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Genetic improvement in glory lily (*Gloriosa superba* L.): A review

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Abstract
Glory lily (*Gloriosa superb* L.) is an herbaceous perennial climber belonging to the family *Liliaceae*. Its tuber and seeds contain an alkaloids *viz., cochicine* and *colchicoside*; among which *colchicine* act as an anti-mitotic agent used for inhibiting mitotic cell division. The variability of genetic stocks could be increased by increasing the collection from diversified origin and geographical distribution. The phenotypic and genotypic variances, estimated from the total variance were used to assess the variability among the genotypes. The genotypic coefficient of variance helps to measure the range of diversity in a character and provides the means to compare genetic variability in quantitative characters. The genotypic coefficient of variance along with phenotypic coefficient of variation was used to ascertain the value of diversity among the genotypes. The variability study revealed that higher phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) were recorded for fresh seed yield per plant, dry seed yield per plant and fresh pod yield per plant. The higher estimates of heritability and genetic advance as per cent of mean were obtained for number of leaves, fresh pod yield and fresh seed yield. Thus, selection for these traits is likely to accumulate more additive genes leading to further improvement in their performance. In context to the associations between characters; positive phenotypic and genotypic correlations of dry seed yield were found with plant height, days to 50% flowering, number of flowers per plant, number of pods per plant, pod length, fresh pod yield per plant and fresh seed yield per plant. These correlated yield components suggested that it may be good selection criteria to improve seed yield of this crop. The traits *viz.,* total sugar, mean leaf area, leaf area index and relative water content exhibited negatively and non-significantly correlation with dry seed yield per plant both at phenotypic and genotypic levels. Fresh seed yield per plant had highest positive effect on seed yield followed by number of pods per plant and fresh seed weight per pod. These yield components suggested that it may be good selection criteria to improve seed yield of glory lily crop. *Gloriosa superba* usually multiply by corm and seeds but due to low germination capability it restricts for the regeneration. Therefore, in order to safeguard and preserve this important plant biotechnological approach would be very useful. Different workers suggested different protocols for *in vitro* regeneration of plant. Now a day's molecular diversity also play a vital role in crop improvement programme. RAPD and SSR markers were frequently used to identify molecular diversity in this crop.

Keywords: Glory lily, heritability, genetic advance, correlation, path analysis, molecular diversity

Introduction
Glory lily (*Gloriosa superba* L.), a high value medicinal crop belongs to the family Colchicaceae found naturally in Africa and Southestern Asia. *Gloriosa* derives its name from the word 'gloriosus', which means handsome and *superba* from the word 'superb' means splendid or majestic. It is called as 'Mauve beauty', 'Purple prince', 'Modest', 'Orange gem', 'Salman glow' and 'Orange glow' (Bose and Yadav, 1989) [5]. It is a perennial herbaceous climber growing up to 3.5 to 6.0 meters in length. It is a native of tropical Africa and is found growing naturally in many countries of tropical Asia including Bangladesh, India, Sri Lanka, Malaysia and Myanmar. It is one of the major medicinal plants in India cultivated for its seeds which are exported to developed countries for pharmaceutical use. In India, it is usually found in Himalayan foot-hills, Central India, Tamil Nadu, Andhra Pradesh and Bengal. Seeds and tubers contain valuable alkaloids *viz*., colchicine and colchicoside as the major constituents, which are used to treat gout and rheumatism (Nadkarni 1978) [25]. Due to the action of colchicine on spindle fibre formation during cell division, the plant has been identified as a potential anti-cancerous drug. In the Indian Systems of Medicine, the tubers are used as tonic,

antiperiodic, antihelmenthic and also against snake bite (Gupta *et al.*, 2005) [16]. The leaf juice of G*loriosa* is used to kill-lice in hair, tubers contain the bitter principles, superbine and gloriosine, which in large doses are fatal; however, in small doses they are used as tonic, antiabortive, and purgatives. The white flour prepared from the tubers is bitter and used as stimulant. It is given with honey in gonorrhea, leprosy, colic and intestinal worms and for promoting labor pains, a paste of tubers is applied over the suprapubic region and vagina. Its warm-poultice is locally applied in rheumatism and neuralgic pains (Samy *et al.*, 2008) [37]. Medicinally, the tuber is used as abortifacient, and in small dose it acts as a tonic, stomachic and anthelmintic. It is also used in the treatment of gout because it contains colchicine. Paste of the tuber is externally applied for parasitic skin diseases.

It is adapted to different soil texture and climatic variation. The plant grows in sandy-loam soil in the mixed deciduous forest in sunny positions. It is very tolerant of nutrient poor soils. It occurs in thickets, forest edges and boundaries of cultivated areas in warm countries upto height of 2530 m. (Neuwinger, 1994) [27]. The plant thrive from Bundelkhand to humid Assam valley. It is known by different names in India such as 'Kalihari', 'Agnishikha', 'Languliata', and 'Nangulika'. There are several associated species of *Gloriosa* including *G. superba*, *G. simplex*, *G. grandiflora*, *G. lutea*, *G. plantii*, *G. lipolidii*, *G. longifolia*, *G. rothschildiana*, *G. virescens*, and *G. sudanica*, etc. These species are distributed mainly in Africa. The genetic variability of Glory lily is low owing to the continued vegetative propagation through tubers which has reduced the vigour, tolerance to biotic and abiotic stress, causing low yields (Rajadurai, 2001) [31].

Genetic variability

Variability refers to the presence of differences among the individuals of plant population. The existence of variability is essential for any crop improvement programme. Selection is also effective when there is presence of ample genetic variability among the individuals in a population. Greater the variation in the material better is the chance for selecting promising and desired types. The genetic variability is calculated from phenotypic observations, which are the results of interaction of genotypes and environment. The better index for measuring the genetic variability is genetic coefficient of variation as described by Burton (1952) [6] to compare the genetic variability present in different characters. Earlier efforts by Johannsen (1909) [9], Nilson-Ehle (1909) [26] and East (1916) [13] had led to the partitioning of total variability into genetic and environmental components. The classical experiment of Johannsen (1909) [9] demonstrated that both heritable and non-heritable agencies contributed to somatic variation in segregating populations and that variation in pure line was entirely due to environment. Nilson-Ehle (1909) [26] and East (1916) [13] further confirmed the work of Johannsen (1909) [9] and demonstrated how such results obtained based on the study on non-segregating populations. Charles and Smith (1939) [7], Powers (1942) [29] and Powers *et al.* (1950) [30] separated genetic variance from total variance. The phenotypic range of variation is not the precise criterion of judging the amount of genetic variation present in population. The genetic parameter like variance components, genotypic and phenotypic coefficient of variation, heritability and genetic advance are important parameters to study the extent of genetic variability more precisely.

Chitra and Kandhasamy (2009) [8] observed leaf-shape variation included ovate, lanceolate and linear in different genotypes while based on tuber-shape, L-shape and V-shape was observed. According to leaf arrangement, mostly opposite, but alternate arrangement was also seen. Leaf lamina colour in *Gloriosa* was predominantly pale-green or dark-green. However, a few accessions showed dark-green lamina with pale-green streaks. They also observed higher variability range for plant height, number of leaves per plant, number of branches per plant, number of flowers per plant, number of pods per plant, number of seeds per pod and dry seed yield per plant. Thus, there existed immense scope for selection based on these characters. Less variability were observed in stem girth, days to flowering, days to 50% flowering, pod length, pod girth and hundred dry seed weight. They also observed significant variation among different genotypes for physiological and biochemical characters. Allard (1960) [2] suggested that the selection should be applied mainly in the lines exhibiting high mean and variability. Finker *et al.*, (1973) [14] suggested that crosses or families with the highest mean could be effectively utilized to identify the superior segregants. The mean performance served as a primary criterion for selecting desirable plants [Kumar *et al.*, (1979)] [21]. They also suggested that GCV (%) was highest for fresh seed yield per plant followed by dry seed yield per plant, number of pods per plant, number of branches per plant, number of flowers per plant and number of leaves per plant. Low GCV (%) was recorded for plant height, stem girth, days to flowering, days to 50% flowering, fresh seed recovery and dry seed recovery. The difference between genotypic and phenotypic variances indicates the contribution of environmental variance (Ram and Singh, 1993) [32]. The smaller the difference in values between phenotypic and genotypic variance, the lesser will be the environmental effect on the character. Similarly, the higher the values, the greater will be the environmental effect. The small difference between PCV and GCV estimated for all characters indicated that variability was primarily due to genotypic difference providing scope for selection.

Selvarasu and Kandhasamy (2017) [38] observed that PCV (%) was higher as compared to GCV (%) for stem girth, plant height, number of primary branches per plant, number of secondary branches per plant, number of leaves per plant, number of flowers per plant, pod length, pod girth, fresh pod weight, dry pod weight, fresh seed weight per pod, number of seed per pod, 100 fresh seed weight, 100 dry seed weight, dry seed yield per plant, tuber length, tuber girth and tuber weight indicated that these traits were influenced by environment.

Heritability and Genetic Advance:

Heritability and genetic advance as percentage of mean are the two important parameters of which heritability is used to estimate the expected genetic advance through selection. According to Johanson *et al.*, (1955) [20] a relative comparison of heritability estimates and expected genetic advance as the percentage of mean will an idea about the nature of gene action governing a particular character. Heritability estimates also have a bearing on the population response to selection (Burton, 1952) [6]. According to Wright (1921) [41] heritability denotes the additive genetic variance in per cent of the total variance. High estimates of heritability for certain traits suggest that they are under genetic control. The higher the value of genetic advance, better and surer the progress will be on the mean in the succeeding generation under directional selection.

Chitra and Kandhsamy (2009) [8] observed that characters like plant height, stem girth, number of leaves per plant, number of branches per plant, days to flowering, days to 50% flowering, number of flower per plant, number of pods per plant, pod setting percentage, pod length, pod girth, number of seeds per pod, fresh pod weight, fresh seed weight per pod, fresh pod yield per plant, fresh pod yield per plant, fresh seed yield per plant, fresh seed recovery, dry seed recovery, 100 fresh seed weight, 100 dry seed weight and dry seed yield per plant registered high heritability indicating that these traits could be governed by additive genes (Panse and Sukhatme, 1978) [28] and therefore these traits could be readily fixed by selection. Even though high heritability estimates represent the heritable portion of variation, they do not indicate the effectiveness with which selection of a phenotype could be based on the phenotypic performance (Johanson, 1955) [20] and thus high heritability could not be considered as an indication of greater genetic gain. High heritability coupled with high genetic advance found in plant height, stem girth, number of leaves per plant, number of branches per plant, number of flowers per plant, number of pods per plant, pod setting percentage, pod length, pod girth, number of seeds per plant, fresh pod weight, fresh seed weight per pod, fresh pod yield per plant, fresh seed yield per plant, 100 fresh seed weight, 100 dry seed weight and dry seed yield per plant. High heritability linked with high genetic advance as percentage of mean of these traits indicates that the expression of these traits is governed by additive genes and improvement could possible through selection. A high heritability value along with high genetic advance as per cent of mean is more useful in predicting genetic progress that would result from selecting the best individuals. In this experiment, high heritability with low genetic advance as percentage of mean were observed for days to 50% flowering, fresh seed recovery and dry seed recovery suggesting that high heritability was not always an indication of high genetic advance. Thus, is appears that during selection of new genotype, heritability and genetic advance as percentage of mean should be consider together. The presence of high heritability and low genetic advance is attributed to the effects of non-additive genes (Panse and Sukhatme, 1978) [28]. In this study, fresh pod yield per plant and fresh seed yield per plant had higher estimates of genetic advance than heritability hence; selection of these characters could be highly reliable.

Selvarasu and Kandhasamy (2017) [38] observed that stem girth, fresh pod weight, fresh seed weight per pod, 100 fresh seed weight, dry seed yield per plant, tuber length and tuber weight exhibited high heritability with high genetic advance indicates that the expression of these traits is governed by additive genes and improvement could possible through selection.

Character associations

The ultimate goal of crop improvement in *Gloriosa* is to achieve improved seed yield and colchicine content. Being a complex trait, the seed yield is largely influenced by many associated traits. The information on strength and direction of correlation of these component characters on seed yield and *inter se* association among them would be useful in designing breeding programmes for yield improvement. Genetic association plays a significant role to study the interrelationship and relative contribution of different characters towards crop improvement. As this investigation would be useful to formulate selection criteria, correlation were studied.

Chitra and Rajamani (2010) [9] observed that dry seed yield exhibited highly positive significant correlation both at phenotypic and genotypic levels for all 19 traits. The remaining two characters *viz*., days to flowering and per cent recovery of dry seed showed negative and non significant association with dry seed yield per plant. The correlation analysis made in this study revealed positive and highly significant association of traits *viz*., number of leaves per plant, number of branches per plant, number of flowers per plant, number of pods per plant, pod length, fresh pod weight, fresh seed weight per pod, fresh seed recovery percentage per plant, hundred fresh seed weight and hundred dry seed weight with dry seed yield per plant in both seasons. Hence, it may be concluded that these traits may be considered as the most important yield contributing attributes in *G. superba*. Days to flowering had negative correlation with all characters. Days to 50% flowering had negative highly significant association with other characters. The percentage recovery of dry seed had negative and non-significant association with hundred fresh seed weight. However, percentage recovery of dry seed had negative and significant association with hundred dry seed weight and dry seed yield.

Anandhi *et al*. (2013) [3] observed that the highest and positive correlation for dry seed yield/plant was observed with number of seeds per pod, number of leaves per plant, dry pod weight, fresh seed weight per pod, fresh pod weight, plant height and number of secondary branches per plant. A positive and significant correlation was exerted by stem girth and number of flowers per plant with dry pod weight and fresh 100 seed weight respectively. Similarly, number of seeds per pod, fresh seed weight per pod and 100 fresh seed weight exhibited a positive and significant correlation with fresh seed weight, 100 fresh seed weight and 100 dry seed weight respectively. Significant correlation in the negative direction was observed for pod girth with 100 dry seed weight and tuber girth.

Selvarasu and Kandhasamy (2017) [38] observed the positive and highest significant correlation for dry seed yield per plant (g) was observed with number of seeds per pod closely followed by number of leaves per plant and dry pod weight which was further followed by fresh seed weight per pod, fresh pod weight, plant height and number of secondary branches per plant. Plant height showed positive significance of inter-correlation (Residual effect-0.3465) for the traits *viz*., number of leaves per plant, number of seeds per pod, tuber length and tuber weight. Positive and significant correlation for number of primary branches per plant was observed with number of secondary branches per plant and plant girth while number of secondary branches per plant exhibited positive and significant correlation with plant girth, number of flowers per plant, number of seeds per pod, 100 fresh seed weight and tuber girth.

Path Analysis

Correlation coefficient between any two characters would not give a complete picture for a parameter like yield which is controlled by several other traits, either directly or indirectly. In such situations, path coefficient analysis furnishes a means of measuring the direct effect of each trait as well as the indirect effect *via* other characters on yield. So information on the direct and indirect effect on yield is important which is explicable by path analysis as proposed by Wright (1921) [41] and illustrated by Dewey and Lu (1959) [12]. The interrelationships of the component characters on yield provide the likely consequences of their selection for simultaneous improvement of desirable characters with yield.

Anandhi *et al.* (2013) [3] revealed that significant direct effects were observed through plant height, number of leaves per plant, number of secondary branches per plant, fresh pod weight, dry pod weight, number of seeds per pod and fresh seed weight per pod for the dry seed yield per plant. The number of seeds per pod exhibited indirect effect *via* plant height, number of leaves per plant, fresh pod weight, dry pod weight and fresh seed weight per pod.

Selvarasu and Kandhasamy (2017) [38] revealed that plant height, number of leaves per plant, number of secondary branches per plant, fresh pod weight, dry pod weight, number of seeds per pod and fresh seed weight per pod for the dry seed yield per plant observed significant direct effects. Similarly, the number of seeds per pod exhibited indirect effect *via* plant height, fresh pod weight, number of leaves per plant, dry pod weight and fresh seed weight per pod. Preference should be given to these characters in the selection programme to isolate superior mutants with genetic potential for improving yield, as the correlation of these characters with yield is positive. The direct and indirect effect of the path analysis revealed that important selection indices for yield improvement are the plant height, number of leaves per plant, number of seeds per pod, fresh pod weight, dry pod weight and fresh seed weight per pod.

***In vitro* studies**

Gloriosa superba usually multiply by corm and seeds but due to low germination capability it restricts for the regeneration. Therefore, in order to safeguard and preserve this important plant biotechnological approaches would be very useful (Sivakumar and Krishnamurthy, 2002) [39]. The conventional method of propagation has many disadvantages as 50% of the yield has to be set aside for raising the next crop, transmittance of soil-borne diseases from one crop to the next, and from one location to another and during the 2-3 month storage period between harvest and the raising of next crop (Mrudul *et al.*, 2001) [23].

Somani *et al.* (1989) [40] reported *in vitro* propagation and corm formation in *G. superba.* The fresh sprouts were excised from corms of *G. superba* and dissected propagules with shoot and root primordia were placed on MS basal medium (Murashige and Skoog, 1962) [24] containing 3% sucrose and 0.6% agar. Explant germinated on the MS medium producing shoot and root, which formed new corm within one month. For shoot and cormlet regeneration, 1-4 mg/L kinetin was added to the medium. Cultures were maintained at 25ºC in white fluorescent light (2500 lux) with an 8- h/day photoperiod.

Samarajeewa *et al.* (1993) [36] studied clonal propagation of *G. superba* from apical bud and node segment of shoot tip, cultured on solidified agar (0.8% w/v) Gamborg's B5 medium containing BA, IAA, Kinetin, NAA, IBA or 2,4-D. The cultures were maintained under fluorescent light at 25-27 ºC. Primary cultures were initiated in solid B5 medium containing 0.5 to 1 mg/L BA and 0.01-0.5 mg/L IAA, IBA, NAA when shoot tip of primary cultures were transferred to shoot multiplication media, shoot proliferation occurred via adventitious bud formation within 4-8 weeks.

Custers and Bergervoet (1994) [11] reported micropropagation of *G. superba* by shoot cuttings and explants from node, internode, leaves, flowers, pedicels and tubers. *G. rothschildiana* (duphur) vs. *G. rothschildiana* (new accession) and *G. rothschildiana* vs. *G. superba* were cultured on MS basal medium with 3% w/v sucrose, 0-10 mg/L Benzyl Adenine (BA) and 0.1 mg lndole Acetic Acid (IAA) and maintained at 24 days under 16 hours photoperiod. Addition of low level of Benzyl Adenine (BA) (1 mg/L) improved plant growth, whereas the high level of BA (10 mg/L) caused proliferation of multiple shoots, from rhizome meristem, by applying alternatively high and low BA level, a method of continued propagation was achieved which resulted in a 4-7 fold multiplication of qualitatively good plantlets every 18 week. The resulting shoots were incubated on MS medium, with 3% sucrose and 0-1 mg/L IAA or NAA. Transplantation into soil was only possible after the plants had formed.

Sivakumar and Krishnamurthy (2002) [39] reported *in vitro* organogenetic responses of *G. superba.* They used MS medium supplemented with ADS and BA, 98%. The callus induction occurred in non-dormant corm bud explants. The maximum number of multiple shoot (57%) was observed in corm-derived calluses.

Hassan and Roy (2005) [17] reported 92% of the cultures of apical and axillary buds of young sprout from naturally grown *G. superba* plants regenerate four shoots per culture in MS basal medium fortified with 1.5 mg/L BA + 0.5 mg/L NAA.

Bharathi and Philomina (2010) [4] investigated the effect of nutritional factors and the precursors on colchicine production in callus cultures of *Gloriosa superba* in order to optimize the colchicines production *in vitro.* Colchicine content in the callus, grown in the medium with sucrose as carbon source and 40 mM ammonium nitrate as nitrate source showed the greatest promise with highest biomass and colchicine content. In addition to this, sulphate ions (40 mM) markedly increased the formation of colchicine. In contrast, highest concentration of phosphate (2.5 mM) and calcium (10 mM) were found to be inhibitory for colchicines formation. Precursors (40 mM tyrosine) also influenced the colchicine content (9.79 mM dry weight) with the above mentioned nutritional effect.

Madhavan and Josef (2010) [22] studied on indirect organogenesis from internodal cultures of *Gloriosa superba* L., and concluded that the callus initiated from the sub epidermal cells. The organogenic and non organogenic calli were the result of hormonal variation in the medium. In non organogenic callus, cells re-differentiated into xylem elements forming clusters of nest like structures. In organogenic callus, cells re-differentiated into nodules of meristemoids which further differentiated into shoot apical meristem.

Ade and Rai (2011) [1] supplemented with 2,4-D (4.52 mM) and BAP (13.30 mM) in MS medium promoted the formation of the maximum number of shoots compared to IAA, IBA and with Gamborg B5 medium supplemented with kinetin, IBA and BAP were found to be superior.

Selvarasu and Kandhasamy (2012) studied on *in vitro* tuberization of glory lily (*Gloriosa superba* L.) by using non-dormant tubers on MS medium supplemented with various concentrations growth regulators. MS medium supplemented with 4.0 mg/l BAP and 1.0 mg/l NAA recorded the highest response for primary tuber (100%) and secondary tuber (100%) formation. This also recorded the maximum number of tubers (1.77) from single explants.

Ritu Mahajan *et al.*, (2016) observed best shoot multiplication in sprouted tubers (88.67%) was observed in MS supplemented with 2.0 mg/l BAP, 0.5mg/l Kn and 1.0 mg/l GA3 with 8.7±0.18 average number of shoots per explant. Long and healthy roots were observed in MS medium supplemented with 2.0 mg/l IBA resulting in 84.50% of root growth and average of 7.47±0.29 roots per explant. Organogenesis was obtained from embryogenic callus in MS medium supplemented with BAP, Kn and IBA. Further sub-culturing of embryogenic callus in MS medium containing

2,4-D, BAP and Kinetin resulted in globular structures while addition of only BAP resulted in heart shaped somatic embryos on the callus.

Molecular Characterization

Gloriosa having well- adaptability to various geographical locations shows variations at both morphological and genetic level (Reddy and Lakshmi, 2016) [33] this leads to varying chemical composition particularly alkaloid content with respect to location. Molecular markers or DNA markers are the best tools and widely used for screening the variation at the genetic level within the accessions or ecotype (Forrest *et al.*, 2000) [15] and also for hybridization of populations and identification of novel genes for further studies and its conservation (Jasmine and Balakrishnan, 2018) [18]. Molecular markers show high polymorphism and independent of environmental effects. RAPD and ISSR methodology has been used in many medicinal crops. ISSR markers are highly reproducible than the RAPD markers (Selvarasu and Kandhasamy, 2017) [38].

Chitra and Kandhsamy (2013) [10] evaluated genetic diversity of eighteen glory lily (*Gloriosa superba* L.) accessions of diverse geographical origin using Random Amplified Polymorphic DNA (RAPD) markers. Fifty eight out of seventy primers screened showed polymorphism across the present set of accessions. A total of 413 amplicons were scored using these 58 primers. Eighty eight per cent of the amplified product showed polymorphism, indicating a fair amount of variation at the DNA level among these accessions.

Selvarasu and Kandhasamy (2017) [38] were used six samples to study the genetic diversity using 12 ISSR primers. The PCR amplification using these 12 primers yielded 444 reproducible amplified bands. The number of amplified bands varied from 12 (UBC 824) to 73 (UBC 807). Out of 444 bands, 116 were found to be polymorphic. Average number of bands and polymorphic bands per primer were 37 and 9.67 respectively. As a relative measure of polymorphism level, Polymorphic Information Content (PIC) value ranged between 0.764 (UBC 810) to 0.947 (UBC 807). Five primers *viz*., UBC 846, UBC 821, UBC 827, UBC 848 and UBC 828 exhibited the PIC value from 0.926 to 0.912 among the primers used in the
study.

Sahana *et al.*, (2019) [35] evaluated genetic diversity among 16 *Gloriosa* accessions collected from different locations in Tamil Nadu was studied by using Inter-simple sequence repeat (ISSR) markers. Thirty six ISSR primers were used, among that 16 primers showed 83.56 per cent polymorphism and produced 213 amplicons. This indicates that there is a high level of variation at the genetic level among these accessions. The primer UBC-807 showed the highest PIC value (0.958), which represented the high efficiency of the individual primer. Gloriosa accessions GSU-6 and GSU-7 are highly similar with 68 per cent and GSU-7 and GSU-16 are distinctly similar with 39 per cent.

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