

## CHANGE IN SPECIFIC ACTIVITY OF PEROXIDASE AND TOTAL PROTEIN CONTENT DURING EARLY PHASE OF SOMATIC EMBRYOGENESIS OF *PINUS KESIYA*

CHITA RANJAN DEB\* AND PRAMOD TANDON

Plant Biotechnology Laboratory, Botany Department, North-Eastern hill University, Shillong 793 022,

Present address of corresponding author: Botany Department, Nagaland University, Headquarters: Lumami, Mokokchung 798 601,

(Received march 2001, Accepted May, 2001)

Change in specific activity of peroxidase and protein content was determined during initiation of somatic embryogenesis of *Pinus kesiya* form Zygotic embryos, secondary needles and apical dome sections. During early phase of the cultures both peroxidase activity and total protein content increased gradually up to day 10 and were maximum at 30<sup>th</sup> of culture and followed declining trend in the entire investigation period. The specific activity of peroxidase and protein content were maximum in zygotic embryos and secondary needles respectively.

**Key words :** *Pinus kesiya*, somatic embryogenesis, peroxidase activity, protein content.

Since 1985 (Hakman *et al.*, 1985) a remarkable progress has been made in the field of somatic embryogenesis in conifers. Despite of growing practical experiences, a lack of biochemical and physiological background knowledge prevents us from move deep understanding the somatic embryogenesis in conifers. Isozyme markers have been applied extensively to study the process of somatic embryogenesis and enzymes like peroxidase, polyphenoloxidase and soluble proteins have been reported to play vital role on initiation of somatic embryogenesis (Pitel *et al.*, 1992). There are several reports pertaining roles of peroxidase activity and change in total protein content on induction of somatic embryogenesis (Tawal and Mehta, 1982, Zhou *et al.*, 1992 Egertsdotter *et al.*, 1993, Donga and Dunstan, 1994). This communication describes the peroxidase activity and change in total protein content during initiation of embryogenic cultures of *Pinus kesiya*.

The tissues for analysis comprised of mature zygotic embryos, Secondary needles and apical dome sections before culture and during the initiation of somatic embryogenesis following protocol given by Deb and Tandon (under publication). The cultured explants were transferred to fresh medium after 20 days of culture initiation and thereafter at every 15 days interval. The tissues were harvested at 5 days interval up to 50 days of culture. About 150 mg tissue was homogenized in 5 ml of chilled 0.2M sodium phosphate buffer (pH-6.0) using a chilled tissue grinder. The

homogenates were centrifuged at 5000g in a Sorval Refrigerated Centrifuge (Model-RC5C) for 30 min at 2- 4°C. The supernatant was used for the assay.

Specific activity of peroxidase (EC 1.11.1.7) was measured following Tandon and Arya (1982). The rate of decomposition of H<sub>2</sub>O<sub>2</sub> by the enzyme with O-dianisidine as hydrogen donor was determined spectrometrically by measuring the color development at 460 nm at 20 sec interval for 3 min. The peroxidase activity is expressed as change in absorbance at 460 nm min<sup>-1</sup> mg<sup>-1</sup> proteins. Protein content was estimated following Lowry *et al.* (1951) from the same supernatant prepared for the assays of peroxidase activity. Protein is expressed as mg g<sup>-1</sup> fresh weight of tissue using a standard curve prepared with bovine serum albumin.

Before culture zygotic embryos, secondary needles and apical dome sections showed a very low specific activity of peroxidase. During culture on initiation medium it increased gradually up to day 10 and subsequently a marked increase was recorded till day 30 of culture age and thereafter showed a slight decline up to day 50 (Fig. 1a). The maximum activity of peroxidase was in zygotic embryos followed by secondary needles and apical dome sections. The maximum protein content was in secondary needles followed by zygotic embryos and apical dome sections before their culture (Fig. 1b). In the former two cases the protein content increased steadily up to day 20 of growth while in apical dome sections, there was a slight

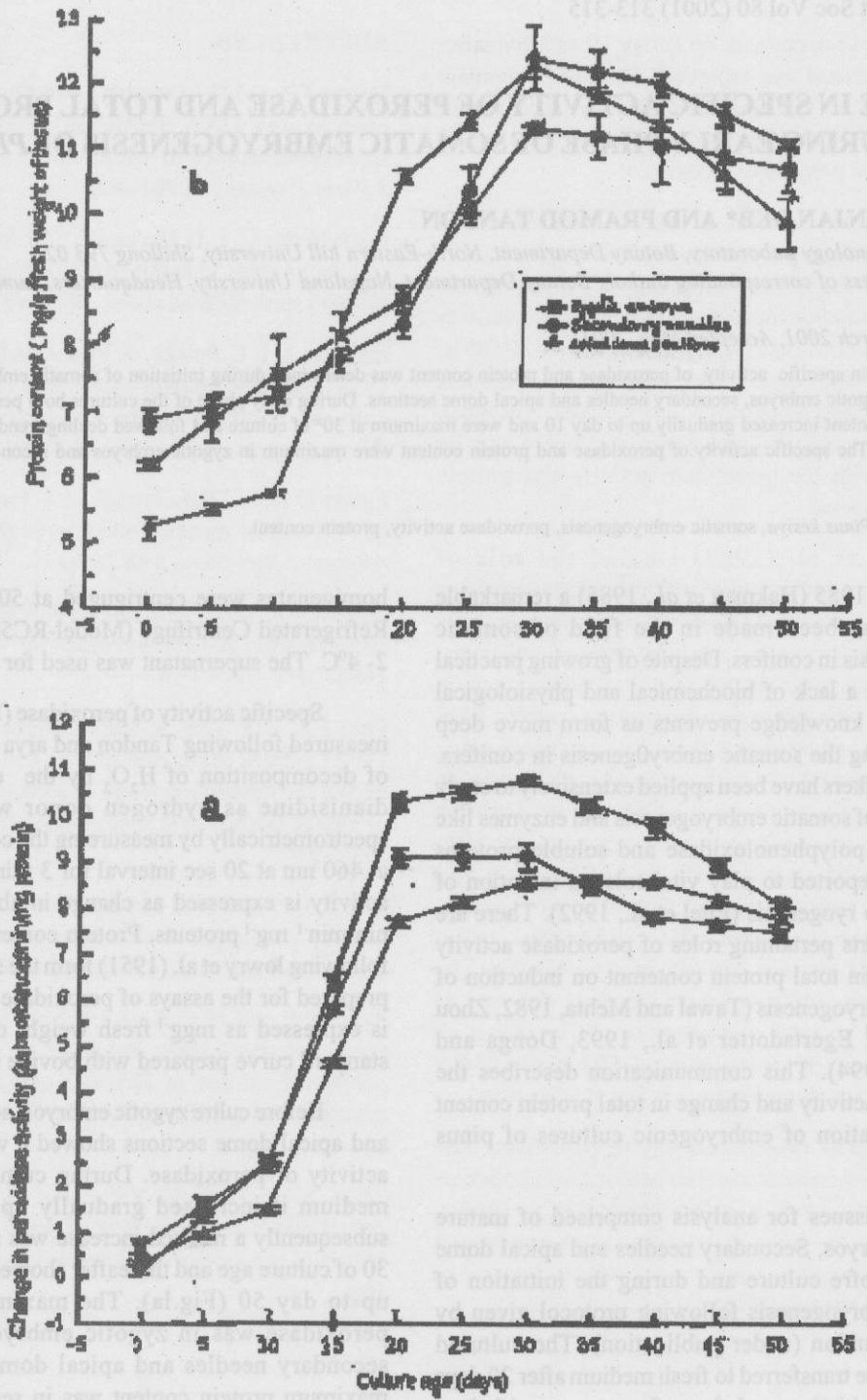


Fig. -1

Fig 1. a. Changes in specific activity of peroxidase during initiation of somatic embryogenesis, b. changes in protein content during initiation of somatic embryogenesis.

increase in protein content up to day 10 and thereafter a marked increase was recorded. The protein content in all the cultures increased considerably up to day 30 of culture age and then showed a gradual decline, which was similar to peroxidase activity.

A very low specific activity of peroxidase and protein content were recorded in all the explants on P. kesiyua before culture initiation. With growth on respective initiation medium, the peroxidase activity and protein content increased gradually up to 30 day of culture. This was the period when embryogenic cultures started forming proembryonal masses. Increase in peroxidase activity and protein content can be correlated with the peroxidase activity and protein content decreased.

Zhou et al. (1992) studied the role of peroxidase on induction of somatic embryogenesis in *Lactuca sativa* and found that peroxidase activity increased prior to visual manifestation of embryos. Till date there is no report on role of peroxidase on induction of somatic embryogenesis in conifer. Pitel et al. (1992) reported that there was no significance in protein content and patterns in non-embryogenic and embryogenic cell lines of *Larix eurolepis* on maintenance medium, but on maturation medium protein content started increasing after day 9 and reached the peak at day 15 followed by gradual decrease in the quantity. Donga and Dunstan (1994) reported that protein rapidly accumulated to the highest value (1109 mg l<sup>-1</sup>) at day 9 at the rate of 94.7 mg l<sup>-1</sup> d<sup>-1</sup> during early stages of initiation of embryogenesis of *Picea glauca*. The intracellular protein content decreased after day 9 of culture.

Change in specific activity of peroxidase and protein content could be used as useful markers to identify the onset of embryogenesis in *P. kesiyua*. Further work on study of iso-peroxidase profile and protein profile and in progress and will be reported in the future communication.

## REFERENCES

- Donga J Z and D I Dunstan 1994 Growth parameters, protein and DNA synthesis of an embryogenic suspension culture of white spruce (*Picea glauca*). *J Plant Physiol* **144** 201-208.
- Egertsdotter U, L H Mo and S Von Arnold 1993 Extra cellular proteins in embryogenic suspension cultures of Norway spruce (*Picea abies*). *Physiol Plant* **88** 315-321.
- Hakman I, L C Fowke, S Von Arnold and T Erikson 1985 The development of somatic embryos in tissue cultures from immature embryos of *Picea abies* (Norway spruce). *Plant Sci* **38** 53-59.
- Lowry O H, N J Rosebrough, A L Fan and R J Randal 1951 Protein measurement with the Folin- Phenol reagent. *J Biol Chem* **193** 265-275.
- Negmani R and J M Bonga 1985 Embryogenesis in subcultured callus of *Larix deciduas*. *Can J For Res* **15** 1088-1091.
- Pitel J A, B Y Yoo, K Kimaszewska and P J Charest 1992 Changes in enzyme activity and protein patterns during the maturation phase of somatic embryogenesis in hybrid larch (*Larix Xeurolepis*). *Can J For Res* **22** 553-560.
- Rawal S K and A R Mehta 1982 Changes in enzyme activity and isoperoxidase in haploid tobacco callus during organogenesis. *Plant Sci Lett* **24** 67-77.
- Tandon P and H C Arya 1982 Association of auxin protectors, Peroxidase, indole acetic acid oxidase and polyphenol oxidase in *Zizyphus* gall and normal stem tissues grown in culture. *Biochem Physiol Pflanzen* **177** 114-124.
- Zhou X, Y Han, W Yang and Xi 1992 Somatic embryogenesis and analysis of peroxidase in cultured lettuce (*Lactuca sativa* L) cotyledons. *Ann Bot* **69** 97-100.