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Full Length Research Paper

Genetic relationship and diversity analysis of *Clitoria ternatea* variants and *Clitoria biflora* using random amplified polymorphic DNA (RAPD) markers

Yeotkar Swati D[1], Malode, S.N[1]*, Waghmare, V.N[2] and Thakre, P.[2]

[1]P.G. Department of Botany, Government Vidarbha Institute of Science and Humanities, Amravati-444604, India.
[2]Crop Improvement Division, C.I.C.R., P. B. No.2, Shankar Nagar, P.O.Nagpur-440010, India.

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***Clitoria ternatea* and *C. biflora* are multipurpose forage legumes. They provide bioactive compounds for medicinal use and also are an ornamental plant. The polymerase chain reaction (PCR) based molecular markers technique; random amplified polymorphic DNA (RAPD) was employed to identify the polymorphism and genetic diversity between four genotypes of different variants A, B and C of *C ternatea* and D of *C. biflora* which belongs to family fabaceae (subfamily- papillionaceae). In RAPD analysis, cluster analysis of 1-0 bivariate data was carried out; 100 random primers revealed a total of 202 polymorphic fragments. The data was further used for constructing dendrogram. The dendrogram indicate that genomic sequences of all four *Clitoria* genotypes were not clustered together even though the origin was same for all the genotypes. OPC11 primer generated the smallest (150 bp) DNA fragment in genotype A, while in the case of genotype 'B', the largest (2500 bp) fragment was obtained in OPB11 primer. In conclusion, the information on polymorphism using RAPD in *Clitoria* genotypes is useful in the assessment of genetic diversity, genetic relationships and phylogenetic analysis.**

Key words: *Clitoria ternatea, Clitoria biflora,* RAPD markers, polymorphism, genetic diversity.

INTRODUCTION

The butterfly pea (*C. ternatea*) is a deep rooted, climbing legume with five leaflets. It is well adapted in a variety of soil types (pH 5.5 to 8.9) including calcareous soils. It survives in both the extended and rainfall regions and in prolong period of drought.

Natural plant propagation occurs through seed. The seeds are normally sown from the beginning until the middle of the wet season in Australia for a forage crop which contains 55% proteins in fresh leaves (Conway et al., 2001). The levels of crude protein and crude fibre in leaves are 21.5 and 21.5 to 29%, respectively (Kalamani and Michael, 2001). Total plant protein ranges from 14 to 20%. The seed contains 25 to 38% proteins, 5% total sugar and 10% oil; however, the seed proteins contain 13 amino acid compositions (Barro and Ribeiro, 1983). *C. biflora* is an erect annual herb, 30 to 50 cm tall, with angular and hairy stems. Leaves are pinnate with five leaflets, 3 to 8 cm, with the last one being larger. Flowers occur in pairs in leaf axils, which explain one of its species names, *biflora*. The flowers, 2.5 cm across, are like those of Butterfly Pea. The standard petal is blue, with white at the base. Its flowering is from August to October.

The objectives of this study were to identify the polymorphism amongst the *C. ternatea* variants (A, B, C) and *C.biflora* (D), and to determine the genetic relationships amongst and within the four genotypes using random amplified polymorphic DNA (RAPD) markers. DNA markers have proven to be valuable in crop breeding especially in the studies of genetic diversity and in cultivar identification. Polymerase chain reaction (PCR) based molecular markers, for example, inter-simple sequence repeat (ISSR), sequence-tagged site (STS), simple sequence repeat (SSR), amplified fragment length polymorphis (AFLP) etc., are useful for various applications in plant breeding. RAPD markers involves the amplification of random DNA segments using arbitrary sequences of

*Corresponding author. E-mail: satishmalode17@gmail.com.

Table 1. Broadly distinguishing characters of all the genotypes (A, B, C, D).

Genotype	Distinguishing character
C. ternatea White (A)	Cultivated; climber; flower 6 to 8 cm, white, yellow neck, petals single layered; pod 8 to 11 cm. leaflets 5 to 9.
C. ternatea Blue (B)	Cultivated; climber; flower 6 to 8 cm, blue white neck, petals single layered; pod 8 to11 cm.; leaflets 5 to 9.
C. ternatea double petalloid (C)	Cultivated; climber; flower7 to 9 cm, bluish purple, petals double layered; pod 8 to 11 cm. leaflets 5 to 9.
C.biflora (D)	Wild; herb; flower 2 to 5cm, faint blue, broad white neck, petals single layered; pod 1.5 to 3.5cm.; leaflets 3 to 5.

10 to15 base pairs without any prior knowledge of DNA sequence (Welsh and McClelland,1990).

For the present molecular diversity analysis, it was observed that out of the four genotypes, the different genotypes coded as: white (A), blue (B) and double petalloid (C), were collected from Amravati region, while *C. biflora* coded as (D) was collected from Melghat, Dist. Amravati; although, it is endemic to Melghat region.

MATERIALS AND METHODS

All Four different genotypes representing family fabaceae were utilized for the present RAPD analysis; differ in their morphological (Table 1 and Figure 1) and biochemical characters. All genotypes exhibited morphological variation in some characters, such as fruit shape, colour, fruit, flower shape, colour, size etc. All plants were grown in the garden, P.G. Department of Botany, GVISH Amravati.

Genomic DNA extraction

Genomic DNA was extracted from 1 g each of the four genotypes (A, B, C, D) 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 for confirmation of its good quality.

Polymerase chain reaction (PCR)

The reaction was carried out in a gradient master cycler for amplification program. Amplification reactions contain sterile distilled water (18.8 µl), Buffer (2.5 µl), $MgCl_2$,(1.0 µl); dNTPs (1.0 µl); Primer (0.5 µl); Taq DNA polymerase (0.2 µl), and DNA (10 ng; 1.0 ng) in a reaction volume of 25 µl. The PCR was carried out with modification in thermal profile after the first step of initial denaturation at 94°C for 6 min, denaturation at 94°C for 1 min, primer annealing at 36°C for 1 min, and primer extension at 74°C for 1 min, and were repeated for 40 cycles. Lastly, final extension was allowed at 74°C for 10 min. The RAPD primers 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 30 µl EtBr (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 100 bp-1 kb DNA ladder. Electrophoresis was carried out at 100 V for 3 h and visualized under geldoc (Alpha Innotech).

Data scoring and statistical analysis

Scoring of RAPD bands were carried out by considering only the clear and unambiguous bands. 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 by the Formula:

$$J = \frac{a}{n - d} \quad \text{or} \quad J = \frac{a}{a + b + c}$$

The genetic associations between genotypes were evaluated by calculating the Jaccard's similarity coefficient for pairwise comparisons based on the proportion of shared band produced by the primers. Unweighted pair group method for arithmetic mean (UPGMA) cluster analysis was performed to develop a dendrogram. Principal coordinate analysis (PCA) of the similarity matrix was also used to estimate relationships among different variants of *Clitoria* variants. Data analysis was performed using the NTSYS-PC (Numerical taxonomy system, Version 2.02, Rohlf, 1990).

RESULTS AND DISCUSSION

Butterfly pea commonly known as Shankupushpam is widely used in traditional Indian systems of medicine as a brain tonic and is believed to promote memory and intelligence. The study conducted on rat revealed that *C. ternatea* root extract increase rat brain acetyl choline content and acetyl choline esterase activity in a similar fashion to the standard cerebro drug pyritinol (Taranalli and Cheeramkuzhy, 2003).

Flavonoids in the petals of several *C. ternatea* lines with different petals were investigated. Delphinidine 3-O-(2”-O-alpha-rhamnosyl-6”-O-malonyl)-beta-glucoside was newly isolated from the petals of mauve line together with three known anthocyanins. Ternatins, a group of 15

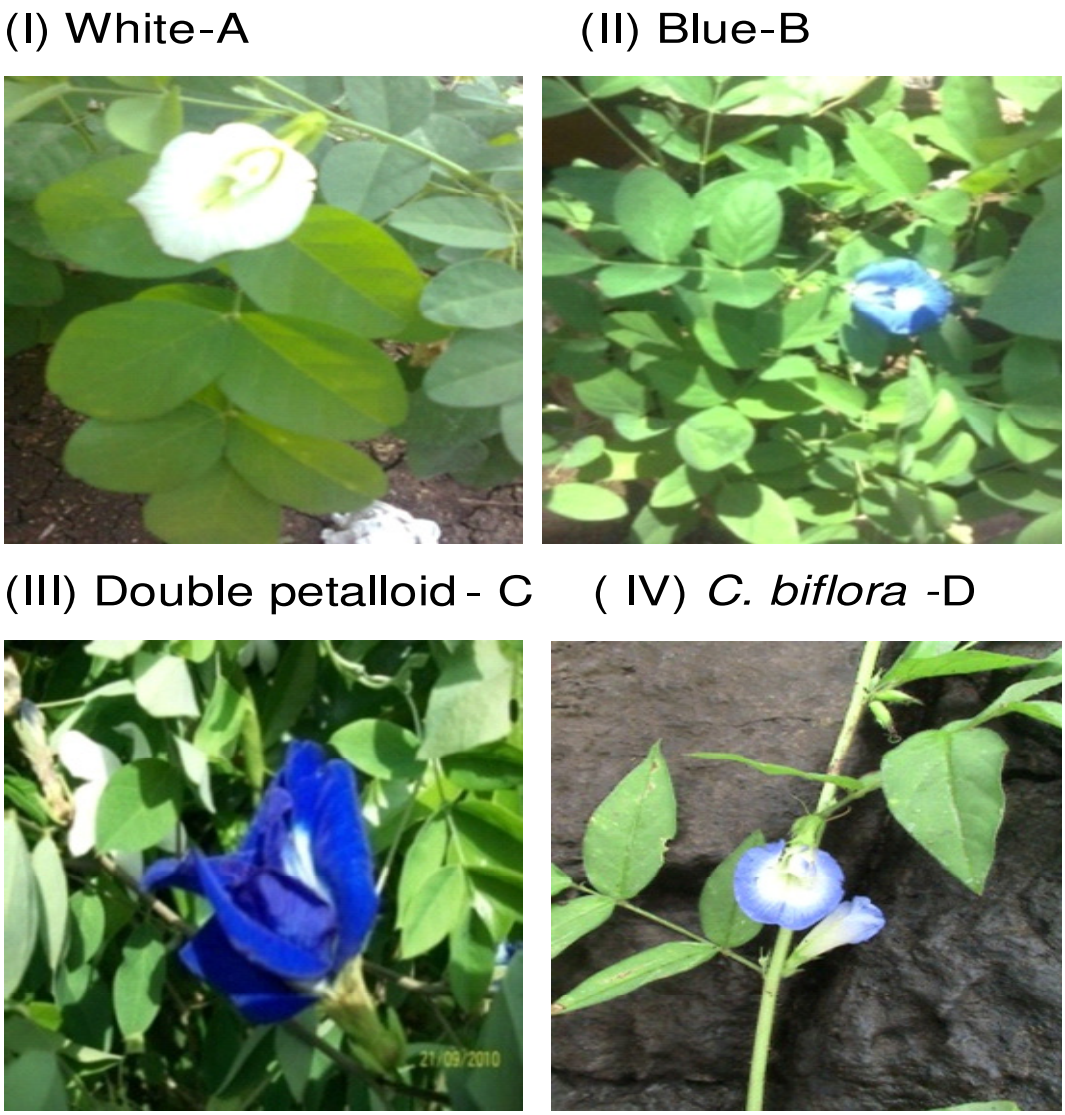

Figure 1. Habits of *C. ternatea* variants (A, B, C) and *C. biflora* (D)

(poly) acylated delphinidine glucosides, were identified in all blue petal lines. White petal line did not contain anthocyanins. The total anthocyanin contents in blue petals and double blue were high, while all the lines contained the same set of 15 flavonol, and glycosides in similar relative ratios. The change in flower colour from blue to mauve was not due to the change in structure of anthocynidin from delphinidin, but to the lack of (polyacylated) glucosyl group substitutions at both the 3'- and 5'- positions. This implies that glucosylation of the 3'- and 5'positions of anthocyanins cause blue petals in *C. ternatea* (Kazuma et al., 2003).

Plant disease resistant genes (R genes) have been cloned and characterized from both mono and dicotyledonous plants (Hammond-Kosack, 1997). *C. ternatea* is resistant to a number of pests and pathogens owing to the presence of a defense protein designated as "finotin". Finotin was found to be highly toxic to insects and important fungal pathogens of plants (Kelemu et al., 2004).

Optimization of various experimental steps were involved during this investigation, starting from DNA extraction protocol to the PCR amplification reaction and gel electrophoresis were done in order to overcome the reported sensitivity problem of RAPD technique. Figure 2 shows the polymorphisms among and within the four genotypes. In total, 120 RAPD primers were screened out to amplify DNA from all four genotypes of which 202 (46.75%) polymorphic bands were detected indicating a moderate gene pool existence in the four genotypes studied. A total of 432 amplified DNA fragments ranging from 150 to 2500 bp were generated by 100 primers with 4.32 as the average number of bands amplified per primer (average polymorphism). The highest numbers of bands (11) were obtained with primer OPF10. Furthermore,

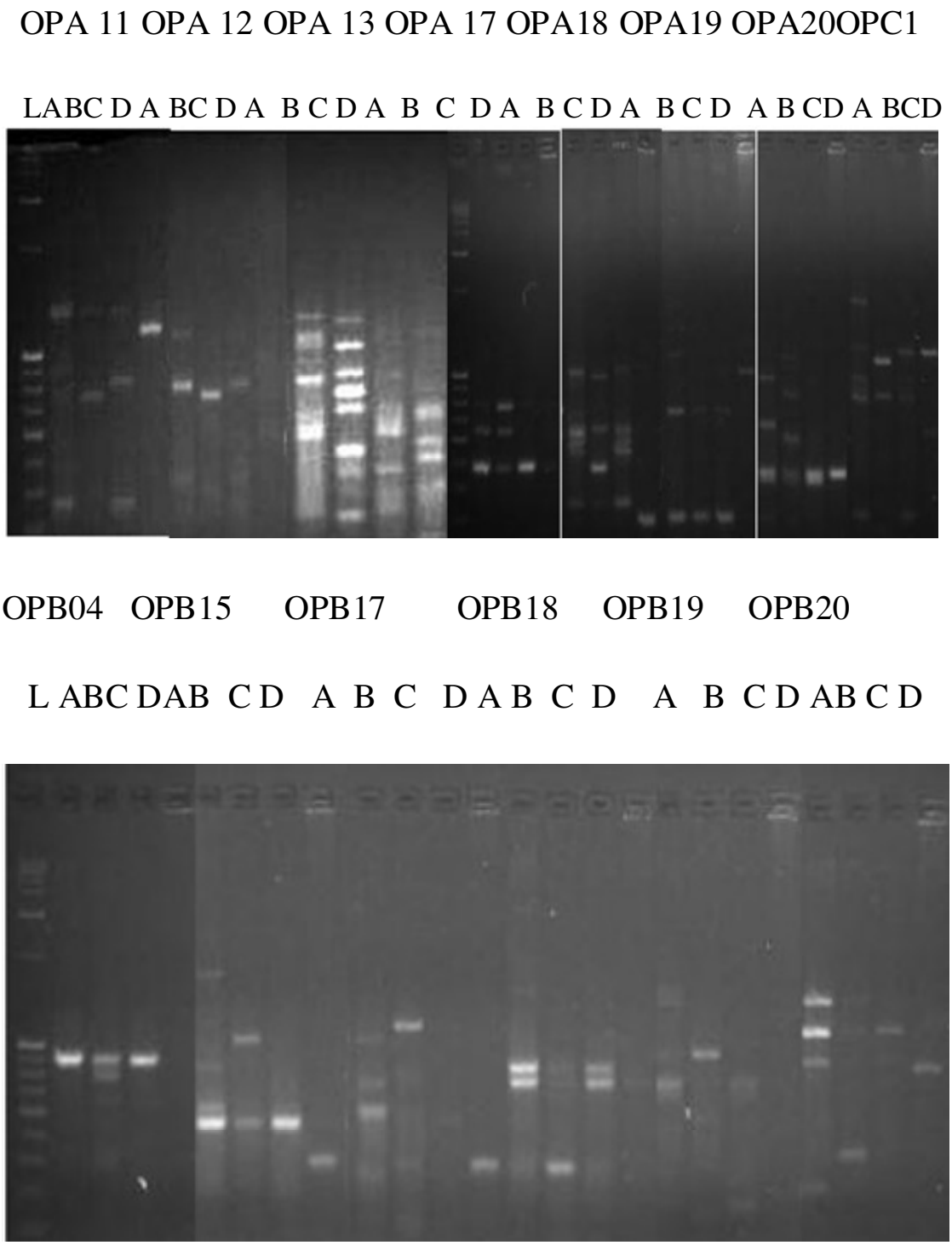

Figure 2. RAPD product generated with different primers showing the differentiating ability of the primers for identifying different genotypes (A, B, C, D).

these findings confirmed that RAPD markers can be used to detect genetic variation at the intraspecific and interspecific level (Kresovich et al., 1992; Wilkie et al., 1993; Abdel-Razzak et al., 2005; Singh et al., 2006; Thormann et al.,1994; Malode et al.,2010).

Thus, the results present in this paper, confirms that RAPDs have high discriminatory power and can be successfully applied to several genetic diversity among all the four genotypes. This is the first report on the use of a DNA based polymorphism assay to assess the level of

Table 2. Jaccard's similarity matrix between *Clitoria* species.

Row/column	A	B	C	D
A	1.0000000	-	-	-
B	0.1951220	1.0000000	-	-
C	0.5799257	0.1863354	1.0000000	-
D	0.1032258	0.1240602	0.1320000	1.0000000

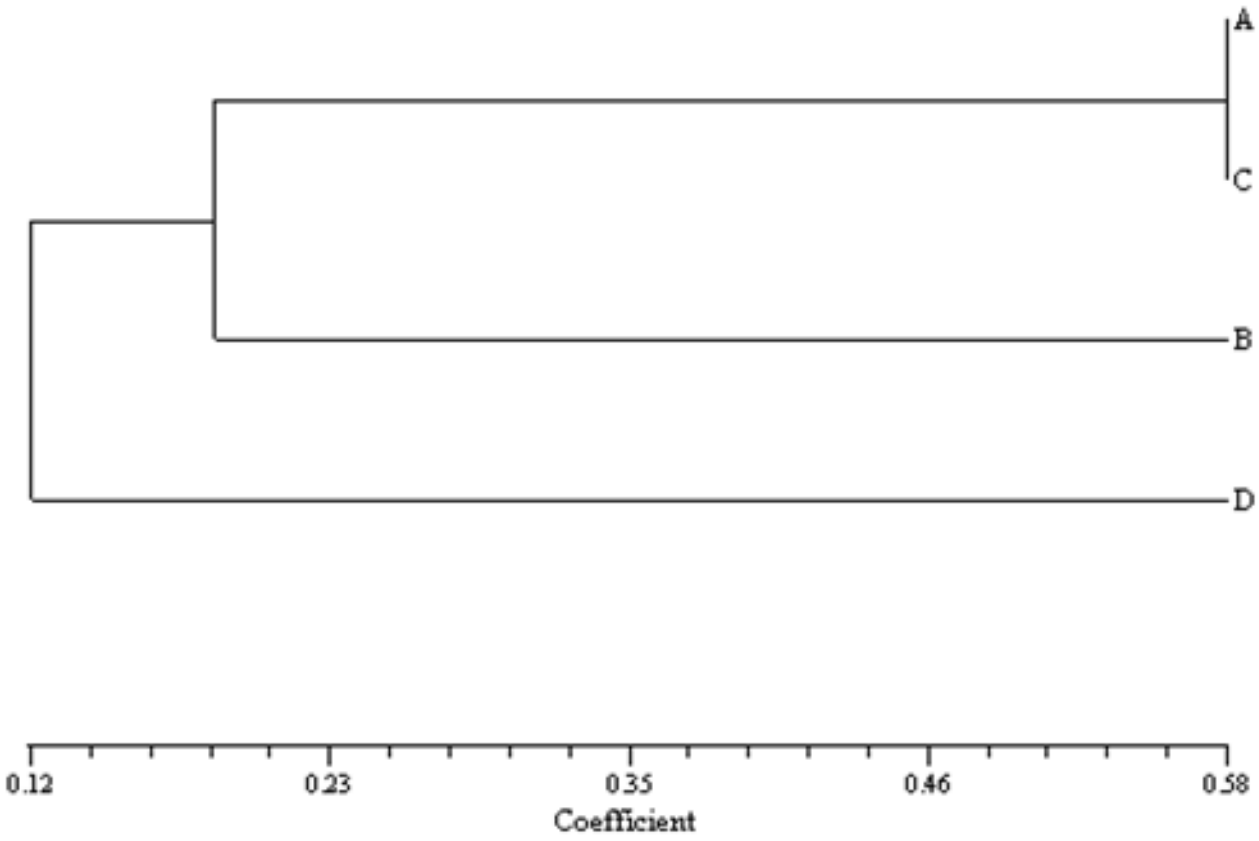

Figure 3. UPGMA dendrogram showing similarity between *Clitoria* species.

variability in four variants of *C. ternatea* (A, B, C) and *C. biflora* (D).

The RAPD data were used to estimate genetic similarities and phylogenetic relationships among the four genotypes. The highest similarity index (0.57) was observed between the A and C genotypes followed by A and B (0.19), B and C (0.18), C and D (0.13), and between B and D (0.12), while the lowest similarity index among the four genotypes was recorded between A and D (0.10) (Table 2). This result clearly supports the utility of RAPD markers *Clitoria* species genome analysis.

The dendrogram for phylogenetic relationships showed that the genomic sequences of all the four genotypes were not clustered together even though the origin was same for all genotypes. D-genotype has independent origin whereas A, B and C, originated from D. A and C has parallel evolution from B having variation in morphological characters. The genetic diversity assessment indicates that wild *C. biflora* is primitive in nature while B is intermediate and A and C evolved parallel from B (Figure 3). It appears that *C. biflora* is a distinct species and is distinguishable from *C. ternatea* by a number of well defined and consistent characters (Table 1). Principal coordinate analysis (Figure 4) shows that *C. ternatea* (single petalloid) and *C. ternatea* (Double petalloid) similar characters were grouped jointly (A and C), while in other cases they were randomly distributed. UPGMA clustering and principal coordinate analysis of similarity indices supporting the above results provided further insight into interrelations and variations among the *Clitoria* variants. Thus, RAPD analysis is found to be useful for the assessment of genetic diversity; genetic

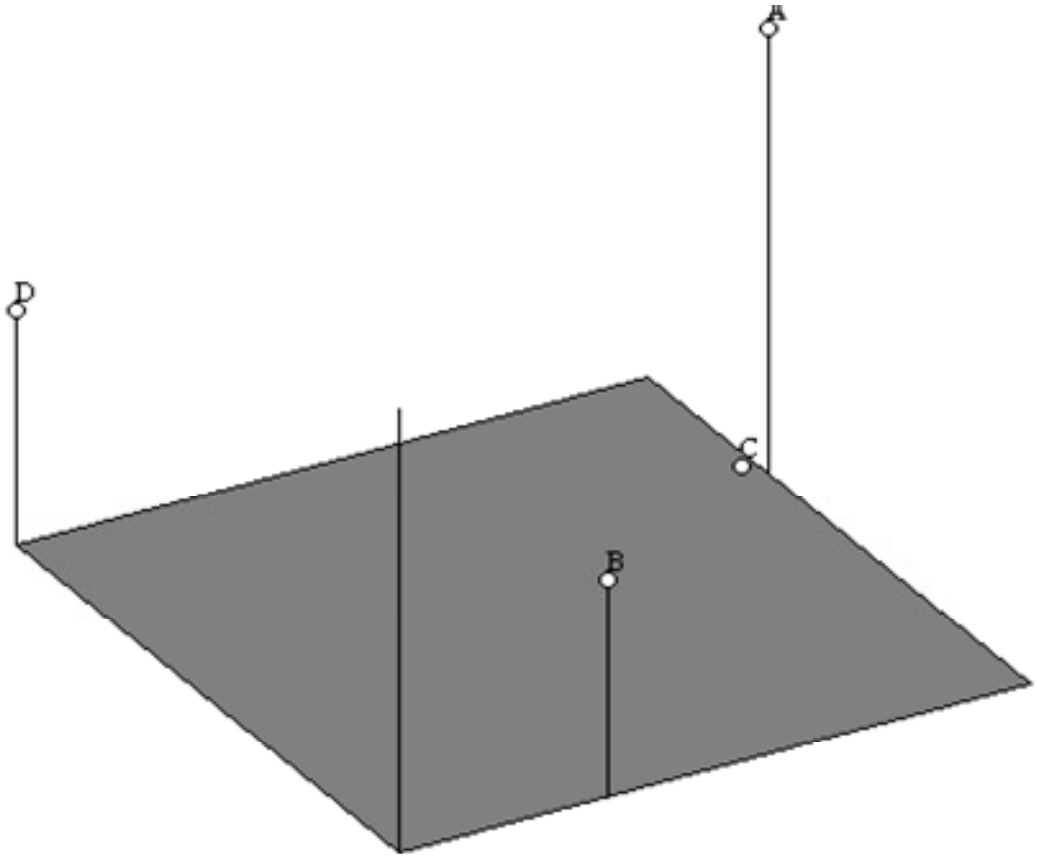

Figure 4. PCA between *Clitoria* species using RAPD markers.

relationships and phylogenetic analysis.

REFERENCES

Abdel-Razzak HS, Ahmed MM, Elmezawy AA (2005).Genetic analysis of some pepper (*Capsicum annum* L.) genotypes using three DNA based markers. Egypt. Sci. Mag. 2(3): 51-58.

Barro C, Ribeiro A (1983).The study of *Clitoria ternatea* L. hay as a forage alternative in tropical countries evolution of chemical composition of at four different growth stages. J. Sci. Food Agric. 34: 780-782.

Conway MJ, McCosker K, Osten V, Coaker S, Pengelly BC (2001). Butterfly pea- A legume success story in cropping lands of central Queensland. Proceedings of the 10th Australian Agronomy Conference, January, Hobart, Tasmania.

Hammond-Kosack KE, Jonas JDG (1997).Plant disease resistant gene. Ann. Rev. Plant Physio. Plant Mol. Bio. 48: 575-607.

Kazuma K, Noda N, Suzuki M (2003). Flavonoid composition related to petal colour in different lines of *Clitorea ternatea.* Phytochemical, 62(2): 229-37.

Kelemu S, Cardona C, Segura G (2004). Antimicrobial and insecticidal protein isolated from seeds of *Clitoria ternatea,* a tropical forage legume. Plant Physio. Biochem. 42(11): 867-873.

Kresovich S, Williams JGK, McFerson JR, Routman EJ, Schaal BA (1992).Characterization of genetic identities and relationships of *Brassica oleraceae* L. via a random amplified polymorphic DNA assay. Theor. Appl. Genet. 85: 190-196.

Malode SN , Shingnapure SM, Waghmare VN, Sutar S (2010).Genetic diversity analysis of some exotic, Indian and mutant *Brassica* sp. through RAPD markers. Afr. J. Biotechnol. 9(26): 3981-3987.

Sadasivam S, Manickam A (1996).Biochemical Methods. NewAge International Publishers, New Delhi. 2:154-155.

Singh AK, Singh M, Singh AK, Singh R, Kumar S, Kalloo G (2006).Genetic diversity within the genus *Solanum* (Solanaceae) as revealed by RAPD markers. Curr. Sci. 90(5): 711-716.

Taranalli AD, Cheeramkuzhy TC (2003). Influence of *Clitoria ternatea* extract on memory and cerebro cholinergic activity in rats. Pharma. Biol. 38:51-56.

Thormann CE, Ferreira ME, Camargo LEA, Tivang JG, Osborn TC (1994). Comparison of RFLP and RAPD markers to estimating genetic relationships within and among cruciferous species. Theor. Appl. Genet. 88: 973-980.

Welsh JO, McClelland M (1990). Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acid Res. 18: 6531-6535.

Wilkie SE, Issac PG, Slater RJ (1993).Random amplified polymorphic DNA (RAPD) markers for genetic analysis in *Allium.* Theor. Appl. Genet. 86: 497-504.