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RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) MARKER ANALYSIS OF RED AND WHITE VARIANTS OF A CLIMBER *ABRUS PRECATORIUS* L.

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ABSTRACT
In this work, the leaves of the red and white *Abrus precatorius* were used for DNA isolation. The variants were separated using the morphological and molecular data. The objective of present study is to identify the polymorphism and genetic diversity between two genotypes of different variants of *Abrus precatorius* (Red seeded -G, White seeded -H) which belongs to family papilionaceae and these are compared with different variants of *Clitoria ternatea* (White flowered single petalloid -A, Blue flowered single petalloid –B, Double petalloid flowered - C, *C. biflora* -D) of family papilionaceae and *Lagenaria siceraria* (Edible-E, Bitter-F) of cucurbitaceae family. In RAPD analysis, cluster analysis of 1-0 bivariate data was carried out, out of 120 primers used, for G, H 90 random primers revealed a total 217 bands out of these shows 96 polymorphic fragments (43.77% polymorphism) while 121 monomorphic bands (56.22% monomorphism) this data was further used for construction of dendrogram by clustering procedure using Unweighed Pair Group Method and Arithmetic Average (UPGMA) algorithm and Principal Coordinate Analysis (PCA) of the similarity matrix. Dendrogram and PCA reveals that G is most nearer to H (0.402) and G, H both variants are more closer to B variant of *Clitoria* sp. (G-0.287, H-0.377) than that of *Lagenaria* sp.

KEY WORDS: Cluster analysis, Principal Coordinate Analysis, RAPD markers.

INTRODUCTION

A. precatorius is a beautiful, much-branched, slender, perennial, deciduous, woody, prickly twining or climbing herb. Stem cylindrical, wrinkled; bark smooth-textured, brown. Leaves stipulate, pinnately compound; leaflets 7-24 pairs, 0.6-2.5 x 0.4-1.2 cm, turgid, oblong, obtuse, truncate at both ends, appressed hairy. Flowers in axillary racemes, shorter than leaves, fascicled on the swollen nodes, pink or pinkish-white; calyx-lobes short, appressed hairy. Pods 1.5-5.0 x 0.8-1.5 cm, turgid, oblong, hairy, with a sharp deflexed beak, silky-textured, 3 to 5-seeded. Seeds elliptic to sub-globose, 0.5 cm in diameter, smooth, glossy, shining red with black blotch around the hilum. Flower and Fruit. August – January (Frohne and Pfander, 1983). It is indigenously found throughout India, even at altitudes up to 1200m on the outer Himalayas. It is now naturalized in all tropical countries (Dwivedi, 2004). It grows in tropical climates such as India, Sri Lanka, Thailand, the Philippine Islands, South China, North America, Tropical Africa and the West Indies. It also grows in all tropical or subtropical areas. It is used as an ornamental throughout North America. According to an estimate of the world health organization (WHO), about 80% of the world population still uses herbs and other traditional medicines for their primary health care need (Agrawal, 2006).

Morphological as well as biochemical markers used in the authentication of herbal drugs have many 25 limitations due to the impact of environmental conditions. DNA based molecular markers however, are important tool in quality assurance and preservation of germplasm of medicinal plant species in the plant kingdom. Our major objective therefore, was to develop DNA based molecular tools for accurate identification of *A. precatorius* variants in local market. Random amplified polymorphic DNA (RAPD) involves the use of a single 'arbitrary' primer in a polymerase chain reaction (PCR) and results in the amplification of several discrete DNA fragments (Kirtikar and Basu, 1990).

MATERIALS AND METHODS

Abrus precatorius (G, H) variants of family papilionaceae (fabaceae) utilized for the present RAPD analysis differ in their morphological (Table 1 and fig. 1) and biochemical characters. All plants (climbers) were grown in the garden, department of botany, GVISH Amravati.

FIGURE 1: Habit of red and white *Abrus precatorius* twigs showing flowers and fruits with seeds

Genomic DNA extraction

Genomic DNA was extracted from 1g. each of the two genotypes (E, F) utilized for RAPD analysis. Genomic DNA isolation was carried out according to the protocol in the manual 'biochemical methods' by Sadasivam and Manickam (1996). The extracted DNA samples were run on 1% agarose gel for confirmation of isolated DNA followed by quantification of DNA by UV-Vis spectrophotometer for confirmation of its good quantity (Table 1).

Polymerase chain reaction (PCR)

The reaction was carried out in a gradient master cycler for amplification program. Amplification reactions contain sterile distilled water, 18.8µl; Buffer, 2.5µl; $MgCl_2$, 1.0µl; dNTPs, 1.0µl; Primer, 0.5µl; Taq DNA polymerase, 0.2µl; DNA, 10 ng; in a reaction volume of 25µl. The PCR was carried out with modification in thermal profile, after first step of initial denaturation at 94^0C for 6 min., denaturation at 94^0C for 1 min., primer annealing at 36 ^{0}C for 1 min., and primer extension at 74^0C for 1 min., were repeated for 40 cycles. At last final extension was allowed at 74^0C for 10 min. The RAPD primers were obtained from Operon technologies, USA were employed for genetic diversity analyses. 2% agarose gel in 1 X TAE buffer (Tris-base, glacial acetic acid, EDTA) with 30µlEtBr (10 mg/ml) per 300 ml of gel volume was prepared. 2 µl of loading dye was added to each PCR tube for sample loading. Sizes of the identified bands were derived relative to 100bp-1 kb DNA ladder. Electrophoresis was carried out at 100V for 3 h. and visualized under geldoc (Alpha Innotech).

Data scoring and analysis

Scoring of RAPD bands were carried out by considering only the clear and unambiguous bands (Fig.2). Markers were scored for the presence (1) and absence (0) of the corresponding band among the different genotypes. Homology of bands was based on distance of migration in the gel. The SIMQUAL (Similarity for qualitative data) program was used to calculate the Jaccard's coefficient. Jaccard's similarity coefficient (J) was used to calculate similarity between genotypes. The genetic associations between genotypes were evaluated by calculating the Jaccard's similarity coefficient for pair wise comparisons based on the proportion of shared band produced by the primers. Data analysis was performed using the NTSYS-PC (Numerical Taxonomy System, Version 2.02, Rohlf, 1990). Dendrogram was constructed using UPGMA (Unweighted pair group method for Arithmetic mean) method. Principal Coordinate Analysis (PCA) of the similarity matrix was also used to study relationships among different variants of *Lagenaria siceraria* (E, F), and to compare with *Clitoria ternatea* (A, B, C, D) and *Abrus precatorius* (G, H).

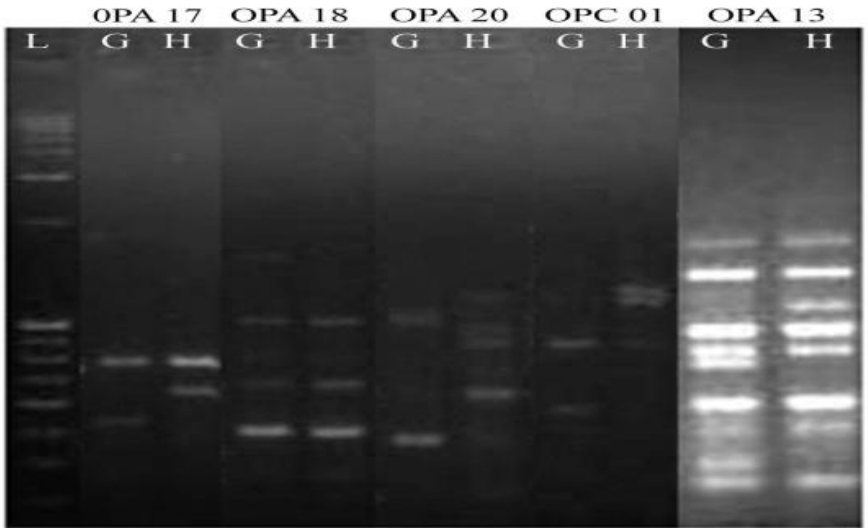


FIGURE 2: Polymorphic bands of red and white *Abrus precatorius* generated by different RAPD primers.

RESULTS

In RAPD analysis, cluster analysis of 1-0 bivariate data was carried out to study presence and absence of bands. Total 120 primers used, out of this, 90 random primers produced reproducible and scorable bands. Out of the total number of 217 amplified, 96 were polymorphic (43.77% polymorphism) while 121 were monomorphic bands (56.22% monomorphism). The RAPD banding pattern in five *A. precatorius* variants using primers OPA13, OPA 17, OPA 18, OPA20, OPC01 is presented in fig. 2.The largest band of 2000 base pairs was observed with primers OPB 01, OPC 19,OPE 02, OPE 07 while the smallest band of 150 base pairs with primer OPC 11. The data was further statistically analyzed and used for construction of dendrogram (fig. 3) by clustering procedure using Unweighted Pair Group Method and Arithmetic Average (UPGMA) algorithm and Principal Coordinate Analysis (PCA) (fig. 4) from the similarity matrix (table II).The *Abrus* variants were grouped under the one cluster. The two variants of *Abrus* shows 0.402 similarity index with each other.

TABLEII : Similarity matrix between variants of *C.ternatea* (A, B, C, D), *L. siceraria* (E, F), *A. precatorius* (G, H).

ROWS/ COLS	A	B	C	D	E	F	G	H
A	1.000							
B	0.195	1.000						
C	0.579	0.186	1.000					
D	0.103	0.124	0.132	1.000				
E	0.107	0.143	0.106	0.119	1.000			
F	0.090	0.118	0.096	0.107	0.796	1.000		
G	0.110	0.287	0.078	0.089	0.103	0.107	1.000	
H	0.114	0.377	0.084	0.130	0.115	0.115	**0.402**	1.000

FIGURE3: Dendrogram showing similarity between variants of *C. ternatea* (A, B, C, D), *L. siceraria* (E, F), *A. precatorius* G, H).

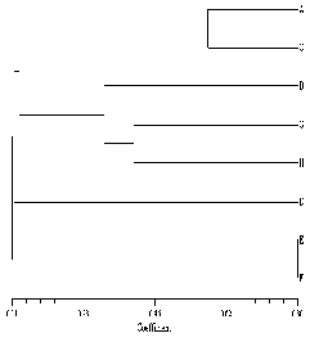
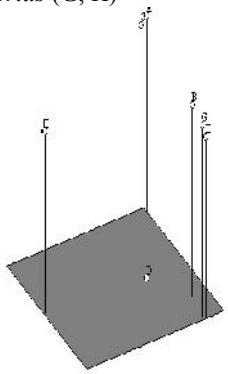


FIGURE 4: PCA between variants of *C .ternatea* (A, B,C,D), *L. siceraria* (E, F), *A. precatorius* (G, H)



DISCUSSION

In this work, the leaves of the red and white *Abrus precatorius* were used for DNA isolation. The variants were separated using the morphological and molecular data. The objective of study is to identify the polymorphism and genetic diversity between two genotypes of different variants of *Abrus precatorius* (Red seeded -G, White seeded -H)which belongs to family papilionaceae and it is compared with different variants of *Clitoria ternatea* A, B, C,D), *Lagenaria siceraria* (E, F). Dendrogram (fig. 3) and PCA(fig. 4) reveals that H is most nearer to G (0.402) and G, H both variants are more closer to B variant of *Clitoria* sp. (G-0.287,H-0.377) than that of *Lagenaria* sp. The PCA analysis is a good indication of genetic relationship among the species and the neutrality test depicts the vulnerability of the variants in the existing natural conditions.

This result clearly indicatesthose genotypes G, H are closer to B variant of *Clitoria* species than E, F species (Yeotkar and Malode, 2010), The dendrogram for phylogenetic relationships showed that the genomic sequences of G, H genotype were clustered together, hence the origin was same for G, H genotype (Fig.3 and 4).Several molecular markers have been widely used to assess the genetic diversity and study of phylogenies in a number of taxa like *Astragalus* (Sanderson and Liston, 1995), *Acacia* (Casiva*et al.*, 2002), *Afgekia* (Prathepha and Baimai, 2003) and *Brassica* (Malode and Shingnapure, 2010) , *Clitoria* (Yeotkar *et al.*, 2010).

In conclusion, RAPD marker proved to be efficient tool for precise estimation of genetic relationship of *Abrus precatorius* (Red seeded -G, White seeded -H) variants. Thus, RAPD analysis is to be useful for assessment of genetic diversity; genetic relationships and phylogenetic analysis. This shows that G, H are closer to B, which all are belongs to same family papilinaceae.

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