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Evaluation of genetic diversity in 21 cultivars of chili pepper (*Capsicum annuum* L.) using isozyme markers

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ABSTRACT

The genus Capsicum commonly known as chili pepper is a major spice crop and is of cosmopolitan in distribution. Assessment of genetic diversity in 21 cultivars of chili peppers was studied by electrophoretic patterns of peroxidase (PO) and polyphenol oxidase (PPO) isozyme marker technique. A maximum of 5(PO) in Ca9 and 4(PPO) in Ca14, Ca15, Ca16, Ca19 and Ca20 were scored respectively. Highest average per cent similarity (100%) was recorded between Ca15&Ca19 and the UPGMA dendrogram represented low genetic diversity. The present study revealed that considerable intraspecific differences were found in the cultivars. Thus the results obtained could be used for fingerprinting the genotypes.

Keywords: Chili pepper, Electrophoresis, PPO, PO, UPGMA.

INTRODUCTION

Chili pepper (*Capsicum* L.) are the most important vegetable cum spice because of their colour, taste, pungency, flavor and aroma belongs to the family Solanaceae grown in tropical and sub tropical regions of the world. Members of the genus are diploids (2n=24) and are either annuals or perennials. Moreover, most of the varieties or cultivars within a species show resemblance with their morphometrics, most of the cases the identification of chili pepper germplasm normally based on visual descriptions of morphological parameters i.e., leaf shape, fruit length, fruit colour and fruit shape etc. Several researchers were made to identify species and varieties of *Capsicum* using seed protein electrophoresis [1-4] however, total proteins composition was affected by a series of environmental and cultural conditions [5]. Now a day the isozyme markers have been useful in determining the genetic relationships among closely related species and cultivars for its relative simplicity because it provides direct visualization of gene products [6] and potentially can provide a unique fingerprint for each genetically distinct clone [7]. Although both morphological traits and isozyme marker systems have limitations, the first due to environmental effects and the latter to selection of markers [8,9]. In chili pepper (*Capsicum*) such studies on isozymes are very limited [10-16]. However, there have been no in depth studies on isozyme polymorphism for ascertaining the intravarietal relationships in chili peppers. Therefore the present investigation an attempt has been made to study the genetic diversity of peroxidase and polyphenol oxidase isozyme profiles for understanding the intravarietal relationships among the 21 cultivars of *Capsicum annuum* L.

MATERIALS AND METHODS

Plant materials

21 cultivars of *Capsicum annuum* ('var. PC1 (*Ca*1)', 'var. X-235 (*Ca*2)', 'var. Pusa Jwala (*Ca*3)', 'var.G4(*Ca*4)', 'var. G5(*Ca*5)', 'var. NP 46A(*Ca*6)', 'var. CA 305(*Ca*7)', 'var.CA 960(*Ca*8)', 'var. LCA, 206(*Ca*9)', 'var.

Paprika(*Ca*10)', 'var. Trupti(*Ca*11)', 'var. Elephants trunk(*Ca*12)', 'var. Surya muchi cluster(*Ca*13)', 'var. Selection 77(*Ca*14)', 'var. California wonder(*Ca*15)', 'var. Anaheim TMR(*Ca*16)', 'var. Triton(*Ca*17)', 'var. Red missile(*Ca*18)', 'var. Masquerade(*Ca*19)', 'var. Kiran(*Ca*20)' and 'var. Hungarian yellow wax(*Ca*21)') were obtained from Sutton seeds, Calcutta, India. They were grown in randomized design with three replicates at the Experimental farm of Andhra University, Visakhapatnam, India. All the cultivars were received similar water and fertilizer treatments. The isozymes were extracted at three different times to test the repeatability and reliability of the biochemical markers.

Isozyme analysis

Three young basal rosette leaves were taken in an ice bucket (4-5 weeks after transplanting) from 3 plants per cultivar. A mixed sample of all leaves per cultivar was used for analysis.

Extraction of isozymes

One gram of leaves from each sample was weighed. The leaves were gently homogenized with the extraction buffer [0.2M phoshate buffer pH 7.5+0.1% PVP+0.1% BSA+10mM $MgCl_2$+14mM β-mercaptoethanol] using mortar and pestle at 4°C. The extracts were centrifuged at 15000rpm for 10 minutes at 4°C using refrigerated centrifuge. 500µl of the supernatant mixed with 250µl of V/V glycerol and bromophenol blue (0.05mg/ml) was added to the extract.

Electrophoresis

The isozymes were resolved on 10% separating gel and 5% stacking gel polyacrylamide slabs using the electrophoretic systems [17]. Electrophoresis was conducted at 50V in 4°C until the bromophenol blue reached the gel end.

Staining and post staining treatments of gels

Detection of peroxidase on gels, the staining solution was prepared by dissolving 500mg of benzidine in 0.5ml of ethanol and 5ml of acetic acid and 95ml of water were added to it. The contents were mixed thoroughly and filtered through cotton. Then 250µl H_2O_2 was added to this just before staining. For the visualization of polyphenol oxidase the substrate solution was prepared by dissolving 0.03M catechol containing 0.05 per cent *p*-phenylene diamine in phosphate citrate buffer (pH 6.0) [18]. After electrophoresis, the gels were carefully removed from the apparatus and carefully washed at 4°C with electrophoretic buffer and then the gels were incubated in the respective staining solutions for few minutes till the clear bands appeared. Staining reaction was stopped by washing the gels 3-5 times with d.H_2O and the gels were fixed with 7% acetic acid solution.

Data analysis

Relative mobility (Rm) values were calculated for each band based on the migration of the band relative to the front or tracking dye and the gels were scored as presence (+) or absence (-) of bands. Depending upon the presence or absence of bands, similarity indices (SI) were calculated [19]. Cluster analysis UPGMA (Unweighted pair group method with arithmetic averages) was performed on the similarity index by using statistical software SPSS for windows package (Version 10).

RESULTS AND DISCUSSION

Agronomic, morphological, biochemical and molecular characteristics are either direct or indirect representations of genetic variability at the DNA level. These are therefore expected to provide inter/intra specific information about genetic relationships. The assessment of genetic diversity is important not only for crop improvement but also for efficient management and conservation of germplasm resources. Peroxidase(PO) and Polyphenol oxidase(PPO) isozymes were used to ascertain the genetic polymorphism in 21 cultivars of chili pepper (*Capsicum annuum* L.). A total of 7 different peroxidases (a range of 1 to 5 bands) with Rm value ranged from 0.266 to 0.850 were recorded (Table-1 & Figure-1). Among the cultivars *Ca*9 expressed maximum peroxidase isoforms (5) numbered as 1,3,4,5 and 6 where as *Ca*5 and *Ca*13 displaced only one band of peroxidase each, numbered as 2 and 1 respectively. Peroxidases are known to exist both in monomeric and dimeric forms [20] and are often encoded by many different loci with the evidence of post translational modifications [21]. It has been studied in *Capsicum* [10-12, 14, 15] in most of these studies, varied numbers of isozyme groups have been reported with number of isozymes ranged from 1 to 3 along with qualitative and quantitative differences. The degree of polymorphism in this isozyme was believed to be due to heterogeneity in its primary structure [22].

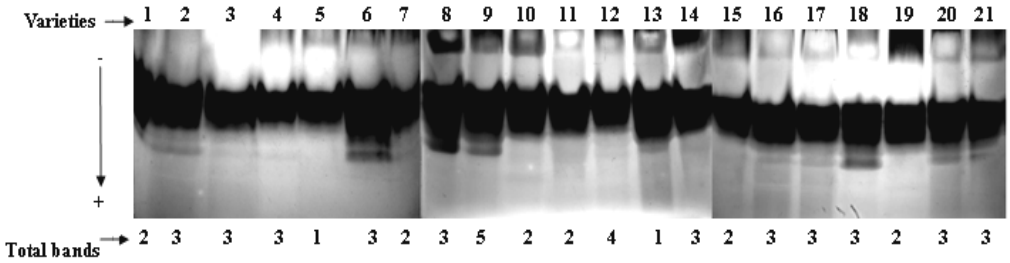

Figure. 1. Peroxidase isozyme patterns of 21 cultivars of chili pepper (*Capsicum annuum* L.) on 10% Polyacrylamide gels (numbers from 1-21 refer to the materials)

Polyphenol oxidase(PPO) has been shown to exist in multiple and interconvertible forms and is widely distributed in plant kingdom. In the present investigation 8 PPO isozyme bands were observed (Table -1 and Figure -2). Among the cultivars *Ca*14, *Ca*15, *Ca*16, *Ca*19 and *Ca*20 expressed four bands each while, twelve cultivars possesses four PPO forms each. Rest of the cultivars i.e., *Ca*3, *Ca*10, *Ca*13 and *Ca*17 displayed 2 PPO isoforms each. The different PPO isozyme patterns could be due to aggregation, differentiation in amino acid composition, variable percentage of covalently linked carbohydrates, partial proteolysis and other post translational modifications [23-26].

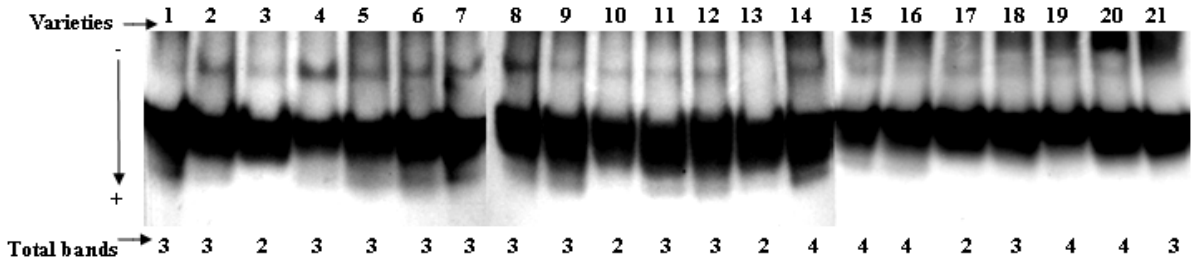

Figure. 2. 10% polyacrylamide gels representing the isozyme Polyphenol oxidase of chili pepper cultivars (numbers from 1-21 refer to the materials)

Similarity index (SI) for 21 cultivars of *Capsicum annuum* L. ranged from 0 to 100% (Table-2). Highest per cent similarity (100%) was recorded between *Ca*15&*Ca*19 and the results are agreement with the earlier report of Gupta et al. [13]. The dendrogram (UPGMA) resulting from cluster analysis segregated the chili pepper cultivars into four groups group I included 2, group II consisted of 7, group III manifested 5 and group IV possesses 7 cultivars respectively Figure (3). The dendrogram as a whole revealed low genetic diversity because most of the cultivars are in the same cluster. Our present results show that 5 (*Ca*6, *Ca*8, *Ca*18 & *Ca*19) and 7 (*Ca*1, *Ca*4, *Ca*7, *Ca*8, *Ca*14, *Ca*20 & *Ca*21) chili pepper cultivars represented demarked PO and PPO profiles respectively. Hence, can be recommended in breeding programs to develop chili pepper varieties.

Table -1. Banding patterns of peroxidase (PO) and polyphenol oxidase (PPO) isozymes from different cultivars of chili pepper (*Capsicum annuum* L.)

Isozyme type (Rm value)	Chili pepper cultivars																				
	C a 1	*C a* 2	*C a* 3	*C a* 4	*C a* 5	*C a* 6	*C a* 7	*C a* 8	*C a* 9	*Ca* 10	*Ca* 11	*Ca* 12	*Ca* 13	*Ca* 14	*Ca* 15	*Ca* 16	*Ca* 17	*Ca* 18	*Ca* 19	*Ca* 20	*Ca* 21
Peroxidase isoforms (PO)																					
PO1(0.266)	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
PO2(0.616)	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
PO3(0.633)	+	+	+	+	-	-	+	-	+	+	+	-	-	-	-	-	+	-	-	-	-
PO4(0.666)	-	+	+	+	-	+	-	+	+	-	-	+	-	+	+	+	-	+	+	+	+
PO5(0.700)	-	-	-	-	-	+	-	+	+	-	-	+	-	+	-	+	+	-	-	+	+
PO6(0.733)	+	+	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
PO7(0.850)	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Polyphenol Oxidase isoforms (PPO)																					
PPO 1(0.200)	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	+	+	+	-
PPO 2(0.250)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
PPO 3(0.283)	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	+	+	-
PPO 4(0.583)	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	+	+	-	+	-	-
PPO 5(0.633)	-	+	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	+	-	+	+
PPO 6(0.650)	-	-	-	-	-	+	-	+	+	-	+	+	+	+	-	-	-	-	-	-	-
PPO 7(0.666)	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PPO 8(0.750)	+	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+

Note: + : presence; - : absence; Ca1- Ca21 refer to the materials.

Table - 2. Similarity matrixes of chili peppers based on peroxidase(PO) and polyphenol oxidase (PPO) isozyme profiles

Ca.Cult.	*C a* 1	*C a* 2	*C a* 3	*C a* 4	*C a* 5	*C a* 6	*C a* 7	*C a* 8	*C a* 9	*Ca*10	*Ca*11	*Ca*12	*Ca*13	*Ca*14	*Ca*15	*Ca*16	*Ca*17	*Ca*18	*C a*19	*Ca*20	*Ca*21
***C a* 1**	100.00	72..72	60.00	72.72	44.44	36.36	80.00	36.36	61.53	44.44	60.00	33.33	25.00	33.33	36.36	33.33	40.00	18.18	36.36	33.33	18.18
***C a* 2**		100.00	90.90	83.33	60.00	50.00	54.54	50.00	71.42	60.00	54.54	46.15	22.22	46.15	50.00	46.15	18.18	50.00	50.00	61.53	50.00
***C a* 3**			100.00	72.72	44.44	36.36	40.00	36.36	61.53	66.66	40.00	33.33	0.00	16.66	36.36	33.33	40.00	36.36	36.36	50.00	36.36
***C a* 4**				100.00	40.00	50.00	54.54	50.00	71.42	40.00	54.54	46.15	22.22	46.15	66.66	6153	54.54	33.33	50.00	46.15	33.33
***C a* 5**					100.00	40.00	44.44	40.00	33.33	50.00	44.44	54.54	28.57	36.36	40.00	36.36	22.22	40.00	40.00	54.54	40.00
***C a* 6**						100.00	54.54	83.33	71.42	20.00	54.54	76.92	44.44	76.92	50.00	61.53	36.36	50.00	50.00	61.53	50.00
***C a* 7**							100.00	36.36	46.15	44.44	54.54	33.33	16.66	33.33	36.36	33.33	40.00	36.36	36.36	33.33	18.18
***C a* 8**								100.00	85.71	40.00	72.72	92.30	66.66	92.30	66.66	76.92	54.54	50.00	66.66	76.92	66.66
***C a* 9**									100.00	50.00	76.92	80.00	54.54	80.00	57.14	66.66	61.53	4285	57.14	66.66	57.14
***C a* 10**										100.00	66.66	36.36	28.57	36.36	40.00	36.36	66.66	40.00	40.00	36.36	40.00
***C a* 11**											100.00	66.66	75.00	66.66	54.54	50.00	40.00	36.36	54.54	50.00	36.36
***C a* 12**												100.00	60.00	85.71	61.53	71.42	50.00	46.15	61.53	71.42	80.00
***C a* 13**													100.00	60.00	44.44	40.00	25.00	50.00	44.44	40.00	44.44
***C a* 14**														100.00	76.92	85.71	50.00	61.53	76.92	85.71	61.53
***C a* 15**															100.00	92.30	54.54	66.66	100.00	76.92	50.00
***C a* 16**																100.00	66.66	61.53	92.30	85.71	61.53
***C a* 17**																	100.00	18.18	54.54	50.00	36.36
***C a* 18**																		100.00	66.66	76.92	66.66
***C a* 19**																			100.00	76.92	50.00
***C a* 20**																				100.00	76.92
***C a* 21**																					100.00

Note: Ca1- Ca21 refer to the materials.

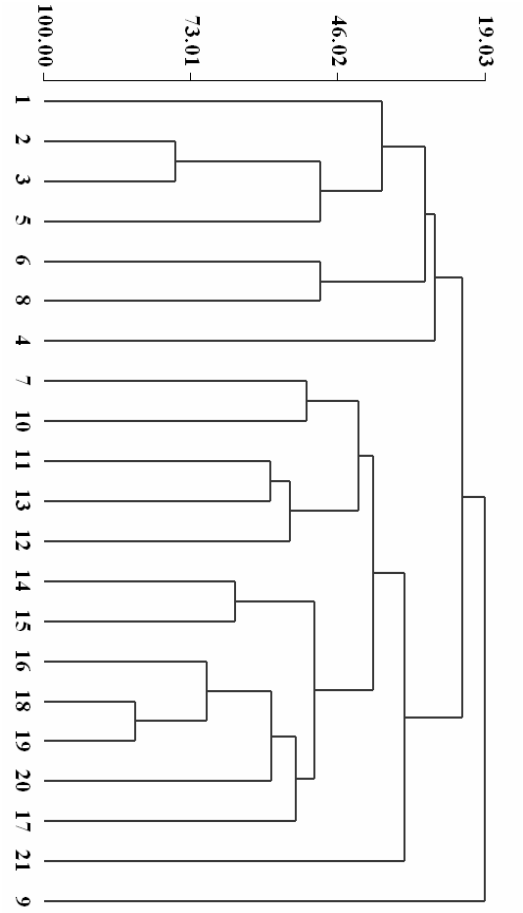

Figure. 3. Dendrogram of isozyme (PO and PPO) profiles of chili pepper cultivars (numbers from 1-21 refer to the materials)

CONCLUSION

It is concluded that peroxidase and polyphenol oxidase isozyme banding patterns in 21 cultivars of chili pepper could be used for cultivar registration and also be helpful for chili breeders.

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