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**By**

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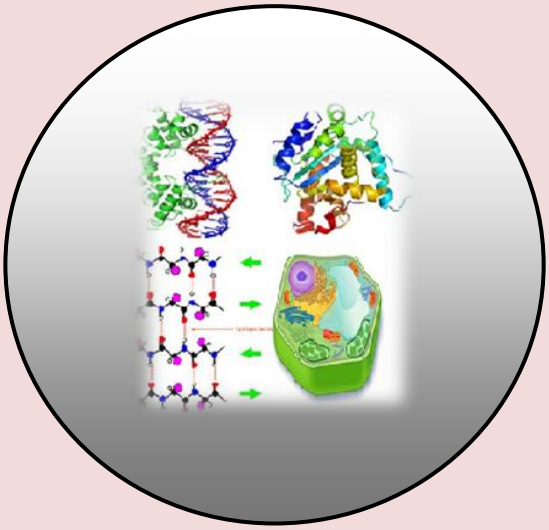

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# Peroxidase and Isoperoxidase Changes in relation to growth and differentiation of *Psoraleacoryli folia* L. *In Vitro*

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## ABSTRACT

***Peroxidases are widely distributed in plant tissues and are of immense importance as they are associated with numerous catalytic functions. Isoenzymes analysis in cultured tissues offers a reasonable and promising tool to understand the biochemical basis of differentiation in vitro. Induction of callus and shoot buds from leaf and stem segments of Psoraleacoryli folia was achieved within 40 days of culture on Murashige and Skoog's (1962) medium supplemented with 3 mg/l (KN) and 0.5 mg/l (NAA) and 3% (w/v) sucrose. The maximum percentage of shoots was obtained when incubated in continuous light for 4 weeks before transfer to the rooting media. Peroxidase activity increased considerably during shoot buds initiation suggesting that peroxidase activity might be involved in shoot buds initiation of Psoraleacoryli folia in vitro.***
***Key words: In vitro, Tissue culture, endangered, Murashige and Skoog's.***

## INTRODUCTION

*Psoraleacoryli folia* Linn (Fabaceae) is a rare and endangered herbaceous medicinal plant, distributed throughout the tropical and subtropical regions of the world (Jain, 1994). It is used as a laxative, aphrodisiac, anthelmintic, diuretic and diaphoretic in febrile conditions. It has been specially recommended for the treatment of leucoderma, leprosy, psoriasis and inflammatory diseases of the skin and prescribed both for oral administration and external application in the form of a paste or ointment (Anonymous,1988) Pharmaceutical companies largely depend upon material procured from naturally occurring stands, but these are being depleted rapidly, raising concern about possible extinction and providing justification for development of in vitro propagation techniques for this species. Plant regeneration via meristem and callus culture of *Psoraleacoryli folia* have been reported previously (Saxena *et al.*, 1987, 1988). Peroxidases are widely distributed in plant tissues and are of immense importance as they are associated with numerous catalytic functions.

Plantperoxidases are have containing monomeric glycoproteins involved in many processes plant growth and development (Rigueleme and Cordemil, 1993). Peroxidases may have significant role in regulation of cell and differentiation (Christensen *et al*., 1998) Plant peroxidases are among the most studied but least understood of all plant proteins (Gaspar *et al*., 1992 and Syros *et al*., 2004, 2005). Their universal nature and availability of many sensitive colorimeter assays have made peroxidases as convenient enzyme marker in genetic and physiological studies (Greppin *et al*., 1986; VanHuystee, 1987). Isoenzymes analysis in cultured tissues offers a reasonable and promising tool to understand the biochemical basis of differentiation *in vitro* (Soni and Swarnkar, 1998). Peroxidase isozymes are widely distributed among higher plants (Scandalios, 1974; Grison and Pilet, 1978 and Šimonovičová, 2004) and are frequently organ and / or tissue specific (Asins*etal*., 1982; Siegel, 1993 and Smith and Walker, 1996). Due to this characteristic, different organs from the some plant may show different peroxidase patterns. Another characteristic of isoperoxidase is the change of electrophoretic patterns in the same tissue or organ, depending upon age, developmental stage or environmental conditions during germination (Kruger and Laberge, 1974; Thorpe *etal*., 1978, Mäder and Füssl,1982).

In the present communication, we report the change in the activities of peroxidase and isozyme pattern of peroxidases during growth and differentiation of shoot buds from callus obtained from leaf and stem explants of *Psoraleacorylifolia.*

## MATERIAL AND METHODS

Wide range of culture experiments were carried out and once a reproducible protocol for callus and shoot buds induction from leaf and stem explants had been developed, the changes in Peroxidase activity and isozyme patterns of peroxidases occurring during growth and differentiation under in vitro conditions were studied.

The changes were recorded after the inoculation of an explant on nutrient medium till callus and shoot buds induction. Cultures were raised from leaf and stem segments of *Psoraleacoryli folia* on Murashige and Skoog's medium (1962) supplemented with different concentrations of Auxins and Cytokinins individually as well as in combinations. (Table 1 & 2, Fig.3.1-3.6). All the culture were incubated under fluorescent tubes in culture room maintained at 26 □ 2$^{O}$C .Changes in peroxidase activity and Isoperoxidases were studied from the day of inoculation Zero day till 40$^{th}$day (the day of shoot buds induction) at a intervals of five days(Fig.2.1-2.6).

**Enzyme Assay**: - Material was homogenized in 0.1 M phosphate buffer, PH 7.0, (10 ml of buffer g$^{-1}$fresh weight) in a pre-chilled mortar and pestle. The homogenate was centrifuged at10,000 □ g for 20 min in high-speed refrigerated centrifuge KR 20000 T, KUBOTA; the supernatant was used for the enzyme assay and after further centrifugation at20, 000 □ g for15 min for polyacrylamide gel electrophoresis. The peroxidase activity was assayed by guaiacol and hydrogen peroxide (Racusen and Foote, 1965).To a clean cuvette with 1.0 cm light path, 2.0 ml of 0.05 m phosphate buffer, pH 7.0, 1.0 ml of 1% guaiacol and 0.2 ml of enzyme extract was added and the absorbance was set zero at 470 nm, 0.2 ml of 0.3% hydrogen peroxide was quickly mixed to start the reaction and changes in absorbance were recorded for every 15 seconds up to 5 minutes. Increase in absorbance per unit time was calculated from the linear phase of enzyme velocity. Enzyme activity was calculated in terms of change of absorbance per sec per mg protein.

**Electrophoresis**:-Isozymes of peroxidase were separated by slab gel on polyacrylamide gel electrophoresis PAGE (Davis,1964) Running Gel (10 %) and Stacking Gel (4.5%) were prepared and a vertical slab gel apparatus as described by Studier (1973), Desatronic 3000/200 power supply and Frigostat, West Germany were used in all electrophoretic work. Peroxidase bands were observed by immersing the gel in equal volume of 1.0 M acetate buffer, pH 4.8 and 1.0 percent guaiacol solution for half an hour. This solution was drained off and the gel transferred to 0.3% hydrogen peroxide solution until the appearance of brown bands (Siegel and Galston,1967).

## RESULTS

Peroxidase activity remained very low in both leaf and stems explants till shoot buds initiation occurred (Fig.1) Enhancement in the enzyme activity was noticed during initiation of shoot buds in both leaf and stem explants.

Isoperoxidases when separated on Polyacrylamide gel electrophoresis showed a varied pattern. Eighteen isoperoxidases were detected throughout the culture period of *Psoraleacoryli folia* (Fig. 2.1-2.6). The number of isozymes was low during the early days of culture. This increased with the culture age. Isozyme I exhibited high intensity during initial days of culture and was present throughout the culture period (Fig. 2.1 & 2.2). Isozyme II appeared newly during shoot buds initiation in both the explants and persisted till shoot bud proliferation stage (Fig. 2.5 & 2.6). Isozyme III showed its appearance during callus formation and callus proliferation stage (15-25 DAI). Isozymes V, VII, VIII, X were absent during earlier days of inoculation in both leaf and stem explants except on day Zero, on which isozyme VIII was present (Fig.2.1&2.2). Isozyme XI disappeared on day 15 and continued its disappearance till last day of culture (Fig.2.3-2.6). Isozyme XI showed high intensity in leaf explants at day 5 & 10 (Fig.2.1&2.2). Isozymes XII, XIII, XIV, XV and XVI were absent in both leaf and stem explants during early days of culture (Fig.2.1&2.2). Isozyme XVII appeared only during callus initiation in both leaf and stem explants (Fig.2.1&2.2). While Isozyme XVIII was present only during first ten days of culture (Fig. 2.1 & 2.2).

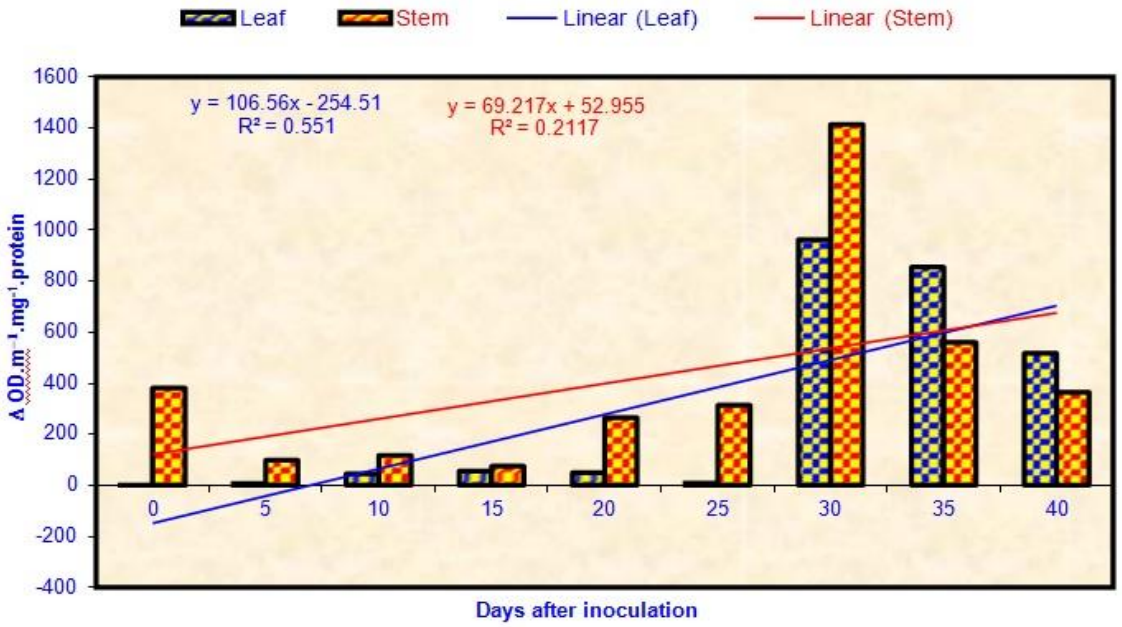


**Figure 1 Peroxidase activity in leaf and stem cultures of *Psoraleacoryli folia* at different days (0-40) after inoculation.**

## DISCUSSION

A significant increase in peroxidase activity was observed during initiation of shoot buds in leaf and stem explants of *Psoraliacoryli folia* suggesting that peroxidase might be involved in shoot bud initiation. Although increase in peroxidase activity prior to shoot bud formation has also been observed in callus cultures (Thorpe and Gaspar, 1978 Hatzilazarou *et al*., 2006), epidermal explants of tobacco (Thorpe *et al*., 1978) and cotyledon explant of *Pinus radiata* (Patel and Thorpe, 1984). The rise in specific activity of peroxidase despite the low soluble protein content in shoot buds forming calli of *Psoraleacory lifolia* indicates the involvement of the enzyme in the process of organogenesis. These results were in accordance to Gaspar *et al*., 1985, 1992.

The isozyme patterns of the leaf and stem explants of the plant studied here was not similar. This was supported by the view that different organs from the same plant may show different peroxidase patterns as for instance, the leaves and roots of Citrus (Button *etal*., 1976; Essen and Sost, 1976), endosperm, leaf, root and coleoptile of Zea Mays (Hamill and Brewbaker, 1969) different organs of *Lycopersicon esculentum* (Evans and Albridge, 1965) and stem and root of *Solanum surattense* (Swarnkar *et al*., 1986) *Solanum nigrum* (Hassanein, 1999).

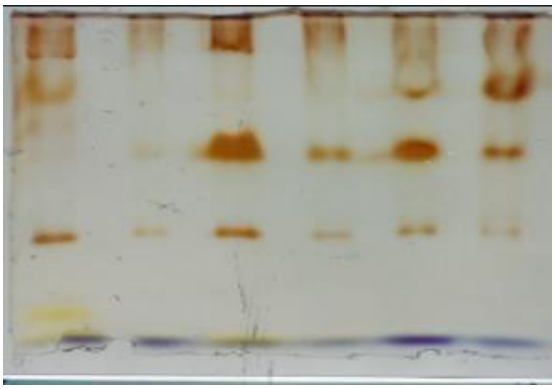

**Fig. 2.1. Isoperoxidases in leaf and stem cultures of *P. corylifolia* at 0,5 and 10 days after inoculation.**

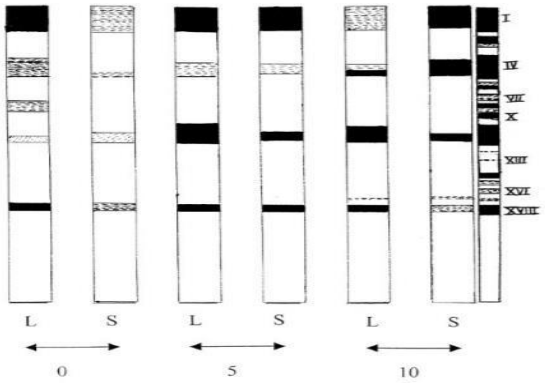


**Fig. 2.2. Zymograms representing isozymic variations in leaf and stem cultures of *P. corylifolia*at 0,5 and 10 days after inoculation.**

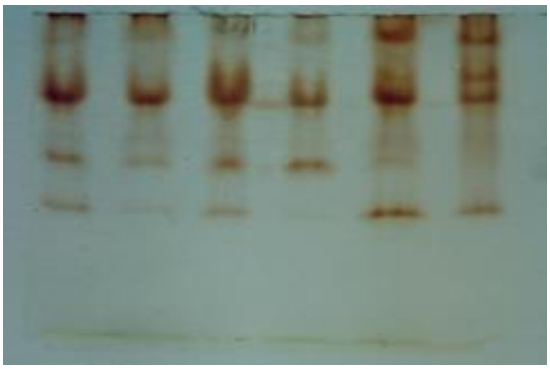

**Fig. 2.3. Isoperoxidases in leaf and stem cultures of *P. corylifolia* at 15,20 and 25 days after inoculation.**

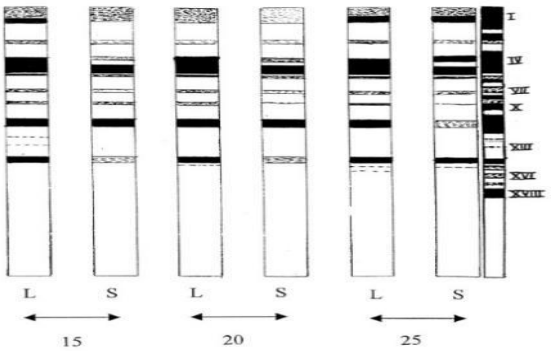


**Fig. 2.4. Zymograms representing isozymic variations in leaf and stem cultures of *P. corylifolia* at 15,20 and 25 days after inoculation.**

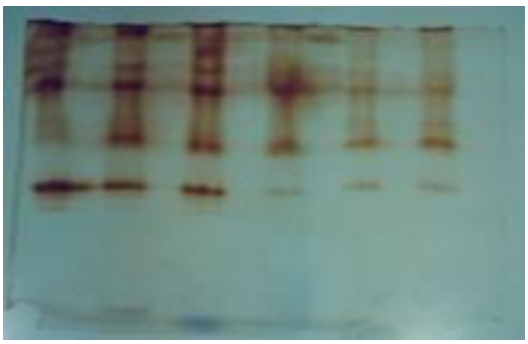

**Fig. 2.5. Isoperoxidases in leaf and stem cultures of *P. corylifolia* at 30,35 and 40 days after inoculation.**

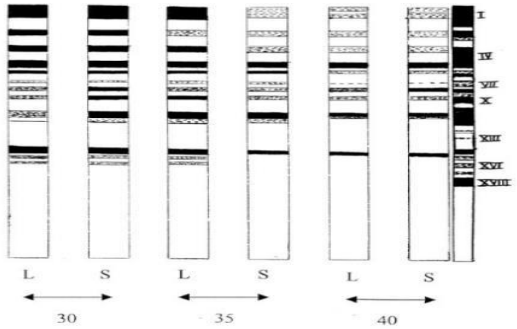


**Fig. 2.6. Zymograms representing isozymic variations in leaf and stem cultures of *P. corylifolia* at 30,35 and 40 days after inoculation.**

**Figure 2.1-2.6.Showing Isoperoxidases and Zymograms of leaf and stem cultures of *Psoraleacoryli folia* at different days after inoculation.**

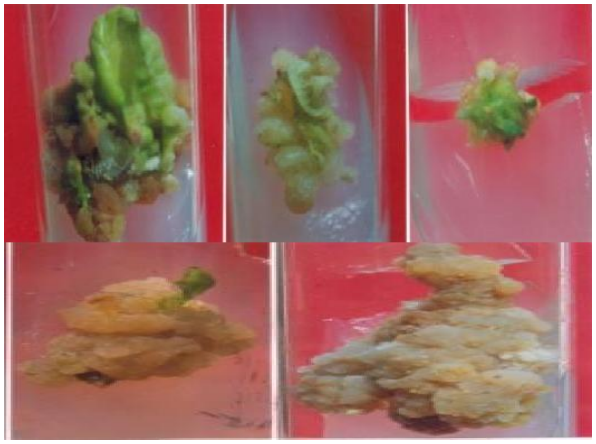

**Fig. 3.1. Response of leaf explants on different growth regulators.**

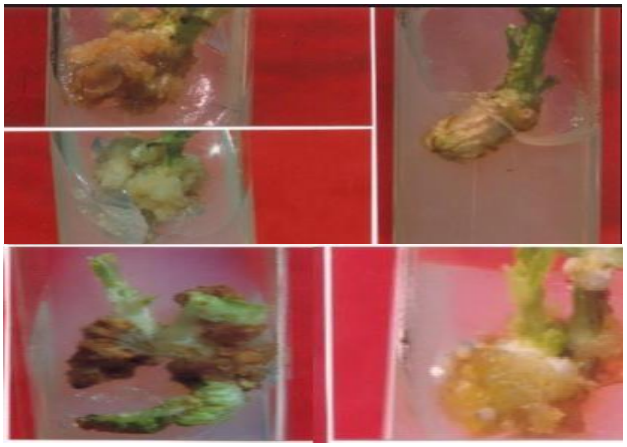

**Fig. 3.4. Response of stem explants on different growth regulators.**

**Fig. 3.2. Shoot buds induction from leaf explants on MS + KN (3mg.l$^{-1}$) + NAA (0.5 mg.l$^{-1}$) considered as 40$^{th}$day.**

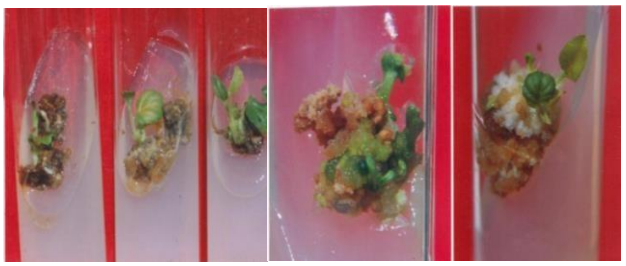

**Fig. 3.5. Shoot buds induction from stem explants on MS + KN (3mg.l$^{-1}$) + NAA (0.5 mg.l$^{-1}$) considered as 40$^{th}$day.**

**Fig. 3.3. Proliferation of shoots from leaf explants.**

**Fig. 3.6. Proliferation of shoots from stem explants.**

**Figure 3.1-3.6. Response of leaf and stem explants of *Psoraleacoryli folia* on different concenterations of Auxins and Cytokinins individually as well as in combination being mentioned in Table 1 & 2 using MS basal medium.**

**Table 1.Effect of plant growth regulators (PGR) on leaf explants of *Psoraliacoryli folia* under *in vitro* conditions.**

| S.No. | PGR in MS medium | Callus Induction | | | Organogenesis | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Caulogenesis | | Rhizogenesis | |
| | | Colour | DAI | Callus Amount | Direct | Indirect | Direct | Indirect |
| 1. | IAA (2 $mg.l^{-1}$) | Cottony White | 19 | $C^{++}$ | - | - | - | - |
| 2. | IBA(2$mg.l^{-1}$) | Creamish | 21 | $C^{++}$ | - | - | - | - |
| 3. | 2,4-D (2$mg.l^{-1}$) | Whitish brown | 16 | $C^{+++}$ | - | - | - | - |
| 4. | NAA (2$mg.l^{-1}$) | Brown | 7 | $C^{+}$ | - | - | - | + + + |
| 5. | Kn(2$mg.l^{-1}$) | Light brown | 9 | $C^{+++}$ | - | - | - | - |
| 6. | BAP(2$mg.l^{-1}$) | Greenish white | 9 | $C^{++}$ | - | - | - | - |
| 7. | BAP(2$mg.l^{-1}$) + 2,4-D (0.5$mg.l^{-1}$) | White | 9 | $C^{+}$ | - | + | - | - |
| 8. | Kn (1$mg.l^{-1}$) + NAA (0.5$mg.l^{-1}$) | Yellow | 10 | $C^{+++}$ | - | + | - | - |
| 9. | Kn (2 $mg.l^{-1}$)+ NAA(0.5$mg.l^{-1}$) | Yellow | 9 | $C^{+++}$ | - | + + | - | + |
| 10. | Kn (3$mg.l^{-1}$) + NAA (0.5$mg.l^{-1}$) | Yellow | 11 | $C^{+++}$ | - | + + + | - | + |

+Poor ++Moderate +++Best

Transient and specific isoperoxidases have been reported by Swarnkar (1985) during callus initiation in stem segments of *Solanum surattense*. Isozyme XVIII appearing only during callus initiation in both leaf and stem segment of *Psoraleacoryli folia* can be considered as marker for callus initiation. Similarly new isozymes appeared during callus induction of *Phaseolus vulgaris* (Arnison and Boll, 1974) and *Sinapsis alba* (Bajaj *et al*. ,1973) tobacco (Faivre-Rampant *et al*., 1998) and during rooting in *Nothofagus nervosa* and *Nothofagus Antarctica* .(Calderón-Baltierra *et al*., 1998) and *Petunia hybrid* (Kotis *et al*., 2009). Though it is not possible to attribute any specific role to these new isozymes, yet it can be said that the new transient and persistent forms might be responsible for induction ofcallus.

The conflicting role of peroxidase in organogenesis is probably due to the occurrence of this enzyme in multi molecular forms. As isozyme II which appeared newly during shoot bud initiation from callus of both the explant, can be considered as marker for shoot bud initiation. Similar isoperoxidases have been used as biochemical markers in leaf and root morphogenesis in barley. (Moncousinand Gaspar, 1983; Coppensand Dewitte, 1990 and Rout *et al*., 2000). The present investigation has also envisaged that changes in peroxidase activity and Isoperoxidase could be used as biochemical markers to understand the growth and differentiation under *in vitro* conditions.

**Table 2.Effect of plant growth regulators (PGR) on stem explants of *Psoraleacorylifolia*under *in vitro* conditions.**

| S.No. | PGR in MS medium | Callus Induction | | | Organogenesis | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Caulogenesis | | Rhizogenesis | |
| | | Colour | DAI | Callus amount | Direct | Indirect | Direct | Indirect |
| 1 | IAA (2mg.l$^{-1}$) | Light yellow | 9 | C $^{++}$ | - | - | - | - |
| 2. | IBA (2mg.l$^{-1}$) | White | 11 | C $^{++}$ | - | - | - | - |
| 3. | NAA (2 mg.l$^{-1}$) | - | - | - | - | - | + + | - |
| 4 | 2,4-D (2 mg.l$^{-1}$) | White | 14 | C$^{+}$ | - | - | - | - |
| 5. | Kn ( 2mg.l$^{-1}$) | Brown | 13 | C$^{+}$ | - | + | - | - |
| 6 | BAP (2 mg.l$^{-1}$) | Brown | 8 | C$^{+}$ | + | - | - | - |
| 7. | BAP (2 mg.l$^{-1}$) + 2,4-D (0.5 mg.l$^{-1}$) | Pale yellow | 13 | C$^{++}$ | - | + | + | - |
| 8. | Kn (2 mg.l$^{-1}$) + 2,4-D (0.5 mg.l$^{-1}$) | Light brown | 11 | C$^{+}$ | - | + | + | - |
| 9. | BAP (1 mg.l$^{-1}$)+ NAA (0.5mg.l$^{-1}$) | Dark brown | 24 | C$^{++}$ | - | + | - | - |
| 10. | BAP (2 mg.l$^{-1}$)+ NAA (0.5mg.l$^{-1}$) | Dark brown | 19 | C$^{+}$ | - | + + | - | - |
| 11. | BAP (3 mg.l$^{-1}$) + NAA (0.5mg.l$^{-1}$) | Dark brown | 17 | C$^{+}$ | - | + + | - | - |
| 12. | Kn (1 mg.l$^{-1}$) + NAA (0.5 mg.l$^{-1}$) | Brown | 15 | C$^{+++}$ | - | + | - | - |
| 13. | Kn (2 mg.l$^{-1}$) + NAA (0.5 mg.l$^{-1}$) | Whitish brown | 14 | C$^{+++}$ | - | + | - | - |
| 14. | Kn (3 mg.l$^{-1}$) + NAA (0.5 mg.l$^{-1}$) | Light brown | 13 | C$^{++}$ | - | + + + | - | - |
| 15. | Kn (4 mg.l$^{-1}$) + NAA (0.5 mg.l$^{-1}$) | Light brown | 15 | C$^{+++}$ | - | + + | - | - |

+Poor ++Moderate +++Best

## REFERENCES


**Anonymous (1988).** The Wealth of India: A Dictionary of Indian Raw Materials and Industrial Products, Vol.II, CSIR, New Delhi, India. pp. 116–118.

**Arnison, P.G. and Boll, W.G. (1974).** Isoenzyme in cell cultures of bush been (Phaseolus vulgaris) Isoenzymatic changes during the callus culture cycle and differences between stock cultures. *Canadian Journal of Botany*, 52: 2621-2629.

**Asins, M.J.Benito, C. and Porezde-la-vega, M. (1982).** Changes and chromosomal location of peroxidase isozyme during hexaploid wheat kernel maturation.*Z. Pflanzen Zuchtg*. 89: 235-241.

**Bajaj, Y.P.S., Bopp, M. and Bajaj, S. (1973).** Pattern of Peroxidases and differentiation in Sinapsisalba. *Phytomorphology* 23:43-52.

**Button , J.,Vardi, A. and Spiegel, P.R. (1976).** Root peroxidase isozymes as an aid in culture breeding and taxonomy. *Theory of Applied Genetics* 47: 119-123.

**Calderón-Baltierra, X., Martínez-Pastur, G.M., Jofre, M.P. and Arena, M.E. (1998).** Activity variation of peroxidase during in vitro rooting of Nothofagus nervosa and Nothofagusantarctica.*Phyton* 62: 137–144.

**Christensen, J., Bauw, G., Welinder, K., Montagu, M. and Boerjan, W. (1998).** Purification and characterization of peroxidases correlated with lignification in poplar xylem. *Plant Physiol.* 118: 125–135.

**Coppens, L. and Dewitte, D. (1990).** Esterases and peroxidase Zymograms from barley(Hordeumvulgare L.) callus as a biochemical marker system of embryogenesis and Morphogenesis. *Plant Science*. 67:97-105.

**Davis, B.J. (1964).** Disc electrophoresis. II. Methods and application to human serum proteins.*Ann. N.Y. Acad. Sci.* 121: 404-427.

**Essen, A. and Sost, R.K. (1976).** Peroxidase polymorphism in Citrus. Journal of Hereditary 67:199-203

Evans, J.J. and Albridge, N.A. 1965 The distribution of peroxidase in extreme dwarf and normal tomato (Lycopersicon esculentum ).*Phytochemistry* 4: 499-503.

**Faivre-Rampant, O., Kevers, C., Bellini, C. and Gaspar, T. (1998).** Peroxidase activity, ethylene production, lignification and growth limitation in shoots of a non-rooting mutant of tobacco. — *Plant Physiol. Biochem*. 36: 873–877.

**Gaspar, T., Kevers, C., Hausman, J.F., Berthon, J.Y. and Ripetti, V. (1992).** Practical use of peroxidase activity as a predictive marker of rooting performance of micropropagated shoots. — *Agronomie* 12: 757–765.

**Gaspar, T., Penel, C., Castillo, F.and Greppin, H. (1985).** A two-step control of basic and acidic peroxidases and its significance for growth and development.— *Physiol. Plant.* 64: 418–423.

**Greppin, H., Penel, L. and Gaspar, T. (1986).** Molecular and physiological aspects of plant peroxidases (Geneva,Switzerland: University of Geneva).

**Grison, R. and Pilet, P.E. (1978).** Analyze critique des peroxydases de la racine de mais. *Plant Science letters.*13: 213-218.

**Hamill, A.E. and Brewbaker, J.L. (1969).** Isozyme polymorphism in flowering plantsIV. The peroxidase isozyme of maize (Zea mays).*Physiology Plantarum* 22: 945-958.

**Hassanein, A.M., Ahmed, A.M., Abed-el-Hafez, A.I. and Soltan, D.M. (1999).** Isozyme expression during root and shoot formation in Solanumnigrum. — *Biol. Plant.* 42: 341–347.

**Hatzilazarou, S.P., Syros, T.D., Yupsanis, T.A., Bosabalidis, A.M. Economou, A.S. (2006).** Peroxidases, lignin and anatomy during in vitro and ex vitro rooting of gardenia (Gardenia jasminoides Ellis) microshoots. — *J. Plant Physiol.* 163: 827–836.

**Jain, S.K. (1994).** Ethnobotany and research in medicinal plants in India.*Ethnobotanical Search New Drugs* 185: 153–168.

**Kotis, M. Yupsanis, T. A., Syros, T. D. and Economou, A. S. (2009).** Peroxidase, acid phosphatase, RNase and DNase activity and isoform patterns during in vitro rooting of Petunia × hybridamicro shoots, *Biologia Plantarum* 53:530-534.

**Kruger, J.E. and Labarge, D.E. (1974).** Changes in peroxidase activity and peroxidase isozyme patterns during kernel growth and maturation. *Cereal Chemistry* 51:345-354.

**Mäder, M. and Füssl, R. (1982).**Role of peroxidase in lignification of tobacco cells. II. Regulation by phenolic compounds. — *Plant Physiol.* 70: 1132–1134.

**Moncousin, C. and Gaspar, T. (1983).**Peroxidase as a marker of rooting improvement of Cynarascolymus L. cultured in vitro. — *Biochem. Physiol. Pflanzen* 178: 263–271.

**Murashige, T. and Skoog, F. (1962).** A revised medium for rapid growth and bioassays with tobacco tissue cultures. — *Physiol. Plant.* 15: 473–479.

**Patel, K.R. and Thorpe and T.A. (1984).** Histochemical examination of shoot initiation in culture cotyledon explants of Radiata pine. *Botanical Gazzete* 145: 312-322.

**Racusen, D. and Foote, M. (1965).** Protein synthesis grown bean leaves. *Canadian journal of Botany*. 43: 817-824.

**Riguemle, A. and Cardemil, L. (1993).** Peroxidase in the cell wall of seeds and seedlings of Aauracariaaraucanna. *Phytomorphology*. 32: 15-20.

**Rout, G.R., Samantaray, S. and Das, P. (2000).** In vitro rooting of *Psoraleacorylifolia* Linn: peroxidase activity as a marker. — *Plant Growth Regul*. 305: 215– 219.

**Saxena, C., Palai, S.K., Samantaray, S., Rout, G.R. and Das, P. (1997).** Plant regeneration from callus cultures of *Psoraleacorylifolia* Linn. *Plant Growth Regul* 22: 13–17.

**Saxena, C., Rout, G.R. and Das, P. (1998).** Micropropagation of *Psoraleacoryli folia*. *Jour. Medicinal and Aromatic Plant Sci.* 20: 15–18.

**Scandalios, J.G. (1974).** Isoenzymes in development and differentiation. *Annual.Review of Plant Physiology*. 25: 252-258.

**Siegel, B.Z. (1993).** Plant peroxidases — An organismic perspective. — *Plant Growth Regul*. 12: 303–312.

**Siegel, B.Z. and Galston, A.W. (1967).** The Isoperoxidases of *Pisumsativum*. *Plant Physiology*. 42:221-226.

**Šimonovičová, M., Huttová, J., Mistrík, I., Široká, B. and Tamás, L. (2004).** Peroxidase mediated hydrogen peroxide production in barley roots grown under salt stress conditions. — *Plant Growth Regul.* 44: 267–275.

**Smith, R.D. and Walker, J.C. (1996).** Plant protein phosphatases. — *Annu. Rev. Plant Physiol. Plant mol. Biol.* 47: 101–125.

**Soni, J. and Swarnkar, P.L. (1998).** Organ specific peroxidases in *Vignaaconiti folia* under stress condition. *Indian J. Plant Physiology*. 3(4): 296-299.

**Studier, F.W. (1973).** Analysis of bacteriophage T7 early RNAs and proteins on slab gels. *Journal of Molecular Biology*. 79: 237-249.

**Swarnkar, P.L. (1985).** Biochemical studies on morphogenesis and differentiation in tissue cultures.Ph.D.Thesis, University of Rajasthan, Jaipur.

**Swarnkar, P.L., Bohra, S.P. and Chandra, N. (1986).** Biochemical changes during growth and differentiation of the callus of SolanumSurattense. *Journal of Plant Physiology* 126: 75-81.

**Syros, T.D., Yupsanis, T.A., Zafiriadis, H. and Economou, A.S. (2004).** Activity and isoforms of peroxidases, lignin and anatomy, during adventitious rooting in cuttings of *Ebenuscretica* L. — *J. Plant Physiol.* 161: 69–77.

**Syros, T.D., Yupsanis, T.A. and Economou, A.S. (2005).** Expression of peroxidases during seedling growth in *Ebenuscretica* L. as affected by light and temperature treatments. — *Plant Growth Regul*. 46: 143–151.

**Thorpe. T.A., Tran Thanh Van, M. and Gaspar, T. (1978).** Isoperoxidases in epidermal layers of tobacco and changes during organ formation in vitro.*Physiology.Plant.*44:388-394.

**Thorpe. T.A. and Gaspar, T. (1978).** Changes in isoperoxidases during shoot formation in tobacco callus. *In Vitro* 14: 522-526.

**Van Huystee, R.B. (1987).** Some molecular aspects of plant peroxidase biosynthetic studies. *Annual.Review of Plant Physiology*. 38: 205-217.

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