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ISOENZYMATIC CHARACTERIZATION OF *ERYNGIUM ELEGANS* CHAM. ET SCHLECHT POPULATIONS OF TUCUMÁN PROVINCE, ARGENTINA

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Abstract

The genus *Eryngium* has about 230 species distributed in temperate and subtropical zones of Europe and America. In South America the genus is presented in Bolivia, Paraguay, Uruguay, Brazil and Argentina. In the northwest of Argentina and specially in Tucumán province, *Eryngium elegans* Cham. et Schlecht is one of the aggressive weed that affects pasture areas as well as economically important crops like sugarcane.

E. elegans is a diploid species with regular meiosis and fertile seeds so that it can easily colonize different ecological areas. The objective of this work was to identify the different populations through the use of isoenzyme patterns of esterases and peroxidases and the application of cluster analysis.

Discontinuous vertical polyacrylamide gel electrophoresis was utilized and Jaccard' Similarity Coefficients was used for making the dendrogram. Profiles of peroxidases, showed a characteristic and distinct pattern in all samples, which explains in part, the phenotypic variation found in the population. This was confirmed by the low value of similarity index showed by the dendrogram. In esterases, a common wide band, of different colour intensity was observed according to the locality. Through the difference in the pattern of peroxidases it was thus possible to determine intraspecific variations in the populations which can be used for characterization of samples.

Introduction

The genus *Eryngium* of the family *Apiaceae* (*Umbelliferae*) has about 230 species distributed in temperate and subtropical zones of Europe and America. In South America it is presented in Bolivia, Paraguay, Uruguay, Brazil and Argentina. *Eryngium elegans* Cham. et Schlecht is an aggressive weed which is little used by the cattle and thus easily established as also reported for *E. paniculatum* (Elizalde *et al.*, 1996; Rochi, & Lallana, 1996). In the northwest of Argentina and specially in Tucumán province, it presents ruderal characteristics with a great invading power. It is found in different soil kinds and ecological conditions, affecting economically important crops like sugarcane and also pasture areas. It is considered as an indicator weed of bad soil management and wrong use of mechanical and chemical cultures.

E. elegans represents characteristic phenotypical variation in natural populations of different provinces which may either be due to a genetic variability or due to varied environmental factors prevailing in different ecological zones. Its dispersion potential and its genetic and cytogenetic characteristics are hardly known. From a cytogenetic point of view a genus basic number of x=8 is given by Darlington & Wyle (1955). Perdigo I Ariso (1981) reported that *Erygium* genus has diploid and tetraploid species (2n=14; 2n=28 and 2n=32). On the other hand, Constance (1982) affirmed that in this genus there are species with n=5 and even n=8, with the possibility of interspecific hybridization. Nasif *et al.*,

1999; reported that *E. elegans* is a diploid species (2n=16), with regular meiosis and a great fertile seed production so that it can colonize different ecological areas.

Isoenzymes, direct products of gene expression are commonly used in taxonomic and evolutionary studies measuring genetic variability within plant populations (Brown & Wies, 1983; Martins *et al.*, 1999), and characterizing different varieties (Farooq *et al.*, 1999; Farooq & Syyed, 1999ab). The cluster analysis, is a statistical tool through which it is possible to interpret band patterns and to estimate variability among populations. The objective of the present study was to estimate and identify possible genetic variability among different populations through variation described in the isoenzyme patterns of esterases and peroxidases and through the use of cluster analysis .

Materials and Methods

The material of *E. elegans* collected from the following Tucumán localities and placed in their respective Departments: El Manantial (Lules), San Javier (Tafí Viejo), Las Talas (Leales), García Fernández (Leales), Campo de Herrera (Famaillá) and Bella Vista (Leales) were used.

Extraction of isozyme: First, second and third rosette central leaf were collected and 200 mg of leaves were macerated in 1 ml of a 20% saccharose solution. The mixture was centrifuged (4000 rpm for 15 min) and the supernatant was preserved for two months at –15°C.

Electrophoresis: The extract was electrophoresed using the discontinuous vertical polyacrylamide gel according to Ornstein & David (1964). The buffer system and the composition of gels used for electrophoresis were different as given in Table 1. The thick pore gel or stacking gel was used for concentration and a thin pore gel or resolving gel was used for separation of isozymes stacks into bands (Table 1). The thin pore gel was polymerized at room temperature (20-25°C) and the thick pore gel in presence of fluorescent light. 40 µl of the sample with a drop of bromophenol blue was used as tracking dye to indicate the migration level.

Table 1. Composition of thick and thin pore gel, buffers, voltage and amperage used in the study.

Gel	Gel buffer	Electrode buffer	Voltage and amperage
Thick pore gel			
2,5% Acrylamide	0,75%		
0,625% Bisacrylamide	Tris -HCl		30 mA
5 x 10^{-4} Riboflavin	0,0617 M		(constant)
20% Saccharose	PH 6,7		(~ 180V)
		Tris Glycine	30 min.
Thin pore gel		pH 8,3 10x	
9% Acrylamide	4,54%		
0,157% Bisacrylamide	Tris -HCl		40 mA
0,30% Ammonium per	0,375 M		(constant)
sulfate 0,30%	pH 8,9		(~ 200V)
TEMED			3-3,5 hs.

Staining Methods

Peroxidases: The gel was incubated in 100 mg. of o-dianisidine, 70 ml. of 75° alcohol, 28 ml. of acetate buffer (0,2 M and pH=5) and 2 ml. of 3% H_2O_2. It was stained for 25 to 60 min, until reddish – brown band appeared. The gels were washed with 70% alcohol and preserved in 10% glycerol mixed with 7% acetic acid.

Esterases: The gel was pre incubated in 0.5 M boric acid for 1 h. in fridge to reduce the pH of the gel to 6. Then it was incubated for 30–60 min at 37°C in a 0.2 M phosphate buffer solution (100 mg. of Fast Blue RR Salt and 2 ml of 1% alpha and beta-naphthyl acetate in 70% ethanol). Gels were washed with distilled water and preserved in mixture methanol: acetic acid: water; 6:1:4 (Brewbaker, 1968). The products formed with alpha-naphthyl acetate gave a blue phenotype and with beta-naphthyl acetate a red phenotype (Saidman & Naranjo, 1982).

The alpha and beta esterases and peroxidases banding patterns were outlined in their respective zymograms using the average distances of each taxonomic unit, measured from the migration front and relatives to a predetermined distance of the gel (10 cm.). The comparison among the populations was made on the basis of the level variations and band intensity called as band 1 to the most fast in the anodal migration.

Cluster analysis: The analysis of the relationships among the different origins was based on numerical techniques. For this, the taxonomic units were formed (constituted by the different origins) and similarity among them was estimated on the basis of their characters or variables (Burghardt, 1996) which in the present study were the bands considering their presence or absence. With these results a data base matrix was made with a binary code to indicate presence (1) or absence (0) of bands for each population as per procedure of Albany *et al.*, (1997). Jaccard' Similarity Coefficients (Sj) were calculated and the resultant matrix was used to make the taxonomic structure: dendrogram according to Crisci & Lopez Amengol (1983). NTSYS pc.2 Computer Program was used for the analysis.

Results

Peroxidases: Populations showed a characteristic and distinct isoenzyme phenotype in all samples (Fig. 1, Fig. 2). Of the 8 bands that were counted: band 1 was presented in San Javier and Bella Vista samples, band 2 in Manantial and San Javier, band 3 in Las Talas and García Fernández, band 4 in Campo de Herrera, band 5 in Manantial, band 6 and 7 in Las Talas and band 8 in Bella Vista. The difference between García Fernández and Campo de Herrera is given by more instense band and it is more wide than the distance.

Dendrogram corresponding to peroxidases showed a little similarity among the populations (Fig. 3). Three groups were delimited: group 1 belonging to Campo de Herrera population, which appears as a unique identity; group 2 formed by García Fernández and Las Talas samples with a value of 0.33%, i.e., 33% of similarity; in group 3 Bella Vista origin joins to Manantial and San Javier at 0.17 i.e., 17% of similarity whereas the latest ones were associated at 33%.

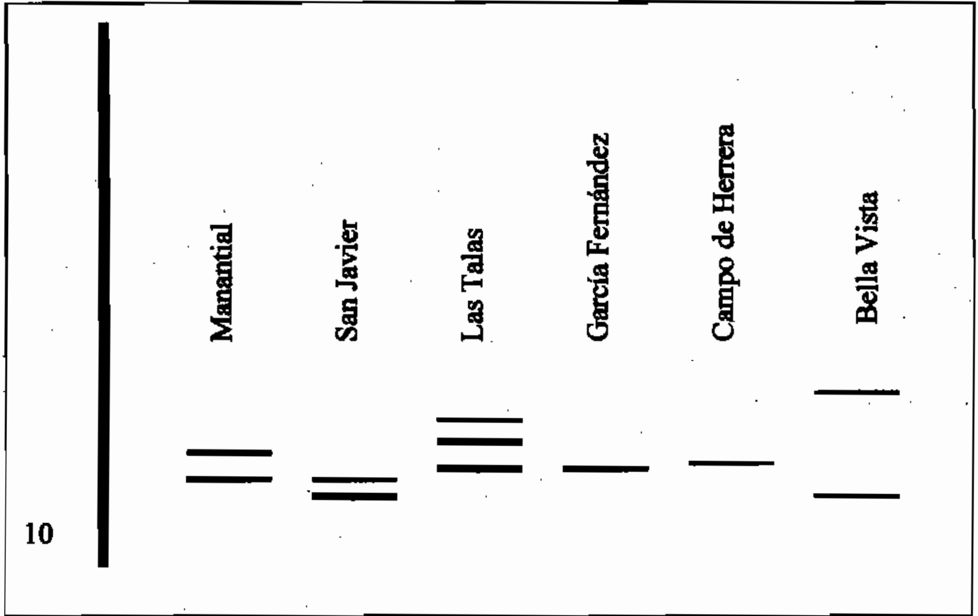

Fig. 1. Zymogram corresponding to peroxidase isoenzymes in *Eryngium elegans* Cham. et Schlecht.

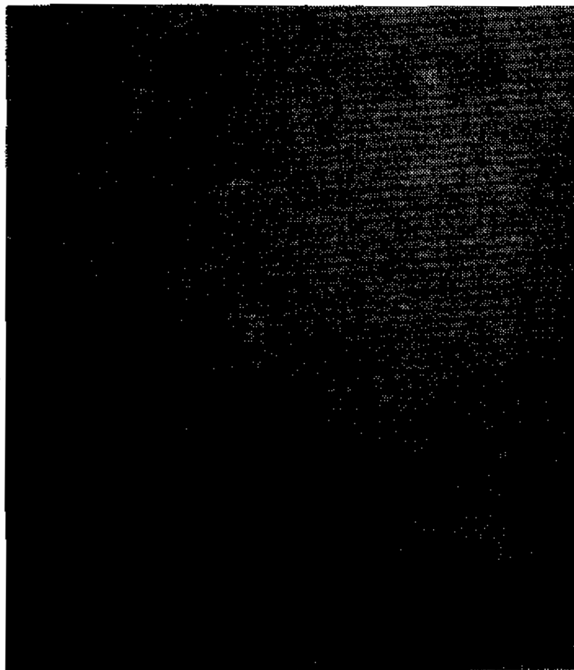

Fig. 2. Profiles of peroxidases in *E. elegans* Cham. et Schlecht.

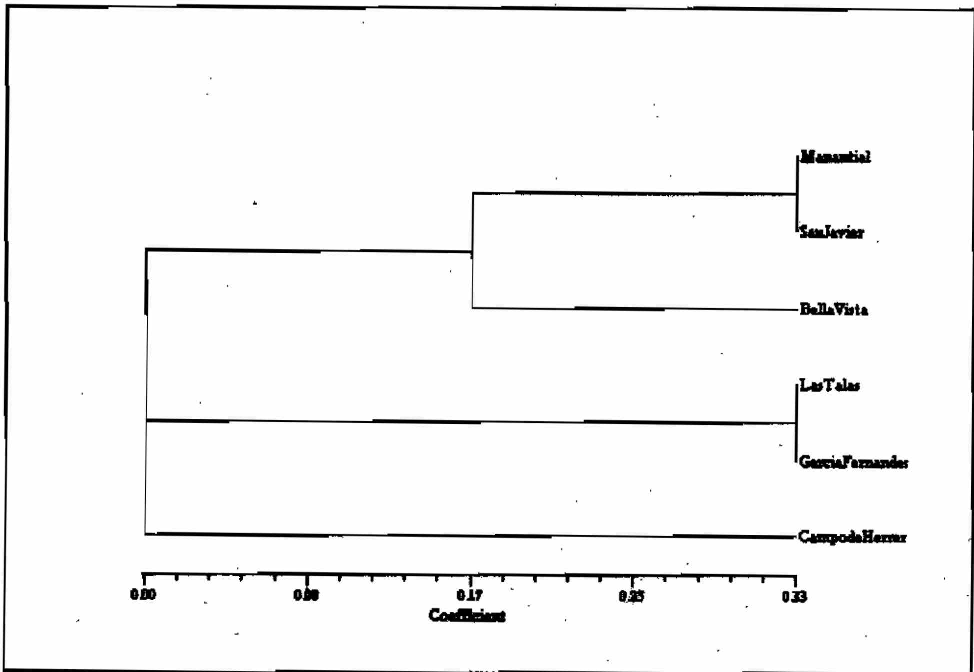

Fig. 3. Dendrogram corresponding to isoenzymes peroxidase in *Eryngium elegans* Cham. et Schlecht.

Esterases: A common wide band was observed with different intensity according to the origin (Fig. 4, Fig. 5). A thinner band was also detected in samples from Las Talas, Campo de Herrera and Bella Vista.

Discussion

The results showed that intraspecific variations does exist in the samples which can be detected by the isozyme of the enzyme peroxidases. Therefore the samples can be characterized by their banding patterns. On the other hand, the low similarity value (maximum of 33%) indicated by the dendrogram may in part be due to the phenotypic variation found in the populations.

Considering the results by Nasif *et al.*, (1999), the successful colonization of *E. elegans* is due to its diploid nature and fertile seed production. Nevertheless the differences in *E. elegans* can be detected through differences in isozyme patterns. Isozyme variation can thus be used as a useful tool to estimate the variability in this species.

In the future, it would be advantageous to put the species belonging to different origins into a common origin and then study the banding pattern in order to detect the genetic differences only by eliminating the environmental effects.

Fig. 4. Profiles of the esterases in *Eryngium elegans* Cham. et Schlecht.

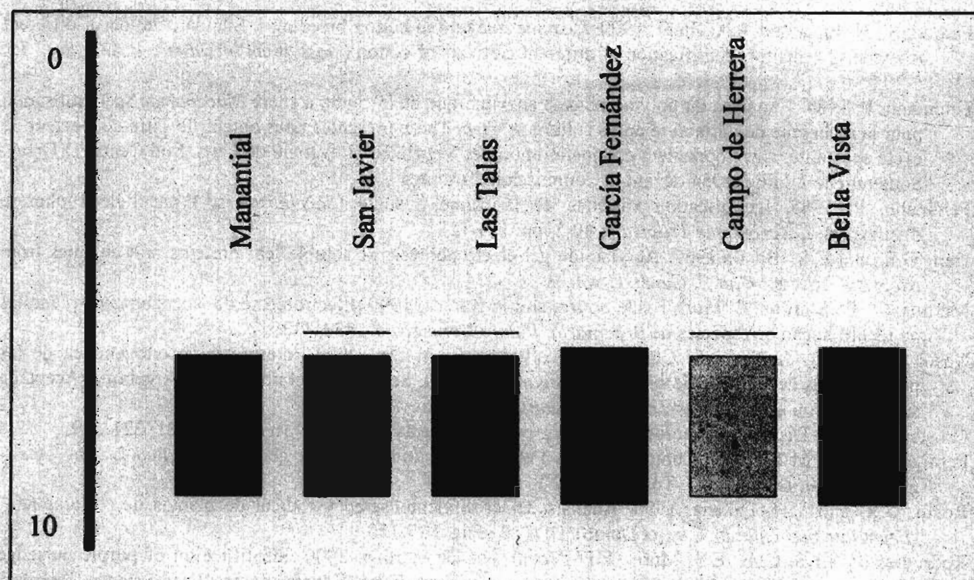

Fig. 5. Zymogram of the isoenzymes esterases in *Eryngium elegans* Cham. et Schlecht.

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