	0.25	94.44	43.15
6.	Number of days taken for initiation of flower spike	1109.49	1110.50	1.00	99.91	153.95
7.	Number of days taken for full emergence of spike	1337.32	1338.77	1.46	99.89	153.43
8.	Length of the floret (cm)	17.54	17.58	0.04	99.76	152.04
9.	Diameter of the floret (cm)	9.76	9.78	0.02	99.75	154.92
10.	Number of florets open at one time	3.79	3.82	0.03	99.22	156.26
11.	Flower length (cm)	709.36	709.60	0.24	99.97	151.95
12.	Flower width in front view	19.11	19.27	0.16	99.17	153.92
13.	Number of florets per spike	15.58	15.62	0.03	99.75	164.27
14.	Field life	37.95	38.10	0.14	99.61	157.19

4.1.5 Correlation analysis

Among the various characters under the study, fourteen characters were selected for the genotypic and phenotypic correlation studies. The fourteen characters includes plant height (cm), number of leaves per shoot, length of the leaf (cm), width of the leaf (cm), internode length (cm), number of days taken for initiation of flower spike, number of days taken for full emergence of the spike, length of the floret (cm), the diameter of the floret (cm), number of florets open at one time, flower length (cm), flower width in front view, number of florets per spike and field life. The significance of genotypic and phenotypic correlations were tested and showed in the Tables 8a and 8b.

High positive correlations at genotypic and phenotypic levels were seen in many vegetative and floral characters. Highly significant positive correlations among vegetative characters were exhibited by length of the leaf with plant height (r_g= 0.9676, r_p= 0.8971).

Among floral characters showed highly significant positive correlations with each other floral characters such as number of days taken for full emergence of spike with number of days taken for initiation of flower spike (r_g= 0.9994, r_p= 0.9992), length of the floret with number of days taken for initiation of flower spike (r_g= 0.9737, r_p= 0.9717), diameter of the floret with number of days taken for initiation of flower spike (r_g= 0.9445, r_p= 0.9434), number of florets open at one time with number of days taken for initiation of flower spike (r_g= 0.9153, r_p= 0.9125), flower length with number of days taken for initiation of flower spike (r_g= 0.976, r_p= 0.9755), flower width in front view with number of days taken for initiation of flower spike (r_g= 0.97, r_p= 0.9649), number of florets per spike with number of days taken for initiation of flower spike (r_g= 0.8746, r_p= 0.8731) and field life with number of days taken for initiation of flower spike (r_g= 0.9284, r_p= 0.9268).

Table 8a. Genotypic correlation of the selected traits under study

	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13	X14
X1	1.00													
X2	0.3009 NS	1.00												
X3	0.9676 **	0.2272 NS	1.00											
X4	-0.3684 NS	-0.1613 NS	-0.166 NS	1.00										
X5	0.0396 NS	-0.1378 NS	-0.1062 NS	-0.3814 NS	1.00									
X6	0.082 NS	0.068 NS	0.0922 NS	0.249 NS	-0.1556 NS	1.00								
X7	0.0663 NS	0.0799 NS	0.0831 NS	0.2543 NS	-0.16 NS	0.9994 **	1.00							
X8	0.106 NS	0.1221 NS	0.1245 NS	0.2444 NS	-0.0792 NS	0.9737 **	0.976 **	1.00						
X9	0.0169 NS	0.0711 NS	0.0294 NS	0.2739 NS	-0.1276 NS	0.9445 **	0.9485 **	0.9842 **	1.00					
X10	-0.0133 NS	0.0205 NS	0.0612 NS	0.2984 NS	-0.0634 NS	0.9153 **	0.9225 **	0.9586 **	0.9505 **	1.00				
X11	0.022 NS	0.0501 NS	0.0531 NS	0.2679 NS	-0.1519 NS	0.976 **	0.9774 **	0.9871 **	0.983 **	0.9662 **	1.00			
X12	0.0951 NS	0.0478 NS	0.1064 NS	0.2432 NS	-0.0615 NS	0.97 **	0.9701 **	0.9952 **	0.9889 **	0.9543 **	0.9847 **	1.00		
X13	-0.086 NS	-0.0627 NS	-0.0027 NS	0.2849 NS	0.0346 NS	0.8746 **	0.8846 **	0.9285 **	0.9304 **	0.98 **	0.9289 **	0.9307 **	1.00	
X14	-0.0193 NS	0.0586 NS	0.0041 NS	0.3296 NS	-0.2001 NS	0.9284 **	0.9325 **	0.9633 **	0.9917 **	0.9287 **	0.9745 **	0.9669 **	0.9106 **	1.00

X1-Plant height, X2-Number of leaves per shoot, X3-Length of leaf, X4-Width of leaf, X5-Internode length, X6- Number of days taken for initiation of flower spike, X7- Number of days taken for full emergence of spike, X8- Length of the floret, X9- Diameter of the floret, X10- Number of flowers open at one time, X11- Flower length, X12- Flower width in front view, X13- Number of florets per spike, X14- Field life. (** significant at 0.05 and * significant at 0.01 levels)

Table 8b. Phenotypic correlation of the selected traits under study

	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13	X14
X1	1.00													
X2	0.2921 NS	1.00												
X3	0.8971 **	0.224 NS	1.00											
X4	-0.237 NS	-0.0429 NS	-0.1362 NS	1.00										
X5	0.0319 NS	-0.102 NS	-0.0975 NS	-0.1325 NS	1.00									
X6	0.0791 NS	0.061 NS	0.0913 NS	0.1626 NS	-0.1492 NS	1.00								
X7	0.0637 NS	0.0681 NS	0.082 NS	0.1725 NS	-0.1515 NS	0.9992 **	1.00							
X8	0.1056 NS	0.1028 NS	0.1181 NS	0.1645 NS	-0.0776 NS	0.9717 **	0.9739 **	1.00						
X9	0.0092 NS	0.0651 NS	0.0319 NS	0.1883 NS	-0.1225 NS	0.9434 **	0.947 **	0.9818 **	1.00					
X10	-0.0284 NS	0.0074 NS	0.0603 NS	0.1924 NS	-0.0627 NS	0.9125 **	0.9193 **	0.9523 **	0.9485 **	1.00				
X11	0.0216 NS	0.042 NS	0.0502 NS	0.1766 NS	-0.1469 NS	0.9755 **	0.9771 **	0.9856 **	0.9815 **	0.9626 **	1.00			
X12	0.0876 NS	0.0231 NS	0.0948 NS	0.1668 NS	-0.0595 NS	0.9649 **	0.9659 **	0.9891 **	0.9826 **	0.9484 **	0.9814 **	1.00		
X13	-0.0765 NS	-0.0522 NS	-0.0018 NS	0.183 NS	0.0353 NS	0.8731 **	0.8834 **	0.9264 **	0.9263 **	0.9719 **	0.9277 **	0.9248 **	1.00	
X14	-0.0259 NS	0.0565 NS	0.005 NS	0.2077 NS	-0.1987 NS	0.9268 **	0.9302 **	0.9591 **	0.9901 **	0.9275 **	0.9726 **	0.9613 **	0.906 **	1.00

X1-Plant height, X2-Number of leaves per shoot, X3-Length of leaf, X4-Width of leaf, X5-Internode length, X6- Number of days taken for initiation of flower spike, X7- Number of days taken for full emergence of spike, X8- Length of the floret, X9- Diameter of the floret, X10- Number of flowers open at one time, X11- Flower length, X12- Flower width in front view, X13- Number of florets per spike, X14- Field life. (** significant at 0.05 and * significant at 0.01 levels)

4.1.6 Principal Component Analysis (PCA)

Principal component analysis was carried out by considering 14 quantitative characters used in the study. This was done to figure out the important characters which contribute to the separation of genotypes in the dendrogram. The Eigen values were recorded the highest values for the first two principal components which correspond to 7.514 and 2.517 Eigen values respectively (Table 9). The first three principal components accounted for 84.312% of the variability among the traits studied.

PC1 accounted for 53.669 % of the variation, which had the number of days taken for initiation of the flower spike, number of days taken for full emergence of spike, length of the floret, width of floret, flower length, flower width in front view, number of florets per spike and field life with high values. The second principal component composed of 17.981% of the variation which includes plant height, length of leaf, width of leaf, internode length, number of florets open at one time, flower length and number of florets per spike. The third principal component represents 12.662% of the variation. This includes characters such as plant height, number of leaves per shoot, length of leaf, width of leaf, internode length, number of florets open at one time and number of florets per spike with high values. The fourth principal component constituted 6.919 % of the variation that includes the number of leaves per shoot, width of leaf, internode length and number of florets open at one time. The fifth principal component has 5.512 % of the variation and the sixth principal component accounted for 1.426% of the variation.

The traits included in PC1, PC2 and PC3 was the number of florets per spike. Plant height, length of leaf, width of leaf, internode length, number of florets open at one time and number of florets per spike was the characters included in PC2 and PC3. Width of leaf, internode length and number of florets open at one time was included in PC2, PC3, PC4, PC5 and PC6. The number of leaves per shoot was included in PC3, PC4, PC5 and PC6. Hence plant height, number of leaves per shoot length of leaf, width of leaf, internode length,

number of florets open at one time and number of florets per spike was significant in distinguishing the genotype.

4.1.7 Similarity matrix and dendrogram of morphological characters

Relationships between the 14 morphological characters of 15 gladiolus varieties were analysed by similarity matrix and cluster analysis as presented in Table 10 and Figure 1 respectively. Values of morphological characters were transformed to 0-1 scale by using the formula as mentioned in the previous section. This transformed data was computed using NTSYS- PC ver 2.02 software for similarity matrix using Jaccard's coefficient (J) and UPGMA based dendrogram.

The coefficient of similarity matrix ranged from zero to 83% (Table 9), indicating a low to high genetic variation in gladiolus genotypes. The highest similarity was between G6 and G10 (83%), while the least similarity was between G5-G4, G5-G9, G5- G13 and G5-G15.

The dendrogram based on morphological data among the 15 gladiolus varieties (Figure 1), formed two main clusters in which the second cluster is an outlier (G5). All other gladiolus varieties were grouped into the first cluster. The first principal cluster is subdivided into clusters at a distance of 0.28. Based on this dendrogram genotypes G6 and G10 have the highest similarity of 83%. Most of the genotypes with superior commercial characters clustered together in the dendrogram, but the clustering of some genotypes was erratic.

Table 9. Principal component analysis in 15 genotypes of gladiolus (Highly loaded variables in combined analysis given in boldface)

Variables	PC1	PC2	PC3	PC4	PC5	PC6
Plant height	-0.008	-0.539	0.352	-0.01	0.105	-0.252
Number of leaves per shoot	0.032	-0.061	0.468	0.492	-0.669	0.259
Length of leaf	0.003	-0.506	0.385	-0.116	0.232	-0.226
Width of leaf	-0.052	0.254	0.104	0.768	0.532	-0.218
Internode length	-0.084	-0.269	-0.53	0.27	-0.397	-0.594
Number of days taken for initiation of flower spike	0.347	-0.105	-0.046	0.082	0.097	0.196
Number of days taken for full emergence of spike	0.336	0.173	0.148	-0.051	-0.015	-0.104
Length of the floret	0.363	-0.008	0.031	0.037	-0.047	-0.062
Diameter of the floret	0.361	0.034	-0.003	-0.002	-0.042	-0.065
Number of flowers open at one time	0.077	-0.483	-0.356	0.242	0.174	0.553
Flower length	0.346	0.152	0.096	-0.091	-0.048	-0.208
Flower width in front view	0.361	-0.058	-0.05	0.044	0.036	0.037
Number of florets per spike	0.328	-0.106	-0.23	0.047	0.009	-0.022
Field life	0.36	0	-0.025	0.012	0.019	-0.097
Eigen values	7.514	2.517	1.773	0.969	0.772	0.2
Percent variation	53.669	17.981	12.662	6.919	5.512	1.426
Cumulative percent	53.669	71.65	84.312	91.231	96.743	98.17

Table 10. Similarity matrix of gladiolus genotypes using morphological characters

	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12	G13	G14	G15
G1	1.00														
G2	0.21	1.00													
G3	0.50	0.30	1.00												
G4	0.54	0.25	0.23	1.00											
G5	0.18	0.14	0.13	0.00	1.00										
G6	0.57	0.55	0.38	0.54	0.08	1.00									
G7	0.55	0.09	0.30	0.36	0.14	0.21	1.00								
G8	0.43	0.50	0.33	0.38	0.22	0.67	0.15	1.00							
G9	0.36	0.56	0.25	0.55	0.00	0.58	0.27	0.31	1.00						
G10	0.69	0.42	0.50	0.54	0.08	0.83	0.31	0.54	0.58	1.00					
G11	0.58	0.17	0.50	0.55	0.11	0.46	0.27	0.42	0.23	0.42	1.00				
G12	0.38	0.63	0.40	0.33	0.13	0.50	0.30	0.33	0.50	0.25	0.38	1.00			
G13	0.54	0.25	0.60	0.50	0.00	0.54	0.50	0.38	0.42	0.42	0.67	0.33	1.00		
G14	0.31	0.71	0.44	0.15	0.14	0.55	0.09	0.50	0.40	0.27	0.42	0.63	0.25	1.00	
G15	0.50	0.45	0.42	0.58	0.00	0.75	0.23	0.58	0.64	0.50	0.75	0.31	0.58	0.45	1.00

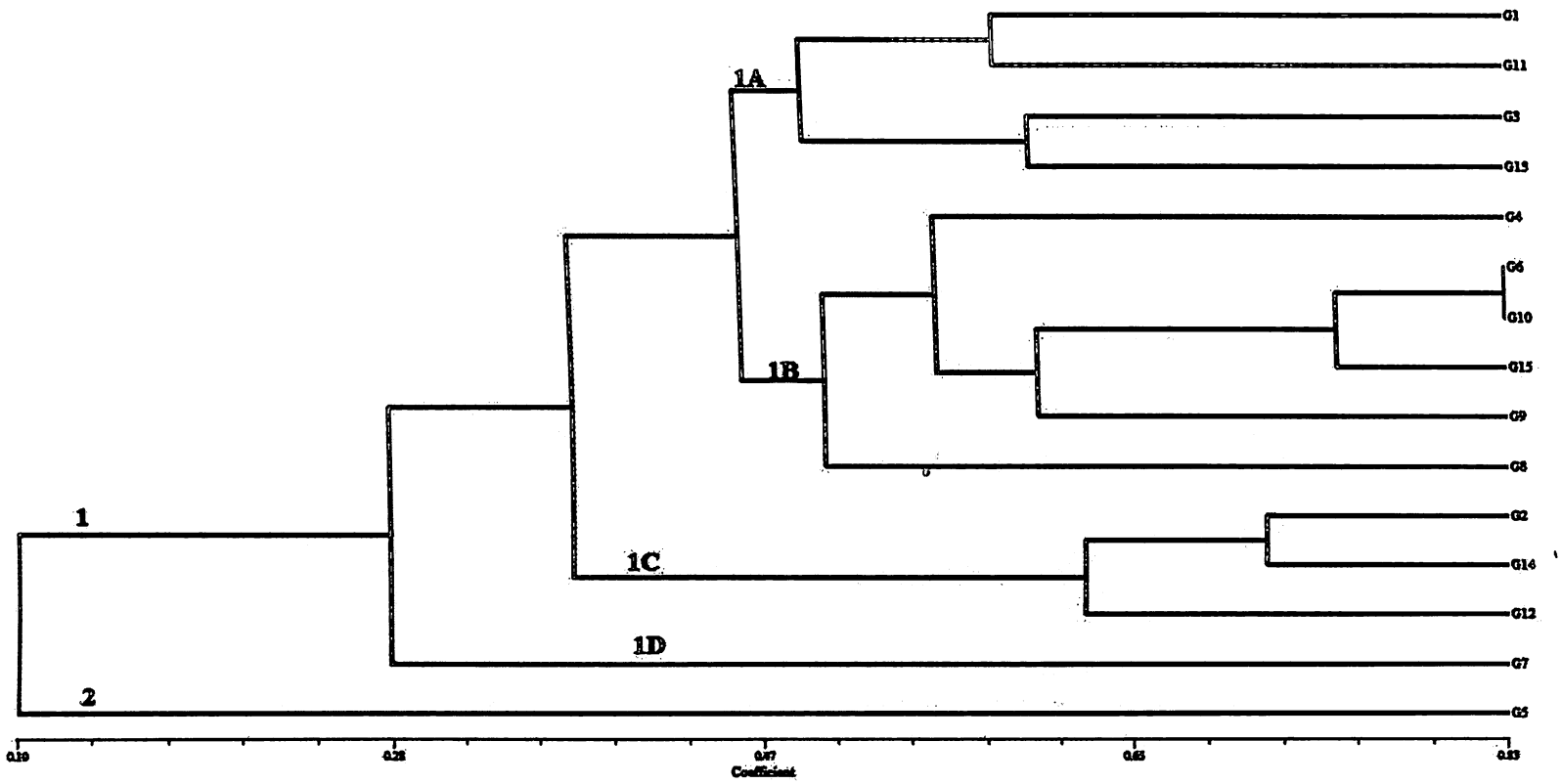


Figure 4. Dendrogram showing genetic relationship among 15 gladiolus genotypes based on morphological traits by UPGMA analysis

4.2 MOLECULAR DATA ANALYSIS

4.2.1 DNA isolation and quantification

DNA of 15 genotypes was isolated using conventional CTAB method with little modifications. The quality of isolated DNA was analysed in 1% agarose gel (Plate 4). The quantities and the purity of the isolated DNA was checked using spectrophotometer and the readings are included in Table 11. The DNA concentration ranged from 1011 ng/µl to 3720 ng/µl.

Table 11. Spectrophotometric readings of DNA isolated

Sl. No.	Sample Name	A260/A280	Concentration (ng/µl)
1.	G1	1.73	1221
2.	G2	1.89	2454
3.	G3	1.87	1992
4.	G4	1.90	2778
5.	G5	1.79	1074
6.	G6	1.82	2508
7.	G7	1.86	2088
8.	G8	1.85	3720
9.	G9	1.63	2487
10.	G10	1.58	1944
11.	G11	1.74	1011
12.	G12	1.89	1338
13.	G13	2.11	3135
14.	G14	1.78	2109
15.	G15	1.85	2235

Plate 4: Checking the quality of DNA of 15 genotypes of Gladiolus using 0.8% Agarose gel electrophoresis

4.2.2 ISSR analysis of gladiolus genotypes

After initial primer screening with 12 ISSR primers, 10 primers which gave reproducible and clear bands were selected for PCR amplification. After PCR, the obtained amplicons were resolved in 2% agarose gel and are shown in Plate 5 to Plate 14.

4.3 ANALYSIS OF MOLECULAR MARKER DATA

4.3.1 ISSR profile

Using 10 ISSR primers (Plates 5-14), a total of 92 bands were generated in which 87 bands are polymorphic. An average of 8.7 polymorphic bands per primer was obtained. Total number of bands per primer ranged from

3(UBC 857) to 14 (UBC 890). A total of 100% polymorphism was obtained in seven primers and rest three primer have 88.89% (UBC 866), 83.34% (UBC 812) and 81.82% (UBC 810) with an average polymorphism of 95.40% (Table 12).

Table 12. Polymorphism detected in 15 genotypes of gladiolus with 10 ISSR primers

Sl. No.	Primer	Total number of bands	Number of polymorphic bands	Percent Polymorphism (%)
1.	UBC 866	9	8	88.89
2.	UBC 857	3	3	100
3.	UBC 807	10	10	100
4.	UBC 812	12	10	83.34
5.	UBC 842	12	12	100
6.	UBC 827	7	7	100
7.	UBC 810	11	9	81.82
8.	UBC 825	6	6	100
9.	UBC 834	8	8	100
10.	UBC 890	14	14	100
Total		92	87	
Mean		9.2	8.7	

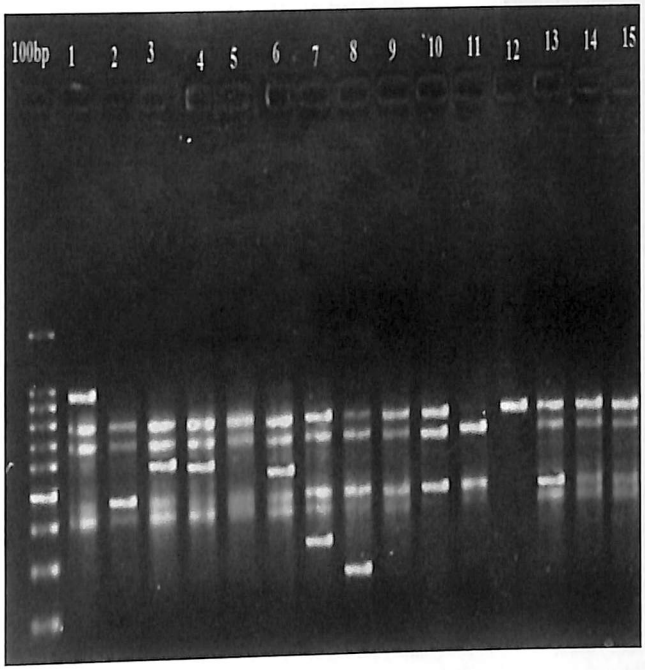


Plate 5: ISSR profile of 15 genotypes of Gladiolus resolved on 2% agarose gel using primer UBC-866

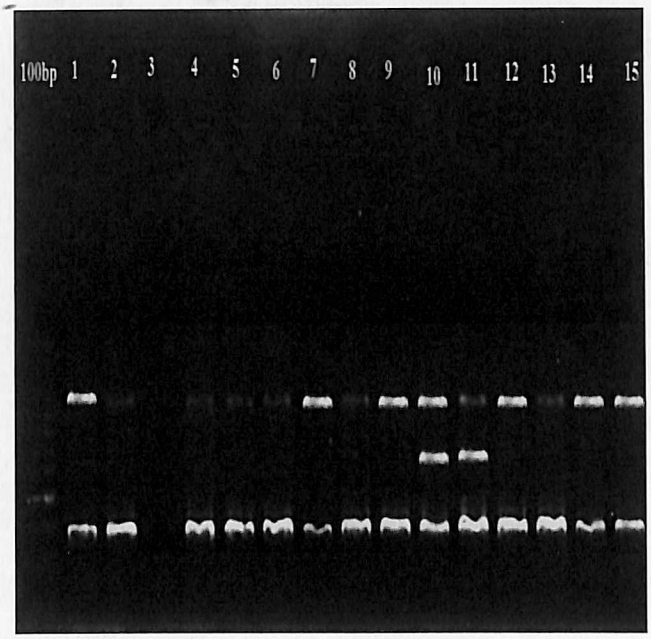


Plate 6: ISSR profile of 15 genotypes of Gladiolus resolved on 2% agarose gel using primer UBC-857

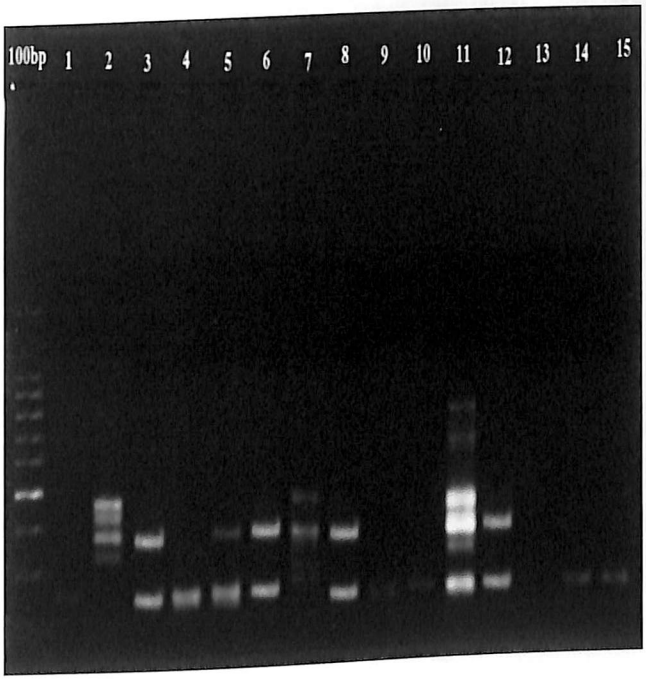


Plate 7: ISSR profile of 15 genotypes of Gladiolus resolved on 2% agarose gel using primer UBC-807

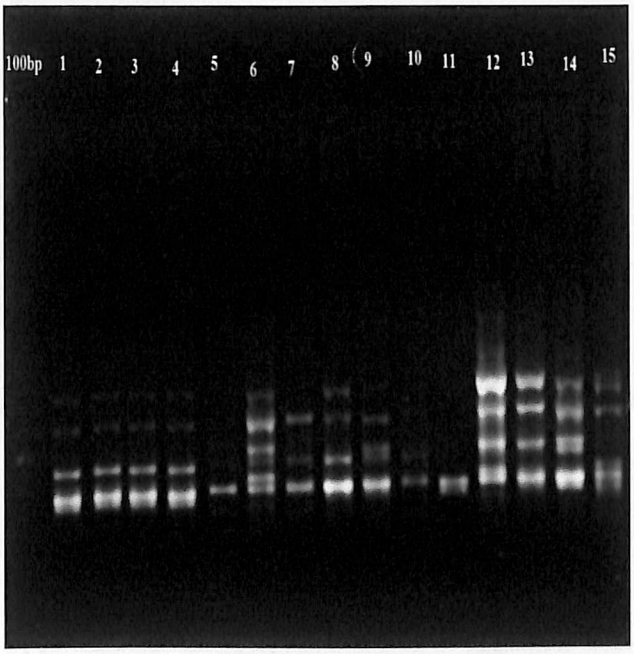


Plate 8: ISSR profile of 15 genotypes of Gladiolus resolved on 2% agarose gel using primer UBC-812

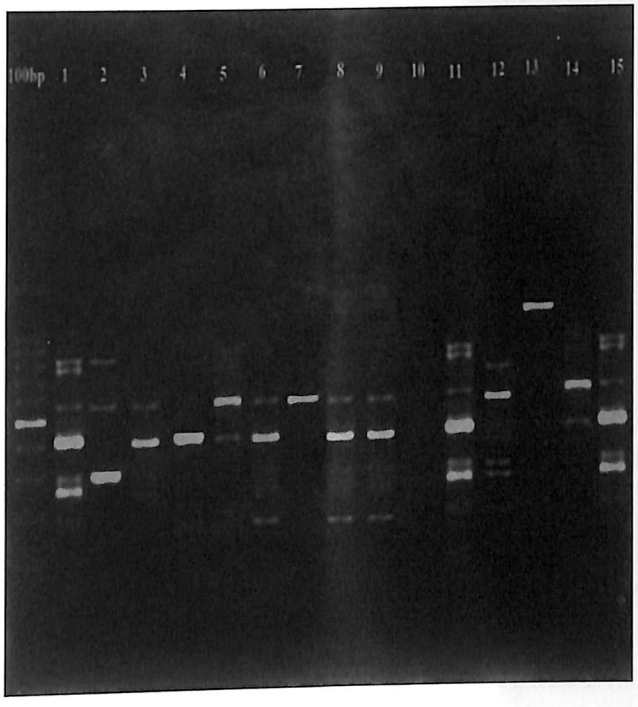


Plate 9: ISSR profile of 15 genotypes of Gladiolus resolved on 2% agarose gel using primer UBC-842

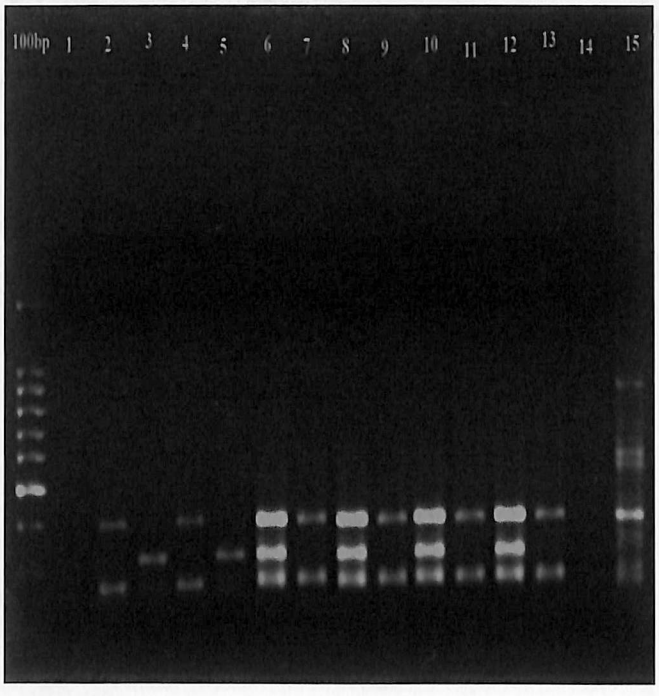


Plate 10: ISSR profile of 15 genotypes of Gladiolus resolved on 2% agarose gel using primer UBC-827

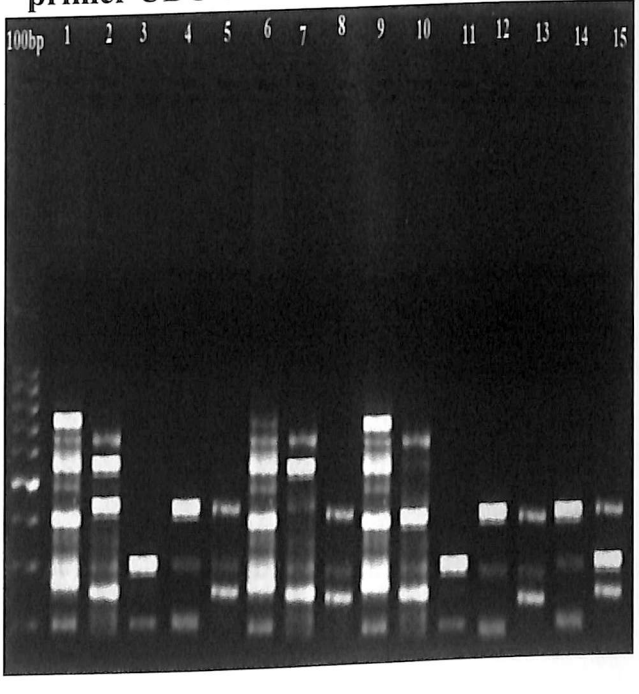


Plate 11: ISSR profile of 15 genotypes of Gladiolus resolved on 2% agarose gel using primer UBC-810

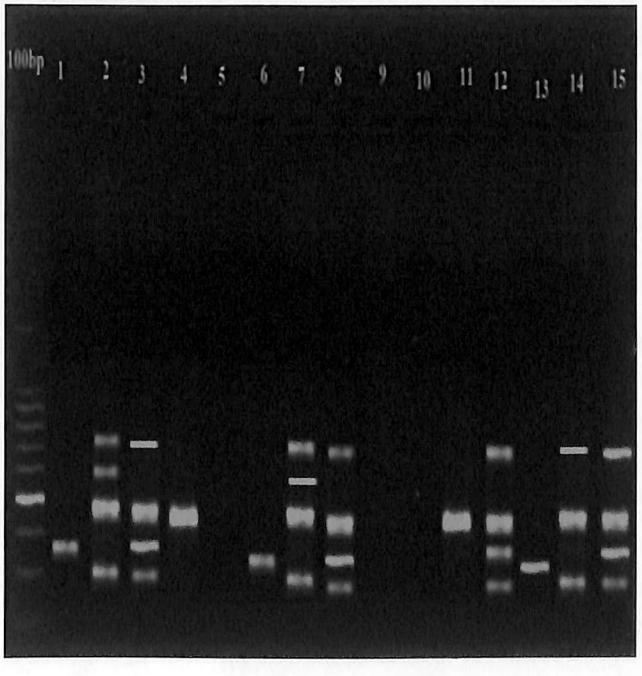


Plate 12: ISSR profile of 15 genotypes of Gladiolus resolved on 2% agarose gel using primer UBC-825

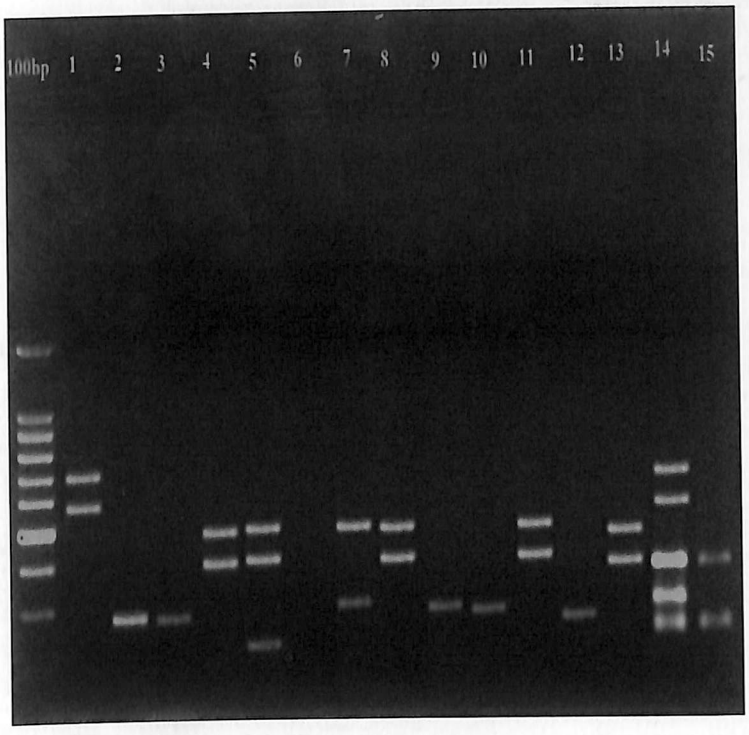


Plate 13: ISSR profile of 15 genotypes of Gladiolus resolved on 2% agarose gel using primer UBC-834

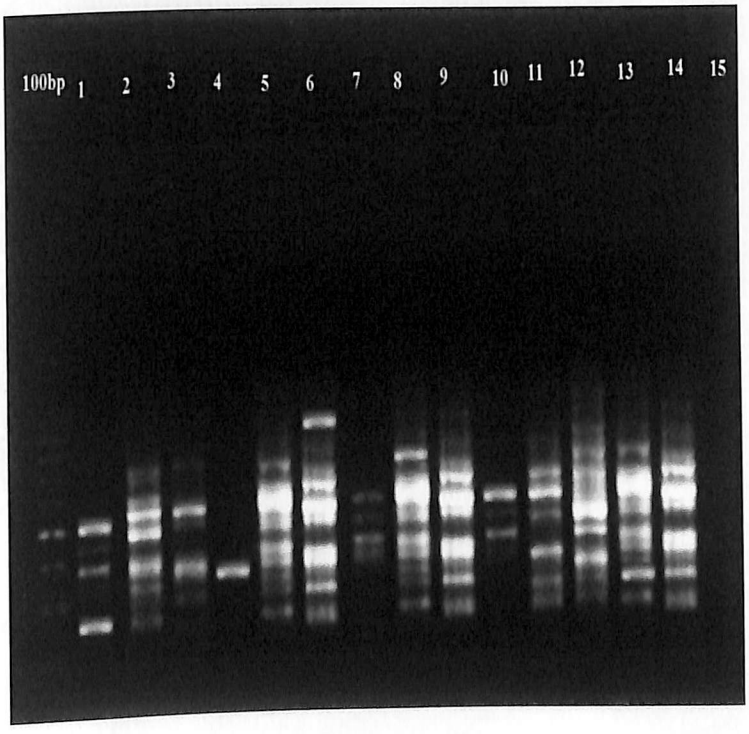


Plate 14: ISSR profile of 15 genotypes of Gladiolus resolved on 2% agarose gel using primer UBC-890

4.3.2 Cluster analysis

The UPGMA dendrogram using Jaccard's similarity coefficient separated the genotypes into two principal clusters at a coefficient 0.46 (Figure 2). Twelve genotypes were included in the first principal cluster. This principal cluster was divided into five sub clusters at a distance of 0.55 (Table 13). These subclusters are again divided into small clusters indicating intraclusteral variability among the genotypes. The second principal cluster is again divided into subclusters. This principal cluster has one outlier (2B) which is G9 and a subcluster which included the genotypes G4 and G13. In the first principal cluster G6 and G10 were grouped together with a similarity of 82%. This set could be considered as the most similar genotypes in the study.

Table 13. Genotypes grouped based on ISSR markers

Clusters	Sub-Clusters	Genotypes
Cluster 1	1A	G1, G6, G10
	1B	G3, G8, G12, G15
	IC	G5, G14
	ID	G11
	1E	G2, G7
Cluster 2	2A	G4, G13
	2B(outlier)	G9

4.3.3 Genetic relationships based on similarity matrix

Estimate of genetic relationships was obtained from the ISSR marker based data on gladiolus genotypes using Jaccard's similarity coefficient. Pairwise comparison of genotypes indicated genetic similarity between genotypes ranging from a minimum of 34% to a maximum of 82% (Table 14).

Table 14. NTSYS-pc similarity coefficients between 15 genotypes (Jaccard's similarity coefficient)

	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12	G13	G14	G15
G1	1.00														
G2	0.47	1.00													
G3	0.45	0.53	1.00												
G4	0.44	0.42	0.43	1.00											
G5	0.44	0.46	0.44	0.46	1.00										
G6	0.64	0.54	0.57	0.45	0.56	1.00									
G7	0.42	0.68	0.42	0.51	0.44	0.47	1.00								
G8	0.51	0.59	0.67	0.58	0.61	0.66	0.55	1.00							
G9	0.42	0.44	0.34	0.53	0.41	0.47	0.54	0.45	1.00						
G10	0.68	0.53	0.49	0.51	0.48	0.82	0.52	0.58	0.54	1.00					
G11	0.46	0.53	0.42	0.47	0.55	0.50	0.43	0.58	0.49	0.36	1.00				
G12	0.46	0.53	0.64	0.44	0.48	0.64	0.43	0.69	0.52	0.39	0.49	1.00			
G13	0.42	0.51	0.42	0.58	0.57	0.51	0.46	0.65	0.50	0.52	0.43	0.43	1.00		
G14	0.53	0.54	0.54	0.49	0.56	0.48	0.47	0.56	0.53	0.40	0.44	0.57	0.47	1.00	
G15	0.48	0.43	0.48	0.46	0.43	0.49	0.38	0.60	0.44	0.38	0.48	0.55	0.38	0.46	1.00

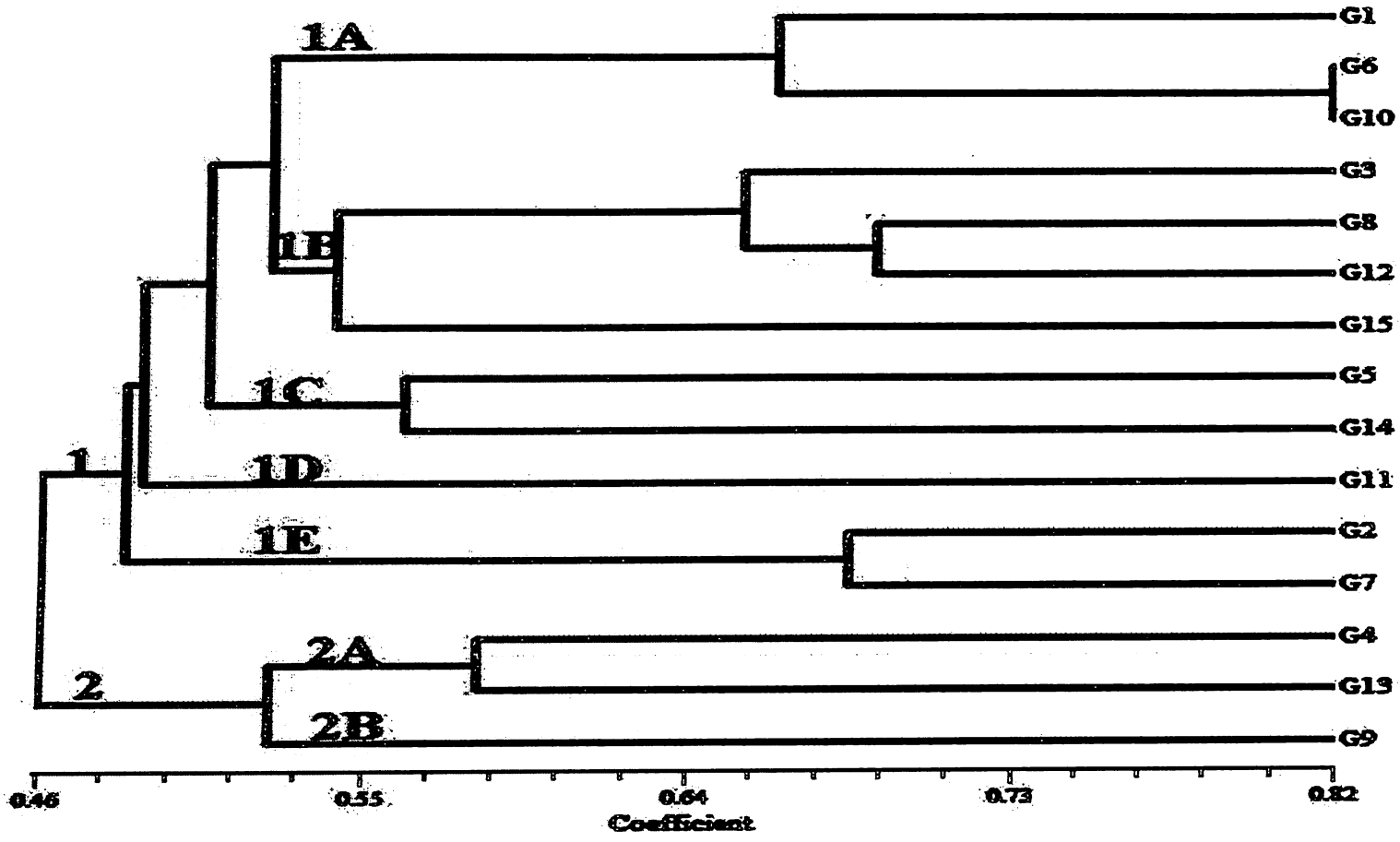


Figure 5. Cluster dendrogram of 15 genotypes based on Hierarchical clustering

DISCUSSION

5. DISCUSSION

Gladiolus is a perennial flowering plant in the family Iridaceae with high economic importance. In the floriculture market, the commercial value of gladiolus has increased because of its local and export market demand. *Gladiolus* is rich in varietal wealth and large variation is seen among the varieties for their traits like growth, shape, spike length, flower colour. Moreover new varieties are analysed using various methods because their performance also depends upon the climatic condition of the region under which they are grown.

Considering these facts, the present study was taken up to identify the diversity within the selected gladiolus varieties (*Gladiolus grandiflorus* L.), suitable for Kerala, using morphological and molecular markers. Genetic diversity within the gladiolus genotypes is helpful for future crop improvement programmes. The understanding of genetic variability and the relation of various characters are significant in planning the breeding programme. The salient findings from the research based on analysis of genetic diversity in *Gladiolus grandiflorus* L. are discussed in this chapter.

5.1 MORPHOLOGICAL CHARACTERIZATION

In the present study, the fifteen *Gladiolus grandiflorus* L. genotypes were studied and a wide range of variations were observed among the vegetative as well as floral characters. A total of nineteen characters from both vegetative and floral traits in gladiolus were analysed.

5.1.1 Variability studies

To evaluate the performance of specific genotypes, the assessment of genetic variability components is essential. In addition to analysis of variance, the estimation of variability is done by assessing the genotypic and phenotypic coefficients of variation. These coefficients are used to recognize the effect of the environment on various traits (Allard, 1960). The GCV computes the range of variation of the different traits that are results of the genetic capacity due to the genotype, while PCV measures the range in total variation.

Among all the selected traits considered in this study the values of GCV was less compared to values of PCV, which indicates that the discernible variation is a result of environmental influence in addition to genotypic difference (Patil *et al.*, 2004, Nimbalker *et al.*, 2007, Kumar *et al.*, 2011a, Venkatapur, 2013).

Slight differences in the genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) show the minimal influence of environment in these characters (Balamurugan *et al.*, 2002, Katewate *et al.*, 2002, Kumar *et al.*, 2011a, Venkatapur, 2013). Characters which showed slight differences between GCV and PCV values were plant height (cm), length of the leaf (cm), internode length (cm), number of days taken for initiation of flower spike, number of days taken for full emergence of spike, length of the floret (cm), diameter of the floret (cm), number of florets open at one time, flower length (cm) and flower width in front view. High GCV with high PCV were recorded for the characters such as number of days taken for initiation of flower spike, number of days taken for full emergence of spike, length of the floret (cm), diameter of the floret (cm), number of florets open at one time, flower length (cm) and flower width in front view. Therefore direct selection could be done for the improvement of these traits. The least values for PCV and GCV among the selected traits were observed for the characters such as plant height (cm), the number of leaves per shoot, length of the leaf (cm), width of the leaf (cm) and internode length (cm). The study of Balamurugan *et al.* (2002) also supports this observation in which lowest value of GCV and PCV was noticed for leaf length and number of leaves per plant. Mishra *et al.* (2014) also reported that PCV was greater than GCV for all characters considered.

5.1.2 Heritability and genetic advance as percent mean

The transfer of a trait from one generation to another generation is called as heritability. This helps in better selection and thus helps in attaining maximum genetic gain in shorter time. Heritability estimation coupled with the genetic advance in percentage mean indicates the resultant effect in selection which could be used for selecting superior genotypes. High heritability was observed in traits such as plant height, number of leaves per shoot, length of the leaf, internode length, number of days taken for initiation of the flower spike, number of days taken for full emergence

of spike, length of the floret, the diameter of the floret, number of florets open at one time, flower length, flower width in front view, number of florets per spike and field life. Selection of the genotypes based on these traits will help in crop improvement in the next generation. The width of the leaf exhibited medium heritability in the range of 30-60%. The characters like 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 and number of spikes per plant exhibited high heritability (Venkatapur, 2013).

In the present study, traits such as plant height, number of leaves per shoot, length of the leaf, internode length, number of days taken for initiation of flower spike, number of days taken for full emergence of spike, length of the floret, diameter of the floret, number of florets open at one time, flower length, flower width in front view, number of florets per spike and field life showed high genetic advance value. The genetic advance value was low for width of the leaf.

High heritability coupled with high genetic advance was noted for characters such as plant height, number of leaves per shoot, length of the leaf, internode length, number of days taken for initiation of flower spike, number of days taken for full emergence of spike, length of the floret, diameter of the floret, number of florets open at one time, flower length, flower width in front view, number of florets per spike and field life. This indicated the least environmental influence on these characters and governed by additive gene action for the direct selection of these characters (Panse and Sukhatme, 1967). Similar results were also reported by Bichoo *et al.*(2002), Katwate *et al.* (2002), Neeraj *et al.* (2005), Pratap and Rao (2006) and Balaram and Janakiram (2009). The present work was also in line with the findings of Nimbalkar *et al.* (2007).

5.1.3 Correlation studies

Correlation studies analyses the magnitude of association and connection of quantitative traits in a population. Knowledge about this interrelationship helps in concurrent upgradation of many characters associated with a particular character of interest, which travels in the same direction of selection. Thus correlation analyses

have predominant influence that could be productively used for formulating effective selection schemes and avoiding inversely related effects during selection.

Fourteen quantitative characters of the gladiolus genotypes were worked out for the genotypic and phenotypic correlations. The maximum influence of environment in the expression of these characters and the presence of powerful inherent association in these characters were concluded from the narrow difference between the phenotypic and genotypic correlation values. Similar correlated values were observed in the study reports conducted by Misra and Saini (1990), Balaram and Janakiram (2009) and Choudhary *et al.* (2011).

In the present study because of positively correlated values, improvement of any of these characters will result in simultaneous improvement of other correlated traits. Plant height had significant and positive correlation with length of the leaf, which showed that plant height along with associated characters could be used for making the selection. Similar correlations were reported by Kumar *et al.* (2011b). Significant and positive correlations was recorded between other characters like number of days taken for full emergence of spike with number of days taken for initiation of flower spike, length of the floret with number of days taken for initiation of flower spike, diameter of the floret with number of days taken for initiation of flower spike, number of florets open at one time with number of days taken for initiation of flower spike, flower length with number of days taken for initiation of flower spike, flower width in front view with number of days taken for initiation of flower spike, number of florets per spike with number of days taken for initiation of flower spike and field life with number of days taken for initiation of flower spike. Similar correlations analysis in related characters was also reported by Hussain *et al.* (2001) and Choudhary *et al.* (2011).

5.1.4 Genetic diversity and relationship based on morphological traits

PCA analysis along with similarity matrix and UPGMA based dendrogram construction were used to produce detailed understanding to the genetic relationships.

In this study, PCA reported plant height, number of leaves per shoot length of leaf, width of leaf, internode length, number of florets open at one time and number

of florets per spike have a significant role in clustering all the genotypes studied. Cluster analysis based on UPGMA with fourteen morphological characters divided the fifteen gladiolus genotypes into two principal clusters at a distance of 0.10 based on Jaccard's coefficient. During comparison between the two principal clusters, the first principal cluster have the most number of genotypes. A similarity of 100% was not observed between any two genotypes in this study. The highest similarity was obtained by G6 (Arka Amar) and G10 (Arka Kumkum) at a distance of 0.83.

Morphological characterization is considered as the first basic step in identifying genetic variability for the separation of varieties. Pragya *et al.* (2010) studied morphological data of 55 gladiolus genotypes with nine morphological traits and reported that Pusa Sarang was found phenotypically close to Pusa Lohit.

5.2 MOLECULAR CHARACTERIZATION

5.2.1 Primer screening for PCR

CTAB method (Doyle and Doyle, 1990) with little modification was used to isolate DNA. The same method is used by Kumar *et al.* (2016) to obtain high quality DNA free of phenolic compounds from the leaves of gladiolus. ISSR markers were screened in the gladiolus genotypes for choosing the primers that give high polymorphism and clear gel profile. In this study 12 ISSR markers were chosen and subjected to primary screening to choose primers that produce reproducible bands. Thus after screening ten ISSR markers were selected that showed good quality, reproducible bands. These 10 ISSR primers are listed below: UBC 866, UBC 857, UBC 807, UBC 812, UBC 842, UBC 827, UBC 810, UBC 825, UBC 834 and UBC 890.

A similar study using 10 ISSR markers was performed by Shufang *et al.* (2010). Preliminary screening of 29 markers, which results in 9 markers that produce reproducible and good bands were also reported by Kumari *et al.*(2015) before the analysis.

5.2.2 ISSR analysis of gladiolus genotypes

Eventhough many physiological and morphological characters show variability in gladiolus genotypes, molecular studies in gladiolus have been restricted to very few varieties with evaluation using RAPD (Sarkhosh *et al.,* 2009; Pragya *et al.,* 2015), ISSR (Kumari *et al.,* 2015; Kumar *et al.,* 2016) and SSR (Piraseyedi *et al.,* 2010) to discover the population dynamics in economically important cultivars. Molecular marker techniques are thus considered as important tools for plant cultivar identification. Closely related individuals are rapidly distinguished using Inter Simple Sequence Repeat amplification technique (Zietkiewicz *et al.,* 1994). ISSR analysis is quicker and simple than many other marker systems. PCR amplification involved the use of single primer that binds randomly to repeats of sequence using one to three bases that is anchored at 3' and 5' end. A major advantage of ISSR analysis is that it does not need prior sequence information of the flanking region and low expenditure compared to other markers.

In this study the ISSR primers used were UBC 866, UBC 857, UBC 807, UBC 812, UBC 842, UBC 827, UBC 810, UBC 825, UBC 834 and UBC 890.These ten ISSR primers generated a total of 92 bands of which 87 bands are polymorphic. The total number of bands per primer ranged from 3 in UBC 857 to 14 in UBC 890. 100% polymorphism was seen in seven markers and rest three markers showed 88.89% (UBC 866), 83.347% (UBC 812) and 81.82% (UBC 810) that corresponds to an average polymorphism of 94.56%.

ISSR could detect high level of polymorphism compared to other marker systems (Kafkar *et al.,* 2006). A total of 75 bands were generated and all the bands were polymorphic that was generated using nine ISSR markers in fifteen gladiolus varieties by Kumar *et al.* (2016). The polymorphism obtained was considered similar to the study of Ahmad *et al.* (2015) in 48 gladiolus genotypes using 48 ISSR markers. Only 40 ISSR markers produced stable and clear polymorphism. Out of a total of 239 bands produced, 216 bands were polymorphic indicating an average of 90.38% polymorphic bands. Jinsang (2008) reported a polymorphism of 93.60% in gladiolus using ISSR marker. All these studies reported that ISSR technique was very efficient in determining gladiolus genetic diversity.

5.2.3 Similarity matrix and Cluster analysis

By using the molecular binary scoring data, dendrogram was constructed using the UPGMA method by NTSYS-PC ver 2.02 software using Jaccard's coefficient. It resulted in the formation of two principal clusters at a distance of 0.46 in which most of the genotypes were grouped in the first principal cluster. The second principal cluster comprised of sub clusters with an outlier and two other genotypes clustered together. The other twelve genotypes were included in the first principal cluster. Pairwise comparison of genotypes indicated genetic similarity between genotypes ranging from a minimum of 34% to a maximum of 82%. The maximum similarity of 82% was obtained between G6 and G10. The least similarity of 34% was observed between G3 and G9.

Kumar *et al.* (2016) employed a genetic diversity analysis of 15 gladiolus genotypes for which hierarchical clustering was done based on UPGMA. This resulted in two principal clusters in which group I had the largest number of cultivars (12) and group II has 3 genotypes. Group II contained 'Arka Amar' and 'Arka Gold' from IIHR and 'American Beauty' from Meerut as an-outlier. Group I was then subdivided to six sub clusters. Geographical locations cannot be considered as a criterion for clustering because genotypes from different regions were found in the same clusters. The main reason for this difference is that the genetic origin of each marker was different and the morphology depends on the state of the plant. Moderate level diversity was reported in the genotypes indicated in the range from 0.68 to 0.90 with an average of 0.84 Jaccard's similarity coefficient.

Similar results were also reported by Ahmad *et al.* (2015) using Jaccard similarity coefficient. Jaccard's similarity coefficient ranged 0.34 to 0.74 from with a maximum similarity between 'Punjab Flame' and 'True Yellow', though they are quite different in morphology. This divided the dendrogram into three principal clusters in which genotypes with the different flower color and dissimilar looks are grouped together, informing that they are genetically similar

5.3 COMPARISON BETWEEN MOLECULAR AND MORPHOLOGICAL CHARACTERIZATION

The cluster analysis done in fifteen gladiolus genotypes revealed that the varieties were grouped into two principal clusters on the basis of both morphological and molecular data. Though there occurred some variations in the grouping of genotypes in both the dendrogram, the grouping is done irrespective of their geographical origin. 'Arka Amar' and 'Arka Kumkum' were the genotypes that showed the highest similarity in both morphology based dendrogram and molecular data based dendrogram. The grouping pattern could be due to common parentage, the similarity of genotype, phenotypic similarity among the genotypes, gene pool and genetic makeup of the genotypes. 'Arka Amar' is the hybrid of 'Watermelon Pink' and 'Aarti' varieties of gladiolus whereas 'Arka Kumkum' is the hybrid of 'Watermelon Pink' and 'Lady John' varieties of gladiolus. This similarity of one parent in these genotypes might contribute to their grouping as the highest similarity genotypes in both the cluster analysis. In the morphology based dendrogram 'Manohar' is the only genotype present in the second principal cluster as an outlier. All other fourteen genotypes were clustered in the first principal cluster with 'Arka Aayush' as an outlier in the first principal cluster. However ISSR analysis showed a different picture. 'Arka Naveen', 'Archana' and 'Arka Gold' were clustered in the second principal cluster with 'Archana' as an outlier. All other genotypes were grouped in the first principal cluster. Taking into consideration the most important benchmark as the genetic results, the loss of accurate indication in morphological classification may be due to the fewer number of morphological traits considered and the influence of morphological expression to the physiological state of the individual plant together with environmental influence.

Kiani and Memariani (2012) reported that classification based on morphological characters may not be similar to ISSR analysis of diversity. Hybrid genotypes that exhibited close similarity with each other may be related close to their common parentage. Jinsang *et al.* (2008) reported in diversity analysis using ISSR markers in *Gladiolus hybridus* Hort. that if the parentage among genotypes is close, varieties with different colours from various places can assemble together in the same cluster. The discrepancy situations between morphology based classification and molecular marker based classification was documented in many studies (Persson *et al.*, 2000;

Wang and Bao, 2005; Kiani *et al.*, 2012; Pragya *et al.*, 2010). A comprehensive analysis of the results of various approaches was suggested to ensure the accuracy of evaluation (Milbourne *et al.*, 1997). To ensure the correctness of the evaluation, only those characters with strong genetic control can be used in morphological character analysis with the effective molecular marker (Wang and Bao, 2005).

In conclusion the findings showed that ISSR analysis together with morphological analysis was more precise than just using phenotypic traits in diversity analysis in gladiolus. A preliminary scientific basis for genetic breeding, selection of parents for hybridization and genotypes that showed good performance in Kerala conditions were identified based on the study by analysing relationships and genetic diversity among the fifteen gladiolus genotypes. Based on the above findings, 'Arka Sapna', 'Arka Nazrana', 'Arka Darshan', 'Arka Amar' and 'Arka Poonam' is recommended as the gladiolus genotypes that showed best performance in Kerala conditions. The variations observed between the cluster diagrams generated by the two methods, could be used as the subject for future research.

SUMMARY

6. SUMMARY

The study titled "Genetic diversity analysis of gladiolus genotypes (*Gladiolus grandiflorus* L.) using molecular markers" was carried out at the Department of Plant Biotechnology and Department of Plant Breeding and Genetics, College of Agriculture, Vellayani, Thiruvananthapuram during 2020-2021. The objective of the study was to analyse the genetic diversity in gladiolus genotypes using morphological and ISSR markers. Fifteen genotypes of gladiolus were selected from IIHR, Bangalore and NBRI, Lucknow for the study. The study was conducted in two sections; morphological and molecular characterization.

Morphological characterization included recording the vegetative and floral characters for gladiolus. The mean performance of gladiolus genotypes for the vegetative and floral characters were obtained and also represented in forms of bar charts. The recorded data were subjected to statistical analysis which included analysis of variance, co-variance and correlations done according to standard procedures (Panse and Sukhatme, 1985). Principal component analysis was done and the first three principal components recorded for 84.312% of the variability. The clustered characters were number of days taken for initiation of flower spike, number of days taken for full emergence of spike, length of the floret, width of floret, flower length, flower width in front view, number of florets per spike and field life with high values in PC1, plant height, length of leaf, width of leaf, internode length, number of florets open at one time, flower length and number of florets per spike in PC2, plant height, number of leaves per shoot, length of leaf, width of leaf, internode length, number of florets open at one time and number of florets per spike in PC3 that resulted in the clustering of gladiolus genotypes. The traits included in PC1, PC2 and PC3 was number of florets per spike. Plant height, length of leaf, width of leaf, internode length, number of florets open at one time and number of florets per spike were the characters included in PC2 and PC3. Width of leaf, internode length and number of florets open at one time were included in PC2, PC3, PC4, PC5 and PC6. Number of leaves per shoot was included in PC3, PC4, PC5 and PC6. Hence plant height, number of leaves per shoot length of leaf, width of leaf, internode length, number of florets open at one time and number of florets per spike were significant in the separation of the genotypes. Further analysis was done using UPGMA (Unweighted Pair Group Method with Arithmetic average) (Sneath and Sokal, 1973) with NTSYS-PC (Numerical Taxonomy and Multivariate Analysis System, Biostatistic, New York, U. S. A.,

Software version 2.02 package) (Rohlf, 1993) using Jaccard's coefficient. Pair wise distance (similarity) matrices were generated using sequential, agglomerative, hierarchical and nested (SAHN) clustering option of the NTSYS. The dendrogram showed the separation of genotypes into two principal clusters at a distance of 0.10.

For the molecular characterization, DNA was isolated from fresh young leaves of the gladiolus genotypes using the conventional CTAB method with slight modifications. The DNA obtained had an A260/A280 ratio of 1.58 – 2.11. A total of twelve ISSR markers were used for the screening of the genotypes. Ten primers that gave reproducible and clear bands were selected for further study. The amplification was obtained at an annealing temperature in the range of 45- 56 °C. The PCR amplified products were resolved on 2% agarose gel and many polymorphic bands were obtained. A total of 100% polymorphism was shown by seven primers and the rest three primers produced 88.89% (UBC 866), 83.34% (UBC 812) and 81.82% (UBC 810) repectively. A total of 92 bands were generated of which 87 bands are polymorphic. An average of 8.7 polymorphic bands per primer was observed. The average polymorphism observed was 95.40%. The total number of bands per primer ranged from 3 for UBC 857 to 14 for UBC 890. NTSYS- PC Version 2.02 was used to generate a dendrogram using molecular marker data, which grouped the genotypes on the basis of Jaccard's similarity coefficient. Thus the fifteen genotypes were grouped into two principal clusters at a distance of 0.46 on the similarity index scale. Most of the gladiolus genotypes were grouped in the first principal cluster. The principal clusters were sub divided into several sub-clusters and there was intra-clusteral variation in the genotypes. The second principal cluster has one outlier (G9) and a subcluster grouping the genotypes G4 and G13. To determine the diversity between the genotypes, pair-wise distance (similarity) matrices was computed using sequential, agglomerative, hierarchical and nested (SAHN) clustering option in the NTSYS-PC. G6 and G10 were considered as the most similarity showing genotypes with a similarity of 82% and the least similarity of 34% was observed between G3 and G9.

'Arka Sapna', 'Arka Nazrana', 'Arka Darshan', 'Arka Amar' and 'Arka Poonam' is recommended as the gladiolus genotypes that showed best performance in Kerala conditions. All gladiolus genotypes included in this study were distinguished by their morphological and molecular characterization. 'Arka Amar' and 'Arka Kumkum' showed the highest morphological similarity concluding that they are closely related. Also at molecular level,

using ISSR analysis they were confined to the same cluster with highest similarity. This grouping pattern may be due to their similarity of genotype, common parentage, phenotypic differences among the genotypes, genetic makeup and gene pool of the varieties. Though some similarity in results existed between the morphological and molecular tools used for identifying the genetic relationships among selected gladiolus varieties in this study, it also revealed some controversies that the varieties were not clearly grouped as separate clusters based on the morphological dendrogram. Considering the most important criteria as the genetic results, the lack of accurate indication in morphological characterization may be due to the less number of morphological traits considered and the dependence of morphological expression on the physiological state of the individual plant along with environmental influence. The suitability of both of these characterizations for diversity analysis was established. By considering the clonal propagation and outcrossing nature of the gladiolus plants, the high genetic diversity between the different gladiolus genotypes could be justified.

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8. APPENDICES

APPENDIX I

1. CTAB extraction buffer

Tris HCl (pH 8.0)	100 mM
EDTA (pH 8.0)	25 mM
NaCl	2 M
β-Mercaptoethanol	1% (v/v)
PVP	2% (w/v)
Distilled water	

APPENDIX II

2. TE buffer (10 X)

Tris HCl (pH 8.0)	10 mM
EDTA	1 Mm

APPENDIX III

3. TBE buffer (10 X)

Tris base	107 g
Boric acid	55 g
0.5 M EDTA (pH 8.0)	40 ml

Final volume made up to 1000 ml with distilled water and autoclaved before use.

APPENDIX IV

4. Chloroform: Isoamyl alcohol

Chloroform	24 ml
Isoamyl alcohol	1 ml

Mix 24 parts of chloroform with 1 part of isoamyl alcohol and store in a tightly sealed container.

APPENDIX V

5. 70% ethanol

100% ethanol	70 ml
Distilled water	30 ml

ABSTRACT

GENETIC DIVERSITY ANALYSIS OF GLADIOLUS GENOTYPES (*Gladiolus grandiflorus* L.) USING MOLECULAR MARKERS

By

KARTHIKA NAIR A. S.

(2016-09-024)

Abstract of thesis

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ABSTRACT

KARTHIKA NAIR A.S. **Date:** 25-10-2021

2016-09-024 **Time:** 2.00 pm-2.30 pm

Characterization of plant genotypes based on crop genetic diversity is important for effective usage and conservation. This is generally achieved by either morphological tools or molecular tools or by using both. This study entitled "Genetic diversity analysis of gladiolus genotypes (*Gladiolus grandiflorus* L.) using molecular markers" was carried out in the Department of Plant Breeding and Genetics, College of Agriculture, Vellayani, Thiruvananthapuram during 2020-2021 with an objective to analyse genetic diversity in the gladiolus genotypes using ISSR as well as morphological markers.

Gladiolus (*Gladiolus sp.)* is a genus of perennial herbaceous cormous flowering plants in the family Iridaceae which is of high economic importance. Fifteen varieties of gladiolus including twelve varieties from IIHR, Bangalore and three varieties from NBRI, Lucknow were selected for this study. The study was divided into two parts- morphological characterization and molecular characterization.

Morphological characterization was done by analysing both vegetative and floral characters. Different tools such as analysis of variance, co-variance, correlations, PCA and dendrogram were used for statistically analysing the recorded data. The dendrogram divided the genotypes into two principal clusters at a distance of 0.10. The major variables that contributed to the clustering of gladiolus genotypes were plant height, number of leaves per shoot, length of leaf, width of leaf, internode length, number of florets open at one time and number of florets per spike as revealed by PCA analysis.

For molecular characterization using ISSR markers the genomic DNA was isolated using CTAB method of DNA isolation with little modifications. Ten ISSR primers were used for screening fifteen gladiolus genotypes. After the final PCR with selected primers, the amplicons were resolved in 2% agarose gel and polymorphic

bands were obtained. Primers showed 94.56% polymorphism and the number of bands obtained ranged from 3(UBC 857) to 14 (UBC 890) with a mean value of 8.7 polymorphic bands per primer. A total of 87 polymorphic bands were obtained. The data analysed using NTSYS PC 2.02 program created a dendrogram, which grouped the genotypes based on Jaccard's similarity coefficient in which the fifteen genotypes were separated into two principal clusters. The first principal cluster consisted of most of the genotypes (12 genotypes). The second principal cluster comprised of 'Arka Naveen', 'Archana' and 'Arka Gold' with 'Archana' as an outlier. In molecular characterization, least similarity of 34% was observed between G3 (Arka Sapna) and G9 (Archana) whereas, maximum genetic similarity of 82% was observed between G6 (Arka Amar) and G10 (Arka Kumkum). The highest morphological similarity was also observed between G6 (Arka Amar) and G10 (Arka Kumkum) at a distance of 0.83 in UPGMA dendrogram based on Jaccard's coefficient.

Though some similarity in results existed between the morphological and molecular tools used for identifying the genetic relationships among selected gladiolus varieties in this study, it also revealed that the varieties were grouped as separate clusters based on morphological dendrogram. This may be due to the dependence of morphological expression on the physiological state of the individual plant along with environmental influence. Self-incompatibility, along with the outcrossing nature together might have contributed to the high variation observed among the gladiolus genotypes. Being a commercial cut flower crop, based on different floral parameters considered 'Arka Sapna', 'Arka Nazrana', Arka Darshan', 'Arka Amar' and 'Arka Poonam' are recommended as the gladiolus genotypes that showed best performance in Kerala conditions.