AMARANTH SPECIES IDENTIFICATION USING ISOZYME POLYMORPHISM FOR ESTERASE (EST)

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ABSTRACT

Isozyme polymorphism for Esterase (EST) enzyme system was studied during *yala* 1998 using 3-4 week old amaranth seedlings and species identification was carried out by investigating the female flower morphology of the same accessions. Results revealed that the four species, *Amaranthus cruentus, Amaranthus tricolor, Amaranthus dubius, and Amaranthus spinosus* have species specific banding patterns. Therefore, isozyme polymorphism for Esterase could be successfully used to identify the above mentioned four species of *Amaranthus* conserved at Plant Genetic Resources Centre using 3-4 week old seedlings.

KEY WORDS: *Amaranthus*, Diversity, Electrophoresis, Esterase, Isozyme, Polymorphism

INTRODUCTION

More than eighty accessions of different amaranth species are conserved at Plant Genetic Resources Centre (PGRC), Peradeniya. However, most of the species of the conserved germplasm are either not identified or in certain instances have been incorrectly identified. During the germplasm collection in the field, thorough investigation of flower morphology is not practical and therefore, species identification is done only by observing the other morphological characters. Some times this leads to incorrect identification of species.

Identification of amaranth species is a tedious process since one has to observe the tiny female flower parts under the microscope to separate into a species. Even under the microscope, differentiation among some amaranth species is difficult because of the close similarity in female flower morphology. Furthermore, identification has to be delayed until the flowering stage is attained to observe the flowers. For certain accessions this period is even more than two months, which is time consuming. Therefore, if we can develop an alternative technique, we can overcome the two major difficulties associated with the traditional method of species identification of amaranths.

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; 1998) and Japanese bunching onion (Haishima *et al.*, 1993). Compared to the conventional method based on flower structure and morphology, isozyme identification has the advantage of making qualitative distinction between phenotype and its environmental stability.

The objectives of this paper are (i) to study the isozyme diversity for Esterase (EST) using polyacrylamide gel electrophoresis (PAGE) and (ii) to ascertain whether isozyme polymorphism for EST could be used for amaranth species identification.

MATERIALS AND METHODS

The complete experiment consisted of two parts: The greenhouse evaluation of amaranth accessions and stereo microscopic investigation of flower morphology for species identification, and laboratory evaluation of amaranth accessions for isozyme polymorphism for Esterase using PAGE.

Seventeen previously identified amaranth accessions belonging to five species (*A. tricolor, A. caudatus, A. spinosus, A. dubius, and A. cruentus*) were used for the study. Fourteen accessions were row sown in the PGRC greenhouse on 19th May 1998 with a spacing of 30 cm between row and 15 cm within row. The length of a row was 2.25 m . Basal fertilizer was applied at the rate of 90 kg urea, 135 kg triple superphosphate and 100 kg of muriate of potash per hectare. The accessions 001522, 001523, 001525, 002376, 007724, and 007728 had very poor germination. Therefore, seeds of those accessions were obtained again from the genebank and sown again on 2nd June 1998 along with three additional accessions 002360, 002375 and 007723. One month after the basal fertilizer application, 45 kg of urea per hectare was applied as top dressing. Female flower morphology was studied for all accessions. Species identification was done based on the female flower morphology according to a provisional key to some edible species of the family amaranthaceae (Grubben and van Sloten, 1981).

Isozyme analysis

Two grams of fresh seedling leaves from each accessions were ground separately with 1 ml of cold extraction buffer in a chilled mortar. Sample extraction buffer with a pH of 7.5 was made with tris- hydroxymethyl amino methane (1.2% w/v), glycerol (20% w/v), polyvinyl pyrrolidon (4% w/v), dithiothretol (0.15% w/v), $MgCl_2$ (0.4% w/v) and distilled water.

The extract was centrifuged at 3500 rpm for 5 min. with a small amount of sepadex G 50 (0.2g) to obtain the soluble protein fraction of the leaf tissues. Each extract was loaded (25 µl) into the well of prepared polyacrylamide gel. The stacking and separating gel consisted of 4.5% and 7.8% acrylamide, respectively. Electrophoresis was carried out using a constant electric current (20 mA DC) for 3 hours until the tracking dye (bromophenol blue) reached the end of gel. The electrode buffer (pH 3.8) contained 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 nepthyl acetate (2% w/v) and fast blue RR salt (0.1% w/v) in 0.1m phosphate buffer (pH 7.2) for esterase enzyme. The stained gels were washed with tap water and fixed in 7% (v/v) acetic acid solution and subjected to photography. Position of bands on gel as indicated by Rf values was calculated as follows and the zymogram was constructed using the average Rf value for two runs.

$$Rf = \frac{\text{Distance the origin isozyme band migrated}}{\text{Distance from the sample application points to the end}}$$

RESULTS AND DISCUSSION

Five species used for the study (based on *yala* 1997 unpublished characterization data) and respective accession numbers along with the collection sites and passport information regarding their species names are indicated in Table 1.

Table 1. Amaranth accessions used for isozyme diversity analysis in *yala* 1998, and their collection sites and species identification data

Accession number	*Collection site*	*Species names (Passport information)*	*Species names (yala 97 identification)*
000445	Kurunegala	*A. spinosus*	*A. caudatus*
002296	Nuwara Eliya	Unidentified	*A. caudatus*
007723	Unknown	*Amaranthus*	*A. caudatus*
001522	Kalutara	*A. dubius*	*A. dubius*
001523	Kalutara	*A. dubius*	*A. dubius*
001525	Kalutara	*A. tricolor*	*A. dubius*
002360	Kalutara	*A. hybidus*	*A. dubius*
002852	Kandy	*A. lividus*	*A. spinosus*
002959	Moneragala	*Amaranthus*	*A. spinosus*
002375	Colombo	Unidentified	*A. spinosus*
005056	Anuradhapura	*A. hybidus*	*A. tricolor*
007722	Unknown	*Amaranthus*	*A. tricolor*
007724	Unknown	*Amaranthus*	*A. tricolor*
007725	Unknown	*Amaranthus*	*A. tricolor*
002376	Colombo	Unidentified	*A. cruentus*
007727	Unknown	*Amaranthus*	*A. cruentus*
007728	Unknown	*Amaranthus*	*A. cruentus*

All accessions except 001523, 002376 and 007727 had a good plant stand. However, the germination percentage of accession 002376 was extremely poor and only one plant was available for the entire row. The accession 001523 and 007727 had 3 and 4 plants, respectively. For the accessions 001523, 002376 and 007727, characterization and species identification was based on 3, 1 and 4 plants, respectively. For all other accessions, prescribed number of plants as stated in the PGRC descriptors was used for characterization. Some of the morphological and agronomical traits of the evaluated accessions are given in Table 2. Among the amaranth species, *Amaranthus spinosus* is the only species which possesses spines in the nodes (Table 2). This character helped to identyfy the mature plants of this species without observing the flower morphology.

Based on yala 1997 characterization data, 3, 3, 3, 4 and 4 accessions of *A. caudatus, A. cruentus, A. spinosus, A. tricolor and A. dubius,* respectively were used for the investigation. Thorough investigation made on female flower morphology revealed that only four species were present among the accessions. This includes 2, 3, 10 and 2 accessions from *A. cruentus, A. spinosus, A. tricolor and A. dubius,* respectively. The identified species and their respective accession numbers are given in Table 3.

The typical characteristics of the female flowers of the identified four species are given in Plate 1.

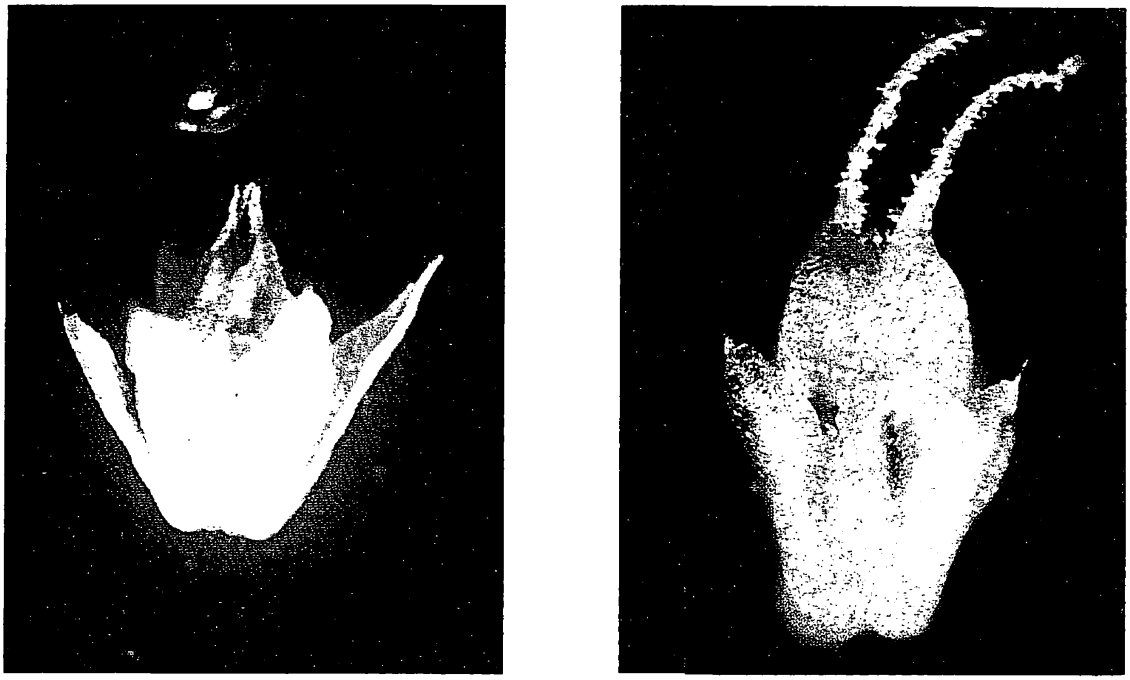
Table 2. Some morphological and agronomical characters of the amaranth accessions

Accession grain number	*Leaf length (cm)*	*Leaf width (cm)*	*Days to 50% flowering*	*Plant height at flowering (cm)*	*Spines in the nodes*	*1000 weight (g)*
000445	19.4	13.3	91	113	absent	0.9
001522	11.9	8.7	33	133	absent	0.3
001523	22.8	14.9	41	156	absent	0.7
001525	22.9	14.1	62	189	absent	0.7
002296	16.4	13.9	96	151	absent	0.8
002360	15.8	8.1	41	80	absent	0.5
002375	8.6	4.6	41	100	present	0.2
002376	18.8	11.6	76	186	absent	0.8
002852	12.1	6.4	48	99	present	0.2
002959	12.7	6.9	48	84	present	0.2
005056	18.4	10.8	96	180	absent	0.8
007722	18.6	12.1	52	162	absent	0.9
007723	15.6	10.4	41	110	absent	0.3
007724	18.6	11.9	73	164	absent	0.8
007725	19.7	14.6	52	130	absent	0.8
007727	19.0	16.6	50	161	absent	0.8
007728	16.4	13.9	41	80	absent	0.9

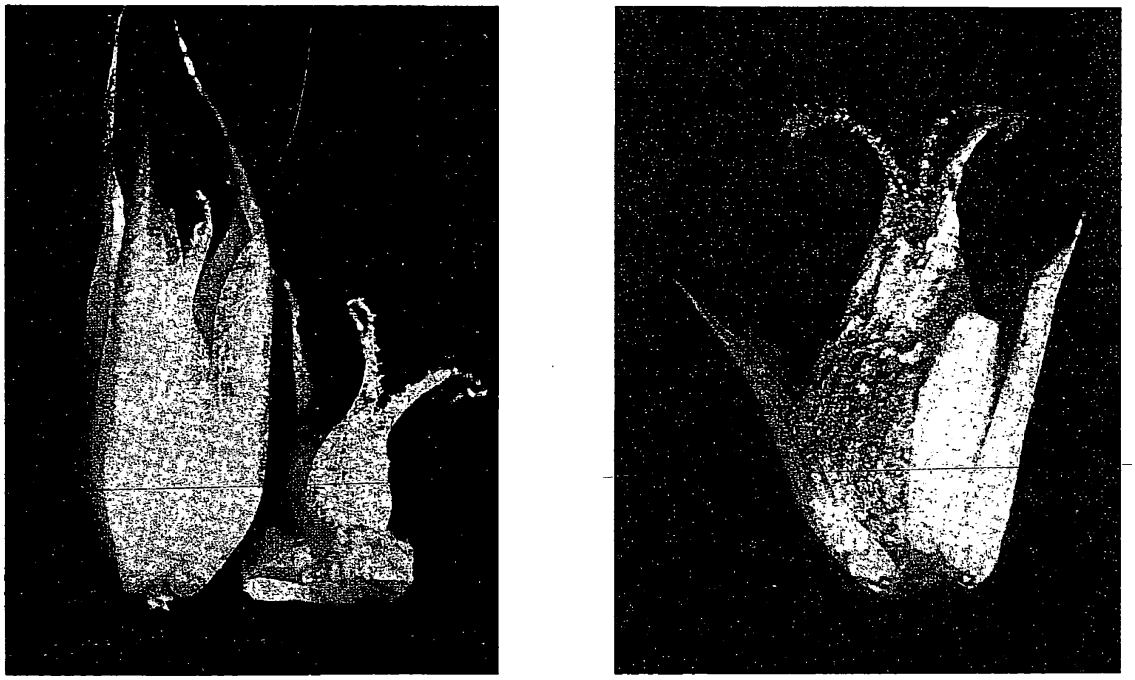
Table 3. Grouping of amaranth accessions into different species based on morphology of female flowers

Species	*Species accession*
A. cruentus	002360, 007728
A. spinosus	002852, 002959, 002375
A. tricolor	000445, 001525, 001523 , 002296, 002376 005056, 007722, 007724, 007725, 007727
A. dubius	001522, 007723

Among the polymorphic banding patterns for Esterase, only four groups of banding patterns were recognised for the eighteen tested accessions (Plate 2 and Fig.1) and the calculated Rf values for the respective species are indicated in Fig.1. However, as indicated in lanes 9 and 10 of the Plate 2 b, accession 002375 (*A. spinosus*) gave a banding pattern which is identical to the *A. dubius*. Closer observation on seedling nurseries of accession 002375 revealed that *A. dubius* seedlings were also present among the *A. spinosus* seedlings. Therefore, after roughing the *A. dubius* seedling out, the same accession was subjected to isozyme analysis again and the banding pattern for this run appeared in lanes 11 and 12 of the Plate 2 c. which is typical of *A. spinosus*. Therefore, the four groups of banding patterns resembled with the four species identified using the female flower morphology. These results clearly indicate that the four Amaranth species, *A. cruentus, A. tricolor, A. dubius, and A. spinosus* have unique species specific banding patterns as indicated in Fig.1 and Plate 2. Therefore, isozyme polymorphism for Esterase could be successfully used to identify the Amaranth species *cruentus, tricolor, dubius* and *spinosus* conserved at PGRC. using 3-4 weeks old seedlings.



a b



c d

Plate 1. Typical flower characteristics of four amaranth species *a. A. cruentus* (x 90) adult female flower and a seed; b. *A. spinosus* (x 250) adult female flower; c. *A. tricolor* (x 200) adult female flower with dehiscent cap; d. *A. dubius* (x 150) adult female flower

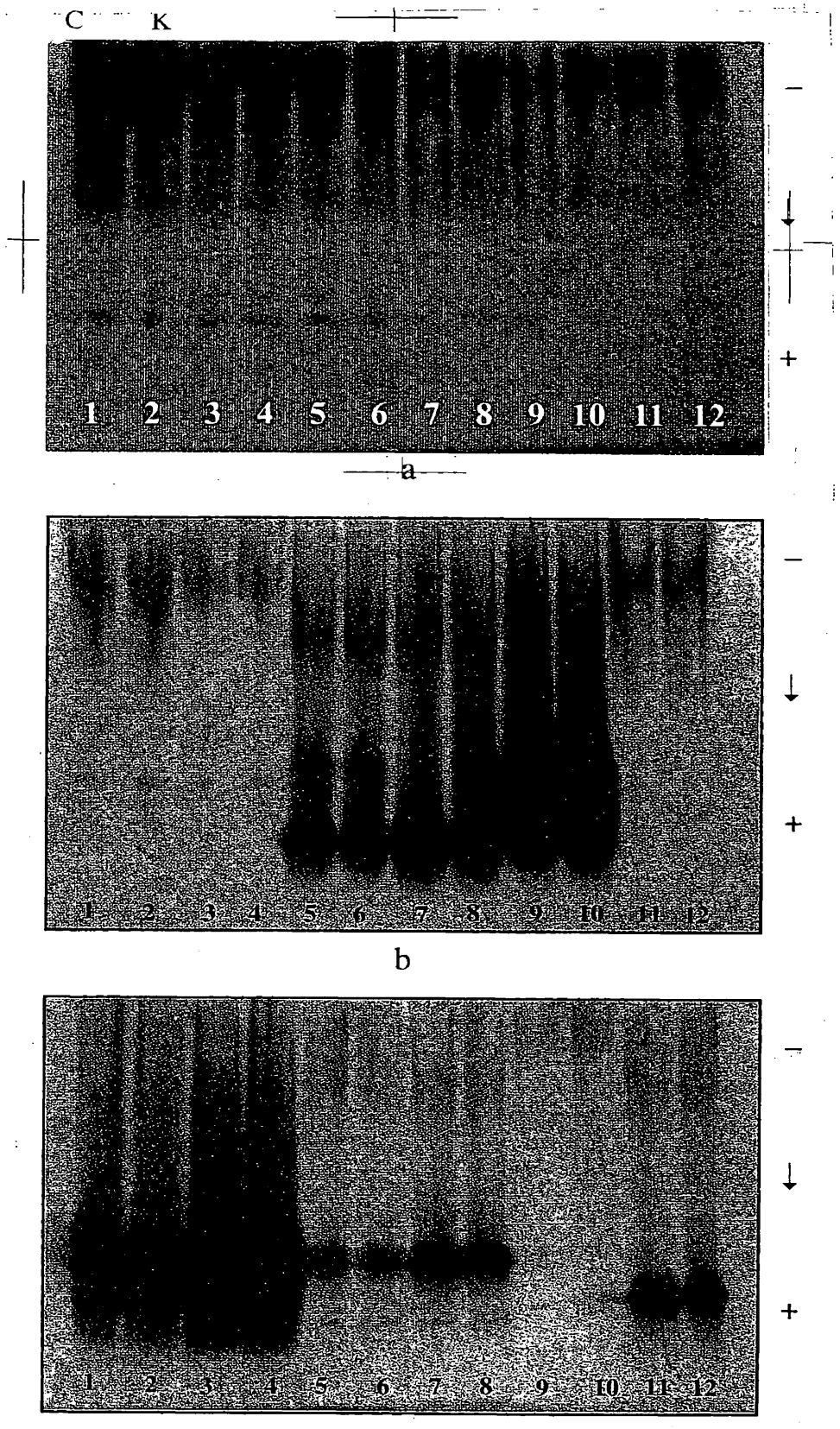


Plate 2. **Examples of resolution achieved for enzyme variants of esterase (2 lanes/accession) a. accessions 005056, 007722, 007724, 007725, 002296, 001525; b. accessions 002376, 000445, 002852, 002959, 002375, 007727; c. accessions 007723, 001522, 007728, 002360, 001523, 002375;**

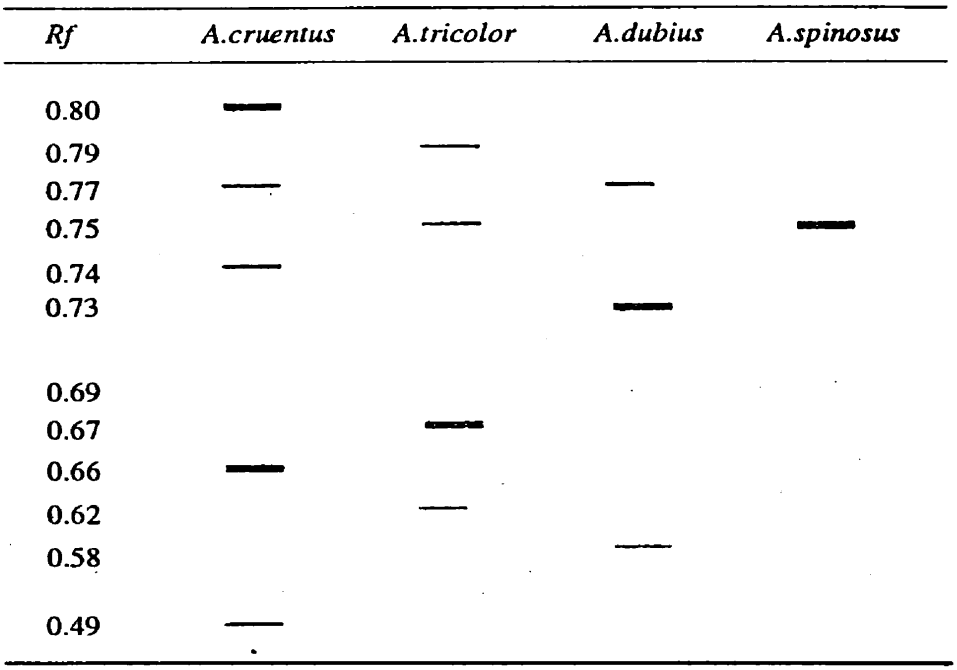


Fig. 1. Schematic representation of the EST banding patterns observed for four amaranth species

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