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POLYMORPHISM AND GENIC EXPRESSION OF ESTERASE AND ACID-PHOSPHATASE ISOZYMES AT DIFFERENT DEVELOPMENTAL STAGES IN *LENS CULINARIS* MEDIKUS

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Esterase and acid-phosphatase isozyme patterns were studied at different developmental stages (0hr, 24 hrs, 48hrs, 96hrs, 120hrs, 144hrs) in four cultivars of *Lens culinaris* Medik). The results of this study show that the total enzyme repertory of an organism changes during development and differentiation which can be detected by the expression of their isozymes at the various developmental stages which are under genetic regulatory control mechanisms. In accordance with this a characteristic isozyme pattern was found in the different varieties of *Lens culinaris* at different developmental stages. Plant populations can also be differentiated genetically as was studied by observing the degree of polymorphism with respect to these two isozymes.

Key words : *Lens culinaris*, isozyme, PAGE, esterases, acid-phosphatases.

Lentils, *Lens culinaris* assume considerable importance due to their high protein content (24-25%) and as a means of improving soil fertility. The use of isozymes as genetic markers has increased dramatically over the last decade as it has a number of important advantages over more conventional morphological markers (Scandalios, 1975).

In order to gain evidence on the existence of polymorphic variation and assessing genetic variation in terms of isozymes, we initiated this investigation on four varieties of *L. culinaris* hypothesizing that genetic variation at molecular level would exist. The study was based on the changes in banding patterns of esterase and acid phosphatase isozymes at different stages of development of seed and seedling growth in four cultivars of *L. culinaris*. Comparisons of

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these isozyme patterns were made between and within the cultivars at different developmental stages in order to observe differential gene expression at different developmental stages as also to determine the extent of genetic variation expressed as polymorphic forms of the above isozymes existing at inter-varietal level.

MATERIALS AND METHODS

Experimental system :

The experiment comprised of the selected four varieties of *Lens culinaris* (Table 1) obtained from the Division of Genetics, Indian Agricultural Research Institute, New Delhi.

Table 1. Variety, parentage, type and their unique characteristics

Variety	Parentage	Type	Unique characteristics
L-4163	L-293 XL-830	Macrosperma	
LC-74-5	L-293XL-830	Macrosperma	
Precoz	A selection from ILL-4605 (Argentinian origin)	Macrosperma	Early maturing
L-4076	Pant L-234X Pant L-639	Microsperma	One of the oldest cultivar released

The anionic system of Davis (1964) was adopted for separation of isoenzymes by disc electrophoresis using polyacrylamide gels (PAGE).

Experimental method :

Preparation of protein extract :

The seeds of different varieties were kept for germination in wet sheets of filter paper after thorough washing of the seeds with 0.1% mercuric chloride. 0.5 gm of seeds were weighed and crushed in a pre-chilled pestle and mortar into a fine paste by adding 3.5 ml extracting buffer (0.2 M Tris-HCl). The ground paste was contrifuged for an hour in a refrigerated centrifuge at 12000 rpm using 10 × 15 ml 300 angle rotor head of IEC-25 high speed refrigerated centrifuge. The supernatant (extract) was drawn for each stage of seed and seedling growth. The protein extract thus drawn was stored in glass vials in the deep freeze and subsequently used for isozyme analysis for esterases and acid-phosphatases.

Electrophoresis and enzyme localization

The stacking and separating gels respectively were prepared according to Davis (1964). The gels were stained for esterases and acid phosphatases. Each isozyme band was characterized by its Rf value.

RESULTS AND DISCUSSION

The morphological variation which is a product of genotype and the environment is no doubt an important parameter but much diversity which remains unexpressed morphologically can be revealed by biochemical methods. Study of isozymic variation is one such important and powerful procedure which has often been employed for this purpose. Isozymic variation has been chosen here to reveal the diversity existing at molecular level in the genepools of *Lens culinaris*.

Isozyme patterns of esterases :

Esterase isozyme pattern obtained on polyacrylamide gels different developmental stages of seeds of four cultivars of *Lens culinaris* Medikus have been presented in the form of zymograms, (Fig. 1A).

It is evident that each variety has its own pattern of increase and decrease in number of bands at different stages of seed development. But in general, it was observed that there was an increase in the number of bands from 24 hours onwards till 72 hours of seed germination and seedling growth after which there was a slight decline in the number accompanied at times by appearance of some new band at a new locus. The only exception to the above mentioned observations was the LC-74-5 variety.

The appearance of isozymes at different stages of development in all varieties could be restricted to three zones, a fast moving zone in which isozymes reach the anodal end, a slow moving zone in which isozymes occupy the cathodal end and an intermediate zone occupying the central region of the gel.

Each zone is occupied by a particular isozyme in the form of a band and is representative of the expression of a particular gene locus coding for that isozyme. In certain stages, in a particular zone more than one distinct band is resolved. These bands could represent allelic isozymes ("allozymes", Prakash et al., 1969), coded by different alleles of the same gene at that locus and thus occupy that particular zone on the gel.

The esterases are a complex and heterogenous group of enzymes catalyzing the hydrolysis of the ester link. The isozyme forms of this enzyme being the primary gene products, gene homology can be deduced with precision by

comparing variation in their expression patterns in different cultivars of *L. culinaris* at different stages of development.

The general pattern of increase in number of zones of activity concomitant with an increase in number of isozymes in three varieties upto 72 hours of seed germination can be explained by the fact that dry seeds (0 hours) contain the nutrients for the initial plant growth and development but carry on all life processes at low metabolic rates. When seeds are kept in moisture for germination, the biological activity is initiated necessitating the production of more isozymes (Singh and Gupta, 1978)

Gradual shifts of isozymes' patterns in seeds of different varieties during the course of development demonstrated the appearance or disappearance, or both, of individual isozymes. The changing pattern during development may be interpreted as evidence for differential timing of gene expression correlated with the physiological changes during germination as observed in other plants (Presely and Fowdson, 1965; Johnson et al., 1973).

The isozymes confined to the anodal zone operational from 0 hours in all varieties and had remained expressed at all stages of development the other two zones. This increased intensity of isozyme bands may indicate either additive effects or enhanced synthesis of these isozymes.

The four varieties of *L. culinaris* under the investigation were also compared amongst each other for their electrophoretic variations in non-specific esterases at different stages of development, (i.e. 0, 24, 48, 72, 96, and 120 hours).

Each variety displayed a characteristic banding pattern in different stages of development which can be compared with other varieties at these particular stages for cultivar identification.

Figure 1A indicates the polymorphic variation of esterase isozymes in the different varieties of Lentils showing an extensive polymorphism existing at inter-varietal level with respect to these isozymes. With the exception of band (Rf-0.75) which is present in all varieties except in variety L-4076 at some stage of development, all other isozyme bands appear to be polymorphic in nature.

Band with Rf value 0.74 was observed in variety L-4163 only, at all stages of development and being an ubiquitous band could be used as a genetic marker for that variety.

Isozyme patterns of acid-phosphatases:

Electrophoretic banding patterns of acid phosphatases in four varieties of *L.culinaris* at different stages of seed germination (0, 24, 48, 72, 120 and 144 hours) are also displayed in the form of zymograms (Fig. 1B).

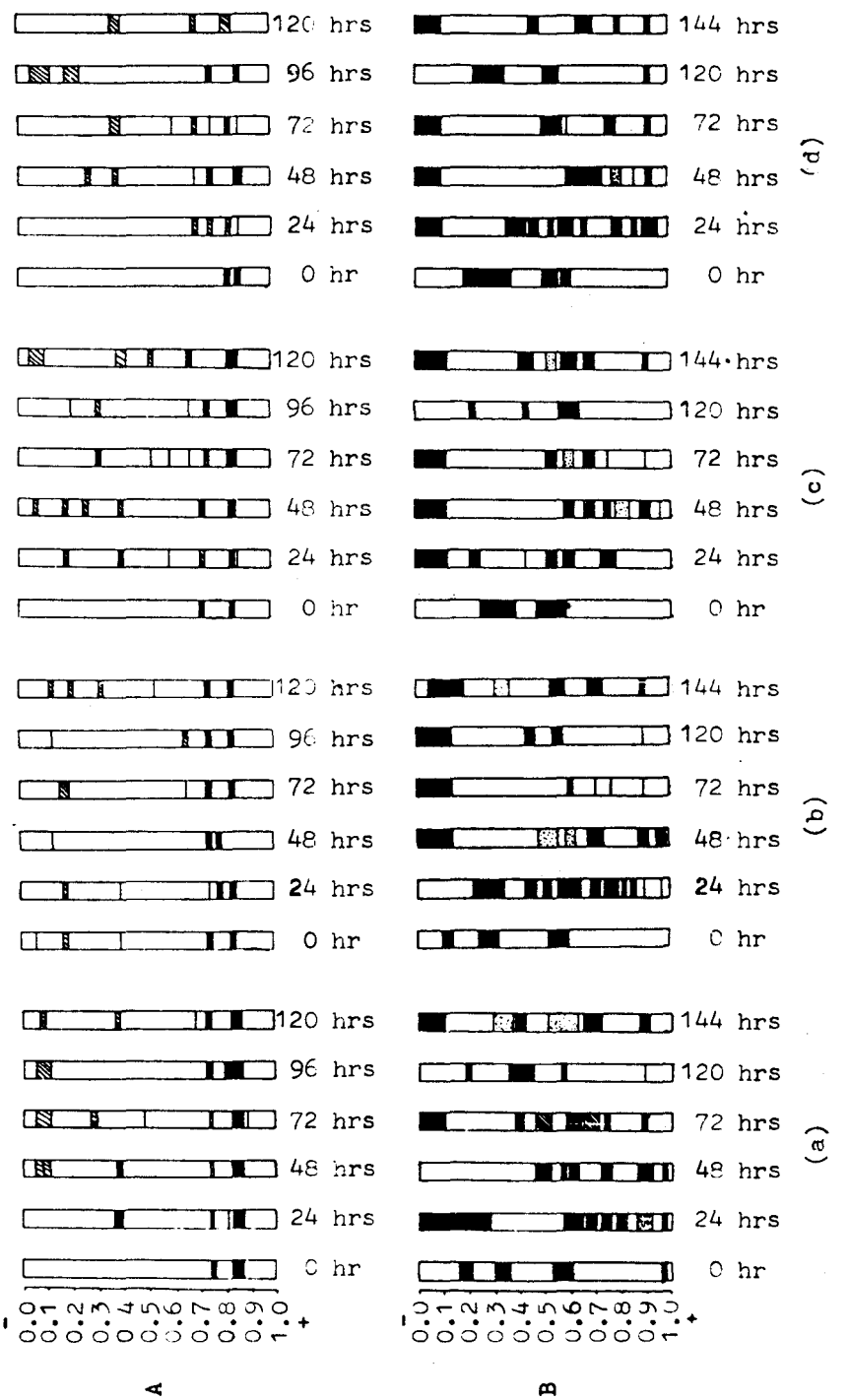

Fig. 1. Represents electrophorograms of isozymes (A) esterases, (B) acid phosphatases at different developmental stages of seed and seedling growth in four cultivars of *Lens culinaris* Medik.; (a) L-4163, (b) LC-74-5, (c) Precoz, (d) L-4076 separated using disc. polyacrylamide gel

The general pattern observed in all varieties was a sudden increase in number of bands from dry seeds stage to 4 hours seed germination stage followed by a slight reduction in the later hours. A further reduction in number of bands at 120 hours developmental stage followed by an increase in the number at 144 hours was also observed.

There are several plant species within which varying numbers of molecular forms of acid phosphatases (orthophosphoric monoester phosphohydrolase, E. C. 3.1.3.2) have been described (Dorn 1965; Brown and Allard, 1969; Cherry and Ory, 1973; Baker and Takeo, 1973) They function in various aspects of cell metabolism such as transport of sugars across membranes (Sauter, 1972), senescence in fruits (De Leo and Sacher, 1970) and in catabolic processes in seeds (Rychter et al, 1972) such as the hydrolysis of phosphomonoesters, important in a variety of biochemical reactions including the formation of sucrose in photosynthesis.

The sudden increase in number of isozymes at 24 hours seed germination can thus be explained on the basis of increase in metabolic activity when seeds are soaked for germination, thereby increasing the production of isozymes. A slight reduction in the later hours, followed by an increase in the number of bands at 144 hours seed germination stage is in consonance with the general belief that metabolic changes occur during seed development and germination (Janardhan et al., 1986).

The general pattern of appearance and disappearance of bands can be explained similarly on the basis of gradual shifts of isozyme patterns in samples taken in the course of development due to differential activation of genes involved in synthesis of these enzymes at the different stages of development (Scandalios, 1969). No unique band present in a particular variety at all stages of development could be located with respect to acid-phosphatase isozymes.

Since the predominant measure of genic polymorphism is currently electrophoretic analysis (Milkman, 1975), consequently the results obtained by determining the extent of genetic variation expressed as polymorphic forms of acid-phosphatases of inter-varietal level shows that extensive polymorphism exists in the different varieties for these isozymes.

The isozyme data obtained and analyzed here can thus express basic trends in the organization of the variations existing in the gene pool of *Lens culinaris* Medik.

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