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Ontogenetic Variability of Isozymes and Proteins in Groundnut

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ABSTRACT. *Peroxidase and esterase isozymes and seed storage protein of different developmental stages of groundnut pod were studied by electrophoresis in polyacrylamide gel for estimating ontogenetic variability of gene expression. The peroxidase isozyme pattern showed that Prx 1 and Prx 2 gene expressed in earlier stages of development but Prx 3 and Prx 4 extended up to 45 days after fertilization (DAF). The esterase isozymes loci showed Est 1 expressed in 20 DAF and not in later stages. The 29 KD protein accumulated up to 30 DAF and later it was converted to other forms. The type of ontogenic variability was observed for both isozyme and protein levels.*

INTRODUCTION

Seed storage protein and enzymes are concerned with the formation of storage products in particular structure or organ within the plant and/or aid for synthesis in particular period during development. Using this knowledge as a starting point, the technologies of molecular biology with the use of DNA enzyme and protein have led to the better understanding of the control of gene expression in a particular organ, which, in turn, is utilized for the marker aided selection for various uses in plant breeding programmes. Hence, the present study was formulated to study the peroxidase, esterase isozyme and storage protein expression during different stages of groundnut pod development.

MATERIALS AND METHODS

The experimental materials consisted of groundnut flowers after fertilization, tagged for different growth stages starting from fertilization

to pod formation. The material taken for isozyme and protein analysis are fertilized ovule from 20 DAF, 30 DAF and 45 DAF. Equal volume of samples (0.5 g) was ground with 0.5 ml of 0.1 M phosphate buffer in ice cold condition using pestle and mortar. The samples were centrifuged at 12,000 rpm for 20 minutes and after centrifugation the fresh supernatant were used for isozyme and protein analysis.

Isozyme analysis

The fresh samples were loaded on native polyacrylamide gel without SDS. After the run was complete, the gel was stained with benezidin staining to visualize the peroxidase isozyme bands and the naphthyl acetate staining for esterase isozyme band. The bands were compared based on Rm values and the enzyme load was assigned.

Protein separation using SDS–PAGE

Polyacrylamide gel electrophoresis (12%) in the presence of SDS was carried out according to Laemmli (1970). After electrophoresis the gel was stained using commasiee brilliant blue R250 and de-stained and the protein bands were compared using standard marker.

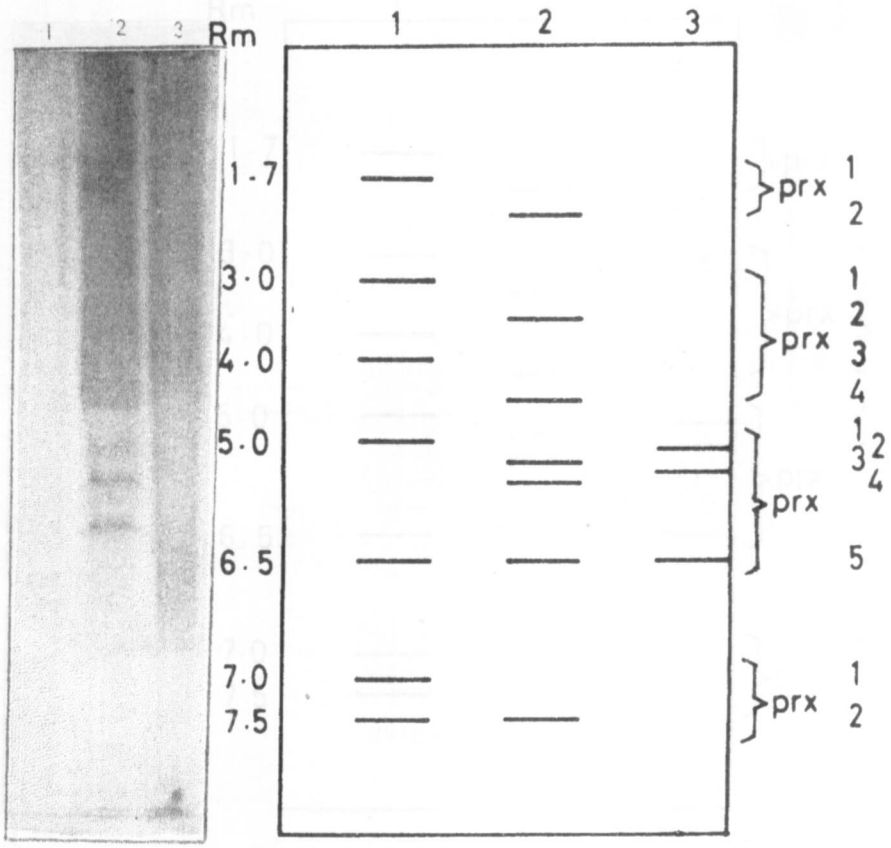
RESULTS AND DISCUSSION

Gene expression obviously changes during growth when particular enzymes formed are active for a short time during the plant's life. Similarly certain protein are deposited in the organs during certain periods of development.

In the present study too the isozyme and storage protein expression variation was observed during developmental stages (Figures 1,2 and 3).

Peroxidase isozyme expression

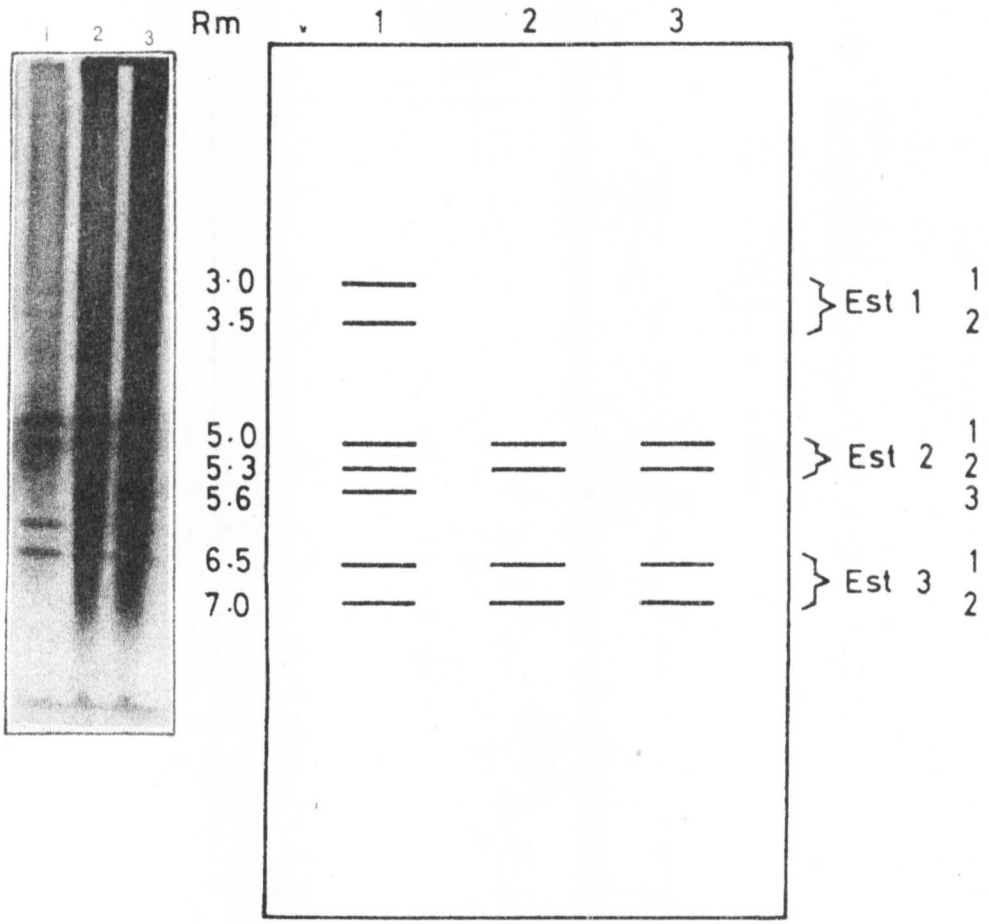
The enzyme expression during developmental stages is shown in Figure 1. The Prx 1 and Prx 2 genes are expressed during 20 DAF and



Lane 1 - 20 days after fertilization
Lane 2 - 30 days after fertilization
Lane 3 - 45 days after fertilization

Figure 1. Peroxidase isozyme banding pattern.

30 DAF which is not expressed during 45 DAF. Cell specific gene expression is also probably always temporally regulated (*i.e.*) found in 20 and 30 DAF and not 45 DAF. In the broadest sense it is limited to the period and existence of the organ (Lyndon and Francis, 1992).

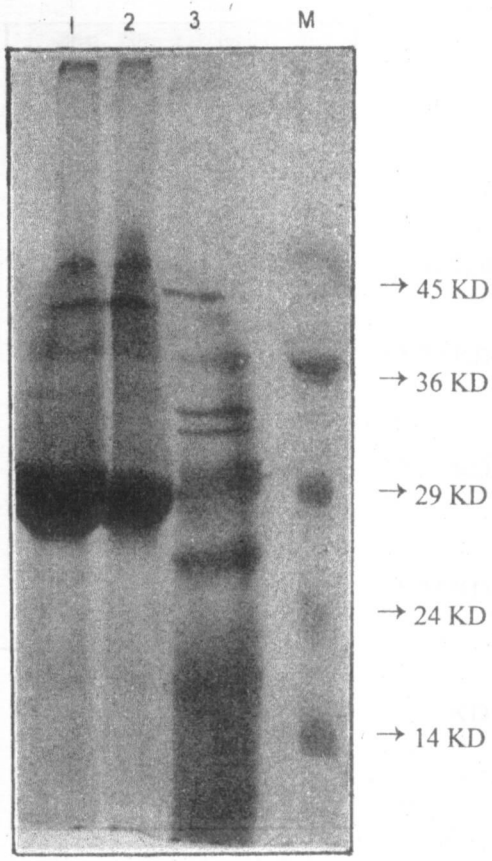


Lane 1 - 20 days after fertilization
Lane 2 - 30 days after fertilization
Lane 3 - 45 days after fertilization

Figure 2. Developmental variation in esterase isozyme banding pattern.

Prx 1 and Prx 2 are time limited than the Prx 3 and Prx 4, where Prx 3 and Prx 4 are expressed even after 30 DAF up to 45 DAF.

Moreover between 20 DAF and 30 DAF, the peroxidase gene loci variation was observed. Earlier Scandalios (1994) reported similar tissue specific isozyme variation in maize.



Lane 1 - Marker
Lane 2 - 45 days after fertilization
Lane 3 - 30 days after fertilization
Lane 4 - 15 days after fertilization

Figure 3. Ontogenetic variation in protein profile during pod maturation in Groundnut.

Esterase isozyme expression

The enzyme expression studied during 20 DAF, 30 DAF and 45 DAF is shown in Figure 2. Here, Est 1 gene which is expressed during 20 DAF is not expressed during later stages (30 DAF and 45 DAF). But Est 2 and Est 3 gene expression started from 30 DAF. This gene expression change during growth is where Est 1, which is active for a short time during the pod development is inactivated very early during development. Earlier Furenkova *et al.* (1995) has reported a similar change in esterase isozyme spectrum during germination.

Storage protein synthesis and accumulation

The storage proteins were fractionated during developmental stages 20 DAF, 30 DAF and 45 DAF. The variation in the protein profile is shown in Figure 3.

The storage protein of 29 KD which accumulated in high amounts up to 30 DAF was not visible during 40 DAF. This shows that the 29 KD protein which accumulated in the early stages may be connected to other forms which may be utilized for the development of organs on specific enzyme activation in the latter stages. During 45 DAF, the low molecular weight polypeptides 29 KD to 14 KD range, accumulated than in the earlier stages, which may have some specific functions and storage properties in groundnut proteins.

CONCLUSIONS

The ontogenetic variability of gene expression and seed protein was observed from peroxidase, esterase isozyme and seed protein in this study. However, the substance which can induce and regulate the levels of gene expression during development of organs has to be studied.

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