Study of indirect organogenesis of *Coccinia grandis* (L.)Voigt by using Fluorescence and Scanning Electron Microscope

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Abstract- *Coccinia grandis*(L.)Voigt is a well-known dioecious plant of Cucurbitaceae.The plant is used as a raw vegetable as well as in the treatment of many diseases like skin disease, bronchitis, diabetes, etc. Different explants such as internode, node, leaf and shoot apex were used for callus induction. In *vitro* regeneration of this plant was obtained by transferring the explants into Murashige and Skoog's (MS) media supplemented with 3.0% sucrose, 0.8% agar. The PGRs used 2,4D, NAA, BAP and Kin at different combinations and concentrations. Maximum root regeneration was obtained from nodal explants derived callus i.e.,9.2 ± 0.27 in 2.0 mg/l NAA. Maximum shoot bud regeneration from callus i.e, 12.3±0.31 was obtained in 0.1mg/l NAA and 1.0 mg/l BAP. Regeneration of shoot bud and root were confirmed by scanning electron microscope. Histological study of regenerated shoot bud with distinct shoot apex was observed through Fluorescence Microscope.

Keywords: MS; NAA; BAP; KIN; Histology; Dioecious

Abbreviations: MS: Murashige and Skoog; NAA: Naphthalene acetic acid; BAP: 6-Benzylaminopurine; Kin:6-furfurylaminopurine

INTRODUCTION

Coccinia grandis(L.)Voigt is a well-known dioecious climber belonging to the family Cucurbitaceae.The plant is economically important as the fruit is used as a vegetable.The roots, stems are used as herbal medicine especially for the treatment of skin diseases, bronchial catarrh,bronchitis, and diabetes (Kothari et al, 1993). It is also used as substitute of insulin(Chopraet.al.,1958).Antioxidant,antidiabetic,antihelminthic,antiimflammatory,analgesic,antitussive, antimicrobial activities were also reported by several workers (Dewanjii et.al.,2007;Umamaheswari and Chatterjee,2008; Niazi et al,(2009); Pattanayak and Sunita,2009; Saheen et al,(2009)Chandira, 2010)Several reports of *in vitro*culture of this plant have been found by some scientist (Patel et al, 2015; Datta et al, 1987; Chattopadhyay& Sharma, 1992). Gulati (1988) used different explants like hypocotyl cotyledon, internodes, nodes and shoot tips from germinating seedling of *Coccinia grandis*. Josekutty et al., (1993) observed both direct and indirect organogenesis from leaf and nodal explants. Indirect organogenesis from nodal explants was also reported byThiripurasundari and Rao(2012). *In vitro* shoot multiplication from the nodal segment was reported by several workers (Datta et al, 1987; Ganthikumar et al, 2014). Direct shoot multiplication of *C.grandis* by using nodal segment was also reported by Amin et al,(2020);Thasani et al(2022)

The objective of this investigation was to study the regeneration of shoot buds and roots from callus tissue by using SEM and as well as the histology of shoot and apex through fluorescence microscope.

MATERIAL& METHOOD

Material: *Coccinia grandis*

Method: Young fresh leaves were collected from the garden of the Biotechnology dept under The University of Burdwan and young leaves, internode, node, leaf and shoot apex were used as explants. These explants were surface sterilized with 0.1% $HgCl_2$ followed by through washing with distilled water. The explants were dissected and inoculated in the culture medium. The basal medium was MS supplemented with 3 % sucrose and 0.8 % agar. The PGRs used for callus induction were 2,4-D, NAA,BAP,Kinetin at different concentrations and combinations and incubated at 25 ± 2 ºC with a 16 hours photoperiod. For regeneration, BAP/Kinetin as cytokinins and NAA as auxin were used either singly or in combinatrion.

For scanning electron microscope, callus pieces with regenerating shoot buds and roots were fixed in 2 % glutaraldehyde for 2 hours at 4ºC. After passing into a series of different grades of ethanol and isoamyl acetate mixture, CPD and gold coating were done and observed under scanning electron microscope (Hitachi S-530) (Chandra & Bhanja, 2002).

For histological study regenerative callus tissue was fixed in formalin-aceto-alcohol (FAA) for 12 hours at 10ºC followed by dehydration, infiltration and paraffin embedding. The paraffin sections were cut and stained with a fluorochrome, Coriphosphine O and observed under fluorescence microscope (Chandra &Bhanja, 2002).

RESULTS AND DISCUSSION

Callus was induced either singly in 2,4-D and NAA or in combination with BAP or Kinetin. Maximum percentage of callus induction was noted in 2,4D and BAP (1:1).). Regeneration of shoot bud was obtained in different concentrations of BAP, but no response was found in Kinetin singly, regeneration of shoot bud was also noted both in BAP & Kinetin in combination with NAA. Maximum shoot bud i.e., 12.3±0.31 obtained in 1.0 mg/l BAP in combination with 0.1 mg/l NAA (Table 1; Figure. 1 a-e) Rhizogenesis was also noted in different explant-derived callus tissue. Maximum root was obtained in nodal segment derived callus tissue i.e., 9.2±0.27 (Table 2,Fig.f) in 2.0mg/l NAA

Report about callus induction and organogenesis has been found by several workers (Datta et al, 1987, Gultti, 1988, Chattopadhyay & Sharma, 71; Josekutty et al, 1993; Chandra et al, 2002). This result of callus induction corroborates with the report of Chattopadhyay and Sharma (1990). Indirect regeneration from nodal explants has also been reported by Thiripurasundari and Rao(2012), in this report, maximum shoot bud regeneration was achieved in 0.1 mg/l NAA,1.0mg/l BAP and0.5mg/l KIN. In the present investigation, more number of shoot bud (12.3 ± 0.31) was obtained by using only BAP in combination with NAA Regeneration was reported from different explants but no report about the regeneration of shoot bud from shoot apex derived callus tissue was found as found in *C.grandis*.. A similar report was found in *Hanabusaya asiatica* (Kim et al,1996)). In this study maximum shoot bud regeneration was observed in BAP (1.0 mg/l) and NAA (0.1 mg/l).

During SEM study undifferentiated callus tissue showed loose cell mass (Fig. 2 a). Some dome-shaped shoot primordia in the form of protuberances covered with an epidermal layer were also noted (Figure. 2b). These structures were surrounded by numerous trichomes (Fig.2 b& c) which were either unicellular or multicellular (Fig. 2d) Scanning electron microscope revealed that profuse prominent root hairs emerged from a single root (Fig.2 e)

In SEM study of *Coccinia grandis* several dome-shaped shoot primordia covered with epidermal layer and surrounded by both unicellular and pluricellular hairs were observed. This report corroborates with the reports of some workers in *Saintpaulia ionantha* and *Theobroma cacao* (Ohki et al, 1994, Santos et al, 1989).Cuticularised epidermis obtained in *C.grandis*.This was also reported by Mishra and Chaturvedi (1994) in regenerated shoot bud of *Rosmarinus officinalis*

Histological section of differentiated shoot bud derived from callus tissue showed a distinct shoot apex with two leaf primordia (Fig.3). A differentiation of tunica and corpus was distinct while observed under fluorescence microscope by using fluorochrome.

Histological study showed regenerated shoot buds and distinct shoot apex with meristemoids and differentiated leaf primordia. Similarly, the differentiation between tunica and corpus by fluorochrome was also reported in some species of *Nicotiana* (Ronchi, 1981) and *Flacourtia jangomas* by Chandra and Bhanja (2002). Histological observation of Plant regeneration was also reported in *Vigna radiata* by Mendoza et al.,(1993)

Therefore, it can be concluded that in the present investigation the regeneration of *Coccinia grandis* from callus tissue has been established and confirmed by histological and scanning electron microscopical studies.

Table 1: Shoot buds regeneration from callus tissue of *Coccinia grandis*

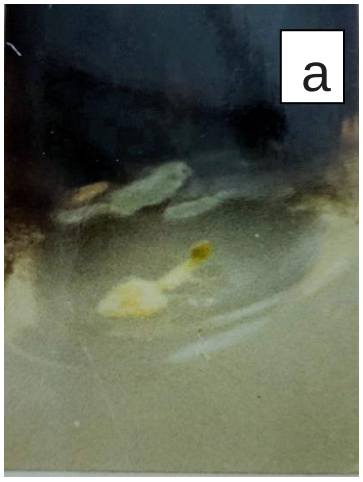
Concentration of PGRs (mg/l)			Number of shoot buds (Mean ± SE)
NAA	BAP	Kin	
--	0.2	--	--
--	0.5	--	4.2 ± 0.20
--	1.0	--	6.0 ± 0.28
--	2.0	--	8.1 ± 0.24
--	5.0	--	6.8 ± 0.21
--	--	0.2	--
--	--	0.5	--
--	--	1.0	--
--	--	2.0	--
--	--	5.0	--
0.1	0.2	--	3.4 ± 0.30
0.1	0.5	--	7.3 ± 0.22
0.1	1.0	--	12.3 ± 0.31
0.1	2.0	--	9.1 ± 0.26
0.1	--	0.2	1.8 ± 0.13
0.1	--	0.5	3.2 ± 0.18
0.1	--	1.0	3.9 ± 0.19
0.1	--	2.0	5.2 ± 0.26

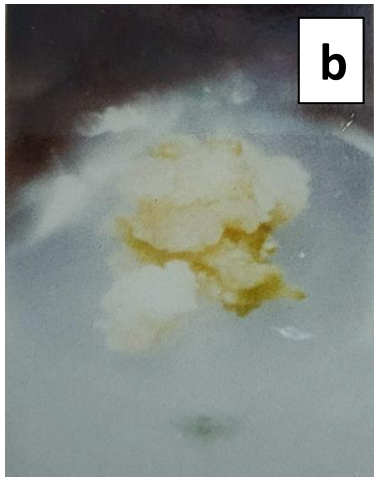
Data represents on the basis of ten replicates each at $p<0.05$

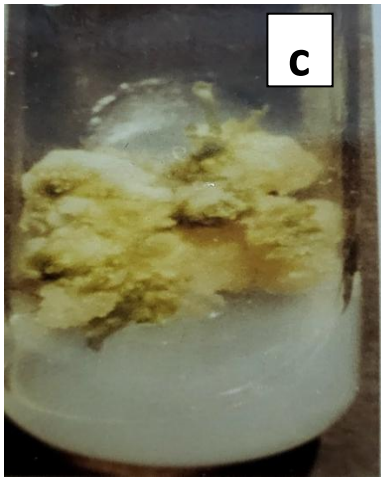
Table2: Rhizogenesis from different explants of *Coccinia grandis*

Concentration of PGRs (mg/l)	Number of the regenerated roots (Mean ± SE)			
NAA	Internode	Leaf	Node	Shoot apex
0.1	--	--	--	--
0.5	1.8 ± 0.11	1.6 ± 0.12	2.6 ± 0.16	2.1 ± 0.17
1.0	3.2 ± 0.16	2.4 ± 0.17	4.4 ± 0.29	3.3 ± 0.20
2.0	7.6 ± 0.26	5.2 ± 0.23	9.2 ± 0.27	8.5 ± 0.34
3.0	4.8 ± 0.24	3.8 ± 0.24	5.6 ± 0.26	5.3 ± 0.26

Data represents on the basis of ten replicates each at $p<0.05$







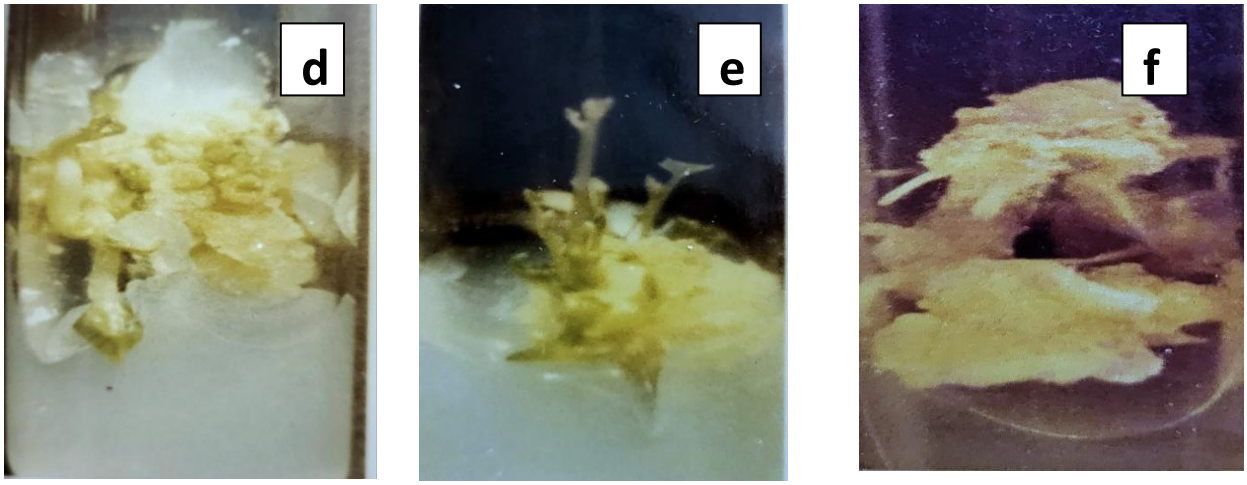


Figure 1 a-b Callus induction from shoot apex explants x3; c- d. Regeneration of shoot buds from callus after 15 & 21 days respectively x 1.3; e. Elongation of few regenerated shoots x1.2; f .Regeneration of root from nodal segment derived callus x1.2

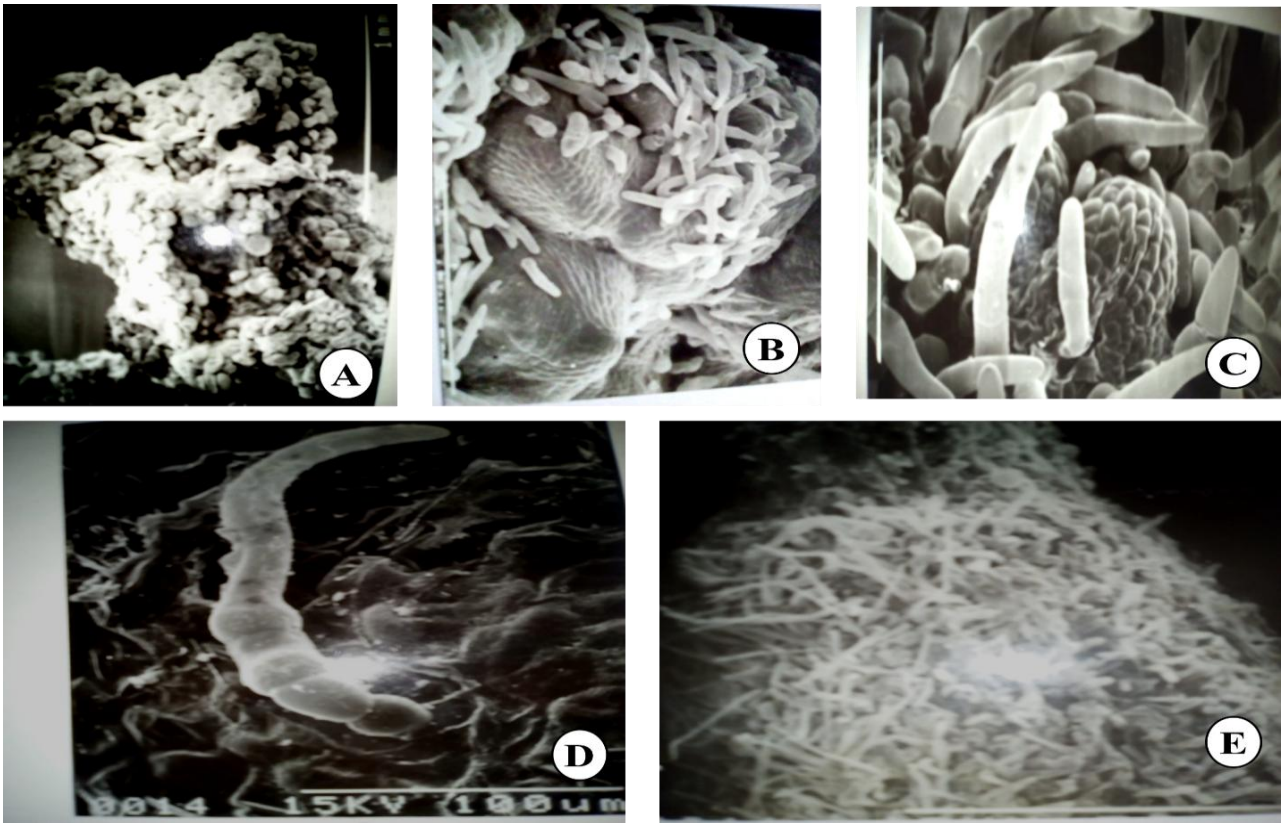


Figure 2a-e .Scanning Electron micrographs : a. *in vitro* grown callus (bar= 1mm), b. Regenerated shoot bud with trichomes from callus (bar= 500 µM) c. Single shoot bud showing distinct epidermis and trichomes (bar= 200 µM) d. Single septate type trichome(bar= 100 µM) e. Regenerated root with profuse root hairs (bar= 200 µM)

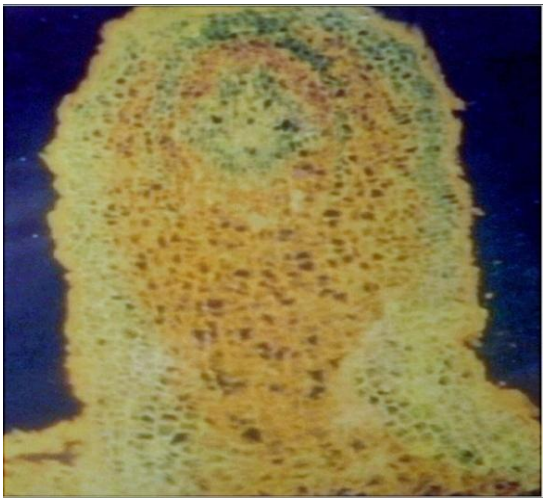


Figure 3 Histological section of LS of a regenerated Shoot bud stained with Coriphosphine O observed Under Fluorescence Microscope (× 190)

ACKNOWLEDGMENT

The author is thankful to UGC, New Delhi for financial assistance. The author gratefully acknowledges Late Prof. P.Bhanja, Ex-Professor, Dept of Botany, The University of Burdwan for his valuable contribution. The author also is thankful to USIC Dept, B.U., and Dr Sumanta Das,Sushobhan Sen and Shibsankar Maiti, Department of Biotechnology, B.U. for their assistance.

Funding
Not applicable.

Conflict of interest
The authors declare that they have no conflict of interest.

REFERENCE

[1] Amin, M., Rao, AVD. D., Kumar, K. R.and Suneetha ,D. R. S.(2020).Culture Establishment of *Coccinia grandis*(L.) through Nodal segments under *in vitro* conditions. Int .J..Curr.Microbio.9(7):2273-2286

[2] Chandira, M. (2010). Studies on anti-stress and free radical scavenging activity of whole plant of *Coccinia indica* Linn.Int.R.J.Pharm.Sci1:50-55

[3] Chandra, I. and Bhanja, P.((2002) Study of organogenesis *in vitro* from callus tissue *Flacourtiajangonas* (Lour.) Raeusch through scanning electron microscopy Current Science 83(4): 476-479.

[4] Chattopadhyay, D. and Sharma, A. K. (1992) Chromosomal variation in Callus tissue in cultures of *Coccinia indica*, a dioecious cucurbit Cytobios, Cambridge Press, Great Britain, 71: 171-180,

[5] Chopra, R. N., Chopra, I. C., Handa, K. L.and Kapur, L.D.(1958)Indigenous drugs of India,2nd ed. ,published byU N Dhar and Sons,Calcutta,:314-316

[6] Datta, K. B., Bag, A .K., De, Sarkar. D. (1987) Auxin-Cytokinin interaction in growth and cytodifferentiation in cultured explants of *Coccinia grandis* (L.) Voigt Cell Chr. Res. 10 (2 & 3) :116-124.

[7] Dewanjii, S., Maiti, A., Kundu, M. and Mandal, S. C.(2007)Evaluation of Antihelminthic activity of crude extract of *Diospyros peregrine*, *Coccinia grandis* and *Schima wallichi* Dhaha University.J.PharmSci.6:121-123

[8] Ghanthikumar, John.,Peter, Arulanandan. and Henr, Joseph. L.(2014) Micropropagation of *Coccina indica*, Wight &Arn.- A medicinal plant, 18:1-4.

[9] Gulati, A. (1988) Tissue Culture of *Coccnia grandis*, *Current Science*, 57 (22): 1232-1235.

[10] Josekutty, P. C., Sha, S. and PrathapaSenan, G. (1993) Direct and Indirect organogenesis in *Coccinia indica.Journal of Hort. Sci*. 68(1): 31-35.

[11] Kim , W .B., Lee, E. A., Choi, K.. S. , Kim, B. H., Kim, J. K. and Lim, H. T.(1996)Plant regeneration from shoot tip of *Hanabusaya asiatica* Nakai, RDA.journal of Agricultural Science Biotechnology38(2):206-211

[12] Kothari, M. J .and Moorthy, S. (1993) *Flora of Raigad District 162 Maharashtra State*, BSI Publishers, Calcutta,.

[13] Mendoza, A.B., Hattori, K.., Nishimura, T. and Futsuhara, Y.(1993)Histological and Scanning electron microscopic observations on plant regeneration in mungbean cotyledon(*Vigna radiata*(L.)Wilczek)cultured *in vitro*. Plant Cell,Tissue Organ Culture 32:137-143

[14] Mishra, P. and Chaturvedi, H. C.(1994), Shoot lead differentiation in leaf segments of *Rosmarinus officinalis* L. *in vitro* histology and scanning electron microscopy**(***Ind. Journal of Exp. Bio*. 32**,:**288-291

[15] Niazi, J., Singh, Parabhdeep., Bunsal, Yogita and Goel, R.K.. (2009) Anti-inflammatory, analgesic and antipyretic activity of aqueous extract of fresh leaves of *Coccinia grandis* Inflammo.Pharmacol.17:239-244

[16] Ohki, S. (1994) Scanning Electron Microscopy of shoot differentiation *in vitro* from leaf explants of an African violet; *Plant, cell, tissue and organ culture*36: 157-162.

[17] Pattanayak, S. P. and Sunita, P. (2009) *In vitro* antitussive activity of *Coccinia grandis* against irritant aerosol and sulfer dioxide–induced cough models in rodants. Bangladesh J. Pharmacol .4:84-87

[18] Patel, A. R. and Ishnava, K. B. (2015), *In vitro*shoot multiplication from nodal explants of *Coccinia grandis* (L.) Voigt and its antidiabetic and antioxidant activity .Asian Journal of Biological Sciences 8:57-71.

[19] Ronchi, V. N. (1981) Histological study of Organogenesis *in vitro* from callus culture of two *Nicotiana* sp. Can. J. Bot**,** 59: 1969-1977.

[20] Saheen, S. Z., Krisna, Bolla., Kandukuri., Vasu. and Singara, Charya. M.A.(2009) Antimicrobial activity of fruit extracts of *Coccinia grandis*. Afri.J.Biotech.8(24):7073-7076

[21] Santos, A.V. P. and Machado, R. (1989), A scanning electron microscope study of *Theobroma cacao* somatic embryogenesis Annals of Botany ,64: 292-296.

[22] Thasani, A., Sarada, S., and Alex, Swapna (2022) Journal of Tropical Agriculture 60(2):222-226

[23] Thiripurasundari, U. and Rao, M.V. (2012). Indirect organogenesis from nodal explants of *Coccina grandis* (L.) Voigt, Indian Journal of Biotechnology,11: 352-354.

[24] Umamaheswari, M. and Chatterjee, T. K. (2008) *In vitro* antioxidant activities of the fractions of *Coccinia grandis*L. leaf extract. Afri.J.Trad.CAM 5(1) : 61-73