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Investigation of Genetic Relatedness of Ten Syngonium Cultivars using RAPD markers

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Abstract- For the first time, genetic variability and differentiation among Ten Synconium white butterfly populations originating from tissue cultured plants maintained in pro-trays under the poly house & green house conditions in Indo American hybrid Seed Company, Bangalore were examined. Random Amplified Polymorphic DNA (RAPD) marker data were obtained and analysed with respect to genetic diversity. The ornamental crops Syngonium generate substantial revenue worldwide in the trade of ornamentals. The understanding in the pattern of genetic diversity of these plants has important implications in breeding programs and in the improvement of plant species. Initially RAPD analysis was used to assess the level of polymorphism, similarities and relationships among Syngonium populations collected from IAHS. RAPD analysis was done by screening 10 varieties of Syngonium using four primers. Totally 10,12, 23 and 25 polymorphic bands were observed in following four primers such as E20, C02, C05 and D18. Maximum and minimum bands were amplified in E20 (80 bands) CO2 (40) primers. While, the number of faded bands also increased in both primers. Based upon the E20 and D18 primer similar as well as maximum polymorphic bands also been observed. Cluster analysis was done based on the UPGMA method using NTSYS software. Dendrogram analysis clearly noticed the Similarity Co-efficient for cultivars in the cluster I, II and II varied from 0.10-0.33, 0.78-1.0, 0.20-0.80 and 0.20-0.90 for E20, CO2, CO5 and E18 respectively.

Index Terms- RAPD markers, Genetic diversity, Protein markers, Cluster analysis, Dendrogram

I. Introduction

Syngonium a genus of ornamental plants in the family Araceae, are woody vines growing to heights of 10–20 m or more in trees. *Syngonium* species also called as arrowhead are often grown as house plants. There are several variegated cultivars, the main differences being in the position and extent of the cream or white markings. Some leaves are almost entirely white, pink or yellow. Propagation is by cuttings or air layering (Bijender Singh *et al*., 2011). The *Syngonium* variety ranking about seventh in the number of foliage plants shipped to market plays a major place in the trade of ornamental plants being provided with molecular and somatic cell technologies which provides an essential underpinning to conventional breeding approaches. Molecular procedures facilitate the identification of novel germplasms for incorporation into breeding programs involving sexual hybridization. Genetic diversity analysis of *Syngonium* varieties helps in the genetic improvement by genetic manipulation of targets including modification of stature and floral characteristics and improved tolerance to pests, diseases, and environmental stress (Simi *et al*., 2012).

Knowledge of genetic relationships in crops is important for genetic resource conservation, plant breeding, variety protection and genetic evaluation (Weising *et al*., 2005). These differences include insertions, deletions, translocations, duplications and point mutations. They do not, however, encompass the activity of specific genes. Information on genetic diversity is essential in optimizing both conservation and utilization strategies (Devos and Gale, 1992). Genetic diversity analysis is an important area of applied research in ornamentals owing to the relatively high value of individual plants compared with the agricultural crops. Knowledge of genetic relationships in crops is important for genetic resource conservation, plant breeding, variety protection and genetic evaluation **(**Michele *et al.*, 2003**).** Co- migration of non-homologous fragments can be a problem at higher taxonomic levels, but is probably negligible at the intraspecific level or when the studied species are closely related (Ashok, 2010). RAPD markers have, despite the above-mentioned limitations, been widely used in plant genetics and breeding, e.g., to estimate genetic diversity, detect phylogenetic relationships, and create genetic maps (Weising *et al.*, 1995, 2005). RAPD markers can analyze genetic diversity and genetic relationships within rather large plant sets due to their technical simplicity and cost-effectiveness. RAPDs have been successfully used in conventional DNA fingerprinting in plants (Caetano-Anolles *et al.*, 1991**).** However a commonly experienced problem with RAPD analysis is its poor reproducibility (Devos and Gale, 1992). However no studies have been conducted to determine the genetic relatedness of Synconium cultivars, hence this work has been undertaken for following objectives; Extraction of high quality DNA from ornamentals – Syngonium using CTAB method, DNA profiling of ornamentals by RAPD markers and analysis of genetic diversity among ornamentals by constructing dendrogram

II. Materials and Methods

The present study was undertaken with the aim of analyzing the genetic variation found among and between Syngonium white butterfly varieties using DNA and protein markers.

All the experiments for the genetic diversity analysis were carried out at Biotech Research Development Lab, Indo

American hybrid Seed Company, Bangalore. All the materials and methods adopted during the study were described below,

Plant Material

All the plant leaves were collected from the tissue cultured plants maintained in pro-trays under the poly house & green house conditions in Indo American hybrid Seed Company, Bangalore, India. Samples of young immature leaves were collected randomly from each of the plant variety using blade & butter.

Genomic DNA extraction

The plant tissues of ornamentals are notoriously difficult material for DNA isolation due to the presence of various Secondary plant products. A number of DNA isolation methods have been developed for different plant groups. A method developed by Doyle & Doyle, 1996 called CTAB method is followed for the genomic DNA extraction. In this method, CTAB extraction buffer is used.

CTAB is a cationic detergent which aids in the lyses of cell membranes and will form complexes with nucleic acids. Sodium chloride aids in the formation of nucleic acid – CTAB complexes, by masking the negative charge of DNA. EDTA will chelate the magnesium ion which is an essential cofactor for DNase, thereby inhibits its activity and β-Mercaptoethanol is a reducing agent which protects DNA from degradation by various oxidants. RNA, protein, polysaccharides, pigments and tannins in plant cells will be removed by treating the extract with RNase, chloroform and phenol, respectively.

After protein is removed DNA is purified by precipitation with ethanol. Isopropanol and absolute alcohol removes water molecules from DNA.

Protocol

2mg of leaf sample was taken and crushed by adding 600µl Extraction buffer containing 100mM Tris-Cl, 1.4M NaCl, 20mM EDTA, 2%CTAB, 2%Mercaptoethanol and 1%PVP using pestle & mortar. It was then taken in eppendorf tubes. The grinded mixture was incubated at 50^0C for 10 mins and 500µl of chloroform isoamyl alcohol (24:1) was added. The mixture was kept in shaker for 15mins and centrifuged at 12,000rpm for 12 mins at RT. About 70µl of supernatant was transferred to new tube, and then equal volume of isopropanol and 0.5V of 5M NaCl was added. The samples were incubated for 2-3hrs at -20^0C to precipitate the DNA. Tubes were centrifuged at 12,000rpm for 12mins at 4^0C to make the pellet. The pellet was washed with 200µl 70% Ethanol (spinned at 12,000rpm for 4mins at RT) and air dried. The DNA pellet was dissolved in 50µl 1x TE buffer and stored at -20^0C

Quality and quantity check of DNA

DNA was checked for its purity and intactness and then quantified. The genomic DNA was run on 0.8% agarose gel stained with ethidium bromide following the protocol of Sambrook *et al*., (1989) and was visualized in a gel documentation system (GelDoc-It 310 Imaging System., UVP, CA, USA). For quality check 3µl of genomic DNA mixed with 2µl gel loading dye was used.

Intact and pure genomic DNA as assessed by agarose gel electrophoresis was quantified with spectrophotometer (UltraSpec III). The absorbance for all accession was measured at 260 nm and 280nm and then the ratio of OD_{260}/OD_{280} was calculated.

PCR Amplification

PCR amplification for RAPD analysis was done using 10 primers supplied by Genei, to get polymorphic bands. The RAPD primers used for diversity analysis are C02, C05, D18 and E20. PCR were performed in 15 µl reaction mixture with 2µl of template DNA using different primers to check for better amplification.

III. RESULTS

In this study explained the genetic organization of ten Synconium white butterfly populations originating from poly house and green house conditions in Indo American hybrid Seed Company, by means of RAPD markers in order to obtain the molecular data on their genetic background. Thus, we have demonstrated the reliability of RAPD analysis to detect DNA polymorphisms and relationships within ten varieties of *Syngonium* populations. The selected primers were highly discriminating since they were characterized by relatively high collective Rp rate (30.00), a high number of polymorphic markers and electrophoretic banding patterns. Totally 10,12, 23 and 25 polymorphic bands were observed in following four primers such as E20, C02, C05 and D18 respectively (Table-2). Out of seven, three primers didn't given any fair amplified product so these three primers were useful for successfully provided deep insights on the genetic background of the above mentioned populations. In RAPD literature, the presence of primers does not allow amplification to occur (Caetano-Annoles 1994), whereas others primers yielding faint banding profiles (Moreno *et al*. 1995, Ortiz *et al*. 1997) were reported. In addition, according to the results forwarded by Devos and Gale (1992), Penner *et al*. (1993) and This *et al*. (1997), some primers are more efficient than others in producing stable and reproducible profiles. The genetic divergence of the populations under investigation was confirmed at the DNA level. In fact, the UPGMA cluster analysis permitted the discrimination of all the genotypes and their sorting into 3 main groups in E20 primer followed by two in CO2 and cumulative nature in D18 primer (2, 4, 8, 10). Thus, population clustering was made dependently from the original region. In the same way, the occurrence of RAPD fingerprints unique to each of the studied populations (Table 2) suggests that RAPD markers may constitute rapid molecular tools for assigning one genotype to its origin. On the other hand, RAPD markers are suitable to perform genetic variation analyses at both intra and inter population levels (Syankumar and Sasikuma 2007; Hadian *et al*. 2008, Zahid *et al*., 2009). According to Shannon's index, high levels of genetic diversity were detected between the populations rather than within populations (Table 2). The existence of low genetic diversity within the species has been mostly attributed to self pollination, unless other environmental pressures are influencing genetic diversity (Archak *et al*. 2002).

In E20 RAPD primer showed fragments ranging from 500bp to 2000bp through all the ten plants. The size of the DNA fragment 1000bp uniformly present in all the nine plants except wb8species Fig. 1. Although, higher 3000bp amplified bands have visibly noticed in wb8 plant with faded manner. Amoung the 10 plants of butterfly varieties analysed clearly (wb4 and wb8) showed indifference among the other plants, while the others resembled the same. Meanwhile intensity of bands such as 2 and 3 were different from other bands. Inorder to that this E20 primer illustrated cluster analysis showed poor similarity coefficient observed on both wb9 and wb10 plant species of butterfly Fig.2. The second primer of CO2 amplification result was showed in Fig.2. It was clearly proved the specified average smallest range to largest amplifying efficiency such as 100 to 1500bp. Despite, dominantly two adjacent thickened bands such as 600 and 1500bp were seen on wb1 to wb10. Moreover, dendrogram analysis clearly noticed highest similarity Co-efficient shared between wb5, 6 and wb9 plants. Additionally poor similarity also been noted on wb4 plants in CO2 primer Fig. 4.

The intra diversity analysis of Syngonium variety white generated 8 allelic banding patterns. The bands 3 to 7 are more intense while the band 1, 2 and 8 are faded nature. The bands 5 and 7 are present in all plants except wb9 plant and less faded in wb6. In cluster analysis identically 0.20% of similarity coefficient was noted on both wb7 and wb8 plant variety. Finally the fourth primer D18 expressed RAPD profile showed that 300 and 600bp were observed in all the ten experimental plants. Although higher molecular weighed fragment of 2000bp have seen only in wb8 plant. Furthermore very smallest range of band 250bp also specifically appeared in wb5 plant Fig.7. While the consistent similarity coefficient noticed between wb7 and wb8 plant (Fig.8).

Fig. 1: RAPD profile of Syngonium variety White butterfly with E20 primer

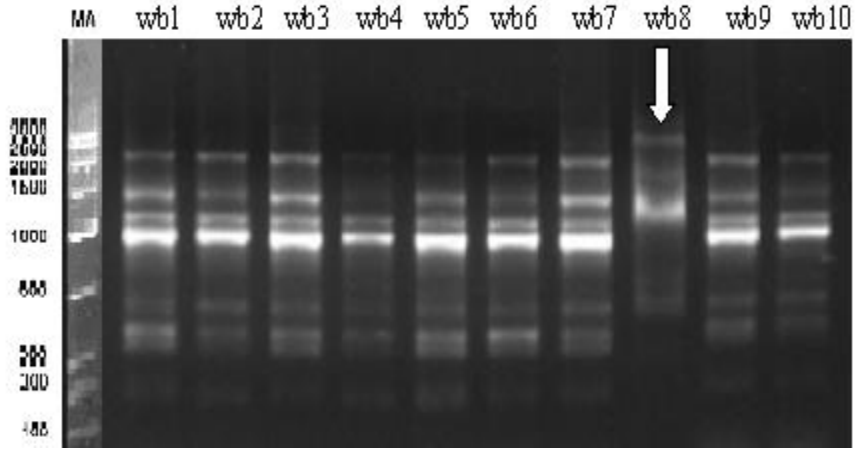

Fig. 1: RAPD profile for Syngonium variety White butterfly with E_{20} primer

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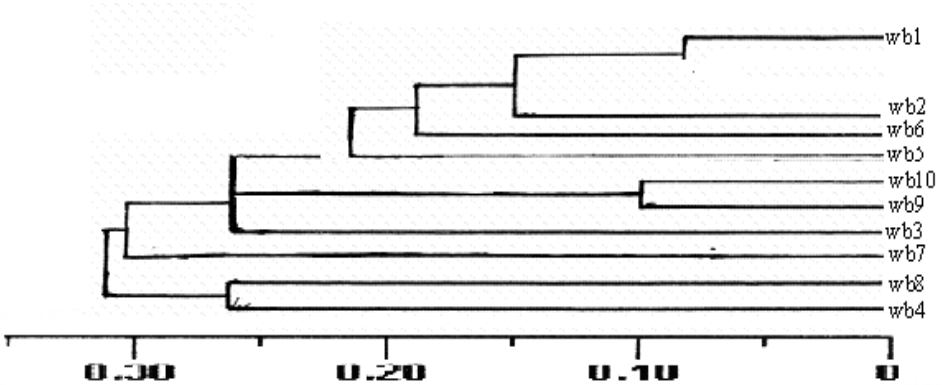
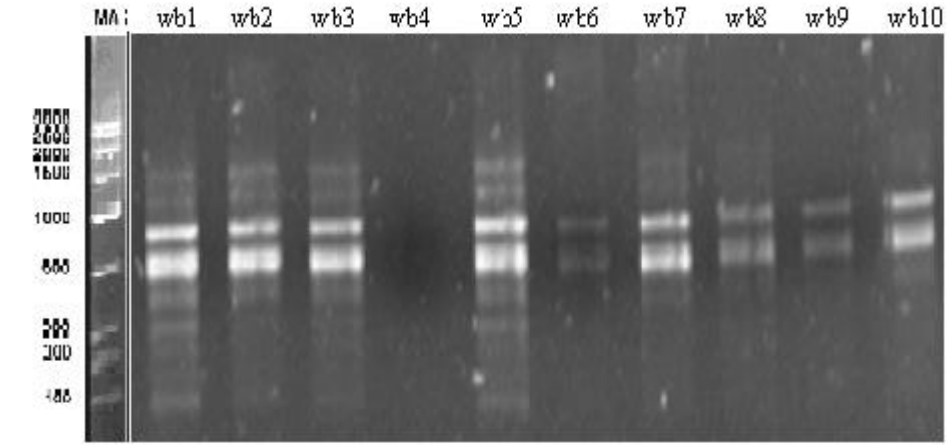


Fig. 2: UPGMA Cluster Analysis using simple matching similarity coefficients with E_{20} primer

Fig. 3: RAPD banding profile generated using C02 primer for Syngonium variety



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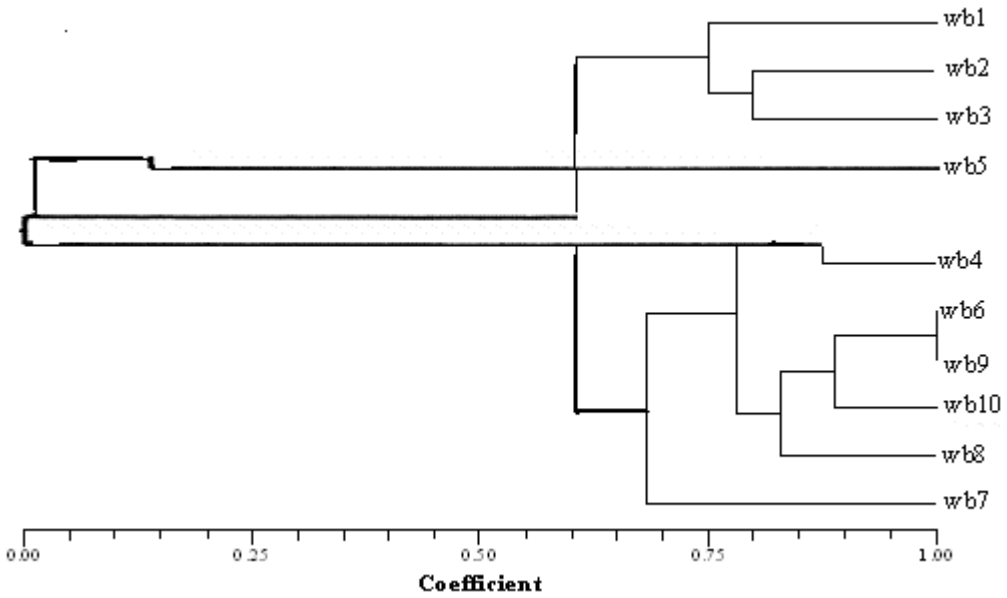

Fig. 4: UPGMA Cluster Analysis using simple matching similarity coefficients with CO_2

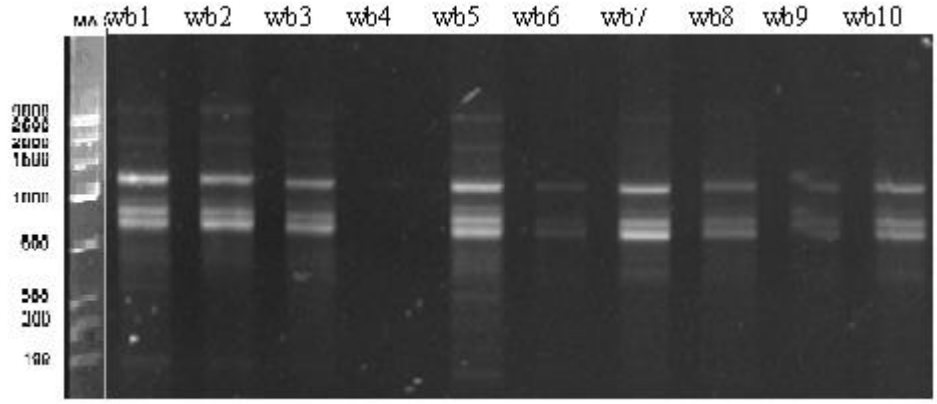

Fig. 5: RAPD banding profile generated using C05 primer for Syngonium variety

. (B)

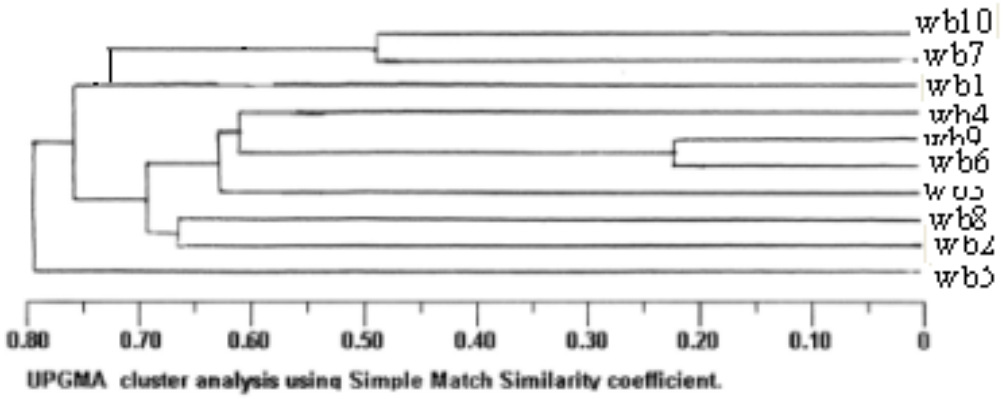

Fig. 6: UPGMA Cluster Analysis using simple matching similarity coefficient C05 primer

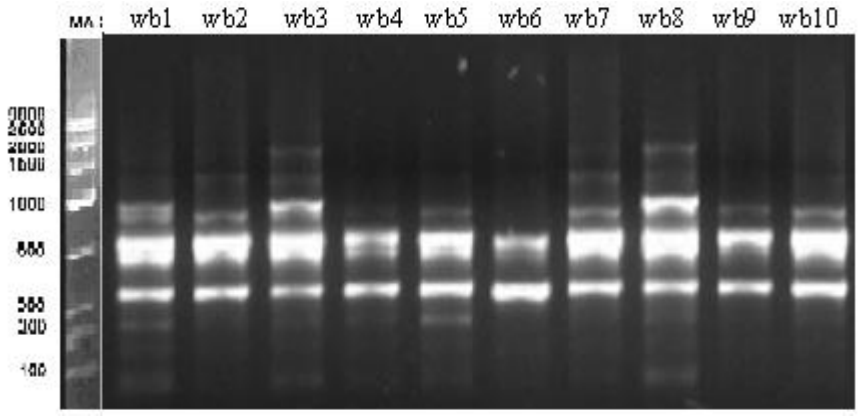

Fig. 7: RAPD banding profile generated using D18 primer for Syngonium variety

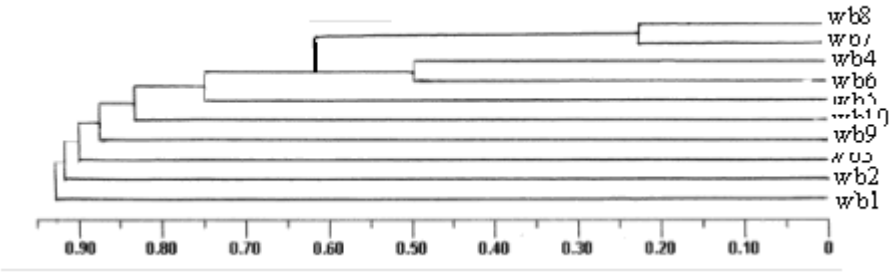

Fig. 8: UPGMA cluster analysis within the ten *Synchonium* white butterfly populations established by means D18 RAPD primer.

Table-1: Genetic diversity analysis through RAPD markers and Shannon's index among ten *Synchonium* white butterfly populations

No	Primer	Sequence (5-3´)	Total Bands	No. of faded bands	Polymorphic bands
1.	E20	5'AACGGTGACC3'	80	35	10
2.	C02	5'GTGAGGCGTC3'	40	27	12
3.	C05	5'GATGACCGCC3'	46	18	23
4.	E18	5'GGACTGCAGA3'	48	25	23

IV. CONCLUSION

To conclude, this is the first report of the genetic diversity assessment and population differentiation analysis in ten Syngonium white butterfly populations originating from tissue cultured plants maintained in pro-trays under the poly house & green house conditions in Indo American hybrid Seed Company, Bangalore, using RAPD markers. This study successfully provided deep insights on the genetic background of the above mentioned populations. Considerable genetic diversity has been detected within the species. However, using more informative RAPD markers and including more populations would allow detecting the genetic background resources in local species of this similar variety too. Therefore, to prevent further substantial loss of genetic diversity, we should conserve as many populations as possible. In this context, that an appropriate strategy for sampling may be formulated when ex situ conservation is required. Besides, the best in situ conservation strategy for Syngoium population is to preserve its natural habitats. We further aim to include wild germplasm in order to select cultivars of economically importance and to transfer important genetic traits from wild to cultivated species by marker-assisted-selection. The dendrogram generated showed the Dieffenbachia and Syngonium varieties show a genetic similarity of 60%. The overall percentage of genetic diversity that exits between all the varieties is 40%.

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