**Annals of Plant Sciences**
ISSN: 2287-688X
Original Research Article

# Low Intraspecific Diversity Suggesting Genetic Drift in *Gloriosa superba* L. (Liliaceae) in Konkan Coast of Karnataka, India

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Received for publication: November 11, 2013; Accepted: November 25, 2013.

**Abstract:** The population of *Gloriosa* species is gradually diminishing in Udupi district of Karnataka, due to their over exploitation for colchicine-the therapeutic alkaloid. The scattered and scant occurrence of this genus is further attributed to their seasonal and truncated seed set in nature. The present study is the first report on polymorphism studies in this plant using AFLP, the dominant molecular marker. Genetic distance based on the AFLP data could authenticate, low intraspecific diversity more efficiently than RAPD. This genetic similarity identified could be correlated with collection sites and altitude. 2C DNA values of the collected accessions ranged between 5.24 and 5.81 pg, when determined by flow cytometer. The study indicated that the genetically isolated smaller population of *G. superba* is experiencing genetic drift within the Udupi district of Karnataka, India and hence must be subjected to suitable conservation measures.

**Keywords:** AFLP, Flow cytometer, *Gloriosa superba*, intraspecific diversity, Nuclear DNA content. RAPD

## Introduction

*Gloriosa superba* L. (Liliaceae) is a weak stemmed climber with tuberous roots rich in alkaloids primarily colchicine and its derivatives which is used for the treatment of gouty arthritis. The genus bears perplexing taxonomy owing to ploidy variations. About 27 species were recognized in this genus which posed difficulty in species identification (Tarar and Viswakarma, 1995). Renewed interest in this plant is due to its high colchicine content after *Colchicum autumnale* and *Iphigenia spp*. Its vegetative propagation is brought about by cultivating tubers which sprout during rainy season. Plants propagated from seeds take three to four years to bloom, whereas from tubers it is faster (5-8 weeks). Flowers undergo both self and cross pollination. An average of six flowers develops on a branch which opens in sequential manner (Gupta and Raina, 2001). The population of *Gloriosa* species is gradually diminishing in Udupi district of Karnataka, because of its wide use for the alkaloid extraction, apart from its low seed set in nature. The study of Ghosh *et al.,* (2008) is the only report on polymorphism studies in India. Ghosh *et al.,* (2008) identified population variations in the plant collected from five regions in North East India (Amtala, Baruipur, Siliguri, Darjeeling and Sikkim). The variations were detected based on the RAPD analysis alone which exhibited 76% polymorphism between populations. Identification of polymorphisms in this plant is relevant as it will suggest whether the plant is threatened due to heavy destruction. Bhat (2003) has reported that '*superba*' is the only species of genus *Gloriosa* found in Udupi district of Karnataka. RAPD fingerprinting is an ideal tool to detect existing variations within and between the plants (Hadrys *et al*., 1992; Lynch and Milligan, 1994; Rieseberg and Swensen, 1996). High reproducibility and high frequency of identifiable polymorphisms make AFLP analysis an authentic and sensitive technique for evaluating individuals from a segregating population (Lin *et al*., 1996). The objective of the study is to examine genetic variations if any, within local *Gloriosa* populations collected from various locations of Udupi district, Karnataka, India.

## Materials and Methods

**Plant Materials:** *G. superba* were collected from five different locations from Udupi district of Karnataka, India (Table 1). Plants collected from the same sites were 200 m apart. Species identification was done with the help of floristic work and also with the help of a taxonomist. We found that the populations of *G. superba* were quite scattered and scant. The plant accessions used for the study were maintained at green

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house of School of Life Sciences, Manipal University. Voucher specimens of *G. superba* were deposited in the herbarium maintained by School of Life Sciences. We collected four plants per site for the study as this was the only available number in each population set.

**Table 1:**

**A.** Flowering Genotypes of *G. superba*, used in the study together with the accession numbers and source

| Code | Accession number | Collection site | Altitude* | Longitude | Latitude | Site Description |
|---|---|---|---|---|---|---|
| **Pop1** | MLSCGS101 | Manipal | 31 | 74°80'E | 13°35'N | Urban |
| **Pop2** | MLSCGS102 | Salmara, Uppoor | 6 | 74°76'E | 13°39'N | Wild |
| **Pop3** | MLSCGS103 | Herga Temple Road (Site 1) | 36 | 74°80'E | 13°37'N | Rural |
| **Pop4** | MLSCGS104 | Herga Temple Arch (Site 2) | 50 | 74°80'E | 13°36'N | Rural |
| **Pop5** | MLSCGS105 | Herga [Kodange Road] (Site 3) | 42 | 74°80'E | 13°36'N | Rural |
| **Pop6** | MLSCGS106 | Vittalwaadi, Parkala (Site 1) | 54 | 74°81'E | 13°35'N | Urban |
| **Pop7** | MLSCGS107 | Vittalwaadi, Parkala (Site 2) | 54 | 74°81'E | 13°35'N | Wild |
| **Pop8** | MLSCGS108 | Vittalwaadi, Parkala (Site 3) | 54 | 74°81'E | 13°35'N | Wild |
| **Pop9** | MLSCGS109 | Vittalwaadi, Parkala (Site 4) | 54 | 74°81'E | 13°35'N | Urban |
| **Pop10** | MLSCGS110 | Perampalli | 11 | 74°77'E | 13°37'N | Urban |

*Altitude (meters from sea level)

**B.** Distance between collection sites of *G. superba* plant populations

| **Population** | **Distance (Kilometers)** | | | | |
|---|---|---|---|---|---|
| | **Manipal** | **Salmara** | **Herga** | **Vittalwaadi** | **Perampalli** |
| **Manipal** | - | 7.17 | 0.58 | 2.31 | 4.13 |
| **Salmara** | | - | 7.07 | 8.97 | 1.62 |
| **Herga** | | | - | 1.43 | 4.4 |
| **Vittalwaadi** | | | | - | 4.6 |
| **Perampalli** | | | | | - |

**DNA Isolation and RAPD PCR Analysis:** Total Genomic DNA was isolated from tender leaves of the plant by CTAB method as prescribed by Doyle and Doyle (1987). The primers for RAPD PCR amplifications were obtained from Sigma Genosys, USA. PCR amplifications were carried out in a final reaction volume of 25 µL containing 100 ng of genomic DNA, 1X PCR buffer (10 mM Tris-HCl, 50 mM KCl and 0.5% DMSO), 0.25 mM each of dNTPs, 0.15 µM of each reverse and forward primers, 1–3.75 mM of $MgCl_2$ and 0.5–1.5 U of *Taq* DNA polymerase (Sigma, USA). Primer and their combinations that gave consistent results are listed in Table.2. The primers used for RAPD analysis are methylation sensitive arbitrary primers proposed by Markl *et al*., 2001.

Amplifications were performed in a Peltier Thermal Cycler PTC-200 (MJ Research, Waltham, USA) with an initial 2 minutes of pre-denaturation at 94°C, followed by 34 cycles of denaturation at 94°C for 2 minutes, an optimum annealing temperature 40°C for 1 minute and extension at 72°C for 2 minutes. The amplified products were concluded with a 10 minutes final extension at 72°C. Amplification products were fractionated on 1.2% agarose gels. The reproducibility of the amplified fragments was assessed by replicating the entire procedure using each combination of primers with three separate DNA isolations per plant. Silver staining on polyacrylamide gel electrophoresis (PAGE) was performed for detecting the fractionated amplified fragments as suggested by Sanguinetti *et al.,* (1994).

**Table 2:**

**A.** RAPD Primer sequences and Combinations used for *G. superba* plant populations (Markl *et al*., 2001)

| Code | Primer | Sequence |
|---|---|---|
| 1 | MGC0 | 5′ AACCCTCACCCTAACCGCGC 3′ |
| 2 | MGE2 | 5′ AACCCTCACCCTAACCCCGG 3′ |
| 3 | GC1 | 5′ GGGCCGCGGC 3′ |
| 4 | GC2 | 5′ CCCCGCGGGG 3′ |
| 5 | TIM1 | 5′ AGCGGCCGCG 3′ |
| 6 | TIM7 | 5′ GAGGTGCGCG 3′ |
| 7 | TIM13 | 5′ CGGGGCGCGA 3′ |
| 8 | MGC0 +MGE2 | 5′ AACCCTCACCCTAACCGCGC 3′+5′ AACCCTCACCCTAACCCCGG 3′ |
| 9 | GC1+GC2 | 5′ GGGCCGCGGC 3′+5′ CCCCGCGGGG 3′ |
| 10 | TIM1+TIM7 | 5′ AGCGGCCGCG 3′+5′ GAGGTGCGCG 3′ |
| 11 | TIM1+TIM13 | 5′ AGCGGCCGCG 3′+5′ CGGGGCGCGA 3′ |

**B.** List of primers that contributed for polymorphic analysis of *G. superba* and the total and their amplification products

| RAPD Primer sets | Amplification products | | electrophoretically identified |
|---|---|---|---|
| | Total | Polymorphic | *G. superba* specific RAPDs (bp) |
| GC1 + GC2 | 102 | 82 | 1500 |
| MGC0 + MGE2 | 238 | 59 | 500, 550, 900, 1500 |
| TIM1 + TIM7 | 95 | 66 | 1500 |
| TIM1 + TIM13 | 50 | 40 | 1500 |
| **Total** | **485** | **247** | - |
| **AFLP Primer sets** | | | |
| MseICTC+EcoRIACC | 260 | 116 | 15, 65,100,120 |
| MseICAG+EcoRIACA | 41 | 20 | 15, 25 |
| MseICAG+EcoR1AGC | 82 | 29 | 240,265 |
| Mse1CAC+EcoRIACA | 224 | 100 | 15, 25 |
| MseICTA+EcoRIAAC | 32 | 9 | 15, 25,165 |
| **Total** | **639** | **274** | - |

**AFLP Analysis:** AFLP profiles were obtained by using the kit purchased from Applied Biosystems comprising Ligation and Pre-selective Amplification module (Part Number 402004) and Amplification Core Mix (Part Number 402005). DNA was double-digested with *EcoRI* and *MseI* and the resulting fragments were ligated to adaptors specific for the *EcoRI* and *MseI* restriction sites. Diluted DNA from the pre-selective amplification was used with twenty primer combinations. The procedure followed was as per the instructions provided by the manufacturer (AFLP® Plant Mapping Manual, Applied Biosystems Protocol, Part Number 4303146E, USA, 2007).

**Loading of AFLP amplified products on Applied Biosystems 3130-16 capillary Genetic Analyzer:** 4 µl of the selective amplified product was diluted with 9.6 µl of loading buffer mix (8µL of deionised formamide along with 1.6 µL of 10 times diluted Gene Scan-500 LIZ) and was heated to 95°C for 5 minutes in a PCR machine. After snap chilling the sample was loaded on a 96 well plate and was subjected to analysis. AFLP electropherograms ranging in size from 15 to 350 bp was analyzed using Gene Mapper Version 4.0 software (PE–Applied Biosystems).

**Data Analysis:** Amplification was repeated three times per primer and resolved fragments with high reproducibility were considered and treated as a unit character with the score of one (present) or zero (absent). All the fragments were scored manually and were used to calculate pair wise genetic distances as suggested by Nei (1972). Clusters were constructed following the UPGMA (Unweighted Pair Group Method using Arithmetic average) method with the aid of the software package POPGENE version 1.6 (Yeh and Boyle, 1997). The robustness of the dendrogram was tested by bootstrap analysis using the software package WINBOOT developed at IRRI (Yap and Nelson, 1996).

**Estimation of Nuclear DNA Content:** Preparation of the samples followed the procedure initially developed by Galbraith *et al.,* (1983) with some modifications necessary for species containing staining inhibitors in their cytosol (Zoldos *et al*., 1998). Leaf blade fragments of *Gloriosa superba* and *Zea mays* of size 0.5–1 $cm^2$ were chopped with a sharp razor blade in a glass petri dish which had 1 mL of nucleus-isolation buffer. The constituents of the nucleus isolation buffer include a combination of Otto I buffer (0.1 M citric acid, 0.5% Tween 20) and Otto II buffer (0.4 M $Na_2HPO_4$) in a ratio of 1:1. *Zea mays* CE-777 (2C = 5.43pg), supplied by Prof. Jaroslav Dolezel (Institute of Experimental Botany, Sokolovska 6, CZ-

77200 Olomouc, Czech Republic), was used as external standard for the flow cytometry analysis. About five leaves (size=0.5-1cm$^2$) from each plant were essential to obtain sufficient numbers of nuclei from intact tissues. This crude suspension was left for 30 minutes in room temperature. After the incubation, it was centrifuged at 1500 rpm for 5 minutes. The entire solution was double filtered using 40 µm nylon filter. The filtrate is collected and centrifuged at 1500 rpm for 30 minutes. The supernatant was discarded and the pellet was washed (1500 rpm for 5 min) using 1:1 Otto I buffer and Otto II buffer (500 µL each). The supernatant was discarded and the pellet is resuspended in 8 µL of RNase A (50 mg mL$^{-1}$). After chopping, the suspension was passed through a 50 µm mesh nylon filter and incubated for 10 minutes at room temperature, then 10 µL of 50 mg mL$^{-1}$ propidium iodide was added and the sample was incubated for another 5 minutes at room temperature. For each sample, about 7000–10,000 nuclei were analyzed using a BD FACS Calibur, analyzed using software viz., Cell Quest Pro equipped with Win2md computer program. The gain of the cytometer was adjusted so that the G0/G1 peak of *Zea mays* was positioned on channel 400. Nuclear DNA content was calculated according to the linear relationship between the ratio of the 2C peak positions of reference and test on histograms of fluorescence intensities. 2C Value = M.P/M.S × 2C-Std; where 2C Value = calculated nuclear DNA amount for the unknown (pg/nucleus), M.P = Modal Channel of the peak for the unknown plant nuclei, M.S = Modal Channel of the fluorescence peak for the external standard and 2C-Std= 2C-Value (pg/nucleus) of the external standard.

## Results

**DNA fingerprinting using RAPD and AFLP:** A total of 14 RAPD primers were randomly tested for their efficiency in generating amplification products in *G. superba*. Among the 14 primers used; 4 primer combinations gave clear consistent resolvable amplification products among the different plants. The banding profiles obtained were used for the assessment of genetic variation in the plant species. Individuals belonging to the same population exhibited RAPD polymorphism which was lower compared to other sites. A representative RAPD pattern generated is shown in Figure 1. Electrophoresis gel resolutions between 1.2% Agarose and 10% Polyacrylamide gels revealed that there were minor differences within the species. However polyacrylamide bands were always used for detection of the separated bands which were unclear in agarose gels; and thus presence and absence of bands could be confirmed. The 4 primers together yielded 485 fragments, of which 247 were polymorphic (Table 2B.). Forty seven amplification products (19.02% of total polymorphism) were specific to the plant collected from 2 different sites code [MLSCGS106] and [MLSCGS107] of Vittalwaadi, Parkala whereas least polymorphism was observed in plant collected from Manipal code [MLSCGS101]. Among the 20 AFLP primer combinations tested, 6 combinations gave consistent results which were used to assess the intraspecific variations. A total of 639 bands were scored out of which 274 were polymorphic. Since the lower and upper values were set it was easy to score the amplicons of even smaller fragments. Eighty four amplification products (13.14% of total polymorphism) were specific to plant collected from Salmara, Uppoor. The AFLP analysis revealed the genetic similarity that existed between the individuals within the population.

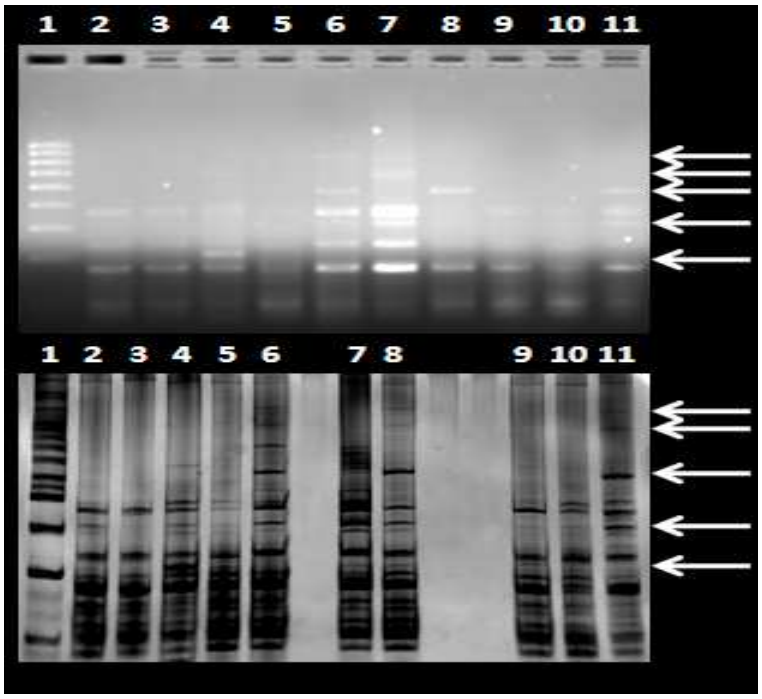



**Figure 1:** Amplification products using primer combination-MGC0 and MGE2 in (A). 2 % Agarose gel (B).10-%PAGE 1. 100 kb ladder 2. MLSCGS101 3. MLSCGS102 4. MLSCGS103 5. MLSCGS104 6. MLSCGS105 7. MLSCGS106 8. MLSCGS107 9. MLSCGS108 10. MLSCGS109 11. MLSCGS110.

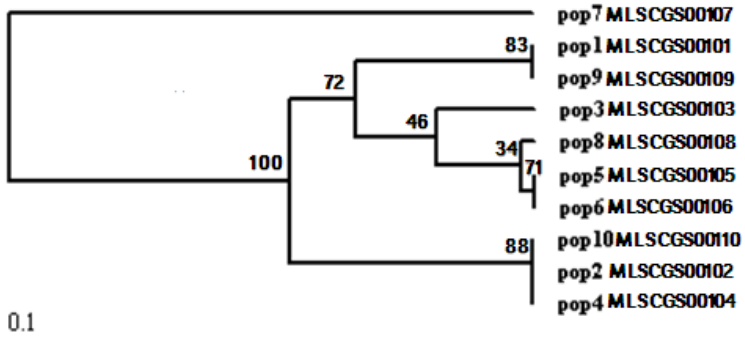
**Genetic Distance:** The pair wise genetic distance matrix was prepared on the basis of RAPD data (Table 3A). The genetic distance varied from 0.0096 to 0.2033 with a mean value of 0.107. The Vittalwaadi plants collected from Site 2 was clearly distinct from other populations. Three other populations from Vittalwaadi showed relatively lower distances among themselves and from other populations. The populations from Perampalli, Uppoor and Herga (Site 3) displayed comparatively lower distances among themselves. But the genetic distance based on the AFLP data could identify the genetically similar plants more efficiently than RAPD. This genetic similarity could be correlated with collection sites and altitude unlike the genetic distance procured from RAPD analysis. Uppoor plants collected from a lower altitude showed higher genetic diversity when compared to plants collected from higher altitudes. Plants collected from median altitude (Perampalli, Herga Site 1) showed similarity among themselves, even though plants from Manipal and Herga (Site 3) were genetically distinct. All the Vittalwaadi plants were genetically identical and were similar to plants collected from Manipal and Herga (Site 3). AFLP based genetic distance varied between 0.0003 and 0.0241 with a mean value of 0.012. Intraspecific similarity that existed among the plants became obvious through AFLP analysis.

**Table 3:** Pair wise Genetic Distance in *G. superba* populations based on (a) RAPD data and (b) AFLP data Population ID corresponds to codes given in Table.1.A.

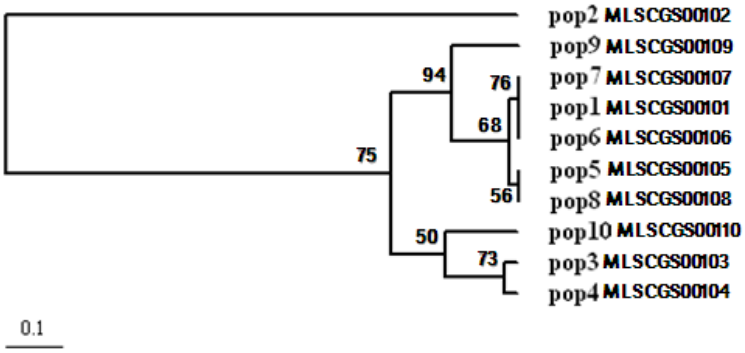
| (A) Pop ID | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | - | | | | | | | | | |
| 2 | 0.0595 | - | | | | | | | | |
| 3 | 0.1003 | 0.0601 | - | | | | | | | |
| 4 | 0.1215 | 0.0248 | 0.0950 | - | | | | | | |
| 5 | 0.0498 | 0.0889 | 0.0679 | 0.1179 | - | | | | | |
| 6 | 0.0786 | 0.0480 | 0.0369 | 0.0421 | 0.0272 | - | | | | |
| 7 | 0.2033 | 0.1461 | 0.0843 | 0.0904 | 0.0897 | 0.0325 | - | | | |
| 8 | 0.0386 | 0.0337 | 0.0369 | 0.0451 | 0.0330 | 0.0113 | 0.0649 | - | | |
| 9 | 0.0122 | 0.0495 | 0.0586 | 0.0854 | 0.0365 | 0.0394 | 0.1194 | 0.0103 | - | |
| 10 | 0.0651 | 0.0096 | 0.0629 | 0.0155 | 0.0573 | 0.0226 | 0.0972 | 0.0240 | 0.0482 | - |
| **(B) Pop ID** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** |
| 1 | - | | | | | | | | | |
| 2 | 0.0241 | - | | | | | | | | |
| 3 | 0.0011 | 0.0176 | - | | | | | | | |
| 4 | 0.0050 | 0.0091 | 0.0017 | - | | | | | | |
| 5 | 0.0020 | 0.0270 | 0.0048 | 0.0095 | - | | | | | |
| 6 | 0.0006 | 0.0226 | 0.0021 | 0.0058 | 0.0006 | - | | | | |
| 7 | 0.0002 | 0.0222 | 0.0009 | 0.0043 | 0.0017 | 0.0003 | - | | | |
| 8 | 0.0011 | 0.0234 | 0.0034 | 0.0073 | 0.0011 | 0.0006 | 0.0013 | - | | |
| 9 | 0.0042 | 0.0113 | 0.0039 | 0.0038 | 0.0049 | 0.0032 | 0.0037 | 0.0026 | - | |
| 10 | 0.0077 | 0.0160 | 0.0043 | 0.0037 | 0.0117 | 0.0078 | 0.0063 | 0.0098 | 0.0060 | - |

**Cluster Analysis:** Cluster analysis performed on RAPD data using UPGMA generated a dendrogram that showed overall genetic relationships among the populations of *G. superba* (Figure 2). Three distinct clusters could be identified. The first cluster comprised Vittalwaadi plants collected from Site 2. The second cluster comprised 20 individuals viz., Vittalwaadi plants collected from sites 1, 3 and 4, Herga plants from Sites 1 and 2 along with plants from Manipal. Manipal accessions were found to be closer to Vittalwaadi accessions collected from Site 4. Uppoor, Perampalli and Herga plants collected from Site 2 were found to be genetically similar. Cluster analysis performed on AFLP data using UPGMA generated a dendrogram showing overall genetic relationships among the populations of *G. superba* (Figure 3). Uppoor plants stood distinct as a separate cluster which was collected from a lower altitude. Plants from Manipal, Vittalwaadi (all 4 Sites) and Herga (Site 3) clustered as a different group. This group mainly comprised of plants from high altitude. Plants from median altitude levels especially from Perampalli and Herga (Site1) stood as a distinct cluster.

**Figure 2:**




**(A)** Pair wise genetic distances among 10 populations of G. superba based on RAPD data.




**(B)** Pair wise genetic distances among 10 populations of G. superba based on AFLP data.

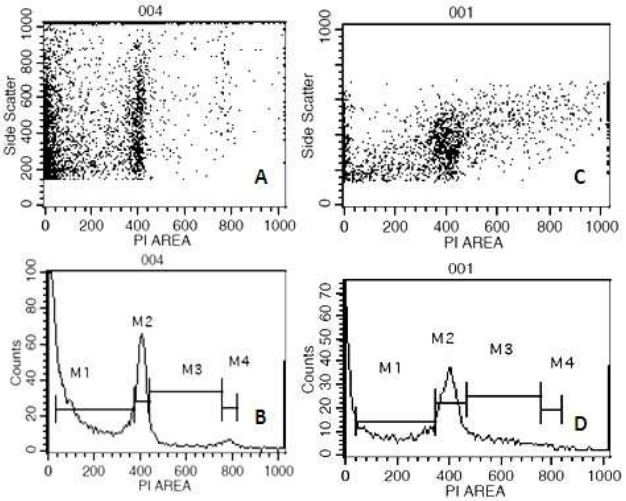



**Figure 3:** Dot plot B. Histogram of the external standard (*Zea mays*) C. Dot plot and D. Histogram of test sample MLSCGS106 displaying cells in G0/G1 set at channel number 400.

**DNA content estimation using flow cytometry:** The coefficient of variation (CV) for the *Gloriosa* peak ranged from 6.74 to 14.76 %, values regarded as acceptable for species containing staining inhibitors in their cytosol. 2C DNA values ranged from 5.24-5.81 pg (Table 4).

**Table 4:** 2C Values for Gloriosa superba determined 8 leaves per sample repeated thrice

| *Sample* | *Mean (pg)* | *SEM* | *CV (%)* |
|---|---|---|---|
| MLSCGS101 | 5.77 | 0.07 | 10.19 |
| MLSCGS102 | 5.51 | 0.11 | 11.68 |
| MLSCGS103 | 5.81 | 0.12 | 10.09 |
| MLSCGS104 | 5.38 | 0.33 | 10.10 |
| MLSCGS105 | 5.40 | 0.41 | 10.48 |
| MLSCGS106 | 5.51 | 0.88 | 6.74 |
| MLSCGS107 | 5.58 | 0.09 | 6.92 |
| MLSCGS108 | 5.69 | 0.01 | 8.08 |
| MLSCGS109 | 5.24 | 0.11 | 7.42 |
| MLSCGS110 | 5.42 | 0.34 | 14.76 |
| *Zea mays* (Standard) | 5.43 | 0.04 | 3.86 |

*SEM*= Standard error of the mean; *CV*= Coefficient of Variation

## Discussion

Restricted and scattered distribution of *G.superba* in the present study strongly resembled a typical endemic dicot plant viz., *Gossypium mustelinum* (Wendel *et al*., 1994). AFLP clustering and genetic distances were more authentic than RAPD as it could be correlated well with geography and collection sites. Closer genetic distances together with low genetic polymorphism showed geography - dependent population differentiation in *G. superba*. This clearly indicated that the species might have diversified locally as a result of natural selection under a micro-ecosystem. It is also suspected, that these plants would have undergone evolutionary sorting while getting acclimated to their respective natural habitats. Higher levels of phenotypic variation (Wilken, 1977) and allozyme polymorphism have been reported earlier in plant populations collected from stressful environments (Nevo *et al*., 1998). The occurrence of genetic variation in plants has been interpreted as a rescue mechanism to increase variability favoring natural selection (Cullis and Creissen, 1987) to tide over stress. This could be the reason for the differential clustering of Herga plants from site 3 and Manipal. It has been suggested that species with impeded gene flow exhibited higher tendency for variation among populations (Govindaraju, 1989; Hamrick *et al*., 1991). The limited scope of gene flow could be one of the possible reasons for low local diversity in *G. superba* in the Konkan region. The two geographical regions Herga and Manipal are not located far apart. Hence differences due to ecological factors are neglected. We presume that altitude might have caused geography-dependent genetic diversity in *G. superba.* Similar altitude related diversity was witnessed in *Clarkia unguiculata* (Vasek, 1964; Vasek and Sauer, 1971; Jonas and Geber, 1999). Studies have shown that species found in isolated groups are susceptible to genetic drift (Godt and Hamrick, 1993; Diaz *et al*., 1999). It is also extremely difficult to categorize the individual effects of selection and genetic drift in species divergence (Briggs and Walters, 1997). In such situations DNA fingerprinting using dominant markers would convey some valuable information. RAPD analysis in *Gloriosa superba* was restricted to one report (Ghosh *et al*., 2008) which showed 76% polymorphism. When compared to the study by Ghosh *et al.,* (2008), polymorphism in the present region was low (50 and 40% respectively). This suggested that the genetic diversity among and within populations in the Konkan region is declining. It has been proven in a number of studies that genetic diversity is comparatively less among rare and endangered plants (Waller *et al*., 1987; Gustafsson and Gustafsson, 1994; Swenson *et al*., 1995). The low level of genetic variation is often correlated with limited population sizes, in which the stochastic processes of genetic drift might pave way to

allele fixation at most loci (Hartl *et al*., 1997) finally causing their extinction. All the plants collected for the present study were diploids, with an approximate genome content of about 5.24-5.81 pg DNA, which also confirmed low intraspecific diversity. Low intraspecific diversity is a rare event in liliaceae. According to Bharathan *et al.,* (1994), the family liliaceae had wide genome contents ranging between 39.8 and 179.0. Amano *et al.,* (2007) evaluated the genome contents of six different genotypes of *Gloriosa*; where *Gloriosa superba* exhibited 1.69 2C value. The high 2C-value (5.2-5.81pg) in the present study could be attributed to environmental cues as well as due to some factors that facilitated cross pollination among the species. Apomixes event is widely attributed to the loss of genetic variability in many species (Palacios and Gonzalez-Candelas, 1997; Koch *et al*., 1998). We feel that cytological evaluation preferably meiotic chromosome behavior is crucial to determine the cause of low diversity in the species. Genetic diversity assessed by RAPD and AFLP along with nuclear DNA content analyses indicated that naturally occurring *G. superba* population is slowly heading towards population decline and must be subjected to suitable conservation procedures. It is likely that the genetically isolated smaller population of *G. superba* is also experiencing genetic drift within the Konkan region of South India.

## Acknowledgements

We thank Professor P. M. Gopinath (Senior Scientist, School of Life Sciences, Manipal University) for his comments and suggestions on the manuscript. We thank Ms. Jyothi Nayak, Technical Staff, School of Life Sciences, for assisting us with FACS analyses. This research was financially supported by TIFAC–CORE,Pharmacogenomics,Government of India and Manipal University.

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**Source of support:** Nil
**Conflict of interest:** None Declared