# Histochemical Studies to Detect the Developmental Stages of Somatic Embryos of Banana cv. Neypoovan

Prathibha K.Y.[1], Geethanjali R.[2], Keshamma. E[3]
[1,2]*Deaprtment of Botany, Maharani's Science College for Women, Bengaluru, Karnataka, India*
[3]*Deaprtment of Biochemistry, Maharani's Science College for Women, Bengaluru, Karnataka, India*

***Abstract*** **- The present study was designed to carry out to evaluate the histochemical changes during the development of somatic embryos from embryogenic callus of male flower buds of banana cv. Neypoovan. The callus of male flower buds was taken at different stages of growth intervals to analyze the parameters for induction of embryogenesis. Histological sections were used for comparison of the histochemical changes occurring during the development of somatic embryos from embryogenic callus of male flower buds of banana cv. Neypoovan. In conclusion, our study findings revealed that the presence of higher amounts of biomolecular substances such as insoluble polysaccharides, proteins and nucleic acids in embryogenic calli and somatic embryos of male flower buds of banana cv. Neypoovan. Furthermore, our study revealed that somatic embryos in banana cv. Neypoovan were formed but failed to germinate into plantlets.**

***Index Terms*** **- Banana cv. Neypoovan, Histochemical changes, Polysaccharides, Protein, Nucleic acids.**

## INTRODUCTION

Banana is perhaps the main food crops on the planet. It is cultivated in excess of 130 nations and is a significant staple yield for a great many individuals in a few developing localities of the world [1]. Banana and plantain involve 10.3 million ha and the total production was assessed at 139 million tons in 2012 [2]. Proliferation through traditional planting materials in banana is moderate paced because of a low number of suckers [3]. On the other hand, rapid production of healthy planting material of wanted clones, inside a brief time frame period, can be worked with by huge scope micropropagation through tissue culture utilizing shoot tips. Despite the fact that few such reports in a banana are accessible, business micropropagation of AAB clones in-vitro is restricted because of helpless augmentation rate when contrasted with AAA clones.

Embryogenic cell suspension (ECS) cultures have been found to display great regeneration response in the distinctive genome bunches in banana. Further cell suspension can bring about enormous scope enlistment of somatic embryo which can be recovered into plants. Micropropagation utilizing banana male flower meristems has likewise been accounted for in before contemplates [4]. In bananas and plantains various sorts of explants, for example, shoot tip [5], zygotic embryos [6], multiplying meristems and scalps [7] and youthful male blossoms [4] have been attempted to create and recover plants from ECS. Of these explants, youthful male blossoms seem, by all accounts, to be the most responsive beginning material for starting ECS and plant recovery. Furthermore, the ECS is the most reasonable material for hereditary control through change [7]. In this association, a comprehension of somatic embryogenesis and the accomplishment in the utilization of biotechnological research can't be accomplished if the morphogenesis cycle isn't all around fathomed. It needs to recognize the cell related with acceptance measure and the arrangement of construction fit for coordinated development and possible advancement into the plant. Somatic embryo induction and progression can be approving utilizing histological and histochemical investigation [8]. With this utilization of the method, it is feasible to assess the development and expansion of calli and suspension by means of somatic embryogenesis [9]. Furthemrore, somatic embryogenesis of banana cultivars of various groups has been effectively accomplished [10-12], nonetheless, the change into plants is as often as

possible low, subsequently restricting its relationship with hereditary change methods. The portrayal of the various phases of the embryogenic cycle can assist with identifying conceivable restricting strides just as to find the embryogenic regions in the explant. This can aid the meaning of methodologies for hereditary control of the material. Hence, the present study was designed to carry out to evaluate the histochemical changes during the development of somatic embryos from embryogenic callus of male flower buds of banana cv. Neypoovan.

## II. METHODS AND MATERIALS

### MATERIALS

The callus of male flower buds were taken at different stages of growth intervals to analyze the parameters for induction of embryogenesis. Embryogenic, non-embryogenic callus and somatic embryos of banana cvs. at successive stages of development were used for the present study.

### HISTOCHEMICAL ANALYSIS

Fixation and killing of the callus were done in FAA (formalin, acetic acid and ethyl alcohol in the proportion of 90:5:5 by volume) for a period of 24 to 48 hours. The fixed material was washed with 70% alcohol and dehydrated using different grades of alcohol such as 70%, 80%, 90% and absolute alcohol for a period of 24 hours in each treatment. They were further dehydrated using ethyl alcohol and n-butanol in the ratio of 3:1, 1;1, 1:3. Paraffin wax of 58-60oC melting point was opted for infiltration and further embedding samples. Thin sections of 10-15 µm thickness were taken with the help of a rotatory microtome. Deparaffinisation is a prerequisite for staining any slide. The slides were deparaffinised using xylene. The deparaffinised sections were subjected to histochemical staining for the localization of different cellular compounds viz., total insoluble polysaccharides, total insoluble proteins and nucleic acids, cytoplasm and nucleus by using standardized protocols and techniques.

## III. RESULTS

Histochemical analysis of embryogenic callus and somatic embryos of banana cv. Neypoovan was done to localize the macromolecules like insoluble polysaccharides, proteins, nucleic acids (Table 1 & 2; Plate 1, 2 & 3).

Table 1: Comparative histochemistry of biomolecules during formation of embryogenic callus from male flower buds of banana cv. Neypoovan

| Biomolecules | Callus cells | Epidermis | Developing proembryos at periphery |
|---|---|---|---|
| DNA | ++ | + | +++ |
| RNA | ++ | + | +++ |
| Total proteins | ++ | + | +++ |
| Total insoluble polysaccharides | ++ | + | +++ |

+++: Intense; ++: Rich; +: Poor

Table 2: Histochemical changes during somatic embryo formation from male flower buds of banana cultivar Neypoovan

| Biomolecules | Globular embryo | Cordate embryo | Mature embryo |
|---|---|---|---|
| DNA | +++ | +++ | ++ |
| RNA | +++ | +++ | ++ |
| Total proteins | +++ | +++ | ++ |
| Total insoluble Polysaccharides | +++ | +++ | ++ |

+++: Intense; ++: Rich; +: Poor

### TOTAL POLYSACCHARIDES

The embryogenic callus and somatic embryos showed intense pink colour at the meristematic zones as well as wall region of the meristemoids. The meristematic zones showed presence of starch in few yellow nodular callus but starch was absent in the meristematic zone of other nodular callus. Some of the non-meristematic parenchymatous regions also showed intense total insoluble polysaccharide. But localization was not uniform in embryogenic callus (plate -1, figure a-d).

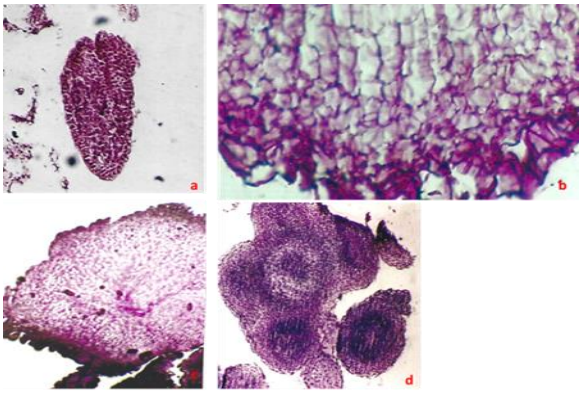

Plate-1

Figs. a-d Sections of somatic embryos stained with per-iodic acid schiffs reagent for localizing insoluble polysaccharides during different developmental stages in banana cv. Neypoovan.

a. Primary globular somatic embryo showing intense accumulation of insoluble polysaccharides.
b. Mitotic cell division in the proliferating somatic embryos showing intense localization of starch.
c. The origin of secondary somatic embryo on epidermal cell of primary somatic embryo.
d. Primary globular somatic embryo showing intense accumulation of insoluble polysaccharides towards periphery and procambial region in the centre.

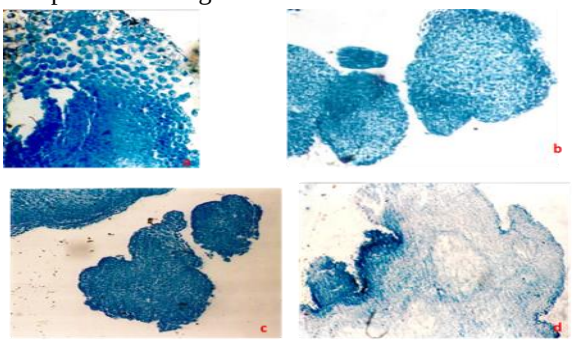

Plate-2

Figs. a-d Sections of embryogenic callus showing origin of proembryos from embryogenic callus, stained with Mercuric Bromophenol Blue for localizing proteins in banana cv. Neypoovan.

a. Intense localization of insoluble proteins in the somatic embryo.
b. Globular and heart shaped epidermised somatic embryos with intense localization of proteins.
c. Intense localization of insoluble protein granules in all the cells of proembryo.
d. Gradual enlargement of proembryos showing insoluble protein accumulation at periphery.

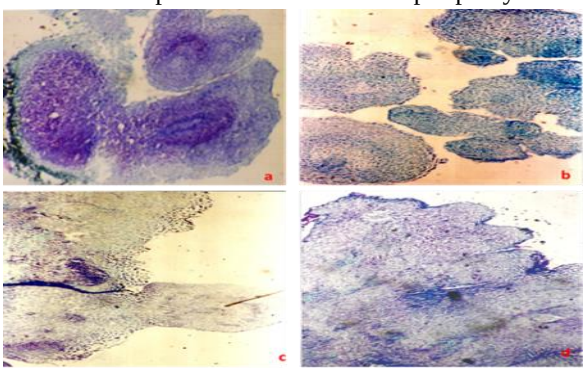

Plate-3

Figs. a-d Sections of somatic embryos showing different stages of development stained with Toluidine blue for localization of nucleic acids in banana cv. Neypoovan.

a. Fused somatic embryos showing provasculature with intense accumulation of RNA and DNA.
b. Somatic embryos of globular and heart shape showing rich localization of RNA and DNA.
c. Somatic embryos showing fused provasculature with Plumule like region.
d. Fused somatic embryogenic cluster showing localization of nucleic acids.

TOTAL PROTEINS

The meristematic embryogenic callus showed intense total insoluble proteins (Plate 2, Figure. a-d). The parenchymatous zone of embryogenic callus and somatic embryos showed intense total insoluble proteins.

NUCLEIC ACIDS

Histochemical analysis of embryogenic callus and somatic embryos showed intense RNA accumulation at the meristematic zones and the parenchymatous regions of embryogenic callus showed good staining for nucleic acids The somatic embryos showed intense staining for nucleic acids (Table 2) (plate-3, figures a-d).

Overall, histochemical observations of embryogenic callus and somatic embryos of banana cv. Neypoovan revealed the intense accumulation of total insoluble polysaccharides, total proteins, and nucleic acids in the embryogenic callus and somatic embryos.

## IV. DISCUSSION

Histochemical localization of insoluble polysaccharides was noted in the fringe locale of embryogenic callus and somatic embryos of male flower buds of banana cv. Neypoovan. Thomas et al believed starch to be a pointer of the improvement of tissue towards somatic embryogenesis [13]. For sure, different works have shown that prior to following organogenesis or embryogenesis the cells orchestrate and store extensive measures of starch [14, 15]. It has been recommended that starch might work as a fuel source during serious meristematic movement or may give osmotica as free dissolvable sugars [16]. Starch

debasement brings about the development of glycolytic intermediates that will along these lines catabolized and yield high measures of ATP [17]. Mikula et al reported that the ultrastructural investigation of embryogenic callus of Gentiana punctata. They noticed distinct accumulation of starch in the fringe region of embryogenic callus which brought about somatic embryos, even in our current work particular starch rich fringe zones in embryogenic callus and somatic embryos were noticed [18]. Dhed'A et al revealed in suspension cultures of banana cv. Bluggoe, starch granules and proteins were seen from single cell to globular and mature phases of substantial undeveloped organisms got from scalp [19]. Notwithstanding, rather than discoveries of our investigation Escalant et al reported absence of starch in the developmental stages and localized presence in the mature embryos [20]. Furthermore, presence of starch in the unicell / multicells which forms somatic embryo has been reported in plants like cork oak [21], sugarcane [22], Cassava [16], highlighting the importance of starch in the ontogeny of somatic embryos. Many reports suggested that starch deposition in the matured somatic embryo is necessary for germination into normal plant as in Norway spruce [23], bamboo [24], in Hevea brazilensis [15].

Neumann reported that a continuous change in the composition of the protein moiety occurs with initiation and termination of protein synthesis of one or other group in a sequential and hierarchical pattern during the induction of somatic embryogenesis in carrot. Similarly, in our work we have noted the presence of protein in embryogenic callus and somatic embryos of banana cv. Neypoovan [25].

According to Misra et al seed storage proteins are the source of amino acids for new proteins needed in germination. Storage proteins are located in protein bodies that may contain amorphic proteins such as enzymes, phytase containing globoids as well as protein crystals [26]. Leal and Misra, considered that accumulation of storage protein to be a marker of zygotic embryo maturation [27]. In embryos, proteins start to accumulate during later stages of rapid growth. Their synthesis declines during desiccations and late embryogenesis abundant (LEA) proteins increases [28]. Presence of proteins in the cells which develop into somatic embryos similar to our work have been reported in cell suspension culture of banana cvs. by number of authors like Dhed'A et al, Escalant et al, Grapin et al [12,19,20]. They have reported that protein rich cells will develop into somatic embryos of banana cvs. Distinct accumulation of proteins at the peripheral meristematic zone, in this study indicate the cells were preparing for cell division and for further growth of somatic embryos in accordance with the report by Michaux-Ferriere et al in Hevea brasiliensis [15].

According to Hu et al, before embryogenic cells were formed the synthesis of RNA was activated first followed with increase of synthesis rates of DNA and protein [29]. The histochemical and ultrastructural properties of meristematic zones during somatic embryogenesis suggest intense RNA synthesis and metabolic activity as stated by Maheshwaran and Williams et al in Trifolium [30]. Similar results were seen in the histochemical sections of embryogenic callus and somatic embryos of banana cv. Neypoovan. In the present histochemical study, RNA localization was seen prominently in the peripheral meristematic zone of embryogenic callus, embryo formation stage showed least localization of RNA. Whereas DNA was seen in embryogenic calli which was in contrast with the findings of Hu, Z. et al. [29]. According to them during formation of globular embryo, DNA synthesis reached peak then the activities of RNA and protein reached the peak. Increased RNA synthesis was also observed ultra-structurally by Mikula et al in Gentiana punctata during somatic embryogenesis [18]. Specific changes in nucleotide biosynthesis during carrot somatic embryogenesis was reported by Claudio Stasolla et al [31].

## V. CONCLUSION

In conclusion, our study findings revealed that the presence of higher amounts of biomolecular substances such as insoluble polysaccharides, proteins and nucleic acids in embryogenic callus and somatic embryos of male flower buds of banana cv. Neypoovan. Furthermore, our study revealed that somatic embryos in banana cv. Neypoovan were formed but failed to germinate into plantlets.

## REFERENCES

[1] Kumar, P. L., Selvarajan, R., Iskra-Caruana, M. L., Chabannes, M., & Hanna, R. (2015). Biology, etiology, and control of virus diseases of banana

and plantain. Advances in virus research, 91, 229-269.

[2] FAO Stat. FAO production statistics for banana and plantain in 2012. 2014.

[3] Sági, L., May, G. D., Remy, S., & Swennen, R. (1998). Recent developments in biotechnological research on bananas (Musa spp.). Biotechnology and Genetic Engineering Reviews, 15(1), 313-328.

[4] Nandhakumar, N., Soorianathasundaram, K., Sudhakar, D., & Kumar, K. K. (2017). Genetic fidelity analysis in the micropropagated banana derived from immature primordial male flower bud.

[5] Kulkarni, V. M., Suprasanna, P., & Bapat, V. A. (2006). Plant regeneration through multiple shoot formation and somatic embryogenesis in a commercially important and endangered Indian banana cv. Rajeli. Current Science, 842-846.

[6] Navarro, C., Escobedo, R. M., & Mayo, A. (1997). In vitro plant regeneration from embryogenic cultures of a diploid and a triploid, Cavendish banana. Plant Cell, Tissue and Organ Culture, 51(1), 17-25.

[7] Tripathi, J. N., Oduor, R. O., & Tripathi, L. (2015). A high-throughput regeneration and transformation platform for production of genetically modified banana. Frontiers in plant science, 6, 1025.

[8] Dai, J. L., Tan, X., Zhan, Y. G., Zhang, Y. Q., Xiao, S., Gao, Y., ... & You, X. L. (2011). Rapid and repetitive plant regeneration of Aralia elata Seem. via somatic embryogenesis. Plant Cell, Tissue and Organ Culture (PCTOC), 104(1), 125-130.

[9] Pauda, M. S., Lima, C. D., Paiva, L. V., Barduche, D., Santos, B. R., & Stein, V. C. (2015). Histological and ultrastructural analysis of the Banana cv. Prata-Anã embryogenic calluses and cell suspension. Revista de Ciências Agrárias Amazonian Journal of Agricultural and Environmental Sciences, 58(2), 168-175.

[10] Novak, F. J., Afza, R., Van Duren, M., Perea-Dallos, M., Conger, B. V., & Xiaolang, T. (1989). Somatic embryogenesis and plant regeneration in suspension cultures of dessert (AA and AAA) and cooking (ABB) bananas (Musa spp.). Bio/Technology, 7(2), 154-159.

[11] Côte, F. X., Domergue, R., Monmarson, S., Schwendiman, J., Teisson, C., & Escalant, J. V. (1996). Embryogenic cell suspensions from the male flower of Musa AAA cv. Grand nain. Physiologia Plantarum, 97(2), 285-290.

[12] Grapin, A., Schwendiman, J., & Teisson, C. (1996). Somatic embryogenesis in plantain banana. In Vitro-Plant, 32(2), 66-71.

[13] Thomas, E., Konar, R. N., & Street, H. E. (1972). The fine structure of the embryogenic callus of Ranunculus sceleratus L. Journal of Cell Science, 11(1), 95-109.

[14] Williams, E. G., & Maheswaran, G. (1986). Somatic embryogenesis: factors influencing coordinated behaviour of cells as an embryogenic group. Annals of Botany, 57(4), 443-462.

[15] Michaux-Ferrière, N., Grout, H., & Carron, M. P. (1992). Origin and Ontogenesis of Somatic Embryos in Heyea Brasiliensis (Euphorbiaceae). American Journal of Botany, 79(2), 174-180.

[16] Stamp, J. A. (1987). Somatic embryogenesis in cassava: the anatomy and morphology of the regeneration process. Annals of Botany, 59(4), 451-459.

[17] Mangat, B. S., Pelekis, M. K., & Cassells, A. C. (1990). Changes in the starch content during organogenesis in in vitro cultured Begonia rex stem explants. Physiologia Plantarum, 79(2), 267-274.

[18] Mikula, A., Tykarska, T. E. R. E. S. A., Zielinska, M., Kuras, M. I. E. C. Z. Y. S. Ł. A. W., & Rybczynski, J. J. (2004). Ultrastructural changes in zygotic embryos of Gentiana punctata L. during callus formation and somatic embryogenesis. Acta Biológica Cracoviensia Series Botánica, 46, 109-120.

[19] Dhed'a, D. B., Dumortier, F., Panis, B., & Vuylsteke, D. (1991). Plant regeneration in cell suspension cultures of the cooking banana cv. Bluggoes'(Musa spp. ABB group).

[20] Escalant, J. V., Teisson, C., & Cote, F. (1994). Amplified somatic embryogenesis from male flowers of triploid banana and plantain cultivars (Musa spp.). In Vitro–Plant, 30(4), 181-186.

[21] Maâtaoui, M. E., Espagnac, H., & Michaux-Ferriere, N. (1990). Histology of callogenesis and somatic embryogenesis induced in stem fragments of cork oak (Quercus suber) cultured in vitro. Annals of Botany, 66(2), 183-190.

[22] Ho, W. J., & Vasil, I. K. (1983). Somatic embryogenesis in sugarcane (Saccharum officinarum L.) I. The morphology and physiology of callus formation and the ontogeny of somatic embryos. Protoplasma, 118(3), 169-180.

[23] Hakman, I. (1993). Embryology in Norway spruce (Picea abies). An analysis of the composition of seed storage proteins and deposition of storage reserves during seed development and somatic embryogenesis. Physiologia Plantarum, 87(2), 148-159.

[24] Godbole, S., Sood, A., Sharma, M., Nagar, P. K., & Ahuja, P. S. (2004). Starch deposition and amylase accumulation during somatic embryogenesis in bamboo (Dendrocalamus hamiltonii). Journal of plant physiology, 161(2), 245-248.

[25] Neumann, K. H. (2000). Some studies on somatic embryogenesis: A tool in plant biotechnology, Inst. für Pflanzenernährung. 43:107-113.

[26] Misra, S., Attree, S. M., Leal, I., & Fowke, L. C. (1993). Effect of abscisic acid, osmoticum, and desiccation on synthesis of storage proteins during the development of white spruce somatic embryos. Annals of Botany, 71(1), 11-22.

[27] Leal, I., & Misra, S. (1993). Molecular cloning and characterization of a legumin-like storage protein cDNA of Douglas fir seeds. Plant molecular biology, 21(4), 709-715.

[28] Han, B., Hughes, D. W., Galau, G. A., Bewley, J. D., & Kermode, A. R. (1997). Changes in late-embryogenesis-abundant (LEA) messenger RNAs and dehydrins during maturation and premature drying of Ricinus communis L. seeds. Planta, 201(1), 27-35.

[29] Hu, Z., Ding, H. B., Wang, X., & Wang, L. S. (1998). A comparative study on the syntheses of DNA, RNA and protein during in vitro organogenesis and somatic embryogenesis of Lycium barbarum L. Shi yan sheng wu xue bao, 31(4), 403-411.

[30] Maheswaran, G., & Williams, E. G. (1985). Origin and development of somatic embryoids formed directly on immature embryos of Trifolium repens in-vitro. Annals of Botany, 56(5), 619-630.

[31] Stasolla, C., Loukanina, N., Ashihara, H., Yeung, E. C., & Thorpe, T. A. (2003). Changes in deoxyribonucleotide biosynthesis during carrot somatic embryogenesis. Plant Physiology and Biochemistry, 41(9), 779-785.