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MOLECULAR DIVERGENCE OF LOCALLY GROWN PUMPKIN (*CUCURBITA MAXIMA*) CULTIVARS THROUGH SOME ISOZYME TESTS

M E Haque, M A Islam, B Sikdar*

Professor Joarder DNA and Chromosome Research Lab, Department of Genetic Engineering and Biotechnology, University of Rajshahi, Rajshahi-6205, Bangladesh

Abstract

Context: Pumpkin (*Cucurbita maxima*) is highly polymorphic vegetable species and its polymorphism can be analyzed by isozyme molecular marker.

Objective: To analyze genetic polymorphism among 10 locally grown pumpkin cultivars by isozyme.

Materials and Methods: Fresh leaves of young plant of different cultivars were used for enzyme extraction. Enzyme extracts were prepared by homogenizing of 2 g sample of each cultivar. Prechilled mortar and pestle nestled in ice along with 1% polyvinylpyrrolidone and 2 ml of chilled extraction buffer were used prior to centrifuge. N-PAGE was conducted for different isozymes and stained the gels with specific chemicals for band development.

Results: Five isozymes (peroxidase, esterase, acid phosphatase, alkaline phosphatase and malate dehydrogenase) were tested for genetic polymorphism analysis of pumpkin cultivars. Among them esterase, peroxidase and alkaline phosphatase showed polymorphism in different cultivars with 75-, 58.33- and 41.18% respectively. But acid phosphatase and malate dehydrogenase did not show any polymorphism. Esterase and peroxidase produced band quickly than others. Relative mobility of first band of esterase, peroxidase, acid phosphatase, alkaline phosphatase and malate deghdrogenase was 0.063, 0.045, 0.262, 0.07 and 0.093 respectively

Conclusion: Out of five isozymes, effective polymorphism was found in esterase and peroxidase test

Keywords: Genetic divergence, isozyme, Pumpkin.

Introduction

Pumpkin (*Cucurbita maxima*) is one of the most important nutritious vegetable in the family cucurbitaceae. Its fruit as well as leaf and stem are very delicious. There has been recent interest in edible pumpkin seed, particularly in genotypes with the hull-less trait (Loy 1990) and good source of secondary metabolites (Chopra *et al*. 1956). This is highly cross pollinated species and mainly propagated by seed. So, it shows much genetic variability in morphologically as well as in molecular level.

Isozymes are the important techniques for genetic polymorphism analysis in pumpkin. Isozymes have been the genetic markers most frequently applied to plant germplasm management (Simpson and Withers 1986). They are proved to be reliable molecular markers in breeding and genetic studies of plant species (Shannon 1968, Glaszmann *et al*. 1989). However isolation of isozymes has become an important technique in molecular biology to conduct research on biological evolution, physiological and biochemistry, taxonomy and genetic science (Rider and Taylor 1980, Luo *et al*. 1999) as well as in agronomy (Tanksley and Orton 1983).

Isoenzymes provide valuable information with respect to hybridization and gene duplication, including polyploidy. In Bangladesh, no report was published on isozyme analysis of pumpkin. In this investigation, peroxidase (POD) E.C. 1.11.1.7; esterase (EST) E.C. 3.1.1.-; acid phosphatase (ACP) E.C. 3.1.3.2; alkaline phosphatase (ALP) E.C. 3.1.3.1 and malate dehydrogenase (MDH) E.C. 1.1.1.37 were used to study genetic polymorphism analysis among 10 locally grown pumpkin cultivars in Rajshahi region of Bangladesh marker.

*Corresponding author *Email:* bsikdar2004@yahoo.com

Materials and Methods

Fresh leaves of young pumpkin plants of different cultivars were used for enzyme extraction. Enzyme extracts were prepared by homogenizing the samples (2 g) of each of the cultivars in prechilled mortar and pestle nestled in ice along with 1% polyvinylpyrrolidone and 2 ml of chilled extraction buffer containing 0.1 M Tris base, 0.25 M Sucrose, 0.1% Ascorbic acid, 1% Cystein HCl, 1 mM EDTA, 1% MgCl2 prior to centrifuge at 15000 rpm for 15 min at 4°C and stored at □20°C. Protein concentration was determined by Lowry method with bovine serum albumin as standard using UV Spectrophotometer (T80 UV/VIS Spectrometer, PG Instruments Ltd). N-PAGE was performed with vertical gel electrophoresis unit (SLAB GEL SYSTEM, BIOTECH, YERCAUD-636601) using 5% stacking gel and 10% resolving gel. Equal amount (15µl) of protein from each sample mixed with sample loading buffer (2X), underwent electrophoretic separation (Tris-glycine buffer, pH 8.3; 80 V for staking gel and 100 V for resolving gel at 4°C) for different isozyme tests and stained the gel with enzyme specific chemicals for band development. The electrophoresis was ended when the distance from staking dye to bottom was 1 cm (Yang and Zeng 1984). Peroxidase activity was performed in a reaction mixture containing 10 mM sodium phosphate buffer (pH 6.0) 50 ml, 30% H2O2 30µl and 20 mM guaiacol.

After completion of electrophoresis, gel was incubated in sodium phosphate buffer for 30 min at 4°C and buffer was changed after 15 min. Then the gel was immersed in staining solution for band formation. Esterase activity was conducted by reaction mixture containing 10 mM sodium phosphate buffer (pH 6.0) 50 ml, α-napthyl acetate 0.2 g, acetone 2 ml, deionized water 2 ml, fast blue RR salt 0.4 g and staining process was similar as peroxidase test. In acid phosphatase test, reaction chemicals were 0.15 M acetate buffer (pH 5.0) 50 ml, α-napthyl acid phosphate sodium salt 0.125 g, fast garnet GBC salt 0.125 g. First the gel was incubated in 0.15 M acetate buffer for 1 h at 4°C. Then the gel was immersed in staining solution at 4°C for overnight for band formation. Alkaline phosphatase was performed by incubating the gel in tris buffer (pH 8.7) 50 ml at 4°C for 1 h and changed the buffer every 15 min interval. Then the gel was immersed in 50 ml tris buffer containing staining chemicals α-Napthyl acid phosphate 50 mg, fast blue BB salt 50 mg, $MgCl_2$ 60 mg, $MnCl_2$ (0.25M) 50 mg at 4°C up to band formation. Malate dehydrogenase isozyme test was done by incubating the gel in 10 mM malate buffer (pH 7.0) 50 ml at 4°C for 30 min and the gel was immersed in 50 ml malate buffer containing MTT 60 mg, NAD 60 mg, PMS 4 mg at 4°C for band formation. Relative mobility (R_f) of each of the band was measured. In the all isozymes tests, 10% resolving gel was used.

Results

Five isozymes (esterase, peroxidase, acid phosphatase, alkaline phosphatase and malate dehydrogenase) were tested for genetic polymorphism analysis of pumpkin cultivars. Among them esterase, peroxidase and alkaline phosphatase showed 75 %, 58.33 % and 41.18% polymorphic bands (Table 1) in different cultivars. Esterase and peroxidase produced red bands and alkaline phosphatase produced gray bands (Table 1, Plate 1, A, B & D). Acid phosphatase and malate dehydrogenase developed purple and blue color band without showing any polymorphism (Table 1, Plate 1, C & E).

Esterase and peroxidase produced bands quickly than others. Relative mobility (R_f) of different bands for different isozymes were 0.045 (POD 1), 0.200 (POD 2), 0.295 (POD 3), 0.364 (POD 4), 0.410 (POD 5), 0.063 (EST 1), 0.167 (EST 2), 0.292 (EST 3), 0.354 (EST 4), 0.420 (EST 5), 0.542 (EST 6), 0.670 (EST 7), 0.792 (ST 8), 0.262 (ACP 1), 0.093 (MDH 1), 0.070 (ALP 1) and 0.390 (ALP 2).

Discussion

In the cucurbitaceous family, isozyme studies have been carried out for intra and interspecific relationships of the cultivated species as well as to classify the wild species and evaluate relationship among the wild and

Table1. Different types of isozymes with their band color, band formation rate, total number of bands, number of polymorphic bands and percentage of polymorphism

Name of isozymes	Band color	Band formation rate	Total number of bands	Number of polymorphic bands	Percentage (%) of polymorphism
Esterase (EST)	Red	Quick	44	33	75
Peroxidase (POD)	Red	Quick	24	14	58.33
Acid phosphatase (ACP)	Grey	Slow	10	-	-
Malate dehydrogenase (MDH)	Blue	Slow	10	-	-
Alkaline Phosphatase (ALP)	Purple	moderate	17	7	41.18

Table2. Different types of isozymes with their band types and relative mobility (R_f)

Name of isozymes	Band types	Relative mobility (R_f)
Peroxidase (POD)	POD 1	0.045
	POD 2	0.200
	POD 3	0.295
	POD 4	0.364
	POD 5	0.410
Esterase (EST)	EST 1	0.063
	EST 2	0.167
	EST 3	0.292
	EST 4	0.354
	EST 5	0.420
	EST 6	0.542
	EST 7	0.670
	EST 8	0.792
Acid phosphatase (ACP)	ACP 1	0.262
Malate dehydrogenase (MDH)	MDH 1	0.093
Alkaline Phosphatase (ALP)	ALP 1	0.070
	ALP 2	0.390

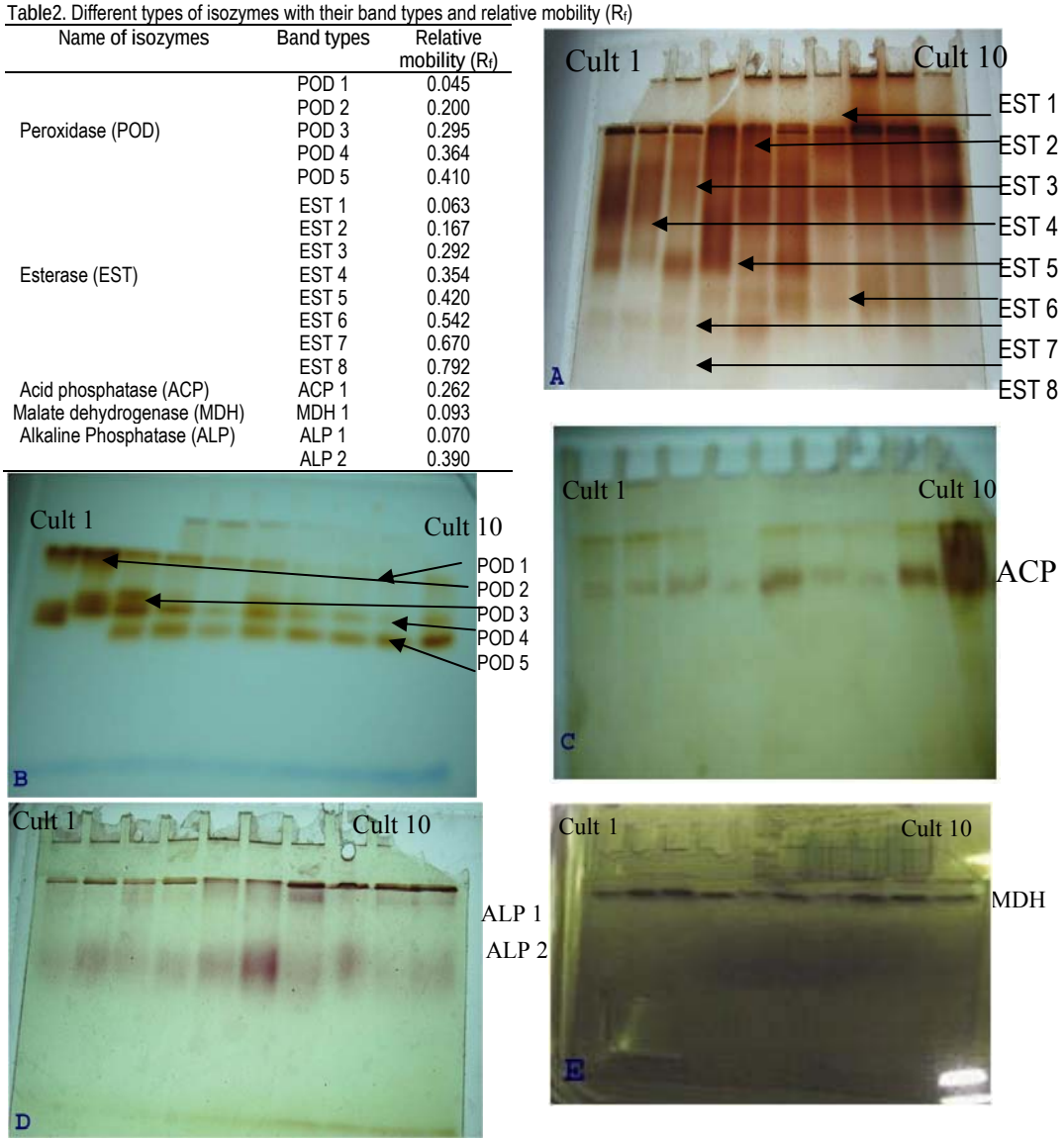

Plate 1- A-Esterase (EST), B- Peroxidase (POD), C- Acid phosphatase (ACP), D- Alkaline phosphatase (ALP), E- Malate dehydrogenase test (MDH); Cult- Cultivar

cultivated taxas (Tanksley and Orton 1983). In the present investigation, pumpkin was shown very reliable cultivated species which were differed from cultivar to cultivar and the differences were analyzed by protein molecular marker-isozyme test. Out of five isozyme tests, esterase produced effective polymorphism that was 75%. Peroxidase test represented 58.33% polymorphism and in alkaline phosphatase test, polymorphism was 41.18%. Another two enzymes did not show polymorphism in ten cultivars. But Ignart and Weeden (1984) found considerable variation among different cultivars of *Cucurbita pepo* by malate dehydrogenase isozyme system. Esterase produced 33 polymorphic bands (out of 44 bands), peroxidase produced 14 polymorphic bands (out of 24 bands) (Table 1). Sujatha and Seshadri (1991) reported 3 polymorphic loci out of 14 by different isozyme tests in melon.

In this investigation, EST 1, POD 3 and ALP 2 were present in every cultivar but other bands were not present. EST 5 and POD 4 were unique polymorphic bands present in cultivar 4 and cultivar 1 and their relative mobility (R_f) were 0.42 and 0.364. Relative mobility of the first band of all the enzymes was not same and it was 0.063, 0.045, 0.262, 0.093 and 0.07 respectively for esterase, peroxidase, acid phosphatase, malate dehydrogenase and alkaline phosphatase. Tao and Jiashu (2008) reported on comparison of protein profile and peroxidases in Bush and Vine type tropical pumpkin by peroxidase isozyme test. They reported that peroxidase activity is lower in leaf tissue than root or internode. Another important report was published by Loy (1972) on a comparison of stem peroxidases in Bush and Vine form of squash (*Cucurbita maxima*). Price *et al. (*2003) reported the molecular and biochemical characterization in lupin (*Lupins alba* L.) by observing peroxidase enzyme pattern. Esquinas-Alcazar (1977) examined 125 *Cucumis melo* populations for variability in six enzyme systems. Polymorphism was observed in all these isozyme systems, indicating that *Cucurbita pepo* possesses a signigficant level of inherent allozyme variation. The several previous investigations on the isozymes of *Cucurbita pepo* have primarily deal with peroxidases (Dvorak and Cernohorska 1967, Denna and Alexander 1975), esterases (Puchalski and Robinson 1978). Lebeda *et al.* (1999) detected peroxidase isozyme polymorphism in *Cucurbita pepo* cultivars with various morphotypes and different level of field resistance to powdery mildew. In this investigation, band formation rate in the five isozyme tests, peroxidase and esterase produced bands quickly and these two isozymes produced polymorphism effectively.

Conclusion

Genetic polymorphism was analyzed by five isozyme tests among ten locally grown pumpkin cultivars. Out of five isozyme tests, esterase and peroxidase showed effective genetic variation.

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