GENETIC DIVERSITY IN BOTTLE GOURD (*Lagenaria siceraria* L.) USING PROTEIN PROFILING AND VARIOUS ISOENZYMES

BHIMANI, DHARTI R. AND *MEHTA, D. R.

**DEPARTMENT OF GENETICS AND PLANT BREEDING,
JUNAGADH AGRICULTURAL UNIVERSITY
JUNAGADH-362 001, GUJARAT, INDIA**

E.mail: drmehta@jau.in

ABSTRACT

A genetic analysis of six diverse parents of bottle gourd (Lagenaria siceraria L.) and their fifteen crosses were performed using protein profiling and isoenzymes like peroxidase, esterase, acid phosphatase and polyphenol oxidase. All 21 bottle gourd genotypes were tested in Native-PAGE at 12 and 18 DAG for the study of genetic diversity among them. Protein profile of bottle gourd genotypes at 12 DAG developed ten bands having Rm values of 0.185, 0.221, 0.297, 0.342, 0.401, 0.478, 0.526, 0.599, 0.689 and 0.748. Out of them, two bands were of monomorphic and others were shared polymorphic. Protein profile of bottle gourd genotypes at 18 DAG developed twelve polymorphic bands having Rm values of 0.201, 0.298, 0.346, 0.409, 0.436, 0.513, 0.577, 0.628, 0.689, 0.718, 0.803 and 0.845. Out of them two bands were monomorphic and rests of the bands were shared polymorphic.Twenty-one bottle gourd genotypes were grouped into two main clusters I and II with an average similarity of 48 per cent. At 12 DAG, five bands of peroxidase isoenzyme were observed having Rm values of 0.078, 0.167, 0.253, 0.403 and 0.437. Out of them four bands were polymorphic, while band of Rm value 0.403 was monomorphic. Six bands of esterase were detected at 12 DAG having Rm values of 0.093, 0.183, 0.264, 0.345, 0.451 and 0.539. Banding pattern showed that all the six bands were present uniformly in all the twenty-one genotypes, indicating absence of esterase polymorphism in 21 bottle gourd genotypes. Total three bands of polyphenol oxidase were observed on PAGE at 12 DAG having Rm values of 0.118, 0.234 and 0.299. Out of three bands, two bands were polymorphic in nature and one band having Rm value of 0.299 was monomorphic. Four bands of Rm values 0.073, 0.133, 0.244 and 0.305 were observed at 12 DAG for acid phosphatase isoenzyme. Band of Rm value 0.073 was present in all the twenty-one bottle gourdgenotypes including monomorphic nature of marker, while rests of bands were polymorphic in nature.

KEY WORDS: Bottle gourd, genetic diversity, isoenzymes, protein profiling

INTRODUCTION

Bottle gourd (*Lagenaria siceraria* L.2n = 22, Cucurbitaceae) is an economically important vegetable crop widely cultivated in Africa, South America, China, Malaysia, Philippines, Indonesia, Sri Lanka and India (Decker-Walters *et al*., 2001). The bottle gourd is a hairy, rapid growing trailing or climbing annual herb, extending 3 to 15 meters in length. The decoction made from the leaves act as medicine for curing the jaundice. The fruit has a cooling effect and is a cardiac tonic and diuretic. The pulp is effective for overcoming constipation, cough, night blindness and as antidote against certain poison (Prajapati *et al*., 2010). Fiber helps in preventing constipation and other digestive disorders like flatulence and piles (Chauhan, 1972).

Several studies have focused on characterizing protein dynamics during plant development as well as the associated genomes and transcriptomes involved (Roberts, 2002; Heazlewood and Millar, 2003; Chen and Harmon, 2006; Gallardo *et al.*, 2007; Dam *et al.*, 2009). Peroxidases are harem-containing glycoproteins found in animal and plant tissues, as well as in microorganisms. There is a family of class III plant peroxidases (POX, EC 1.11.1.7) encoded by a large multigene family that comprises a number of peroxidase isoenzymes. Esterases are the family of enzymes that catalyze the hydrolysis of variety of esters, peptides, amines and halides. The function of esterase is unclear, but it is believed that it take part in detoxification of a wide range of exogenous substances (Thimmaiah, 1999).

Polyphenol oxidase (o-diphenol: oxygen oxidoreductase, EC 1.10.3.1) has been found in higher plants, and is responsible for enzymaticbrowning in raw fruits and vegetables. This reaction is important in food preservation and processing, and is generally considered to be an undesirable reaction because of the unpleasant appearance and concomitant developmentof an off-flavor (Mathew and Parpia, 1971). Ascorbic acid is a natural inhibitor of PPO (Polyphenol Oxidase) (Weaver and Charley, 1974). The enzyme acid phosphatase is present in all plant cells. Acid phosphatase is enzyme that hydrolyzes the terminal phosphate of phosphor monoesters that releasing inorganic phosphate. These enzymes are ubiquitous in bacteria, fungi, animals and plants. The acid phosphatase from various plant sources plays an important role during the solubilization of macromolecular organic phosphate in soils and mobilization of the phosphorous reserves during germination (Yenigun and Guvenilir, 2003).

Isoenzymes are useful in evolutionary study, gene activity and establishing genetic purity of seed, etc. In the present experiment, 21 bottle gourd genotypes were used for studying the variation through four isoenzymatic peroxidase, esterase, polyphenol oxidase and acid phosphatase pattern at 12 and 18 days after germination.

MATERIALS AND METHODS

The experimental material comprised of twenty-one genotypes of bottle gourd (6 diverse parents and their 15 crosses made following half diallel mating design) utilized in the present investigation are given in Table 1.

Protein profiling

For extraction of protein, 0.5 g fresh tissue was extracted in 1 ml of 0.2M Sodium phosphate buffer (pH 7.2) with the help of mortar and pestle and transferred it into centrifuge tube. The content was centrifuged at 10,000 rpm for 10 min. Supernatant was collected and 20µl of gel loading dye was added as described by Agarwal *et al*. (1998). For preparation of separating gel (10%), take 10 ml of Acrylamide from stock solution and 8.0 ml of 1.5 M Tris buffer was added in the beaker, followed by 12 ml of distilled water. The content was degassed up to five minutes. After degassing, 150 µl of freshly prepared ammonium per sulphate was added followed by 50 µl TEMED. The flask was shaken well and contents transferred quickly into the PAGE unit chamber between the glass plates. The gel was left for polymerization (15 – 20 minutes).

For preparation of stacking gel (4%), take 1.35 ml Acrylamide solution into a beaker followed by adding of 2.5 ml of 0.5 M Tris buffer and 6.0 ml distilled water. After degassing for 10 minutes, 50µl ammonium per sulphate and 30 µl TEMED were added and mixed well. The solution was poured in the PAGE unit chamber containing polymerized separating gel. The comb was placed in the stacking gel and allowed to set for 20 minutes. The comb was removed after complete polymerization without distorting the shape of the well. Load 35 µl of sample into each well with the help of micropipette. The electrophoresis was carried out on vertical PAGE. The current was turned on allowing 30 mA 120 volts for initial 20 minutes until the sample travels through the stacking gel (Sadasivam and Manikcam, 1992). After complete separation of molecules, power supply was turned off. The gel was gently removed from the space between the plates, immersed in bromophenol blue staining solution contained in a tray. The tray was periodically shaken for uniform staining and this was continued for at leastonehour. The gel was distained by putting in distaining solution. The relative mobility of the different protein bands was recorded manually.

Isoenzymes

Enzyme estimation is done in cold phosphate buffer (pH- 7.2). Prepare 0.2 M solution of monobasic sodium phosphate (27.8 g in 100 ml) and 0.2 M solution of dibasic sodium phosphate (53.65 g of Na_2HPO_4, $7H_2O$ or 71.7 g of Na_2HPO_4, $12H_2O$ in 100ml) separately and designated as A and B, respectively. Finally, add 28.0 ml of A and 72.0 ml of B and final volume was adjusted to 200 ml with distilled water. Immediately after electrophoresis, the gels were incubated in the respective substrate solution (Sadasivam and Manikam, 1992). The zones where the enzymes are located in the gel were visualized due to the appearance of colored reaction products. After sufficient incubation period, the reaction was stopped by adding appropriate stop solution followed by photography of zymogram. Enzyme extraction and staining procedure for various isoenzymes is described as under.

1. **Esterase:** Native PAGE of the sample extracts was carried out. The gel was incubated in the 2.8 g Sodium dihydrogen phosphate, 1.1 g Disodium hydrogen phosphate, 0.2 g Fast blue RR salt, 0.03 g Alpha-naphthyl acetate and 200 ml distilled water at 37^0C for 20-30 minutes, preferably in dark enzymatic reactions were stopped by adding a mixture of methanol: distilled water: acetic acid: ethyl alcohol in the ratio 10: 10: 2: 1 (Desborough and Peloquin, 1967).
2. **Peroxidase:** Native PAGE of the sample extracts was carried out. The gel was incubated in the 2 g Ortho-dianicidine, 10 ml Acetic acid, 40 ml Methanol and 100 ml distilled water at 37ºC for 25-30 minutes in dark. Hydrogen peroxide (3%) was carefully added drop by drop in the gel up to the appearance of brown band. When the bands were stained sufficiently, the reaction was arrested by immersing the gel in 7 per cent acetic acid solution for 10 minutes (Van Loon, 1971).
3. **Acid phosphatase:** After electrophoresis the gel was incubated in the 50 mg α-Naphthyl phosphate, 50 mg Fast Blue RR Salt, 1 g Sodium chloride, 0.5 ml 0 % Magnesium chloride and 50 ml 0.1M

Acetate buffer (pH 5.0) at 37^0 C for 20-30 minutes, in dark conditions. Reddish brown bands were visible in the gel. Fix the gel using 50 per cent ethanol.

4. **Polyphenol oxidase (PPO):** After electrophoresis the gel was equilibrated for 30 min in 0.1 per cent p-Phenylenediamine prepared in 0.1 M Sodium phosphate buffer (pH 7.0) followed by addition of 10 mM catachol (MW-110 g/mole) in the same buffer. After staining the gel is washed with Millipore water and photographed for further analysis (Van Loon, 1971).

Data analysis

The relative mobility (Rm) of each band was measured in each zymogram for every genotype tested as per formula given by Eeswara and Peiris (2001).

RESULT AND DISSCUTION

Protein profiling

Protein profiling of bottle gourd genotypes was done at 12 and 18 days after germination (DAG). Protein profile at 12 DAG of bottle gourd genotypes developed ten bands. Out of them two bands were of monomorphic and others were shared polymorphic.Protein profile of bottle gourd genotypes at 18 DAG developed twelve polymorphic bands. Two bands were monomorphic and rests of the bands were shared polymorphic. Thus, a total of 22 bands were generated by protein profile at different DAG (12 and 18 days) in which four bands were monomorphic and rests of them were polymorphic (Figure 1). Twelve and eighteen days PIC value were 0.893 and 0.913, respectively. Twenty-one bottle gourd genotypes were grouped into two main clusters, clusters I and II with an average similarity of 48 per cent (Figure 2). The cluster I consisted of twenty genotypes, while Cluster-II consisted only one hybrid (Pusa Naveen x NDBG-104). The formation of several sub clusters within cluster I suggested the presence of wide genetic diversity among the twenty-one bottle gourd genotypes studied. The geographical diversity was not associated with genetic diversity.

Isoenzymes

Total five bands of peroxidase isoenzyme were observed having Rm values of 0.078, 0.167, 0.253, 0.403 and 0.437 (Figure 3). Out of five bands, four bands were polymorphic, while band of Rm value 0.403 was monomorphic.. Six bands of esterase were detected at 12 DAG. Banding pattern showed that all the six bands were present uniformly in all the twenty-one genotypes, indicating absence of esterase polymorphism in 21 bottle gourd genotypes. A total three bands (isoforms) of polyphenol oxidase were observed on PAGE at 12 DAG having Rm values of 0.118, 0.234 and 0.299. Out of three bands, two bands were polymorphic in nature and one band having Rm value of 0.299 was monomorphic. Four bands of Rm values 0.073, 0.133, 0.244 and 0.305 were observed at 12 DAG for acid phosphatase isoenzyme. Band of Rm value 0.073 was present in all the twenty-one bottle gourd genotypes including monomorphic nature of marker, while rests of bands were polymorphic in nature.

The protein profile through electrophoresis provides very good means for cultivar identification and widely accepted in the breeding program. Thus, it can be concluded that biochemical markers like isoenzymes and protein can be used as an important tool to distinguish parents and hybrids (Shukla and Saxena, 2005).

CONCLUSION

From the ongoing discussion, it can be concluded that protein profile through electrophoresis provides very good means for cultivar identification and formulation of breeding programme. Biochemical markers like isoenzymes and protein can be used as an important tool to study the genetic diversity as well as to distinguish the parents and hybrids.

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Table 1: List of bottle gourd genotypes used in study

No.	Name of Genotypes	No.	Name of Genotypes	No.	Name of Genotypes
1.	ABG-1	8.	ABG-1 x PBOG-90	15.	Pusa Naveen x JBOGL-01-6
2.	Pusa Naveen	9.	ABG-1 x VR-2	16.	PBOG-90 x VR-2
3.	PBOG-90	10.	ABG-1 x NDBG-104	17.	PBOG-90 x NDBG-104
4.	VR-2	11.	ABG-1 x JBOGL-01-6	18.	PBOG-90 x JBOGL-01-6
5.	NDBG-104	12.	Pusa Naveen x PBOG-90	19.	VR-2 x NDBG-104
6.	JBOGL-01-6	13.	Pusa Naveen x VR-2	20.	VR-2 x JBOGL-01-6
7.	ABG-1 x Pusa Naveen	14.	Pusa Naveen x NDBG-104	21.	NDBG-104 x JBOGL-01-6

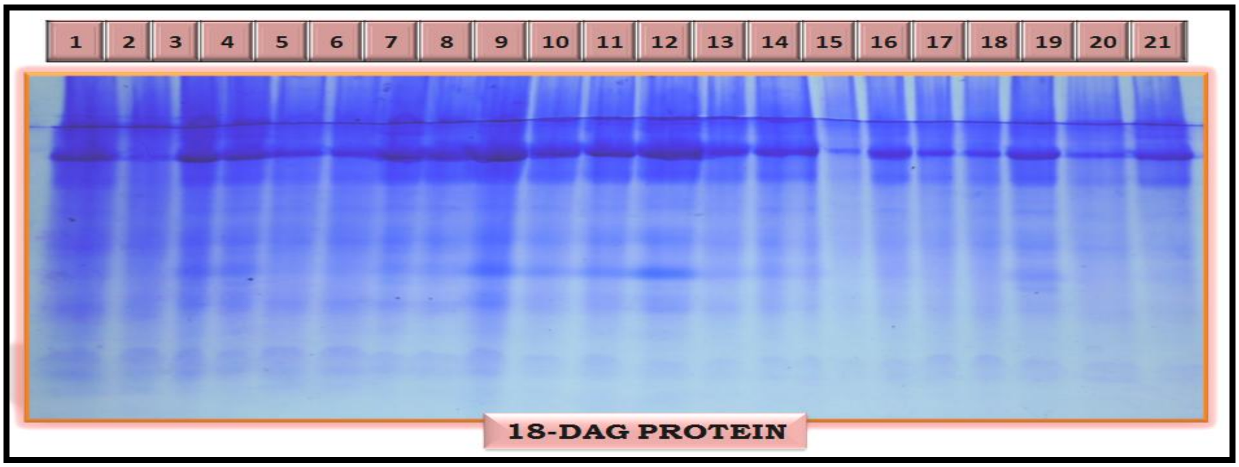

Figure 1: Protein profile generated using polyacrylamide gel electrophoresis at 12 and 18 days after germination

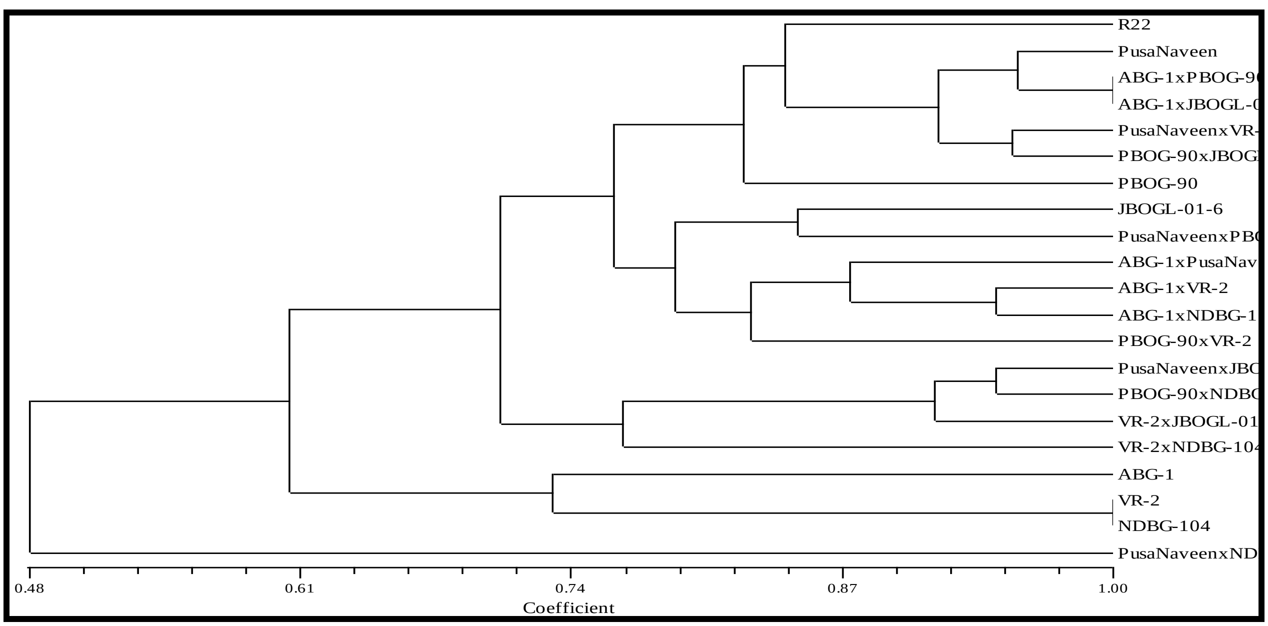

Fig. 2: Dendrogram depicting the genetic relationship among 21 bottle Gourd genotypes based on 12 DAG and 18 DAG protein profile data

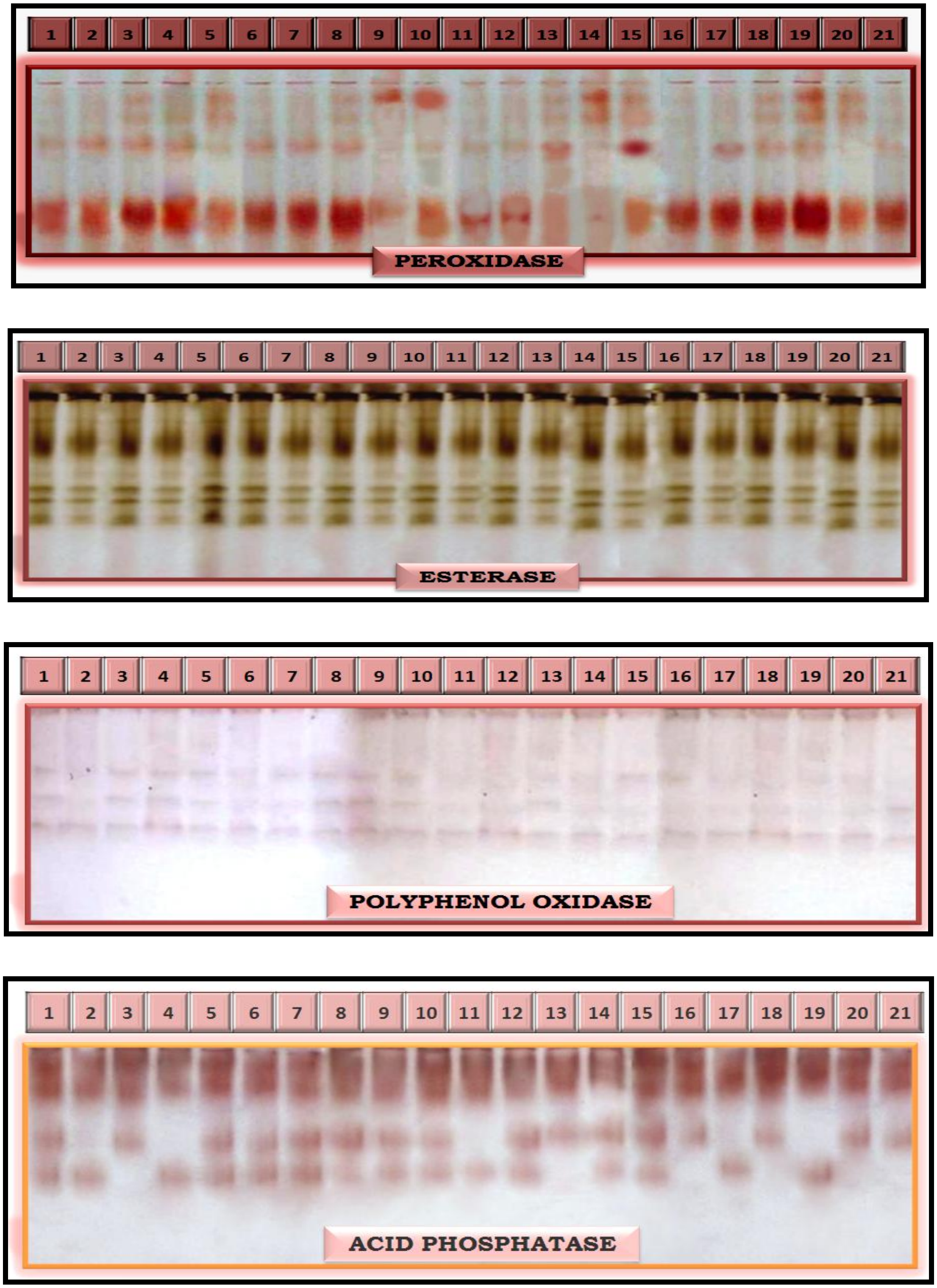

Fig. 3: Polyacrylamide gel electrophoresis of Peroxidase, Esterase, Polyphenol Oxidase and Acid Phosphatase isoenzyme at 12 days after germination.

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