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Assessment of genetic diversity in a highly valuable medicinal plant *Catharanthus roseus* using molecular markers

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ABSTRACT – *Genetic diversity was evaluated among 14 cultivars of* Catharanthus roseus *using RAPD and ISSR markers. The RAPD primers resulted in the amplification of 56 bands, among which 46 (82%) bands were polymorphic Four ISSR primers amplified 31 loci out of which 17 were polymorphic and 14 are monomorphic. The Jaccard's similarity derived from the combined marker system showed that the varieties First Kiss Coral and Cooler Orchid were the most closely related cultivars, with 98% similarity. In the dendrogram constructed on the basis of both RAPD and ISSR data two clear clusters were obtained. The smaller cluster included* C. roseus *Cv Blue Pearl and* C. roseus *Cv. Patricia White and the larger cluster was subdivided into two sub clusters with* C. roseus *Cv. First Kiss Polka Dot isolated from the rest of the cultivars. This may be useful for breeding for improved quality.*

Key words: ISSR, RAPD, *Catharanthus roseus*, Genetic variation.

INTRODUCTION

Catharanthus roseus (L.) G. Don (family Apocynaceae) is one of the most interesting groups of ornamental plants in the world with a very high medicinal value. (Papon etal. 2005). The plant is known to control major diseases such as leukemia and diabetes (Chattopadhyay et al. 1992, Singh et al. 2001, El-Sayeed and Verpoorte 2005). Initially, the plants were however grown as garden plants because of their beautiful flowers of different colors, such as pink, deep rose, scarlet red, white, white with red eye, and lavender blue with white eye. Nowadays, new and improved varieties of *Vinca* are also available.

Catharanthus roseus (Vinca rosea) is native to Madagascar and spread throughout the tropics and subtropics. Though considerable variation can be observed in gardens around the world, no information on the origin is available not even attempts have been made so far to study the genomic relations among *Vinca*. The polymerase chain reaction (PCR) based on molecular markers such as random-amplified polymorphic DNA (RAPD) and inter simple sequence repeats (ISSRs) are being extensively used to study the genetic diversity in a number of plant species at the species as well as cultivar level. To date, no report is available on applications of molecular markers in studies on the genetic diversity of different *Catharanthus roseus*

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varieties. In this investigation, a first attempt was made to study the genetic diversity among different cultivars of *Catharanthus roseus* using RAPD and ISSR markers.

MATERIAL AND METHODS

For this study 14 cultivars of *Catharanthus roseus* (L.) G. Don were collected in the botanical garden of the Regional Plant Resource Centre, Bhubaneswar, Orissa, India (Table 1). Fresh and young leaf samples were collected to isolate genomic DNA

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Isolation of genomic DNA

Genomic DNA was isolated from tender young leaves by the standard CTAB (Cetyl trimethyl ammonium bromide) method (Doyle and Doyle 1990) with slight modifications. Insoluble polyvinylpolypyrrolidone (PVPP) was added to the leaf tissue prior to grinding. RNA was removed by RNaseA treatment. The DNA concentration was determined by comparison with a known concentration of ë-phase DNA, as described by Sambrook et al. (1989). The DNA quality as well as quantity was also checked by an UV-VIS Spectrophotometer (Analytik Jena, Germany). After quantification, the DNA was diluted with $T_{10}E_1$ buffer (Tris 10mM and EDTA 1 mM, pH 8.0) to a working concentration of 25 ng mL^{-1} for PCR analysis.

Random Amplified Polymorphic DNA (RAPD) analysis

The RAPD analysis was performed following the methodology of Williams et al. (1990). Each amplification of 25 mL reaction volume contained 2.5 mL 10X assay buffer (100 mM Tris-Cl, pH 9.0, 500 mM KCl, 15 mM $MgCl_2$ and 0.1% gelatin), 200 mM of each dNTPs (dATP, dTTP, dCTP and dGTP) (MBI Fermantas, Lithuania) 15 ng primer, 0.5 unit of Taq DNA polymerase (Bangalore Genei Pvt. Ltd, Bangalore, India) and 25 ng of DNA template. The amplification reaction was carried out using a thermal cycler (GeneAmp PCR System 9700, Applied Biosystems, USA). Amplification was performed in three steps. In the first, the sample was maintained at 94°C for 5 minutes for complete denaturation of the template DNA. The second step consisted of 42 cycles, each cycle with three temperature regimes, i.e. at 92°C for 1 min for denaturation of template DNA, at 37°C for 1 min. for primer annealing and at 72°C for 2 min. for primer extension followed by complete polymerization at 72°C for 7 min. The soaking temperature was 4°C.

Table 1. Name, petal color and eye color of 14 cultivars of *Catharanthus roseus* (L.)G. Don

Sl. No.	Name of cultivar	Petal color	Eye color
1.	First Kiss Coral	Crimson Red	Large white
2.	Cooler Orchid	Pinkish Red	White eye with yellow cente
3	First Kiss Orchid	Pinkish Red	Star-shaped eye with large yellow center
4.	Experimental Deep Pink	Deep Pink	dark pink eye with w dark pink eye with white centre
5.	First Kiss Peach	Whitish Pink	Pink radiating eye with yellow centre
6.	Cooler Peppermint	White	Star shape red eye with white centre
7.	Cooler Peppermint improved	White	Small red eye with yelbw centre
8.	Cooler Icy Pink	Light pink	White eye
9.	First kiss Cherry red	Deep crimson	Large radiating white eye
10.	Experimental Rose Pink With Eyes	Pale pink	Radiating red eye
11.	Victory Red	Deep red centre	Dark red eye with yellow
12.	Blue Pearl	Purple blue	large radiating white base with yellow center
13.	First Kiss Polka Dot	Milky white	Red eye with yellow centre
14.	Patricia White	Off white	Small yellow centre

After completion of the PCR, 2.5 mL of 6X loading dye (MBI Fermantas, Lithuania) was added to the amplified product, which was deep-frozen at –20°C for future use.

Inter Simple Sequence Repeat (ISSR) analysis

For ISSR analysis, four selected inter simple sequence repeats (Bangalore Genei Pvt. Ltd., Bangalore, India) [(AGG)6, (GA)9T, (GACA)4, and T (GA)9] were used for PCR amplification. Each amplification reaction mixture of 25 mL contained 25 ng template DNA, 2.5 mL of 10X assay buffer (100mM Tris-HCl pH 8.3, 500mM KCl and 0.1% gelatin), 1.5mM MgCl2, 200mm of each dNTPs, 15ng primer and 0.5U Taq DNA polymerase (Bangalore Genei Pvt. Ltd., Bangalore, India). The amplification was carried out in a thermal cycler (GeneAmp PCR System 9700, Applied Biosystem, USA). The first step consisted of complete denaturation of the sample template DNA at 94°C for 5 minutes. The second step consisted of 42 cycles, each cycle with three temperature ranges: denaturation of template DNA at 92°C for 1 minute, primer annealing at 45-54°C for 1 min and primer extension at 72°C for 2 min. followed by complete polymerization of the sample at 72°C for 7 min.. The soaking temperature was 4°C. After completion of the PCR, 2.5 mL of 6X loading dye (MBI Fermentas, Lithuania) was added to the amplified product, which was deep frozen at –20°C for future use.

Agarose Gel Electrophoresis

The amplicons were separated in 1.5 and 2 % agarose gel for RAPD and ISSR, respectively. Electrophoresis was performed at a constant voltage at 60 V for 3 hours. The amplicons were visualized under UV light and photographed. The gel was also documented by Gel Doc 2000 (Bio-Rad, USA) for scoring the bands. The amplicon size was determined by comparison with the ladder (Gene Ruler 100 bp ladder plus). The entire process was repeated at least twice to ensure reproducibility.

Data scoring

The data were scored as '1' for band presence and '0' for absence for each primer genotype combination for RAPD and ISSR analysis. All bands (monomorphic and polymorphic) were considered to avoid under/over estimation of the genetic similarity (Gherardi et al. 1998). The data were used as discrete variables in a binary matrix.

Statistical data analysis

Jaccard's similarity coefficient (Jaccard 1908) was measured as well as a phylogram based on similarity coefficients generated by the unweighted pair group method using arithmetic means (UPGMA) (Sneath and Sokal 1973) and SAHN clustering. The most informative primers were detected by comparing all primers with those of the pooled data using Mantel Z statistics (Mantel 1967). Most informative primers were used for a diagrammatic representation of the banding pattern of 14 individual specimens of *Catharanthus roseus*. The entire analysis was performed using the statistical package NTSYS pc 2.02e (Rohlf 1997). The resolving power of the RAPD and ISSR primer was calculated according to the method of Prevost and Wilkinson (1999). Resolving power (RP) was calculated as RP=SIb (Ib= Band informativeness) =1-[2´(0.5-P)], where P is the proportion of the 18 plant specimens containing the band. The RAPD Primer Index (RPI) was calculated based on the Polymorphic index. This index (PIC) was calculated as PIC = $1-\text{å}P^2_i$; P_i is the band frequency of the i^{th} allele (Smith et al. 1997). In case of RAPDs and ISSRs, the PIC was considered to be $1-p^2-q^2$, where p is band frequency and q is no band frequency (Ghislain et al. 1999). The PIC value was then used to calculate the RAPD primer index (RPI). RPI is the sum of the PIC of all markers amplified by the same primer.

RESULTS AND DISCUSSION

In this investigation, 25 random decamer oligonucleotide primers were used for fingerprinting of 14 *Catharanthus roseus* cultivars. Of these 25 primers, the amplifications of only 14 primers (OPA03 OPC-11, OPC-12, OPC-13, OPC-16, OPD-20, OPAF05, OPAF06, OPAF10, OPAF11, OPAF14, OPAF15, OPN15, and OPN16) were satisfactory and reproducible. The reason for the non-amplifications of the other 11 primers could not be explained. Probably the sample DNA did not have any binding site for the primers. A similar non-amplification of decamer primers was reported by Hosaka et al. (1984), Cisneros and Quiros (1995), Sosinski and Douches (1996) and Mattagajasingh et al. (2006), in different plant species. The amplification pattern is shown in Figure 1 (a) and the details of the

RAPD analyses in Table 2. All these 14 primers resulted in the amplification of 56 bands, of which 46 bands (82%) were polymorphic while only 10 bands (18%) were monomorphic, indicating the presence of a high degree of genetic variation in the studied cultivars. The mean number of amplified bands per primer was only four. All primers obtained a wide range of amplicons, ranging from 300 bp to 3000 bp. The largest amplicon (3000 bp) in this study was amplified by the primers OPC13 and OPAF15 and the shortest (300 bp) by OPN16. The highest number of amplicons (9) was observed for primer OPAF05 and the lowest (1) for the primers OPN16 and OPAF10. The primers OPN15, OPAF05 and OPAF15 produced a maximum number of polymorphic bands (7) and OPN 16, OPAF10 and OPAF14 a minimum number. The primers OPAF05, OPC11, OPC12, OPD20 produced 2 and OPA03 and OPAF14, produced 1 monomorphic band, respectively. It is noteworthy that primer OPAF11 produced two exclusive bands while OPD20, OPAF06 and OPAF15 produced one exclusive band each. In this investigation the resolving power (RP) was highest for primer OPAF05 (9) and lowest for primer OPAF10 (1.57) (Table 2). The primer index (RPI) was highest in case of OPN15 (2.44), and lowest in case of OPN16 (0.13). The similarity index ranged from 0.4 to 0.97 with a mean similarity of 0.57 indicating reasonable variability as obtained by RAPD markers (data not shown). The probability that DNA Fingerprints of two cultivars are identical by chance using RAPD primers is very low (5.37×10^{-8}).

In this investigation, RAPD markers were successfully used to differentiate 14 cultivars of *Catharanthus roseus* from each other. Thus, on the basis of RAPD, the findings of this study are similar to observations of Rajaseger et al. (1997 and 1999). Our

Figure1. RAPD (1a) and ISSR (1b) banding pattern in 14 cultivars of C. roseus (M = MBI 100 bp DNA ladder plus, 1-14 are different cultivars as listed in Table-1)

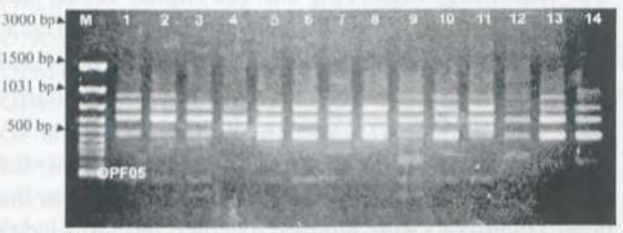

Fig. 1a

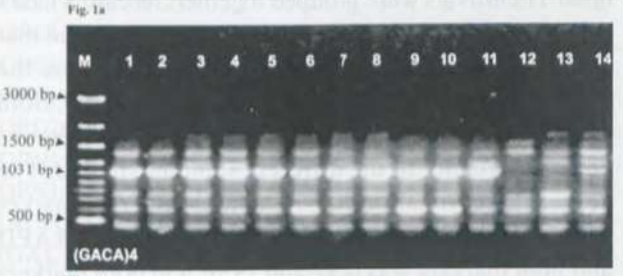

Fig. 1b

Table 2. Details of primers and banding pattern of RAPD analyses in 14 *C. roseus* cultivars

Primer	Nucleotide sequences	Range of Ampli cons(in bp)	Total bands	No.of polymorphic bands	No. of monomorphic bands	No. of exclusive bands	Resolving power
OPA03	AGTCAGCCAC	700-1600	4	3	1	0	5.428571
OPC11	AAAGCTGCGG	600-1400	2	0	2	0	4
OPC12	TGTCATCCCC	1030-2700	5	3	2	0	8.285714
OPC13	AAGCCTCGTC	1030-3000	4	4	0	0	4
OPD20	AACCCGGTCA	700-1200	4	2	2	1	4.428571
OPN15	CAGCGACTGT	350-2000	7	7	0	0	3.285714
OPN16	AAGCGACCTG	300	1	1	0	0	1.857143
OPAF05	CCCGATCAGA	450-1800	9	7	2	0	9
OPAF06	CCGCAGTCTG	600-1700	5	5	0	1	3.714286
OPAF10	GGTTGGAGAC	2500	1	1	0	0	1.571429
OPAF11	ACTGGGCATC	750-1500	3	3	0	2	2
OPAF14	GGTGCGCACT	1200-1900	2	1	1	0	3.285714
OPAF15	CACGAACCTC	650-3000	7	7	0	1	6.714286
TOTAL		**300-3000**	**56**	**46**	**10**	**5**	

results also agree with findings of Loh et al. (1999) who used AFLP markers to study genetic diversity in *Caladium bicolar*.

Four ISSR primers amplified 31 loci of which 17 (55%) were polymorphic and 14 (45%) monomorphic. The mean number of bands amplified per primer was 7.75. The amplicons were amplified in a range from 2200 bp to 600 bp (Figure 1b). The resolving power and primer index for ISSR were nearly the same as for RAPD markers (Table 3). The highest number of bands (10) was resolved for primer $(GACA)_4$ and the lowest number (5) for T $(GA)_9$. The highest number of polymorphic loci (6) was detected by primer $(GACA)_4$ and the lowest (2) by primer $(AGG)_6$. The resolving power of primer $(GA)_9T$ was maximum (16.) and lowest (8.71) for $T(GA)_9$. Contrastingly, the primer index was maximum (1.65) in case of $(GACA)_4$ and minimum (0.96) for $(AGG)_6$. The similarity index ranged from 0.57 to 1.0 with a mean similarity of 0.81, indicating considerable variability detected by ISSR markers. The lowest similarity was observed between varieties First Kiss Polka Dot and Victory Red (0.571), and the highest between varieties First Kiss Coral and Cooler Orchid, First Kiss Orchid & Experimental Deep Pink and varieties First Kiss Peach & Cooler Peppermint (data not shown). The probability that DNA Fingerprints of two cultivars are identical by chance using ISSR primers is very low (6.664676×10^{-3}). The genomic relations detected by ISSR markers were almost similar to those by RAPD markers, with few exceptions. A deviation of results was expected since only four ISSR primers were used against 14 primers in the RAPD analysis. However, the mean number of bands amplified per ISSR primer was higher. In the analysis by ISSR, the mean similarity index was 0.811, and 0.57 by RAPD.

The number of bands amplified in a single variety was highest in First Kiss Coral and First Kiss Cherry (58) and lowest in Blue Pearl (46) (Table-4). The mean number of bands amplified per cultivar was 54.06 when using all primers. The probability of DNA Fingerprints of two cultivars being identical by chance when using both RAPD and ISSR primers was very low (3.94×10^{-10}).

The combined markers of RAPD and ISSR detected high degrees of variations in genetic relationship. The similarity index ranged from 0.51 (between varieties Victory Red and Patricia White and varieties Blue Pearl and Experimental Deep Pink) to 0.98 (between varieties First Kiss Coral and Cooler Orchid). The mean similarity was reasonably high, i.e., 0.67. A similar result was expressed in the dendrogram (Figure 2) where two clear clusters were obtained. In cluster 1, Varieties Blue Pearl and Patricia White shared the same node at a similarity level of 57%. Cluster 2 was subdivided again into 2A and 2B at a similarity level of 61%. 2A was represented by a single cultivar, First Kiss Polka Dot, whereas the other 11 cultivars were grouped together. Jaccard's index derived from the combined marker system showed that varieties First Kiss Coral and Cooler Orchid were the most closely related cultivars with 98% similarity. Both marker types were equally important to investigate the genetic diversity in the different samples studied. The degrees of correlation (0.57) between the two marker types were moderate. The correlation between RAPD and both markers was 0.92 and ISSR and both markers 0.84. Both RAPD and ISSR were found to be equally effective in distinguishing the 14 *C. roseus* cultivars. The 14 primers produced 82.14% polymorphic bands indicating the presence of a high degree of genetic diversity in the 14 cultivars. The result of the dendrogram was similar although it was observed that two distinct clusters were formed where the cultivars Blue Pearl and Patricia White shared the same node with 57% similarity. The highest similarity was observed

Table 3. Details of primers and banding pattern of ISSR amplifications in 14 *C. roseus* cultivars

Primer	Nucleotide sequences	Range of Ampli cons(in bp)	Total bands	No.of polymorphic bands	No. of monomorphic bands	Resolving power	Primer index
(AGG)6	(AGG)6	850-2200	7	2	5	11.71429	0.959184
(GA)9T	(GA)9T	600-1500	9	5	4	16	1.102041
(GACA)4	(GACA)4	450-1800	10	6	4	11.42857	1.653061
T(GA)9	T(GA)9	700-2100	5	4	1	8.714296	1..071429
TOTAL		**450-2200**	**31**	**17**	**14**		

Table 4. Number of bands amplified in each C. roseus cultivar using 14 RAPD and 4 ISSR primers

Primers	First Kiss Coral	Cooler Orchid	First Kiss Orchid	Experimental Deep Pink	First Kiss Peach	Cooler Peppermint	Cooler Peppemint improved	Cooler Icy Pink	First kiss Cherry	Expt. Rose Pink With Eyes	Victory Red	Blue Pearl	First Kiss Polka Dot	Patricia White
OPA03	4	4	2	3	4	2	3	4	2	2	2	2	2	2
OPAF05	5	5	4	3	4	4	5	4	6	6	6	5	4	5
OPAF06	2	2	3	2	0	2	2	1	2	2	2	3	1	2
OPAF10	1	1	1	1	1	1	1	1	1	1	1	0	0	0
OPAF11	1	1	3	1	0	1	1	0	1	1	1	1	1	1
OPAF14	2	2	2	1	1	2	1	1	2	2	2	1	2	2
OPAF15	2	2	2	2	5	5	4	6	4	4	0	2	5	4
OPC11	2	2	2	2	2	2	2	2	2	2	2	2	2	2
OPC12	4	4	4	5	5	4	4	5	4	4	4	3	5	3
OPC13	3	3	2	2	1	4	2	3	3	2	0	1	2	0
OPC16	2	2	2	2	0	1	1	1	1	1	0	0	1	2
OPD20	2	2	2	2	2	2	2	2	2	2	3	3	2	3
OPN15	2	1	2	2	1	2	2	3	2	1	2	0	1	2
OPN16	1	1	1	1	1	1	1	1	1	1	1	0	1	1
$(AGG)_6$	6	6	6	6	6	6	5	5	6	6	6	6	6	6
$(GA)_9T$	9	9	8	8	8	8	9	8	9	8	7	9	5	7
$(GACA)_4$	5	5	6	6	5	5	5	5	5	5	6	6	7	9
$T(GA)_9$	5	5	5	5	5	5	5	4	5	4	3	2	4	4
Total	**58**	**57**	**57**	**54**	**51**	**57**	**55**	**56**	**58**	**54**	**48**	**46**	**51**	**55**

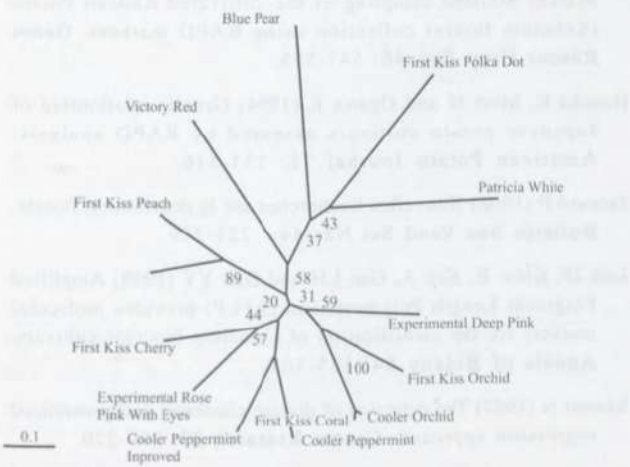

Figure 2. Genomic relations among 14 cultivars of *Catharanthus roseus* as revealed by 14 RAPD and 4 ISSR primers using Jaccard's similarity co-efficient and SAHN clustering method (numerical values indicate 100 bootstrap value)

between First Kiss Coral and Cooler Orchid with 97% similarity, which were the most closely related cultivars. However each cultivar had a mean similarity index of 0.5738.

The dendrogram constructed from combined RAPD and ISSR analysis successfully differentiated all cultivars from each other. The 18 RAPD and ISSR primers in this investigation produced 63 polymorphic bands, which unambiguously discriminated 14 cultivars. This demonstrates the power of RAPD and ISSR fingerprinting to study the genetic diversity in closely related taxa. A similar result was reported by Raina et al. (2001) in *Arachis hypogea*. The bootstrap value indicated the stability of relationship between two varieties. High bootstrap values express a strong statistical support of conclusions drawn based on genetic analyses. In this study, the bootstrap value

strongly supported the differentiations of 14 cultivars in 4 distinct groups.

In this study, it was found that RAPD analysis produced more polymorphic bands than the ISSR analysis. The result was also expressed in the primer index where the RAPD was higher than the ISSR primer index (Table3). Raina et al. (2001) reported higher values of the RAPD than of the ISSR primer index in *Arachis hypogea*. The principal coordinate analysis supported the similar grouping, as can be seen in the cluster diagram (figure not shown). The correlation among different markers suggested that the correlation between RAPD and ISSR is lower, i.e., 0.57. However, RAPD and ISSR are highly correlated with the combined markers suggesting that all markers are relevant to study the genetic diversity in *Catharanthus roseus*.

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Avaliação da diversidade genética de uma planta de alto valor medicinal, *Catharanthus roseus*, usando marcadores moleculares

RESUMO – *A diversidade genética foi avaliada entre 14 cultivares de* Catharanthus roseus *usando marcadores RAPD e ISSR. Os primers RAPD resultaram na amplificação de 56 bandas, entre as quais 46 (82%) foram polimórficas. Quatro primers ISSR amplificaram 31 locos dos quais 17 foram polimórficos e 14 foram monomórficos. A similaridade genética de Jaccard derivado da combinação de dois grupos de marcadores mostrou que as variedades First Kiss Coral e Cooler Orchid foram as mais próximas com 98% de similaridade. No dendrograma construído baseado nos dados de RAPD e ISSR foram obtidos dois grupos. O menor grupo inclui as cultivares* C. roseus *Cv Blue Pearl e* C. roseus *Cv. Patricia White e o maior grupo foi subdivido em dois subgrupos com* C. roseus *Cv. First Kiss Polka Dot isolado do resto dos cultivares. Esses resultados podem ser úteis para o melhoramento da qualidade.*

Palavras-chave: ISSR, RAPD, *Catharanthus roseus*, variação genética.

REFERENCES

Chattopadhyay RR, Banerjee RN, Sarkar SK, Ganguly S and Basu TK (1992) Antiinflammatory and acute toxicity studies with the leaves of Vinca rosea Linn in experimental animals. **Indian J Physiol Pharmacol 36:** 291-2.

Cisneros PL and Quiros (1995) Variation and phylogeny of the triploid cultivated potato Solanum chaucha based on RAPD and Isozyme markers. **Genetic Research and Crop Evolution 42:** 373-386.

Doyle JJ and Doyle JL (1990). Isolation of plant DNA from fresh tissue. **Focus 12:** 13 -15.

El-Sayed M and Verpoorte R (2005) Methyljasmonate accelerates catabolism of monoterpenoid indole alkaloids in Catharanthus roseus during leaf processing. **Fitoterapia 76:** 83-90.

Gherardi M, Mangin B, Goffinet B, Bonnet D and Huguet T (1998) A method to measure genetic distance between allogamous populations of alfalfa (Medicago sativa) using RAPD molecular marker. **Theoretical Applied Genetics 98:** 406-412.

Ghislain MD, Fazardo ZD, Huaman Z and Hismans RH (1999) Marker assisted sampling of the cultivated Andean Potato (Solanum fureja) collection using RAPD markers. **Genet Resour Crop Evo 46:** 547-555.

Hosaka K, Mori M and Ogawa K (1994) Genetic relationship of Japanese potato cultivars assessed by RAPD analysis. **American Potato Journal 71:** 535-546.

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

Loh JP, Kiew R, Kee A, Gan LH and Gan YY (1999) Amplified Fragment Length Polymorphism (AFLP) provides molecular markers for the identification of Caladium biocolor cultivars. **Annals of Botany 84:** 155-161.

Mantel N (1967) The detection of disease clustering and generalized regression approach. **Cancer Research 27:** 209-220.

Mattagajasingh I, Mukherjee, AK, and Das P (2006) Genomic Relations Among 31 Species of Mammillaria Haworth (Cactaceae) Using Random Amplified Polymorphic DNA. **Z Naturforsch 61:** 583-591.

Papon N, Bremer J, Vansiri A, Andreu F, Rideau M and Creche J (2005) Cytokinin and ethylene control indole alkaloid production at the level of the MEP/terpenoid pathway in Catharanthus roseus suspension cells. **Planta Med 71:** 572-4.

Prevost A and Wilkinson M (1999) A new system of comparing PCR primers applied to ISSR fingerprinting of Potato cultivars. **Theor Appl Genet 98:** 107-112.

Rajasegar G, Tan HTW, Turner IM and Kumar PP (1999) Random Amplified Polymorphic DNA variation among and within selected Ixora (Rubiaceae) populations and mutants. **Annals of Botany 84:** 253-257.

Rajaseger G, Tan HTW, Turner IM and Kumar PP (1997) Analysis of genetic diversity among Ixora cultivars (Rubiaceae) using random amplified polymorphic DNA. **Annals of Botany 80:** 355-361.

Raina SN, Rani V, Kojima T, Ogihara Y, Singh KP and Devarumath RM (2001) RAPD and ISSR fingerprints as useful genetic markers for analysis of genetic diversity, varietal identification and phylogenetic relationship in peanut (Arachis hypogea) cultivars and wild species. **Genome 44:** 763-772.

Rohlf FJ (1997) **NTSYS-Pc. Numerical taxonomy and multivariate analysis system version 2.02e.** Exeter Software.New York.

Sambrook J, Fritsch EF and Maniatis T (1989) **Molecular cloning a laboratory manual.** 2nd ed., 3: 18-47.

Singh SN, Vats P, Suri S, Shyam R, Kumria MM, Ranganathan S and Sridharan K (2001) Effect on antidiabetic extract of Catharanthus roseus. On enzymic activities in streptozotocin induced diabetic rats. **J Ethnopharmacol 76:** 269-77.

Smith JSC, Chin ECL, Shu H, Smith OS, Wall SJ, Senior ML, Mitchell SE, Kresovich S and Ziegle J (1997) An evaluation of the utility of SSR loci as molecular markers in maize (Zea mays L.): Comparison of data from RFLPs and pedigree. **Theor Appl Genet 95:** 163-173.

Sneath PHA and Sokal RR (1973) **Numerical Taxonomy.** W. H. Freeman and Company, San Francisco, California.

Sosinski B and Douches DS (1996) Use of PCR based DNA amplification to fingerprint North American potato cultivars. **Horticulture Science 31:** 130-133.

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA and Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. **Nucl Acids Res 18:** 6531-6535.