VARIETAL IDENTIFICATION USING ISOZYME POLYMORPHISM FOR ESTERASE (EST) AND GLUTAMATE OXALOACETATE TRANSAMINASE (GOT) OF SOME LOCALLY AVAILABLE BIG ONION (*Allium cepa* L.) VARIETIES.

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ABSTRACT

Varietal identification using enzyme polymorphism for Esterase (EST) and Glutamate oxaloacetate transaminase (GOT) of some of the onion varieties available in Sri Lanka was studied. Between the two isozymes studied, EST was found to be the more useful isozyme for discrimination of most of the varieties with similar morphological traits. GOT is of little use in differentiating the onion varieties. Morphologically distinct Poona Red and Henry's special showed a similar banding pattern for EST. Similarly, Rampuram and Red Granex also showed an identical banding pattern for EST though they are morphologically distinct from each other. Therefore, EST isozyme polymorphism together with morphological characteristics can be used for the discrimination of different onion varieties used for this experiment.

KEY WORDS: Electrophoresis, Isozyme, Morphological, Allium cepa L., Polymorphism.

INTRODUCTION

Most of the onion varieties available in the Sri Lankan market, especially in Dambulla area, are imported from India by local traders. Although many onion varieties are available in the market, names of some of the varieties are not even listed in the variety catalogues issued by the producers or the institutions responsible for breeding and releasing of onion varieties. Reliable identification of big onion varieties based on morphological traits is difficult because of the existence of varieties with similar morphological traits. Recently, isozyme polymorphism has been successfully used in elucidating taxonomic grouping and identifying varieties in many crops. Some recent examples include banana (Liyanage *et al.*, 1995), Japanese bunching onion (Haishima *et al.*, 1993), Japanese persimmon (Tao and Sugiura, 1987), cassava (Hussain *et al.*, 1987) citrus (Hirai, 1986) and apple (Weeden and Lamb, 1985). Compared to the conventional methods, isozyme patterns have some advantages such as their availability to make qualitative distinction between phenotypes and their environmental stability. In addition, isozyme also provides markers for the genetic evaluation of Mendelian inheritance. Genetic polymorphism would also provide the basic information for breeding and germplasm evaluation. The objective of this paper was to study the isozyme diversity for Esterase (EST) and Glutamate oxaloacetate transaminase (GOT) using PAGE in big onion and to ascertain whether isozyme polymorphism for EST and GOT could be used together with morphological and chemical characteristics to distinguish between big onion varieties.

MATERIALS AND METHODS

The following experiments were conducted.

1. Field evaluation of onion varieties for identification of important morphological and chemical characteristics which can be used for varietal identification.
2. Laboratory evaluation for isozyme polymorphism.

Field evaluation was done at Regional Agricultural Research and Development Centre (RARDC), Aralaganwila in 1995 Yala season and the laboratory analysis was performed at the Plant Genetic Resources Centre (PGRC), Gannoruwa, Peradeniya.

Field evaluation for morphological and chemical chafacteristics

Seeds of the varieties, Poona Red, Pusa Red, Rampur, Rampuram, Nasik Red, Nasik Rose, Dark Red, and four hybrid varieties, Henry's special, Red granex, Rojo and Dessex were row sown in 3 m x 1 m nursery beds with the basal fertilizer mixture of 15 g urea, 30 g concentrated super phosphate and 15 g of muriate of potash per nursery bed. Four weeks after sowing, 15 g of urea was dissolved in water and applied to the nursery.

Field experiment was laid out in a Randomized Complete Blocks Design (RCBD) with three replications and the plot size was 1 m x 1 m. Forty three day old seedlings were transplanted in the experimental plots on 9th June 1995 with the plant and row spacing of 10 cm. Basal dressing was applied at the rate of 50 kg urea, 100 kg triple superphosphate (TSP) and 50 kg of muriate of potash (MOP) per hectare. Three weeks after transplanting (WAT), 50 kg of urea was applied six weeks after planting, 50 kg of urea with 25 kg of MOP per hectare were applied. Hand weeding was carried out at 2,4 and 6 WAT.

Bulb skin colour and foliage colour was recorded as morphological characters. The total soluble solids (TSS) content, a chemical character, was measured using a refractometer. TSS content was analyzed using "MSTAT" statistical software and the Duncan's Multiple Range Test was performed to separate the means.

Laboratory analysis

Eleven onion varieties were grown in the field and the variety N-53 were grown in the PGRC greenhouse during October 1995. Two grams of fresh leaf tissue of each variety was ground separately with 1 ml of cold extraction buffer in a chilled mortar. Sample extraction buffer with 7.5 pH was made with tris (1.2% W/V), glycerol (20% w/v), polyvinyl pyrrolidon (4% w/v), dithiothreitol (0.15% w/v), $MgCl_2$ (0.4% w/v) and distilled water.

The extract was centrifuged on 3500 rpm for 5 min. to obtain the soluble protein fraction of the leaf tissues. Each extract was loaded (15 µl and 25 µl) in to the wells of the prepared polyacrylamide gel. The stacking and separating gels consisted of 4.5% and 7.8% acrylamide respectively. For electrophoresis, 15 mA DC current was employed for 3 hours. The electrode buffer (pH 3.8) was prepared with Tris 0.3% w/v, glycine 1.45% w/v and distilled water.

Subsequently the gel was incubated for one hour in the staining solution of naphthyl acetate (2% w/v) and fast blue PR salt (0.1% w/v) in 0.1 m phosphate buffer (pH 7.2) for esterase enzyme. Gel was stained for GOT with D-L aspartic acid (0.2% w/v), Ketoglutaric acid

(0.125% w/v), 0.5 M tris-HCl buffer (pH 8.5, 40 ml), pyridoxal 5 phosphate (0.025% w/v) and fast blue BB salt (0.16% w/ v). Position of bands on gel as indicated by Rf values were calculated as follows.

$$Rf = \frac{\text{the origin isoenzyme band migrated}}{\text{distance from the sample application point to the end}}$$

RESULTS AND DISCUSSION

Isozyme polymorphism

Two regions labelled as EST-1 and EST-2 were identified on gels stained for esterase activity (Fig. 1). EST-2 region with slow mobility was stained as a single band zone of activity when sample was loaded with 25 µl/well (Fig. 2). This zone was resolved into 5 bands for varieties Nasik Red, Pusa Red, Poona Red, Henry's special and Dessex when sample was loaded at 15 µl/well (Figs. 1 and 3). Glutamate oxaloacetate estarate did not show any polymorphism (Fig. 4).

Among the varieties tested, Nasik Red, Nasik Rose, Rampur, Rampuram, Dark Red, N-53 and Pusa Red are morphologically very much similar. Considering the isozyme profile of EST-2 region, the tested varieties can be divided into two groups by the presence or absence of the band at Rf 0.16 (Fig. 1). Nasik Red, Poona Red, Pusa Red, Henry's special and Dessex belong to one group and the rest of varieties to the other group (Fig. 1). However, the band present at Rf 0.46 of EST-1 region in Nasik Red was not seen in Poona Red (Figs. 1 and 2).

Table 1. Some of the morphological and chemical characteristics of the onion varieties evaluated at RARDC, Aralaganwila in Yala 95.

Variety	Morphological		Chemical
	Bulb skin color	Foliage color	TSS %
Poona Red	DP	BG	10.6 AB
Pusa Red	P	G	10.8 AB
Rampur	P	G	9.9 AB
Rampuram	P	G	11.0 A
Nasik Red	P	G	10.3 AB
Nasik Rose	P	G	10.4 AB
Dark Red	P	G	9.5 B
Henry's special	CW	G	5.8 C
Red Granex	DP	G	6.9 C
Rojo	DP	BG	6.8 C
Dessex	CW	G	6.3 C

Means followed by the same letter are not statistically diferent at 0.01. TSS - Total soluble solids
DP - Dark purple P - Pink BG - Bluish green CW - Creamy white G - Green

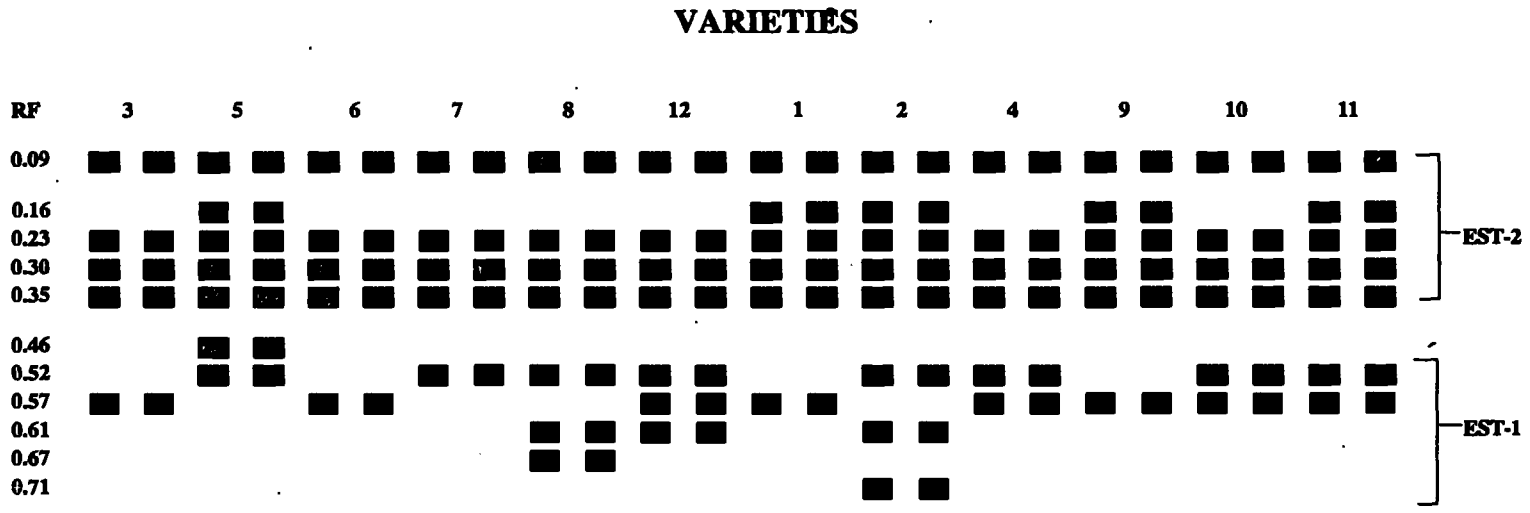

Fig. 1. Interpretive drawing of the EST banding patterns observed in Onion cultivars.

Varieties - 1. Rampur 2. Nasik Red 3. Nasik Rose 4. Dark Red 5. N-53 6. Rojo 7. Poona Red 8. Pusa Red 9. Rampuram 10. Henry's Special 11. Red Granex 12. Dessex
(2 lanes/ cultivar)

Therefore, these two varieties can be differentiated using the band at Rf 0.46. However, the variety Poona Red cannot be distinguished from the variety Henry's special only by using the esterase enzyme profile (Figs. 1 and 2). Morphological traits such as bulb colour and the leaf colour can be used to differentiate these two varieties. In addition to the above mentioned morphological characters, TSS content of variety Poona Red is significantly higher than that of Hybrid Henry's Special (Table 1). The absence of band which was present in variety Dessex at Rf 0.52 in Henry's special is an indication for the differences between these two varieties (Figs. 1 and 2). Dessex and Poona Red differ due to the presence of band which was absent in Poona Red at Rf 0.52 in Dessex. Similarly Dessex differs from Nasik Red due to the presence of band at Rf 0.46 only in Nasik Red. Variety Nasik Red differs from Nasik Rose and Rampur because of the presence of band at Rf 0.16 in Nasik Red. Rampur and Nasik Rose differ due to the absence of band at Rf 0.52 in Rampur. Band at Rf 0.57 seen in Nasik Rose was not seen in Dark Red and therefore these two varieties are different from each other. Two fast migrating bands at Rf 0.61 and 0.67 present in variety N-53 were absent in Dark Red and therefore, these two varieties are genetically different from each other. Since the variety Rojo does not possess the band at Rf 0.64 it is different from N-53. Among all the varieties, fastest migrating band at Rf 0.71 was found only in the variety Pusa Red (Figs. 1 and 2). Pusa Red differs from Rampur because band present in Rampur at Rf 0.57 was not present in Pusa Red. However, Rampuram and Red Granex cannot be differentiated using isozyme polymorphism because both varieties showed a similar banding pattern. However, these two varieties varied in bulb colour (Table 1). TSS content also was significantly higher in variety Rampur when compared with the variety Red Granex (Table 1). Therefore this chemical property will also be useful in differentiating these two varieties.

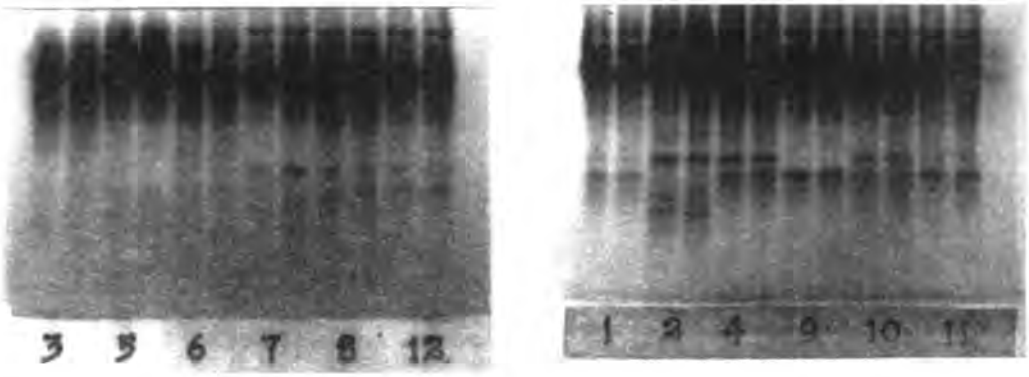

Fig. 2. EST banding patterns for onion varieties when extracts were loaded with 25 µl per well (2 lanes per variety)

Varieties: 1. Rampur 2. Nasik Red 3. Nasik Rose 4. Dark Red 5. N-53 6. Rojo 7. Poona Red 8. Pusa Red 9. Rampuram 10. Henry's special 11. Red Granex 12.Dessex

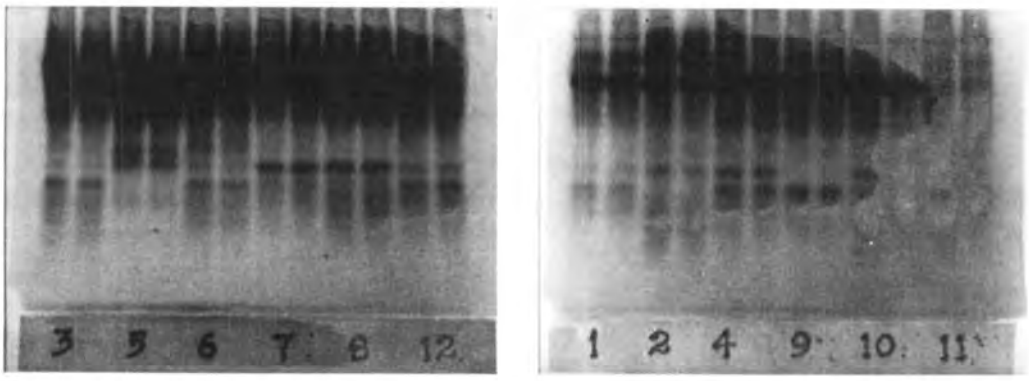

Fig. 3. EST banding patterns for onion varieties when extracts were loaded with 15 µl per well (2 lanes per variety)

Varieties: 1. Poona Red 2. Pusa Red 3. Rampur 4. Rampuram
5. Nasik Red 6. Nasik Rose 7. Dark Red 8. N-53
9.Henry's special 10. Red Granex 11. Dessex

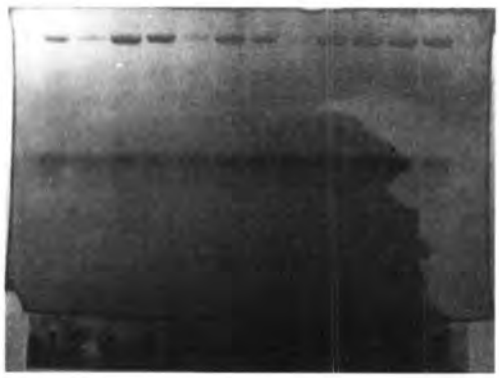

Fig. 4. GOT banding patterns for onion varieties when extracts were loaded with 25 µl per well (2 lanes per variety)

Varieties: 1. Poona Red 2. Pusa Red 3. Rampur 4. Rampuram
5. Nasik Red 6. Nasik Rose 7. Dark Red 8. N-53
9. Henry's special 1 10. Red Granex 11. Dessex

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