Environment & Ecology 29 (1A) : 389—392, 2011
 ISSN 0970-0420

Evaluation of Glory Lily (*Gloriosa Species*) Using Morphological Anatomical and Molecular Parameters for Genetic Resource Conservation

H. K. SARKAR*, TRIPARNA LAHARI, T. K. MAITY[1] AND SOMNATH BHATTACHARYYA

Department of Genetics, [1]*Department of Vegetable Crops*, *Bidhan Chandra Krishi Viswavidyalaya Mohanpur 741252, India*
E-mail : hemensarkar@rediffmail.com
**Correspondence*

Abstract

Four morphotypes of glory lily (*Gloriosa superba* L.) and another species of *Gloriosa rothschildiana* of family Colchicaceae, one of the important endangered medicinal plants and rich source of different derivatives of colchicines used as antitumor and anti cancerous drugs were evaluated using morpho-anatomical and molecular parameters. In spite of having close resemblance, the morphotypes of *Gloriosa superba* L. and *Gloriosa rothschildiana* were different from each other in respect of flower color and leaf characteristics, stem anatomical features including vascular bundles. Protocols for rapid propagation of different morphotypes from seeds in addition to the conventional slow multiplication through rhizomes were developed/ standardized. The two species of *Gloriosa* were different from each other in respect of stem anatomical features. DNA polymorphism study using RAPD and ISSR primers indicated that the types were genetically diverse in nature. Highest level of similarity (59%) was observed between type-1 and type-3 whereas type-2 was found to be most diverse among others as it exhibited only 21% similarity with type-4, 27% similarity with type-5, 35% with type-1 and 37% with type-3 respectively. Among the 15 primers used, TGC CGA ACTG showed the highest PIC value. The present study lend adequate support to the potentiality of using molecular markers as supplementary to morphological descriptors in the identification process of different morphotypes and species of *Gloriosa*. The results also helped in identifying and isolating different morphotypes as distinct genetic materials to be conserved suitably.

Key words : *Gloriosa superba, Gloriosa rothschildiana,* RAPD and ISSR Markers, Polymorphism, Molecular data.

Global demand for biological resources from the wild has been continuously on the rise. The annual demand of the botanical raw drugs in India is estimated to be 319,500 MT in 2008. Six thousand plants are currently being used by the herbal drug industry in some from or the other. About 960 medicinal plants constitute the source of 1,289 botanical raw drugs with 178 species being consumed in volumes exceeding 100 MT per year. Most of this demand is met from the wild populations without any assessment of their actual availability on a sustainable basis. This dependency on wild populations of plants for life saving drugs will not continue for a long time to come for at least two reasons—non-availability of technologies to domesticate large set of species, and growing competition between cultivation and developmental activities (1). Indiscriminate harvestings of medicinal plants for drugs and phar-

Table 1. Sequence of primers and amprification profile.

	Primer Sequence	Marker	Tm (c)	PIC value	Marker index
1	5' GAG AGA GAGA AGA GAG AC 3'	ISSR	45	0.32	0.96
2	5' GTG TGT GTG TGT GTG TC 3'	ISSR	47	0.373	0.397
3	5' AGC GCC ATT G 3'	RAPD	36	0.365	1.251
4	5' CAT CCG TGC T 3'	RAPD	36	0.373	0.895
5	5' CAG GCC CTT C 3'	RAPD	36	0.248	0.859
6	5' TGC CGA GCT G 3'	RAPD	36	0.44	1.672
7	5' GTG ATC GCA G 3'	RAPD	36	0.32	1.042

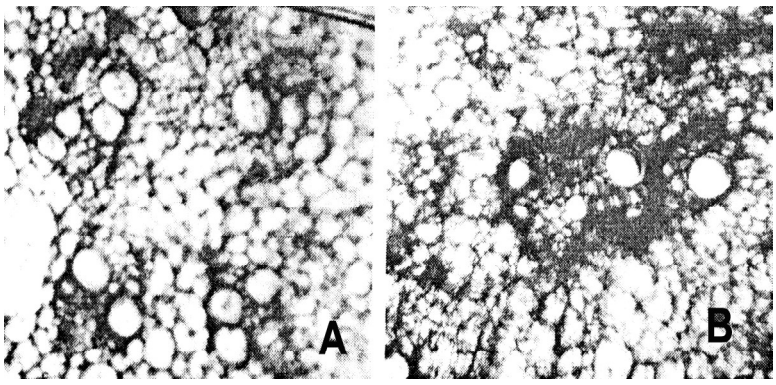

Figure 1. TS of stems of *Gloriosa rothschildiana and Gloriosa superba.*

maceutical products created a serious threat to diversity of important medicinal plant species. Suitable method of propagation and conservation may help in widening the biodiversity and an increasing scope of availability of different species of glory lily (*Gloriosa* species), endangered species of Asia and Africa (2). The species is not only used as substitute plant for *Colchicum autumnale* for the alkaloid colchicines (3) but also has antitumor, anti carcinogenic, and nematicidal properties (4). It is the national flower of Rhodesia, Zimbabawe and Sri Lanka suggesting its importance as an important ornamental plant inspite of having wild habitat. Development / standardization protocol for conservation and easy rapid propagation after identification as distinct genetic material using both morpho-anatomical and molecular descriptors is almost an unexplored area and hence needs serious attention of scientists to save this endangered medicinal plant. Propagation through rhizome is an easy choice but scope is limited because a few number of new plants can be generated within a very short period of time. Propagation through mature seeds harvested from mature capsule is not always suitable because of seed dormancy. Clonal propagation met with limited success (5, 6). An attempt was therefore made to develop a protocol for easy and dependable propagation from mature seeds to have large plant population within a very short time for genetic resource conservation. Glory lily was then could be evaluated using molecular parameters for genetic resource conservation.

Methods

Gloriosa rothschildiana and four morphotypes of *Gloriosa superba* L. constituted the experimental materials. Mature seeds were sown to earthen pots in July and pots were irrigated intermittently throughout the season and healthy plants could be had in June next year. Large numbers of plants so recovered were transplanted to soil for getting flower and fruit as usually. The plants come to flowering after four years. There exist a conspicuous difference between two species of glory lily like *Gloriosa superba* L. and *Gloriosa rothschildiana* in respect of stem anatomical features including vascular bundles (Fig. 1). Transverse section of stems of both the species were stained with pyronin and methyl green for studying the stem anatomical features particularly the arrangement of vascular bundles. *Gloriosa rothschildiana* having roundish stem whereas *Gloriosa superba* L. having angular stem with permanent furrows and ridges. Molecular characterization was done as per the following schedule.

Gloriosa DNA was extracted from 40 mg of fresh

Table 2. Similarity matrix of *Gloriosa* morphotypes based on Jaccard's similarity coefficient using molecular data.

	G_1	G_2	G_3	G_4	G_5
G_1	1.000				
G_2	0.3590	1.000			
G_3	0.5938	0.3714	1.000		
G_4	0.5313	0.2105	0.5172	1.000	
G_5	0.4000	0.2778	0.4194	0.5000	1.000

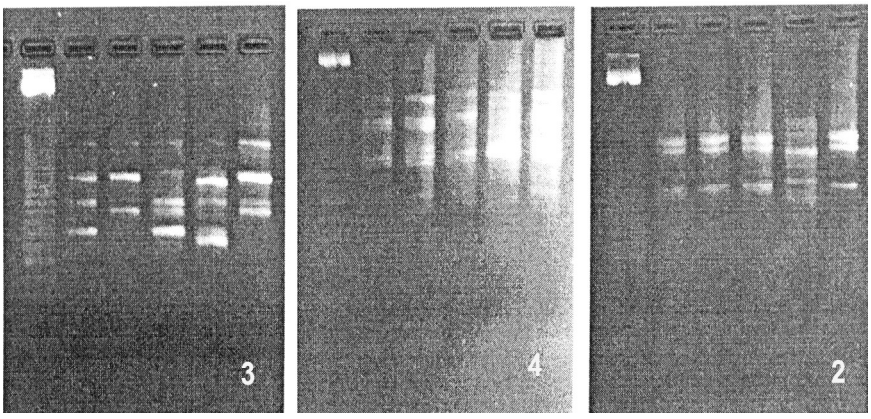

Figure 2. Internal simple sequence repeat marker amplification profile with primer : 5' GTG TGT GTG TGT GTG TC 3'. **Figure 3**. Random amplified polymorphic DNA amplification profile with primer : 5' AGC GCC ATT G. **Figure 4.** Random amplified polymorphic DNA amplification profile with primer : 5' TGC CGA GCT G 3.

green tender leaves following standard method. Then subjected to PCR amplification, using the selected ISSR and RAPD markers with 25 µl of PCR mixture comprised of 2 µl (20 ng) template DNA, 2.5µl 10X PCR Buffer, 3.5 µl – 4 µl 2.5 mM dNTPs, 1 µl (100 ng) ISSR primer (Fig. 2), 2µl (100 ng) RAPD primer (Figs. 3, 4), 1U Taq polymerase enzyme and 14—15.5µl double distilled sterile water as volume make up. Gel electrophoresis was done in 1.5% agarose gel.

Allelic Diversity Analysis

The frequency of polymorphism was calculated based on presence (1) or absence (0) of common bands. Polymorphism information content (PIC) values were calculated as

$$PIC = 1/n \; \Sigma \; 2F \; (1 - F)$$

Where F = Proportion of particular allele among genotypes.

MI = PIC × Proportion of polymorphic bands × Average number of loci per assay unit. It is the resolving power of a molecular marker that indicates their ability to generate polymorphism for different genotypes.

The genetic associations were evaluated from Jaccard's similarity coefficient. Dendogram (Fig. 5) was generated with unweighted pair-group method arithmetic average (UPGMA) algorithm, using NTSYS-pc Version 2.1 software (7).

Results and Discussion

Leaf breadth is wider in *G. rothschildiana* contrast to narrower leaves of *G. superba* with longer vine length. With the age of the flower, color of petals changed from yellow to deep red starting from apex to base in *G. superba* and on the contrary slightly pinkish color develops along the middle part of the petal and finally turns into pinkish violet to violet in case of *G. rothschildiana.* The stem anatomy revealed the vascular bundles as scattered throughout the pith in *G. superba.* However, in addition to peripheral vascular bundles, there were prominent four vascular bundles inside the pith in *G.*

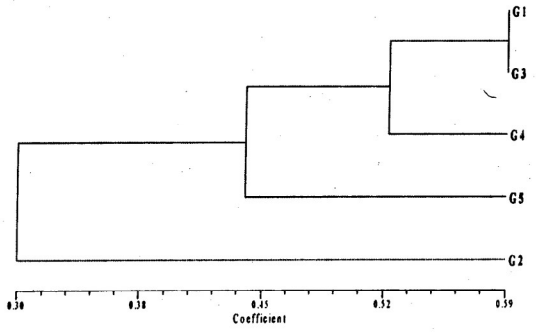

Figure 5. Dendogram of clustering of gloriosa morphotypes by UPGMA using molecular data.

rothschildiana (Fig. 1). The materials consisting of 4 morphotypes of *Gloriosa superba* and *G. rothschildiana* were screened with around 15 ISSR and RAPD primers. Among those around 5 RAPD and 2 ISSR had shown polymorphism in their amplification profile in 1.5% agarose gel. Table 1 shows the sequences of those primers and information of those primers and their amplification profile in details is provided. Among all used primers 5' TGC CGA GCT G 3' showed highest polymorphism information content value (0.44) among the morphotypes and species. The highest marker index is observed in this same RAPD marker.

Clustering of Gloriosa superba L. Morphotypes and Gloriosa rothschildiana by UPGMA Using Molecular Data. Clustering of five *Gloriosa* morphotypes through Jaccard's similarity coefficient (Table 2) revealed maximum level of similarity of 59% between G_1 and G_3. The most diverse morphotype among all is G_2 (*G. rothschildiana*) which showed only 21% similarity G_4, 27% with G_5, 35% with G_1 and 37% with G_3.

The two species of *Gloriosa* are not only separable by morphological descriptors but also may be distinguished by stem anatomical characteristics and genetical parameters i.e. DNA polymorphism study by RAPD and ISSR. Maximum similarity could be observed between G_1 and G_3. So conservation of any one of the two may serve the purpose. G_2 i.e. *Gloriosa rothschildiana* is the most diverse type among various morphotypes. It is most distinctly related to other morphotypes of *Gloriosa superba* and may be used as a better parent for crossing for exploiting heterosis.

References

1. Swarup R., S. Sarin, S. Nitish and K. N. Gawshaiah. 2009. Mapping life. *Biotech. News* 4 : 18—22.
2. Siva Kumar G. and K. V. Krishnamurthy. 2000. Micropropagation of *Gloriosa superba* L. and endangered species of Asia and Africa. *Curr. Sci.* 78 : 30—32.
3. Bharthi P., D. Philomina and S. Chakkaravarthi. 2006. Estimation of colchicines in six different species of gloriosa grown in vivo. *Ind. J. Farm Sci.* 68 : 806—809.
4. Panday R. and A. Hassep. 1988. Studies on the toxicity of extract of certain medicinal plants to root knot nematode, *Meloidogyne incognata. Ind. J. Pl. Path.* 6 : 184—186.
5. Sharma P., Y. D. Sharma, S. R. Dhiman and Y. C. Gupta. 2003. Studies on clonal propagation of Gloriosa. *J. Ornam. Hort. (New Series)* 6 : 344—351.
6. Hassan A., K. M. Sayeed and S. K. Roy. 2005. Micro-propagation of *Gloriosa superba* L. through high frequency shoot proliferation. *Pl. Tiss. Cult.* 15 : 67—74.
7. Rohlf P. J. 1990. *NTSYS-pc. Numerical taxonomy and multivariate analysis system, version 2.02.* Appl. Biostat., New York, USA.