Histochemical localization of starch, protein, lipid and lignin in the callus, field-grown and in vitro raised plants of Scopariadulcis L.

ThusharaT ,Devipriya V

Abstract: *Scopariadulcis*L.(Scrophulariaceae), commonly known as bitter broom or kallurukki, is much valued in tradition medicine to treat respiratory , gastric and hepatic disturbances, kidney stones, diabetes and inflammations. Histochemical localization of starch, protein, lipid and lignin was done in the calli and regenerated plants of *S. dulcis*grown in MS medium supplemented with 0.1 mg/l IBA, and also in the field-grown plants. Starch grains were abundant in the cortex and pith of field-grown plants and also in the calli, while the *in vitro* raised plants had lesser starch content. Intense accumulation of proteins was observed in the calli and *in vitro* raised plants. Both the field-grown and *in vitro* raised plants showed accumulation of lipids in the cortex and pith, and heavy deposition of lignin in the xylem elements. The calli showed a greater deposition of starch, proteins, lipids and lignin, when compared to both the field-grown as well as *in vitro* raised plants. The study reveals the potential of utilizing calli in herbal formulations of the species, as this may yield better results including improved nutraceutical value.

Index Terms:-Histochemical analysis, lignin, callus, *in vitro culture*,Dichlorophenoxyaceticacid,Indole Butyric acid,cytokinin,Benzyl Adenine

INTRODUCTION

In vitro culture system is used for rapid plant multiplication, germplasm conservation, elimination ofpathogens, genetic manipulation and for secondary metabolite production(O'Riordain, 1999). The histochemical analysis of the *in vitro* raised plants further confirms the presence of biomolecules in relation to the field-grown plant. So the economic benefits can be enhanced by cutting down the cost of production per plant by applying low cost tissue culture.Histochemistry is a powerful technique for localization of trace quantities of trace quantities of substances present in biological tissues (Pearse 1988; Krishnamurthy 1998). Histochemical techniques have been employed to characterize structure and development, and to study time course of deposition and distribution of major storage compounds such as protein, lipid, starch, phytin and minerals such as calcium, potassium and iron in rice grains (Krishnan et al.2001; Krishnan and Dayanandan 2003).

1.**Thushara T** *Post Graduate & Research Department of Botany, SreeNarayana College, Kollam, Kerala.*
2. **Devipriya V** *Department of Botany, SreeNarayana College, Chempazhanthy, Thiruvananthapuram, Kerala.*

E-mail: nayadithaa@gmail.com

MATERIALS AND METHODS

The standard MS medium (Murashige and Skoog, 1962) was used for the experiments conducted. Concentrated stock solutions of macronutrients, micronutrients, vitamins and aminoacids were prepared separately in double distilled water and stored in dark colored bottles and kept in refrigerator. Adequate quantities of the different stock solutions were taken mixed together and the final volume was made up by adding double distilled water. 3% sucrose was added to the medium as the carbon source. In addition to basal medium, the callus and plants were regenerated in medium containing three hormones (two auxins- 2,4-Dichlorophenoxyaceticacid,(2,4- D) &Indole Butyric acid(IBA) and one cytokinin – Benzyl Adenine (BA) through the culture of four explants internode, leaf, node and shoot tip. The hormones were prepared in five different concentrations (0.1mg/l,0.5mg/l,1mg/l,3mg/l and 5mg/l)and thehistochemical analysis was done for studying starch, protein, lipid and lignin in callus and regenerated plant for each culture, using the following procedures :**Starch** (Johansen, 1940) : Sections of the sample were placed in iodine-potassium(0.2g of iodine dissolved in 2% potassium iodide solution) for two minutes and rinsed in distilled water. They were then mounted in glycerine jelly, observed and photographed. Starch grains appeared blue-black.**Total Proteins** (Mazia et al., 1953): Sections of the materials were immersed in the dye solution (10g of mercuric chloride and 100mg of bromophenol blue dissolved in 100ml of water) for 15 min. The sections were then washed in 0.5% acetic acid for 20 min to remove the excess dye. They were then washed in water for 15 mi and mounted in glycerin jelly, observed and photographed. Proteins stain blue.**Lipids**(Ruthman., 1970): Sections of thematerial were pretreated with 70% ethanol for 1-2 min

and stained in a freshly filtered solution of Sudan Black B at 60^0 C for one hour. The sections were then rinsed in 70% ethanol for 1 min, washed in water, dried and mounted in glycerine jelly, observed and photographed. Lipids are stained black.

RESULTS AND DISCUSSION

Maximum response was obtained on supplementing the medium with0.1-0.5mg/l IBA. The starch grains stained bluish black with iodine –potassium iodide solutions, and were abundant in the cortex and pith of the field-grown plant. The starch grains occurred not only inside the cortical and pith cells but also in the intercellular spaces. The callus was also observed to be rich in intracellular starch grains. However the *in vitro* raised plants had lesser starch content, compared to field-grown plant. Here the starch grains were observed in the intracellular spaces alone, both in the cortex as well as the pith.

The cellular proteins appeared blue due to mercuric bromophenol staining. The sections taken from the field-grown plant showed accumulation of proteins in the cortex and pith. The callus showed a very heavy deposition of proteins.Intense accumulation of proteins was also observed in the *in vitro* raised plants, especially in regions of vasculature and cortex. Lipids were stained black by Sudan B. The outer cortex and pith of the field-grown plant showed accumulation of lipids. The callus was also abundant in lipids. In the *in vitro* raised plants, there was intense accumulation of lipids in the region of vasculature, middle cortex and pith. The outer cortex did not show heavy lipid deposition.

Lignin appeared magenta- red upon staining with Schiff's reagent. Both the field-grown and *in vitro* raised plants showed heavy deposition of lignin in the xylem elements. The callus also showed a moderate presence of lignin.

Histochemical studies have been relied upon to study deposition and distribution of major storage comounds such as protein, lipid, starch, protein and minerals in plant tissues (Krishnan &Dayanandan 2003).Bose et al (1992) reported the presence of newly synthesized starch bodies and cells of different shapes and sizes in the suspension culture callus cells of *Vigna radiate* based on the Fluorescent microscopic study. The present study also showed abundant intracellular starch grains in callus culture. Bhatnagar*et al.* (21) had reported that regenerated shoots of *Solanumlaciniatum*yielded higher solasodine content than undifferentiated as well as organogenic callus.However, in the present study,the calli showed a greater deposition of starch, proteins, lipids and lignin, when compared to both the field-grown as well as *in vitro* raised plants. The study reveals the potential of utilizing calli in herbal formulations of the species, as this may yield better results including improved nutraceutical value.

Lignin(Mc Lean and cook, 1941): Sections of the material were stained in Schiff's reagent for a few minutes. The sections were then rinsed in water, dried and mounted in glycerine jelly, observed and photographed. Lipids stain pink or magenta.

CONCLUSION

The present study focuses on the histochemical localization of starch, protein, lipid and lignin in callus and *in vitro* raised plants in relation to field grown field-grown plant. The increased accumulation of proteins and lipids in the *in vitro* raised plants and calli highlight the advantages of utilizing *in vitro* techniques for enhanced production or storage of beneficial biocompounds in the living system itself, so that such *in vitro* systems may serve as mines for the continued and large scale production of high quality biomass and valuable phytochemical constituents, using minimal space and resources. The potential of utilizing calli in herbal formulations of the taxon may lead to improved nutraceutical value.

ACKNOWLEDGEMENTS

The authors are thankful to the University of Kerala for financial support and the Principals, SreeNarayana Colleges, Kollam and Chempazhanthy for facilities provided.

References

Krishnan S &Dayanandan P 2003.Structural and histochemical studies on grain filling in the caryopsis of rice.J.Bisci. ***28:****455-469*

Murashige T. &Skoog F. 1962.Arevised medium for rapid growth and bioassays with tobacco tissue cultures.Physiol. Plant, ***15****: 473-497*

O'Riordain F. 1999. Directory of European plant tissue culture laboratories, 1996-97.COST Action, vol.822. Brussels: Commission of the European Communities.

Krishnamurthy K. V.1998. Methods in Plant HIstochemistry.S. Viswanathan, Chennai

Pearse A.G.E. 1988. Histochemistry:TheoreticalansApplied.Vol. 1, 4th ed. Longman, London

*Krishnan S., Ebenenzer G. A. I &Dayanandan P. 2001. Hostochemicallocalisation of storage components in caryopsis of rice (oryzasativa L.)Curr.Sci.****80****:567-571*

*MaziaD.,Brewer P.A. &Alfert M. 1953. The cytochemical staining and measuremet of protein with mercuric bromophenol blue. Biol.Bull.****104****:57-67*

Ruthman.A.C.1970. Methods in Cell Research.Cornell University Press, Ithaca, Newyork.

Mc Lean R.C. & Cook W.R.I1941.Plant Science Formulae.macmillian, London.

Bose M., Sarker R.H.,Hoque M.I &Haque M.M. 1992 . Investigation intothe Possible Causes for Failure of In vitro Regeneration in Mungbean. Plant Tissue cult. ***2****(2) : 81-88*

*Bhatnagar P.,Bhatnagar M., Nath A.K & Sharma D.R. 2004. Production of Solasodine by Solanumlanciniatumusing plant tiisue culture technique .Ind.J.Exp.Biol.****42*** *: 1020-1023*

J.B.Harborne phytochemical method, a guide to modern techniques of plan tanalysis: 3rd ed.1998.Chapman & Hall, Thomson Science, London 151,235

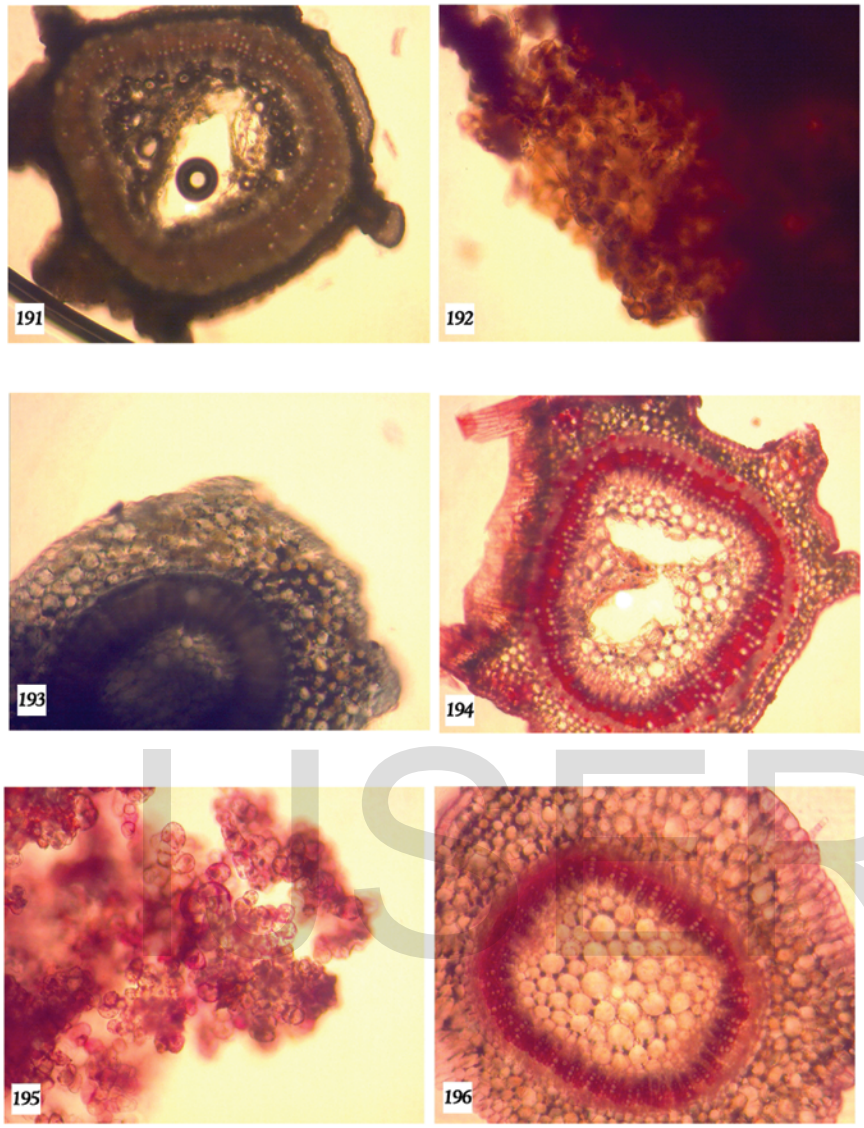


Fig: 191 - Localization of lipids in the cortex and pith of mother plant.

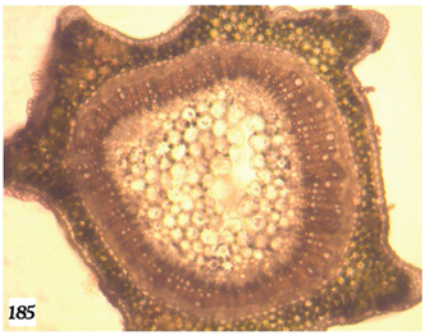
Fig: 192 - Intense accumulation of lipids in the callus.

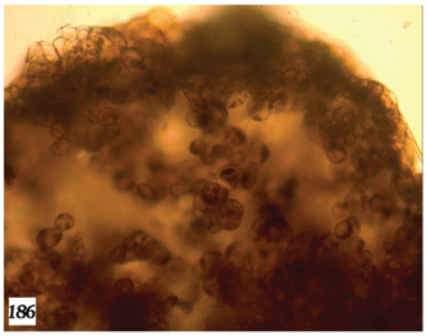
Fig: 193 - Intense accumulation of lipids in the stelar region of the in vitro raised plant.

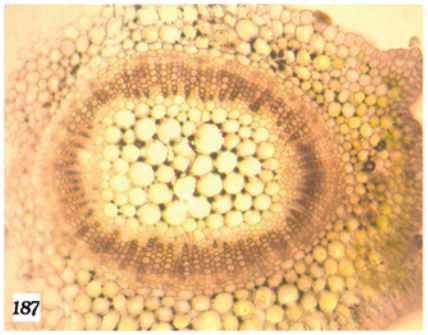
Fig: 194 - Localization of lignin in the xylem vessels of the mother plant.

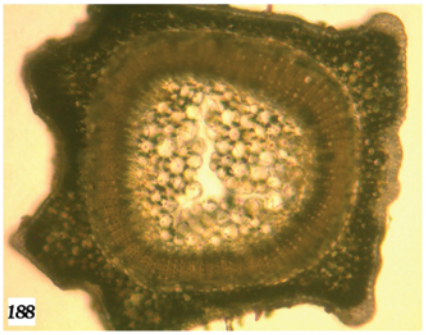
Fig: 195- A section of callus showing the presence of lignin.

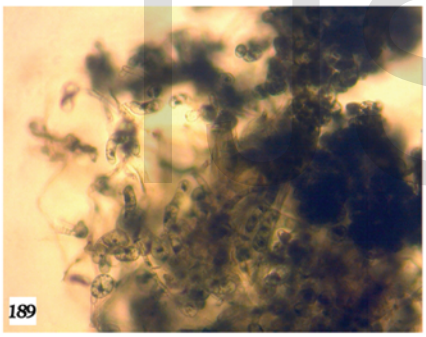
Fig: 196- Localization of lignin in the xylem vessels of the in vitro raised plant.











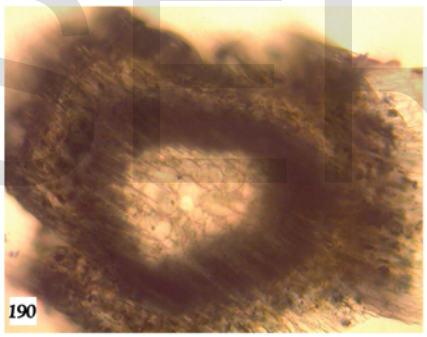


Fig: 185 - Localization of starch grains in the cortex and pith of mother plant.

Fig: 186 - Intense accumulation of starch grains in the callus.

Fig: 187- Localization of starch grains in the in vitro raised plant

Fig: 188- Localization of proteins in the cortex and pith of mother plant.

Fig: 189 - Intense accumulation of proteins in the callus

Fig: 190- Intense accumulation of proteins in the cortex and vascular tissues of the in vitro raised plant.