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Genetic Variation of Isozyme Polyphenol Oxidase (PPO) Profiles in Different Varieties of *Capsicum annuum* L.

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Abstract

The genus *Capsicum* commonly known as chilli pepper is a major spice crop and is of cosmopolitan in distribution. Native polyacrylamide gel electrophoresis (Native PAGE) was used to study the polyphenol oxidase (PPO) isozyme variation in 21 varieties of *Capsicum annuum* L. A maximum of 4 PPO bands were scored in five varieties i.e., Ca14, Ca15, Ca16, Ca19 & Ca20, while the minimum (2 bands) was observed in four varieties (Ca3, Ca10, Ca13 & Ca17). 15 pair wise combinations showed highest average per cent similarity (100%) and the UPGMA dendrogram represented low genetic diversity. The present study revealed that considerable intraspecific differences were found in the varieties. Thus the results obtained could be used in fingerprinting the genotypes.

Keywords: chilli pepper, electrophoresis, PPO, UPGMA

Introduction

Chili peppers (*Capsicum* L.) are the most important vegetable cum spice because of their colour, taste, pungen¬cy, flavor and aroma belongings 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. Although morphological traits and isozyme marker analysis have been used to distinguish cultivars, both systems have limitations, the first due to environmental effects (Bhat *et al.*, 1992a,b) and the latter to selection of markers. Several researchers were made to identify species and varieties of *Capsicum* using seed protein electrophoresis (Aniel Kumar and Subba Tata, 2010; Anu and Peter, 2003; Odeigah *et al.*, 1999; Panda *et al.*, 1986). However, total proteins composition affected by a series of environmental and cultural conditions (Stegemann, 1979). The isozyme markers have been useful in determining genetic relationships among closely related species and cultivars etc. Isozyme electrophoresis is chosen for its relative simplicity because it provides direct visualization of gene products (Brewer and Sing, 1970) and potentially can provide a unique fingerprint for each genetically distinct clone (Lebot *et al.*, 1991).

Isozyme polymorphism has been successfully used for demonstrating inter and intragenic variations in different species and cultivars (Angelov and Ivanova, 2012; Barta *et al.*, 2003; Johnson *et al.*, 2010; Mukhlesur *et al.*, 2004). In chili pepper (*Capsicum*) such studies on isozymes are very limited (Barrera *et al.*, 2005; Gupta *et al.*, 1997; Jensen *et al.*, 1979; Loaiza-Figueroa *et al.*, 1989; Mc Leod *et al.*, 1979; Onus and Pickersgill, 2000). Polyphenoloxidase (PPO) (E.C. 1.14.18.1) is one such enzyme and has been shown to exist in multiple and interconvertible forms and is widely distributed in plant kingdom. It is well known that the enzyme plays an important role in the browning reaction in fruits and vegetables (Mayer and Harel, 1973). It has been suggested that the enzyme might be associated with many important physiological functions such as growth and differentiation (Gordon and Paleg, 1961). So far, the literature concerned only Nitesh *et al.* (2010) reported varietial identification of chilli peppers by PPO isozyme profiles. However, there have been no in depth studies on its polymorphism for assessing intravarietal relationships in chilli peppers (*C. annuum* L.). In this communication an attempt has been made to study the diversity of polyphenol oxidase isozyme for understanding the intravarietal relationships and variations among 21 varieties of *C. annuum* L.

Materials and methods

Plant material

21 varieties of *C. annuum* ('PC1 (Ca1)', 'X-235 (Ca2)', 'Pusa Jwala (Ca3)', 'G4 (Ca4)', 'G5 (Ca5)', 'var. NP 46A (Ca6)', 'CA 305 (Ca7)', 'CA 960 (Ca8)', 'LCA, 206 (Ca9)', 'Paprika (Ca10)', 'Trupti (Ca11)', 'Elephants trunk (Ca12)', 'Surya muchi cluster (Ca13)', 'Selection 77 (Ca14)', 'Cali-

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fornia wonder (Ca15)', 'Anaheim TMR (Ca16)', 'Triton (Ca17)', 'Red missile (Ca18)', 'Masquerade (Ca19)', 'Kiran (Ca20)' and 'Hungarian yellow wax (Ca21)') 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 varieties were received similar water and fertilizer treatments. The polyphenol oxidase isozymes were extracted at three different times to test the repeatability and reliability of the biochemical marker.

Isozyme analysis

Three young basal rosette leaves were taken in an ice bucket (4–5 weeks after transplanting) from 3 plants per variety. A mixed sample of all leaves per variety 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 MgCl2+14mM β – mercaptoethanol) using mortar and pestle at 4°C. The extracts were centrifuged at 15000 rpm for 10 minutes at 4°C using refrigerated centrifuge. 500µl of the supernatant was then mixed with 250µl of V/V glycerol and bromophenol blue (0.05 mg/ml) was added to the extract.

Electrophoresis

The enzymes were resolved on 10% separating gel and 5% stacking gel polyacrylamide slabs using the electrophoretic systems of Davis (1964). Electrophoresis was conducted at 50V in 4°C until the bromophenol reached the gel end.

Staining and post staining treatments of gel

For the detection of polyphenol oxidase isozymes on gel, staining solution was prepared by dissolving 0.03M catechol containing 0.05 per cent p-phenylene diamine in phosphate citrate buffer (pH 6.0) (Vallejos, 1983). 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 staining solution for few minutes till the clear bands appeared. Staining reaction was stopped by washing the gel 3-5 times with d.H2O 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. The gels were scored as presence (+) or absence (-) of isozyme bands and their staining intensities i.e., faint, medium and intensed. Depending upon the presence or absence of bands, similarity indices (SI) were calculated (Nei and Li, 1979). 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. Polyphenol oxidase (PPO) isozyme marker was used to ascertain the genetic polymorphism in 21 varieties of chilli pepper (*C. annuum* L.). Data on the PPO isozyme variations in 21 varieties of *Capsicum annuum* L. are complied in Tab. 1.

Tab. 1. Banding patterns of polyphenol oxidase (PPO) isozyme from different varieties of chilli pepper (*C. annuum* L.)

S. No.	Chilli pepper	Band No. (Rm value)								
		1 (0.200)	2 (0.250	3 (0.283)	4 (0.583)	5 (0.633)	6 (0.650)	7 (0.666)	8 (0.750)	Total bands
1.	C a1	-	-	+(F)	-	-	-	+(I)	+(M)	3
2.	C a2	-	-	+(M)	-	+(I)	-	-	+(F)	3
3.	C a3	-	-	+(F)	-	+(I)	-	-	-	2
4.	C a4	-	-	+(I)	+(I)	-	-	-	+(F)	3
5.	C a5	-	-	+(M)	-	+(I)	-	-	+(F)	3
6.	C a6	-	-	+(M)	-	-	+(I)	-	+(F)	3
7.	C a7	-	-	+(M)	-	-	-	+(I)	+(M)	3
8.	C a8	-	-	+(I)	-	-	+(I)	-	+(F)	3
9.	C a9	-	-	+(F)	-	-	+(I)	-	+(F)	3
10.	C a10	-	-	+(F)	-	+(I)	-	-	-	2
11.	C a11	-	-	+(F)	-	-	+(I)	-	+(F)	3
12.	C a12	-	-	+(F)	-	-	+(I)	-	+(F)	3
13.	C a13	-	-	-	-	-	+(I)	-	+(F)	2
14.	C a14	+(I)	-	+(M)	-	-	+(I)	-	+(M)	4
15.	C a15	+(I)	-	+(F)	+(I)	-	-	-	+(F)	4
16.	C a16	+(I)	-	+(F)	+(I)	-	-	-	+(F)	4
17.	C a17	-	-	+(F)	+(I)	-	-	-	-	2
18.	C a18	+(I)	-	-	-	+(I)	-	-	+(F)	3
19.	C a19	+(I)	-	+(F)	+(I)	-	-	-	+(F)	4
20.	C a20	+(I)	-	+(F)	-	+(I)	-	-	+(F)	4
21.	C a21	-	+(I)	-	-	+(I)	-	-	+(F)	3

All the 21 varieties produced a total of 8 different PPO forms (with a range of 1 to 4 bands) Rm value ranged from (0.200 to 0.750). Among the cultivars Ca14, Ca15, Ca16, Ca19 and Ca20 exhibited four bands each, while twelve varieties expressed three PPO forms. However four chilli

pepper varieties i.e, Ca3, Ca10, Ca13&Ca17 displayed two bands each (Fig. 1).

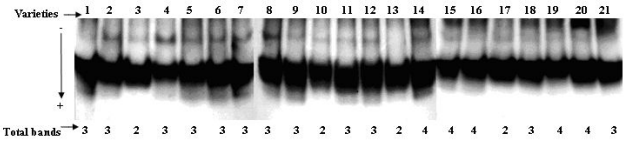

Fig. 1. Polyphenol oxidase isozyme (PPO) profiles in 21 varieties of chilli pepper *(C. annuum* L.) (Numbers from 1-21 refer to the materials)

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 (Chock *et al.*, 1980; Lodish, 1981; Paulson, 1989; Wold, 1981). Similarity index for 21 varieties of *Capsicum annuum* L. ranged from 0 to 100%. Highest per cent similarity (100%) was recorded in 15 pair wise combinations viz., Ca2&Ca5, Ca2&Ca7, Ca3&Ca10, Ca6&Ca8, Ca6&Ca9, Ca6&Ca11, Ca6&Ca12, Ca8&Ca9, Ca8&Ca11, Ca8&Ca12, Ca9&Ca11, Ca9&Ca12, Ca11&Ca12 Ca15&Ca16 and Ca15&Ca19. The dendrogram (UPGMA) resulting from cluster analysis (Fig. 2) segregated the chilli pepper varieties into three groups. Group I included two varieties, group II consisted of three varieties and Group III manifested sixteen varieties. The dendrogram as a whole revealed low genetic diversity because most of the varieties are in the same cluster. With reference to PPO band intensity, our results suggest that seven varieties i.e., Ca1, Ca4, Ca7, Ca8, Ca14, Ca20 and Ca21 can be recommended in breeding programs to develop chili pepper varieties.

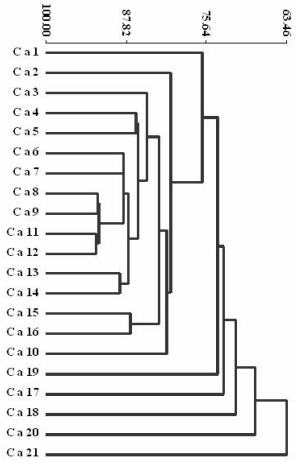

Fig. 2. Dendrogram of 21 varieties of *C. annuum* L. from polyphenol oxidase (PPO) isozyme profiles

Conclusions

It is concluded that polyphenol oxidase isozyme banding patterns in varieties of chilli pepper could be used for varietal registration and also be helpful for chilli breeders.

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References

Angelov GB, Ivanova TA (2012). Isoenzyme variation and genetic affinities among four species of the genus *Festuca* L. (Poaceae). Biodiv Res Conserv 28:3-8.

Aniel Kumar O, Subba Tata S (2010). SDS-Page Seed Storage Protein Profiles in Chili Peppers (*Capsicum* L.). NSB 2(3):86-90.

Anu A, Peter KV (2003). Analysis of seed protein of 29 lines of *Capsicum annuum* L. by polyacrylamide gel electrophoresis. Genetic Resources and Crop Evolution 50:239-243.

Barrera LQ, Garcia MC, Giraldo MC, Melgarejo LM (2005). Isozyme characterization of *Capsicum* accessions from the Amazonian Colombian collections. Revista Colombiana De Biotecnologia 1:59-65.

Barta J, Curn V, Divis J (2003). Studies of biochemical variability of potato cultivars by soluble protein, isoesterase and isoperoxidase electrophoretic patterns. Plant Soil Environ 49(5):230-236.

Bhat KV, Bhat SR, Chandel KPS (1992a). Survey of isozyme polymorphism for clonal identification in Musa I. Esterase, acid phosphatase, and catalase. J Horti Sci 67(4):501-508.

Bhat KV, Bhat SR, Chandel KPS (1992b). Survey of isozyme polymorphism for clonal identification in Musa II. Peroxidase, superoxide dismutase, shikimate dehydrogenase, and malate dehydrogenase. J Horti Sci 67(6):737-743.

Brewer GJ, Sing CF (1970). An Introduction to Isozyme Techniques. Academic Press, New York.

Chock PB, Rhee SG, Stadtman ER (1980). Interconvertable enzyme cascades in cellular regulation. Annu Rev Biochem 49:813-841.

Davis B (1964). Disc electrophoresis. I. Method and application to human serum proteins. Ann NY Acad Sci 121:404-427.

Gordon SA, Paleg LG (1961). Formation of auxin from tryptophan through action of polyphenols. Plant Physiol 36:838-845.

Gupta ChG, Lakshmi N, Srivalli T (1997). Analysis of isozyme patterns (peroxidase) of *Capsicum hybrids* through PAGE. Capsicum and Eggplant Newsletter 16:64-67.

Jensen RJ, McLeod MJ, Eshbaugh WH, Guttman SI (1979). Numerical taxonomic analysis of allozymic variation in Capsicum (Solanaceae). Taxon 28(4):315-327.

Johnson M, Usha Raja AN, Malar TRJJ (2010). Isozyme varia-

tions and genetic relationships among three *Plumbago* species. J Ecobiotechn 2(5):54-59.

Lebot V, Aradhya MK, Manshardt RM (1991). Geographic survey of genetic variation in kava (*Piper methysticum* Forst. F. and *P. wichmanii* C. DC.). Pacific Sci 45(2):169-185.

Loaiza-Figueroa F, Ritland K, Jose ALC, Tanksley SD (1989). Patterns of genetic variation of the genus *Capsicum* (Solanaceae) in Mexico. Pl Syst Evol 165:159-188.

Lodish HF (1981). Post translational modification of proteins, enzymes. Microb Technol 3:178-188.

Mayer AM, Harel E (1973). Polyphenol oxidases in plants. Phytochemistry 18:193-215.

Mc Leod MJ, Eshbaugh WH, Guttman SI (1979). An electrophoretic study of Capsicum (Solanaceae): The purple flowered taxa. Bull Torrey Bot Club 106(4):326-333.

Mukhlesur RM, Hirata Y, Alam SE (2004). Genetic variation within Brassica rapa cultivars using SDS-PAGR for seed and isozyme analysis. J of Biol Sci 4(2):239-242.

Nei M, Li WH (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc Natl Acad Sci USA 76:127-5273.

Nitesh L, Dilraj K, Patel NJ, Talati JG (2010). Varietal identification of Chilli (Capsicum annuum L.) by Electrophoretic technique. Indian J Agric Biochem 23(1):36-40.

Odeigah PGC, Oboh B, Aghalokpe IO (1999). The characterization of Nigerian varieties of pepper, *Capsicum annuum* and *Capsicum frutescens* by SDS-polyacrylamide gel electrophoresis of seed proteins. Genetic Resources and Crop Evolution 46:127-131.

Onus AN, Pickersgill B (2000). A study of selected isozymes in *Capsicum baccatum*, *Capsicum eximium*, *Capsicum cardenasii,* and two interspecific F1 hybrids in Capsicum species. Turk J Bot 24:311-318.

Panda RC, Aniel Kumar O, Raja Rao KG (1986). The use of seed protein electrophoresis in the study of phylogenetic relationships in chilli pepper (*Capsicum* L.). Theoretical and Applied Genetics 72:665-670.

Paulson JC (1989). Glycoproteins: what are the sugar chain for?. Trends Biochem Sci 14:272-276.

Stegemann H (1979). The Biology and Taxonomy of Solanaceae, p. 279-284. In: Hawkens JG, Lester RN, Skelding AO, (Eds.). Characterization of proteins from potatoes and the " Index of European varieties". Academic Press, London.

Vallejos CE (1983). Enzyme activity staining, p. 469–516. In: Tanksley SD, Orton TS (Eds.). Isozymes in Plant Genetics and Breeding. Elsevier, Amsterdam.

Wold F (1981). In vivo chemical modification of proteins. Annu Rev Biochem 50:783-814.