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Assessment of genetic diversity of *Gloriosa superba* L. accessions detected by random amplified polymorphic DNA analysis

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Genetic diversity of eighteen glory lily (*Gloriosa superba* L.) accessions of diverse geographical origin was studied 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. Cluster analysis delineated the accessions into two groups.

Key words: Genetic diversity, *Gloriosa superba*, random amplification of polymorphic DNA (RAPD), unweighted pair group method using arithmetic averages (UPGMA).

INTRODUCTION

Gloriosa superba L., a climber belonging to the family Liliaceae is a major highly valuable medicinal crop. It is one of the major medicinal plants in India cultivated for its seeds and tubers 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, namely, colchicine and colchicoside as the major constituents, used to treat gout and rheumatism (Gupta et al., 2005). It is traditionally used for the treatment of bruises, colic, chronic ulcers, haemorrhoids and cancer (Nadkarni, 1978). Duke (1985) also reported the abortifacient action of the plant rhizome.

Gloriosa was found in the wild on natural fences a decade back, but now it has been domesticated for economic gain in as much as all parts of the plant having medicinal value. In nature, less seed germination with poor viability is responsible for its diminishing population size. The poor propagation coupled with over-exploitation companies has put this plant into acutely threatened by the local population as well as pharmaceutical species (Yadav et al., 2012). Therefore, tracing successfully adapted variants at genetic level of *G. superba* is of immediate necessity for their long-term preservation of these species.

For efficient conservation and management, the genetic composition of the species in different geographic locations needs to be assessed. Due to technical simplicity and speed, RAPD methodology has been used for diversity analysis in many red listed plants (Li et al., 2002; Fu et al., 2003; Prasad et al., 2007). The objective of the present study is to assess genetic diversity among the accessions using random amplification of polymorphic DNA (RAPD) markers to provide genetic data and a theoretical basis for protection of the species. Hence, an attempt has been to investigate variation among eighteen accessions of *G. superba by* using RAPD markers. RAPD markers are based on the amplification of unknown DNA sequences using single, short, random oligonucleotide primers, therefore, RAPD

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Table 1. Glory lily accessions subjected to RAPD analysis to determine genetic diversity.

S/N	Accessions	Name of the germplasm
1	GS 01	Nallampalayam cultivated
2	GS 02	Kallimanthayam cultivated
3	GS 03	Sathyamangalam wild
4	GS 04	Aruppukotai wild
5	GS 05	Aruppukotai cultivated
6	GS 06	Kankayam cultivated
7	GS 07	Kallimanthayam wild
8	GS 08	Ottanchadram cultivated
9	GS 09	Moolanur cultivated
10	GS 10	Jeyankondam cultivated
11	GS 11	Udangudi cultivated
12	GS 12	Viralimalai cultivated
13	GS 13	Pudukottai cultivated
14	GS 14	Andhra cultivated - I
15	GS 15	Andhra wild
16	GS 16	Z-Melur cultivated
17	GS 17	Poondurai wild
18	GS 18	Andhra cultivated -II

Wild: Sourced from natural habitat; Cultivated: sourced from farmers' field of respective location.

polymorphism is the reflection of variation of the whole genomic DNA, and would be a better parameter to measure the pattern of genetic diversity of the rare and endangered plants.

MATERIALS AND METHODS

Plant and DNA extraction

Tubers were collected from different locations of Tamil Nadu and Andhra Pradesh and grown in the field at the Medicinal Plants Unit, Botanical Garden, Tamil Nadu Agricultural University, Coimbatore (Table 1). The leaf materials were used for DNA isolation.

DNA was extracted from one-month-old young furled leaves following modified CTAB method of Wilkie (1997). DNA quality and quantity was checked on agarose gel with λ*Hin*d III DNA marker.

RAPD analysis

RAPD marker amplifications were carried out in a 25 µl reaction volume containing 30 ng of genomic DNA, 2.5 µl of 10X polymerase chain reaction (PCR) buffer (including 15 mM $MgCl_2$), 0.5 µl of 10 mM each of dATP, dTTP, dGTP and dCTP, 1 µl of 20 pMol of primer, 1 unit of Taq DNA polymerase (Bangalore Genei Pvt Ltd, Bangalore). Amplifications were performed in Bio-Rad (MyCycler thermal cycler) programmed for an initial denaturation at 94°C for 5 min, 44 cycles of 1 min denaturation at 94°C, 1 min annealing at 37°C and 2 min extension at 72°C and a final extension of 10 min at 72°C and then at 4°C till storage.

PCR amplified products (12.5 µl) were subjected to electrophoresis in a 1.5% agarose gel in 1X Tris-borate-EDTA (TBE) buffer at 100 V for 3.5 h using submarine electrophoresis unit. The ethidium bromide stained gels were documented using Alpha Imager TM 1200-Documentation and Analysis system of the Alpha Innotech Corportion, USA. Sizes of the identified bands were determined relative to 1 kb ladder (Fermentas, Germany).

Analysis of data

Bands were scored for the presence and absence of the corresponding band among the different accessions. The scores '1' and '0' were given for the presence and absence of bands, respectively. The data obtained by scoring the RAPD profiles of different primers were subject to cluster analysis. Similarity matrix was constructed using Jaccard's coefficient (Jaccard, 1908) and the similarity values were used for cluster analysis and dendrogram was constructed by unweighted pair-group method using arithmetic averages (UPGMA) with the sequential agglomerative hierarchical and nested (SAHN) function (Sneath and Sokal, 1973). Similarity coefficients were also subjected to Principal Coordinate (PCO) analysis. Data was analysed using NTSYS-PC version 2.02i (Rohlf, 1994).

RESULTS AND DISCUSSION

Progress in crop breeding requires the exploitation of genetic variation among races and gene pools. Careful selection of primers and a high total number of polymorphic bands are essential for germplasm discrimination analysis. RAPD analysis in the 18 accessions of *G. superba* using 58 primers produced a total of 413 bands/amplicons, of which 370 were polymorphic (Table 2). This resulted in a very high polymorphism of 87.53%. Thirty seven out of fifty eight primers used, gave 100% polymorphism. The band size ranged from 100 to 3000 bp. Total number of bands per

Table 2. List of primers along with their sequences and amplification details.

Primer	Sequence (5'–3')	No. of amplified fragments	No. of polymorphic bands	Polymorphism (%)	Molecular weight range (bp)
OPA-01	CAGGCCCTTC	4	2	50.00	600-1200
OPA-02	TGCCGAGCTG	3	3	100.00	750-1500
OPA-05	AGGGGTCTTG	6	5	83.33	750-2000
OPA-07	GAAACGGGTG	9	9	100.00	500-2500
OPA-08	GTGACGTAGG	7	7	100.00	500-2000
OPA-10	GTGATCGCAG	4	4	100.00	250-1500
OPA-13	CAGCACCCAC	9	9	100.00	100-2000
OPA-15	TTCCGAACCC	8	8	100.00	500-2000
OPB-03	CATCCCCCTG	7	4	57.14	500-2500
OPB-04	GGACTGGAGT	6	6	100.00	500-3000
OPB-06	TGCTCTGCCC	9	7	77.78	600-3000
OPB-13	TTCCCCCGCT	7	7	100.00	500-2000
OPB-15	GGAGGGTGTT	8	8	100.00	500-3000
OPB-17	AGGGAACGAG	6	6	100.00	1000-3000
OPB-19	ACCCCCGAAG	7	5	71.43	500-2000
OPC-02	GTGAGGCGTC	7	7	100.00	250-1500
OPC-08	TGGACCGGTG	5	5	100.00	100-3000
OPC-09	CTCACCGTCC	6	6	100.00	750-2000
OPC-10	TGTCATCCCC	5	5	100.00	500-3000
OPC-15	GACGGATCAG	5	5	100.00	250-3000
OPC-16	CACACTCCAG	11	8	72.73	750-3000
OPC-17	TTCCCCCCAG	7	6	85.71	500-2000
OPC-18	TGAGTGGGTG	8	8	100.00	100-3000
OPD-07	TTGGCACGGG	5	5	100.00	500-3000
OPD-13	GGGGTGACGA	6	6	100.00	100-1500
OPD-16	AGGGCGTAAG	14	14	100.00	100-3000
OPF-01	AGCGATCCTG	9	9	100.00	800-3000
OPF-02	GAGGATCCCT	7	5	71.43	500-1500
OPF-03	CCTGATCACC	10	8	80.00	500-2500
OPF-04	GGTGATCAGG	6	5	83.33	600-3000
OPF-05	CCGAATTCCC	13	12	92.31	600-3000
OPF-06	GGGAATTCGG	14	10	71.43	600-3000
OPF-07	CCGATATCCC	11	9	81.82	350-1500
OPF-08	GGGATATCGG	11	11	100.00	250-2500
OPF-09	CCAAGCTTCC	7	7	100.00	600-2000
OPF-11	TTGGTACCCC	9	9	100.00	500-1000
OPF-12	ACGGTACCAG	2	2	100.00	750-1500
OPF-13	GGCTGCAGAA	7	6	85.71	600-2000
OPF-14	TGCTGCAGGT	4	3	75.00	250-1500
OPF-15	CCAGTACTCC	8	8	100.00	600-2000
OPF-16	GGAGTACTGG	6	4	66.67	500-1500
OPF-17	AACCCGGGAA	7	6	85.71	750-2000
OPF-18	TTCCGGGTTT	4	4	100.00	600-2000
OPF-19	CCTCTAGACC	7	4	57.14	500-1500
OPF-20	GGTCTAGAGG	4	2	50.00	500-1400
OPS-11	AGTCGGGTGG	10	9	90.00	600-2000
OPZ- 03	CAGCACCGCA	2	2	100.00	350-2500
OPZ- 04	AGGCTGTGCT	8	8	100.00	750-2000
OPZ- 05	TCCCATGCTG	5	5	100.00	250-3000

Table 2. Contd.

OPZ- 06	GTGCCGTTCA	8	8	100.00	150-2500
OPZ- 07	CCAGGAGGAC	11	11	100.00	750-2000
OPZ- 08	GGGTGGGTAA	3	2	66.67	250-1000
OPZ- 09	CACCCCAGTC	5	5	100.00	500-2500
OPZ- 10	CCGACAAACC	9	9	100.00	750-3000
OPZ- 11	CTCAGTCGCA	5	5	100.00	250-2000
OPZ- 12	TCAACGGGAC	5	5	100.00	500-2500
OPZ- 13	GACTAAGCCC	8	8	100.00	250-3000
OPAW- 03	CCATGCGGAG	5	4	80.00	500-2000

primer ranged from 2 (OPF-12 and OPZ-3) to 14 (OPD-16 and OPF-6). The primers, namely, OPD-16 and OPF-6 showed the maximum number of bands (14). The average number of bands produced per primer was 6.88. The number of polymorphic bands ranged from 2 (OPA-1, OPF-12, OPF-20, OPZ-3 and OPZ-8) to 14 (OPD-16) (Figure 1).

This shows that RAPD is a good marker system for genetic diversity analysis in the crop under study due to its highly informative nature. Mossi et al. (2007) used 180 polymorphic bands from 14 RAPD markers for categorization of *Maytenus ilicifolia* cultivars; likewise, Jayaram and Prasad (2008) used 188 polymorphic bands from 40 RAPD markers for characterization of *Oroxylum indicum* accessions. Obviously, the more bands that are scored and the more plants that are studied, the higher will be the statistical significance of the calculation. About 100 bands should be enough to obtain statistically significant results (Kocsis et al., 2005).

The percentage of polymorphism, that is, 87.53% was higher in comparison to other endangered plants, e.g. *Lactoris fernandeziana* (Lactoridaceae) (24.5%) (Brauner et al., 1992), *Paeonia suffruticosa* (22.5%) and *Paeonia rockii* (27.6%) (Pei et al., 1995), *Cathaya argyrophylla* (32%) (Wang et al., 1996), *Dacydium pierrei* (33.3%) (Su et al., 1999) and *Oroxylum indicum* (49.61%) (Jayaram and Prasad, 2008). This shows that the species genetic diversity by itself is higher when compared with other endangered species as stated earlier and it should be able to adapt to the environmental variation.

Cluster analysis revealed that 18 accessions were grouped in two major clusters (Figure 2). The clusters A and B contain 16 and 2 accessions, respectively. The genetic similarity coefficient (GS) values based on RAPDs for all the possible 153 pairs of accessions ranged from 0.66 (between Andhra and Poondurai wild) to 0.88 (between Kallimanthayam cultivated and Sathyamangalam wild) with a mean of 0.77. The results of principal coordinate analysis are as shown in Figure 3. The variation accounted in the first, second and third components accounted for 26.25, 11.41 and 8.46% of total variation, respectively while the total variation was 46.12%. The results of principal coordinate analysis

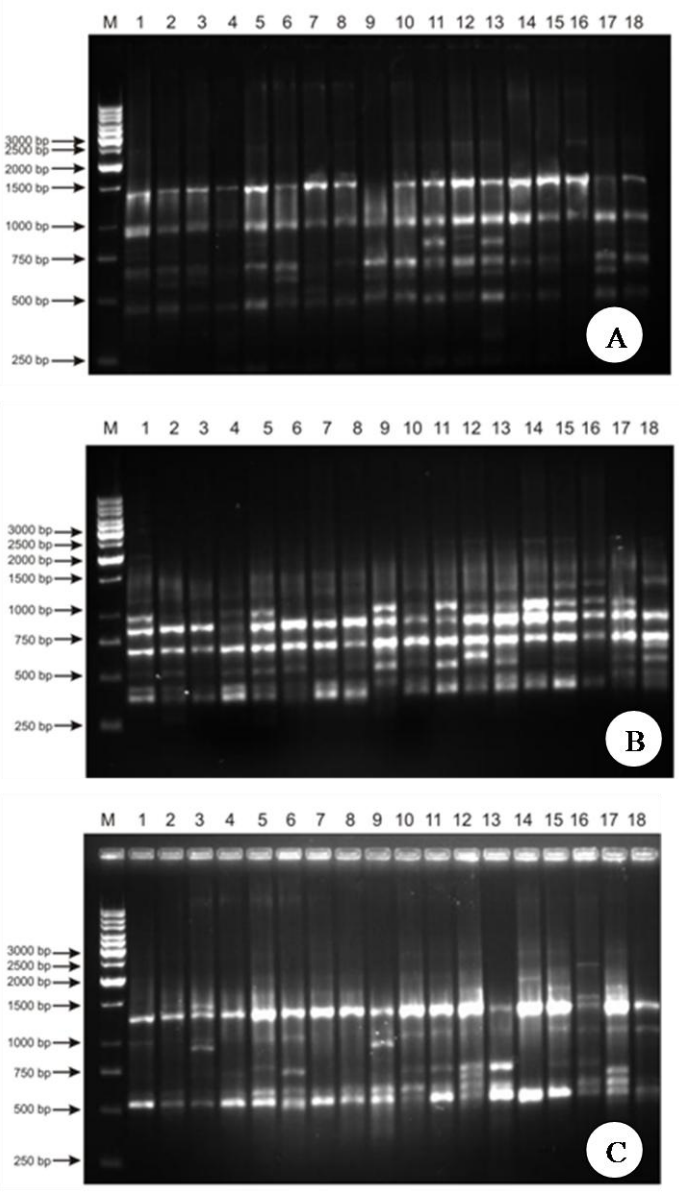

Figure 1. PCR amplification patterns showing polymorphism detected by an RAPD primer OPB-3 (A), OPF-7 (B) and OPF-4 (C) in different accessions of glory lily. M =1 kb ladder. Numbers 1-18 represents different varieties as given in the Table 1.

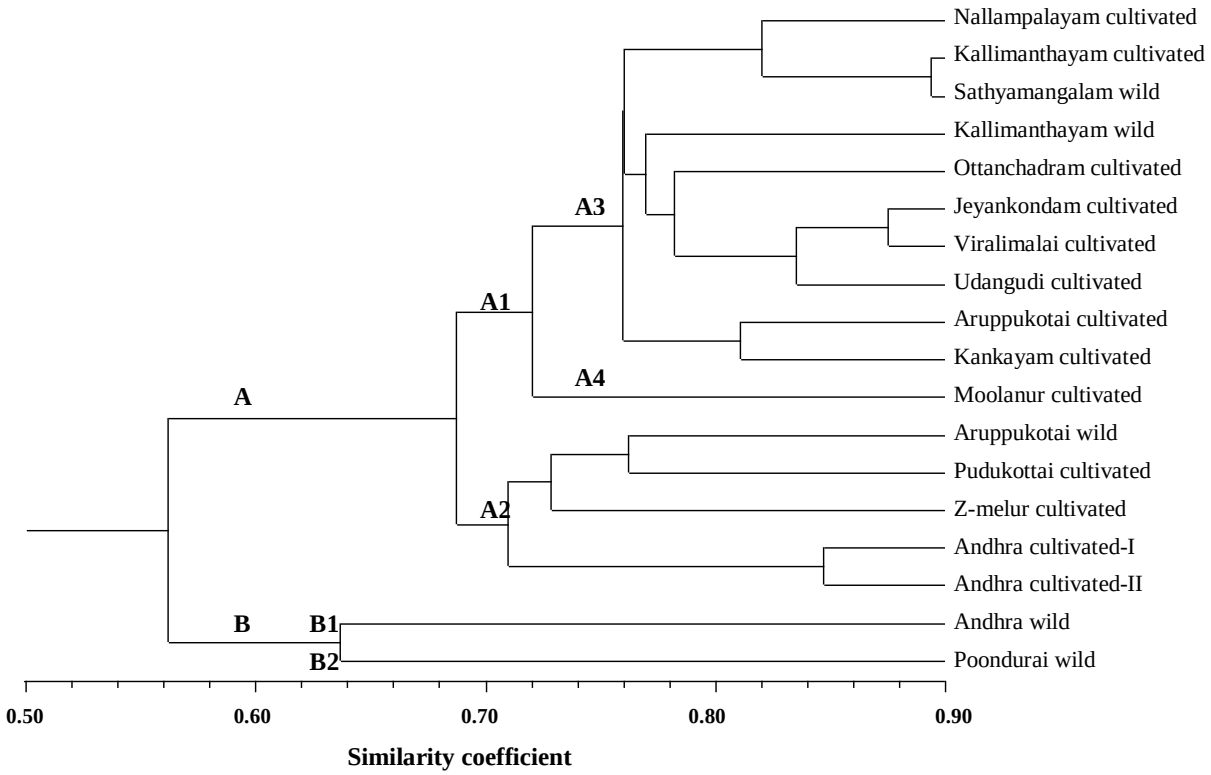

Figure 2. Dendrogram showing clustering pattern of 18 glory lily accessions based on genetic similarity values obtained from the RAPD data. Numbers 1-18 represents different accessions as given in Table 1.

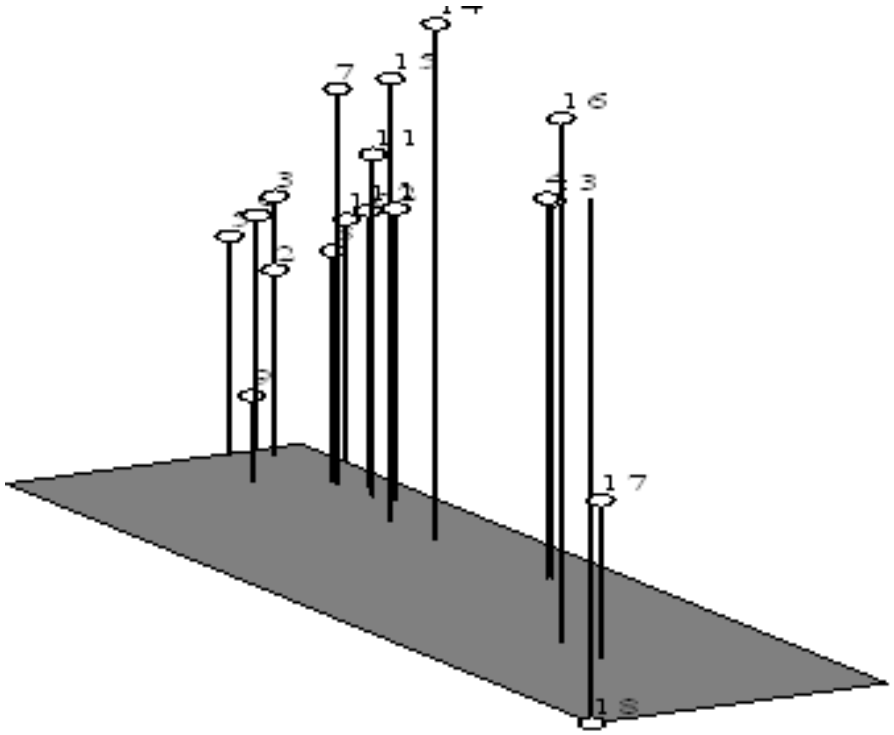

Figure 3. Patterns of relationships among 18 glory lily accessions revealed by principal coordinate analysis based on RAPD data. Numbers 1-18 in figure represents different accessions as shown in Table 1.

(PCA) corresponded well with cluster analysis obtained through UPGMA. The present results support the earlier views that RAPD analysis is a desirable technique for detection of genetic diversity in glory lily. Results of cluster analysis and PCA clearly showed the RAPD primers used in the present study can easily demarcates the accessions. The overall results indicated that a considerable diversity exists in a set of accessions analysed in the present investigation. This information can be used for selection of desirable parents for improvement of glory lily.

ABBREVIATIONS

RAPD, Random amplification of polymorphic DNA; **UPGMA,** unweighted pair group method using arithmetic averages; **PCA,** principal coordinate analysis.

REFERENCES

Brauner S, Crawford DJ, Stuessy TF (1992). Ribosomal DNA and RAPD variation in the rare plant family Lactoridaceae. Am. J. Bot. 79:1436-1439.

Duke JA (1985). Handbook of medicinal herbs. CRC Press, USA.

Fu C, Qiu Y, Kong H (2003). RAPD analysis for genetic diversity in *Chagium smyrnioides* (Apiaceae), an endangered plant. Bot. Bull. Acad. Sin. 44:13-18.

Gupta LM, Rana RC, Raina R, Meenakshi G (2005). Colchicine content in *Gloriosa superba* L. SKUAST-J. J. Res. 4:238-241.

Jaccard P (1908). Nouvelles recherches sur la distribution florale. Bull. Soc. Vaud. Sci. Nat. 44 :223-270.

Jayaram K, Prasad MNV (2008). Genetic diversity in *Oroxylum indicum* (L.) Vent. (Bignoniaceae), a vulnerable medicinal plant by random amplified polymorphic DNA marker. Afr. J. Biotech. 7:254-262.

Kocsis M, Jaromi L, Putnoky P (2005). Genetic diversity among twelve grape cultivars indigenous to the Carpathian Basin revealed by RAPD markers. Vitis 44:87-91.

Li Q, Xu Z, He T (2002). *Ex situ* genetic conservation of endangered *Vatica guangxiensis* (Dipterocarpaceae) in China. Biol. Conserv. 106:151-156.

Mossi AJ, Cansian RL, Leontiev-Orlov O, Zanin EM, Oliveira CH, Cechet ML, Carvalho AZ, Echeverrigaray S (2007). Intra and inter populational genetic variability in *Maytenus ilicifolia* Mart.through RAPD markers. Braz. J. Biol. 67:957-961.

Nadkarni KM (1978). Indian Materia Medica, Popular Prakashan, Mumbai, India P. 1.

Pei YL, Zou YP, Yin Z, Wang XQ, Zhang ZX, Hong DY (1995). Preliminary report of RAPD analysis in *Paeonia suffruticosa* subsp. *Spontanea* and *P. rockii*. Acta Phys. Sin. 33:350-356.

Prasad MNV, Padmalatha K, Jayaram K, Raju NL, Teixeira DA, Silva JA (2007). Medicinal plants from Deccan ecoregion, India: Traditional knowledge, ethnopharmacology, cultivation, utilization, biotechnology and conservation-Opportunities and impediments. Med. Arom. Plants Sci. Biotech. 1:155-208.

Rohlf FJ (1994). NTSYS-PC: Numerical taxonomy and multivariate analysis system. version 2.2. State University of New York, Stony Brook, New York.

Sneath PHA, Sokal RR (1999). Numerical taxonomy. W.H.Freeman. (ed.) San Fransisco.

Su YJ, Wang T, Huang C (1999). RAPD analysis of different population of *Dacydium pierrei*. Acta Bot. Sin. 40:169-175.

Wang XQ, Zou YP, Zhang DM, Hong DY (1996). RAPD analysis for genetic polymorphism in *Cathaya argyrophylla*. China. Science 26:437-441.

Wilkie S (1997). Genomic DNA isolation, Southern blotting and Hybridization. In: Plant Molecular Biology - a Laboratory Manual. (ed).Clark, M.S. Springer- Verlag, New York pp. 3-53.

Yadav K, Aggarwal A, Singh N (2012). Actions for *ex situ* conservation of *Gloriosa superba* L. - an endangered ornamental cum medicinal plant. J. Crop Sci. Biotech. 15:297-303.