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Diversity of polyphenoloxidase isozyme in cashew (*Anacardium occidentale* L.) cultivars using PAGE method

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ABSTRACT

The experiments were conducted at Department of Horticulture, Dr. B.S.Konkan Krishi Vidyapeeth, Dapoli, and Molecular Biology Laboratory Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola to estimate the genetic diversity of cashew hybrids and their parents using polyphenoloxidase isozyme marker.Electrophoretic pattern of 8 hybrids and their parents of cashew genotype were analysis by PAGE. The polyphenoloxidase activity carried out on PAGE, peak density and relative front (Rf) value were evaluated in the Gel Docunmentation system. Polyphenoloxidase activicity of PAGE exhibited total 23 bands. The peak density observed was found in the range of 94.70 to 131.50. The range of Rf value was found 0.146 to 0.277. The polyphenoloxidase activity was not much more polymorphic to differrentiate cashew genotype and activity were only at anodal side.Dendrogram constructed base on UPGMA dice coefficient exhibited major three clusters on the basis of polyphenoloxidase activity in cashew hybrid and it's parents.

Key words : Polyphenoloxidase activity, Cashew diversity

Cashew (*Anacardium occidentale* L.) is one of the most important dollar earning crops among the major horticultural and plantation crops of our country.Though,it is export oriented premier crop in Indian commerce, first among the horticultural commodities, however, this crop was neglected and was treated as a forest tree for aforestration and wasteland development. Cashewnut originally belonging to South Eastern Brazil and introduced in the first half of the sixteenth century by the Portuguese first in Goa and Malbara hill and then, it spreads slowly to other parts of the country. Presently, cashew cultivations is confined mostly in coastal regions of Kerela, Karnataka, Goa and Maharashtra in the west coast and Andhra Pradesh, Tamil Nadu, Orissa and West Bengal (Mandal, 2000). Further improvement programme, as well as for the introduction of new variety, needs proper identification. The morphological differences in this crop are not sufficient to establish variety description suitable for plant breeding. Like other crops, the research efforts in cashew are mainly directed towards enhancing the yield potential and improving the production technology.Greatest impact of enzymes has been based on the development in the area of modern biology. Extensive studies in the field of enzymology have ushered in the modern era of molecular biology, genetic engineering and biotechnology. Isolation, cloning, characterization and manipulation of genes, creation of transgenic plant or animal utilization of molecular markers for crop improvement, fingerprinting of an organism or an individual at the molecular level etc. can not be envisaged without the help of enzyme at one stage or the other. Changes in coding base sequence will result in corresponding replacement in the amino acids and thus in the primary structure of proteins and enzymes. In the presence of electric field and while passing through a semiporous gel medium, these differences cause dissimilar forms of a protein / enzyme (Markert and Moller, 1959). Isozymes are considered to be particularly useful for this purpose as the expression of isozyme loci is co-dominant which not only allow the detection of the true hybrid but also identifies the contaminating parental seed or any other off type. Since many of the isoenzymes are expressed in seeds/ seedlings these are found very useful for genetic purity testing (Dadlani and Varier, 1993).

MATERIALS AND METHODS

The experiments were conducted at Department of Horticulture,Dr. B.S. Konkan Krishi Vidyapeeth, Dapoli, RFRS, Vengurla and in collabration with Biotechnology Centre, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola during 2005-2006. Eight hybrids and their parents have been used which were developed at RFRS Vengurla, Dr. B.S. Konkan Krishi Vidyapeeth, Dapoli (M.S.) as shown in Table 1.

Polyacrylamid Gel Electrophoresis method was followed for the seperation of total seed protein enzyme for the identification of cultivar with a pocedure described by Dadlani and Varier (1993). Sample extraction medium used was 2 x treatment buffer 0.125 m Tris cl, 20%

Table 1 : List of cashew cultivars included for study with abbreviations

Sr. No.	Variety / hybrids	Abbreviation	Parents
1.	Vengurla –1	V-1	Selection (Ansur Early – 1)
2.	Vengurla -2 Collection	V-2	Selection West Bengal Deepal
3.	Vengurla -3	V-3	Vengurla-1 (Ansur Early – 1) x Vetore-56
4.	Vengurla –4	V-4	Midnopur Red x Vetore-56
5.	Vengurla –5	V-5	(Vengurla - 1)Ansur Early -1 x Mysore Kotekar
6.	Vengurla –6	V-6	Vetore - 56 x Vengurla-1 (Ansur Early1)
7.	Vengurla –7	V-7	Vengurla 3 x M-10/4 (VRI-1) Vriddhachalam-I
8.	Vengurla –8	V-8	Vengurla 4 x M-10/4 (VRI-1) Vriddhachalam-I
9.	Vetore–56	Vt-56	Parental genotype
10.	Midnapur Red	MR	Parental genotype
11.	Mysore Kotekar	Mykt	Parental genotype
12.	Vriddhachalam-I	VRI-I	Parental genotype

glycerol, 0.2 M dithiothereitol, 0.02 % Bromophenol blue at pH 6.8. Equal part of enzyme sample and 2 x treatment buffer were combined in a test tube and placed the sample on ice until ready for use. Centrifuged at 8000 rpm for 30 min, supernatant was removed and used the clear supernatant for electrophoresis as enzyme source just before loading the gel. SE 600 vertical slab gel unit was assembled in the dual gel casting stand with gel thickness 1.5 mm. 4x running gel buffer for 1.5 and 0.5 mm thick gel 10%, containing 36.39 Tris (FW 121.1), pH 8.8, 10% ammonium persulphate and TEMED. 4x staking gel mixture solution contained 4 % acrylamide, 0.5 m Tris cl pH 6.8, 10 % ammonium persulphate and TEMED. Electrode tank buffer used Trisglycine pH 8.3, Fixing solution used 15 % trichloroacetic acid. Electrophoresis was carried out after loading the sample with each well 5 to 10 ul sample. The samples was electrophroesed with adjust power supply of 60 MA and voltage should stand about 70 to 80 V. After completion of electrophoresis, removed buffer disassembled the sandwiches put the gel into staining solution for band appearance and then stored in water for further analysis.

The isozyme of polyphenoloxidase was localized on polyacrylamide gel as per the procedure suggested by Park *et al*. (1980). The gel was incubated in 0.03 M catechol containing 0.05 per cent phenylene diamine in citrate phosphate buffer, pH 6.0 (0.1 M solution citric acid, (2 : 10 g in 100 ml) + 0.2 M solution of dibasic sodium phosphate (3.56 g in 100 ml) for one hour.

The migration was expressed as the ratio of distance band from the top of its lane to the total length of the lane. This factor is known as relative front (Rf).

$$\mathbf{Rf} = \frac{\textbf{Distance of bands from the top of its lane}}{\textbf{Total length of the lane}}$$

Analysis of peroxidose isozyme was carried out after development of specific isozyme pattern on native PAGE. The Rf value, peak density of isozymes were subjected to analysis by gel documentation system (Bio-Rad) make Gel. Doc. Eq 4.5.0 software. Similarity, index constructed by Nei's and Li's (1979) method and dendrogram constructed by UPGMA method based on dice coefficient.

RESULTS AND DISCUSSION

The procedure of PAGE followed for staining gel of polyphenoloxidase isozymes was adopted for identifying their activity in the cashew hybrids and their respective parents. The polyphenoloxidase activity was carried out on PAGE and the each band peak density and Rf value evaluated in Gel documentation system (Bio Rad Quantity one). Peak density and relative front value of polyphenoloxidase activity are presented in Table 2. Polyphenoloxidase activity of PAGE exhibited total 23 bands. The peak density observed in Table 2 was found in the range of 96.80 to 131.50. The hybrid V-1, V-3, V-4, V-5, V-7, V-8 and parents Vt-56, MR and Mykt produced maximum 2 band except hybrid V-2 and V-6 which produced one band each and parental genotype VRI-1 produced 3 bands. The hybrid V-1 exhibited only 2 band and slightly change in peak density 110.90 and 110.26. The hybrid V-2 exhibited only one band having peak density 103.20. The hybrid V-3 exhibited 2 band of peak density 104.40 and 107.40, respectively and its parents V-1 and Vt-56 produced 2 band each. The hybrid V-4 exhibited 2 band of peak density 109.40 and 110.40 while their parents Midnapur Red and Vt -56 exhibited 2 bands each. The hybrid V-5 exhibited two band showing 105.10 and 96.80 peak density and its parent V-1 and Mykt also produced the two bands. The hybrid V-6 exhibited 1 band of peak density of 94.70 while its parents

Table 2 : Peak density and Rf value of the polyphenoloxidase profile pattern (PAGE) of cashew cultivars

Cultivars	Lane no.	Band no.	Peak density	Rf values
V-1	1	1	110.90	0.201 PPO-4
		2	110.20	0.259 PPO-6
V-2	2	1	103.20	0.213 PPO-4
V-3	3	1	104.40	0.204 PPO-4
		2	107.40	0.277 PPO-6
V-4	4	1	109.40	0.207 PPO-4
		2	110.40	0.254 PPO-6
V-5	5	1	105.10	0.201 PPO-4
		2	96.80	0.265 PPO-6
V-6	6	1	94.70	0.210 PPO-4
V-7	7	1	105.00	0.198 PPO-3
		2	100.10	0.254 PPO-6
V-8	8	1	119.20	0.175 PPO-2
		2	121.90	0.238 PPO-4
Vt-56	9	1	127.10	0.190 PPO-3
		2	127.30	0.242 PPO-5
MR	10	1	125.70	0.192 PPO-3
		2	127.70	0.245 PPO-5
Mykt	11	1	122.20	0.152 PPO-1
		2	119.90	0.216 PPO-4
VRI-1	12	1	129.00	0.146 PPO-1
		2	131.50	0.267 PPO-6
		3	130.00	0.254 PPO-6

Vt-56 and V-1 exhibited 2 band each. The hybrid V-7 exhibited 2 band of peak density 105.00 and 100.10 while its parent V-3 produced 2 band and VRI-1 produced 3 bands. Hybrid V-8 showed 2 bands having peak density 119.20 and 121.90, while compared to its parents V-4 produced 2 bands and VRI-1 produced 3 bands. The parental genotype Vt-56 produced two bands with peak density of 127.10 and 127.30. The tenth lane loaded enzyme extract of genotypes MR showed two bands with peak density of 125.70 and 127.70. The genotype Mykt showed the two bands of 122.20 and 119.90 peak density. The last lane having loaded sample of VRI-1 showed maximum 3 bands with peak density 129.00, 131.50 and 130.00. as compared to all other hybrids and parents.

Rf value of the each band of polyphenoloxidase shown with its values are given in Table 2. The range of Rf value was found are 0.146 to 0.277. The polyphenoloxidase activity was not much more polymorphic to differentiate the cashew hybrids and their parents. The polyphenoloxidase activity was only at anodal side and it showed the PPO-1, PPO-2, PPO-3, PPO-4 and PPO-5 isozyme. The PPO-1 exhibited only in the parental genotype Mykt and VRI-1 having Rf value of 0.152 and 0.146. The PPO-2, isozyme was present only in hybrid V-8 with Rf value of 0.175. The PPO-3 isozyme was present in genotype V-7 and parental genotype Vt-56 and MR with Rf value of 0.198, 0.190 and 0.192, respectively. The PPO-4 isozyme observed in all hybrids except V-7 and parental genotype Mykt only with range of 0.201 to 0.216. PPO-5 was present in parental genotype Vt-56 and MR having Rf value 0.242 and 0.245. Isozyme PPO-6 was present in V-1,V-3, V-4, V-5 and V-7 produced each band and in parental genoytpe VRI-1 having two bands of Rf value 0.259, 0.277, 0.254, 0.265, 0.254 and 0.267 and 0.254, respectively.

Dendrogram (Fig. 1) based on the UPGMA dice coefficient exhibited major three cluster forms on the basis of polyphenol activity in cashew hybrids and parents. The hybrid V-5 and V-1 were very closely related and found in first cluster while 2nd cluster consisted subcluster formed by Vt-56 and MR which were closely related than V-8. In the third major cluster the 3 subcluster consisted showing the close relationship between the hybrid V-4 and VRI-1 genotype and V-2 and V-6 but the hybrid V-7 and V-3 were different from above gentoype. The PPO enzyme activity was expressed more or less or different tissue but it was also undetectable in young leaf in cashew rather than that enzyme it showed highest activity in PAL and TAL. Gilber Vela *et al*. (2003) reported PPO activity during ripening of chilling stress in Manilo mangoes.

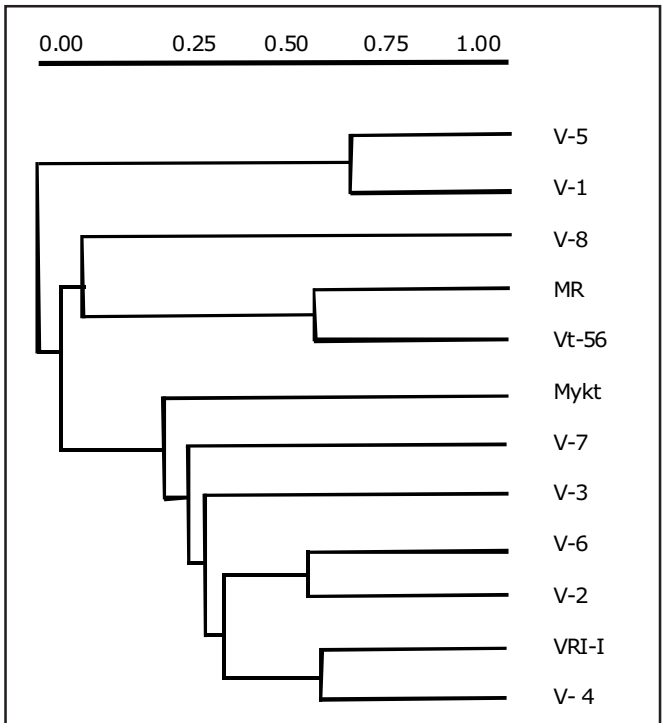

Fig. 1 : Dendogram showing on the basis of polyphenoloxidase isozyme profile pattern of cashew genotypes by UPGMA method using Dice coefficient

Jayaramraja *et al.* (2002) screened certain tea cultivar for productivity and drought hardiness using PPO and PO as biochemical marker and it was imperative that PPO had major role in tea quality but not in drought tolerance. In the present study, Polyphenol banding pattern exhibited that the activity was very less and hence no polymorphism of polyphenol may be due to use of seed tissue cotyledon which took long period and may reduce the PPO activity. The cluster pattern revealed that the species were interspersed between the varieties of common parents indicating that the genetic diversity with cashew hybrids and its related parents was very narrow which may necessitate the use of technique/procedures like wide hybridization. Low moderate genetic variability earlier observed by same hybrids and its parents in protein marker by SDS – PAGE which showed 0.47 % mean average, similarity in co-efficient (Raut *et al.,* 2009). The VRI-1 collected from Puttur, NRCC showed the highest activity among all the genotypes studied which may be due to environmental effect. The polyphenol oxidase activity was not able to oxidize phenolic compounds and it possesed that product of such oxidation which might inhibit pathogen development (Retig, 1974). In the studied genotype, the polyphenoloxidase activity may possibly show tissue specific variation and it is present in the particular tissue to inhibit the pathogens that attack rather than seed cotyledon tissue.

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