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Callus formation from different explants of *Chlorophytum borivilianum* (Safed musli)

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SUMMARY

Stem disc, shoot bud, root disc and seed of *Chlorophytum borivilianum* Santapaw and Fernandes (Safed Musli) were cultured on different MS basal media having different concentrations and combinations of auxins (IAA, NAA and IBA) and cytokinins (BAP and KIN). The different cultured explants showed variation in establishment of swelling and callus formation. Besides explants, these responses were also found to be media dependent. The formed calli were largely friable and of yellow green colour suggesting their undifferentiated nature and indicating the possibility of induction of somaclonal variations. Further, shoot differentiation was also observed from different explants. The work showed the possibility of the use of somaclonal variation in the improvement of this important medicinal plant.

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Key words : *Chlorophytum borivilianum*, Tissue culture,Callus

Chlorophytum borivilianum Santapau and Fernandes is an important medicinal plant, which is grown for its white tubers, commonly known as Safed musli. Safed musli is one of the most important drugs in Indian system of medicine particularly for its aphrodisiac and sex tonic properties (Ram and Pandey, 2004). It is an integral part of more than 100 Ayurvedic formulations (Singh *et al.,* 2004). The medicinal properties of Safed musli are due to presence of saponins, which are found in the tuberous roots. These medicinal saponins have their highest content in the plants grown under natural environment found in forest. Under artificial cultivation the saponin content declines. Plant tissue and cell cultures technologies are seen as a tool for channelizing the resources of nature for benefit of mankind by conservation of elite, endangered plants and ecofriendly production of drugs and drug intermediates. Improved cell and tissue culture technology would help in producing the active compounds *in vitro* without cutting down the natural resources (Heble, 1993). Callus cells show lots of variations, many of which can be selected and used for the improvement of the plant. These cells can also be selected for their ability to produce higher concentration of saponins. Growing such selected cell lines in large fermenters will help in production of these medicinally important saponins on an industrial scale without harvesting and killing the plants and will help in saving the plant from being extinct. The callus formation from different explants particularly the root disc and identification of cell lines having capacities to produce higher amount of saponin will help in industrial exploitation of this important medicinal species without harvesting and killing of the plants of *Chlorophytum borivilianum.*

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MATERIALS AND METHODS

Stem disc, shoot bud, root disc and seeds of *Chlorophytum borivilianum* were used as explants for tissue culture studies. These explants were washed and pretreated in a mixture solution of 0.1% streptomycin and 0.1% bavestin for 30 minutes. The pretreated explants were surface sterilized with 0.2% $HgCl_2$ solution for 5 to 10 minutes. The sterile explants were inoculated on different MS media having different concentrations and combinations of auxins (IAA, NAA, IBA) and cytokinins (BAP and KIN).The culture were incubated at 25±2^{0}C under continuous fluorescent light of 1 k lux.

RESULTS AND DISCUSSION

The findings of the present study as well as relevant discussion have been summarized under following heads:

Selection of explant:

Explant plays an important role in the success of plant tissue culture. Explants with young and meristematic tissues are more responsive than those with mature and differentiated tissues (De Bruyn, 1997). The stem is reduced to the stem disc in *Chlorophytum borivilianum* . These stem disc on germination give rise to shoot buds. Therefore, both the stem disc and shoot buds have large portion of juvenile and meristematic tissues, which make them more responsive in tissue culture particularly for callus formation (Purohit *et al.,* 1994; Joshi *et al.,*1999 and Pudake and Dhumale , 2003).Seed and seedling also have large amount of juvenile tissues and are good for callus formation and other responses during tissue culture (Gaikwad *et al.,* 2003). Storage tuberous root disc mainly consists of a layer of uniseriate epidermal cells followed by a large zone of cortex. Both the epidermal cell and cortical cell are good originating point for the formation of callus (Gaikwad *et al.,* 2003). Thus, stem disc, shoot bud, root disc and seed were selected as explant for callus formation in *Chlorophytum borivilianum*.

Selection of medium:

The four selected explants stem disc, shoot bud, root disc, and seed were cultured on twelve different MS media having different combinations and concentrations of auxins NAA, IAA and IBA and cytokinins KIN and BAP. Response of these explants were observed only on two media MS + 5.37 ìM NAA + 4.65 ìM KIN (M_1) and MS +2.68 ìM NAA + 4.65 ìM KIN (M_2) on which there were callus formation from the explants.

Among the two selected media, medium M_1 was slightly better than the medium M_2. The superiourity of medium M_1 for callus formation was mainly due to higher concentration of auxin in it. Callus formation from cultured stem disc of *Chlorophytum borivilianum* on a medium containing an auxin (2,4-D) and a cytokinin (BAP) at lower concentration(Joshi *et al.,* 1999). However, callus formation obtained on a medium with higher concentrations of NAA irrespective to the concentration of cytokinin in the media (Pudake and Dhumale , 2003).

Establishment and swelling of the explants:

The establishments of aseptic culture of the four explants were followed by their swelling. For the establishment of the explant and their swelling stem disc showed the best response followed by root disc, seed and shoot bud, respectively. The two media M_1 and M_2 showed more or less similar response for the establishment and swelling of the explant. The swelling of the explant was observed the earliest in cultured shoot bud (after 2 to 3 days), followed by root disc (after 4 to 6 days), stem disc (after 5 to 7 days) and seed (after 8 to 10 days). Swelling was more prominent at the basal region of the explant compared to the apical region in case of stem disc and root disc, while in case of shoot bud and seed entire explant swelled. The swelling of explant was the first response observed during tissue culture. It was largely due to imbibition of water from the medium and the result of cell elongation and initiation of the cell division of the explants. The cultured stem disc and root disc had more dry matter compared to other explants, which resulted in their basal regions imbibing more water from the medium and thus showing more swelling. Further, the concentrations of the phytohormones were also high in their basal regions, which resulted in more cell divisions leading to more swelling.

Callus formation:

Callus formation was observed after differentiation of shoots from the cultured stem disc, shoot bud, root disc and seed of *Chlorophytum borivilianum* on only

Table 1 : Callus formation from different explants of *Chlorophytum borivilianum*.

Explants	Medium	% cultures showing establishment and swelling of explant	Callus formation			
			% cultures showing callus formation	Nature of callus	Colour of callus	Callus growth
Stem disc	M_1	95.23	38.09	Friable + compact	Yellow green	+++
	M_2	90.47	28.57	Friable + compact	Green yellow	++
Shoot bud	M_1	50.00	31.81	Friable	Yellow	++
	M_2	47.36	26.31	Friable	Yellow green	+
Root disc	M_1	86.95	17.39	Friable	Green	+++
	M_2	86.30	23.80	Friable + compact	Green	++
Seed	M_1	80.00	30.23	Friable + compact	Yellow	++
	M_2	82.60	31.22	Friable + compact	Green yellow	+++

two media M_1 and M_2. The frequency of callus formation ranged from the highest 38.09 per cent of cultured stem disc on medium M_1 to the lowest 17.39 per cent of cultured root disc on medium M_1 (Table 1).

Callus, which is a mass of undifferentiated parenchymatous cells formed by continuous cell division, is generally formed by the influence of phytohormone content of the medium as well as the internal phytohormone content of the explant. The transformation of the cultured explant into a proliferating mass of callus is dependent on the change in basic architectural pattern of the explant by cell division. Loss of certain cell types, development of new types and quiescent cell becoming metabolically active, are some changes that result in callus formation (Thorpe,1980 and Wagle *et al.,* 1987). Callus, generally considered as aberrant in tissue culture, however, is important as store house of variations. The variations of callus cells, when taken upto regenerated plants are known as somaclonal variation and had been used in the improvement of many plants. The main advantages of such somaclonal variants are, the high frequency with which such variants occur and the possibility of obtaining variation for resistance against biotic and abiotic stresses, useful agronomic traits and in improvement of the overall quality and property of the plant including medicinal characteristics (Evans,1989 and Heinz *et al.*, 1977) .

REFERENCES

De Bruyn, M.H. (1997). Micropropagation of *Amaryllis*. In: *Biotechnology in agriculture and forestry,* Vol. 40 (Ed. Bajaj YPS). Springer Verlog, Berlin, Heidelberg, pp. 3-31.

Evans, D.A. (1989). Somaclonal variation -genetic basis and breeding applications. *Trends Genet.*, **5** : 46-50.

Gaikwad, P.D., John, S.C. and Billore, M. (2003). Callus induction and regeneration studies in Safed musli (*Chlorophytum borivilianum* Sant and Fern). *Res. Crops*, **4** (2) : 249-253.

Heble, M.R. (1993). High value chemicals by tissue culture Chem..Ind Digest, 3rd Quarter: 113-118.

Heinz, D.J., Krishnamurthy, M., Nickell, L.G. and Muretzki, A. (1977). Cell tissue and organ culture in sugarcane improvement. In: *Applied and fundamental aspects of plant cell, tissue and organ culture* (Eds. Reinert J and Bajaj YPS). Springer- Verlog, Berlin, pp. 1-17.

Joshi, A, Ganesh, R, Sharma, A. and Sharma, G.S. (1999). Plant regeneration from stem-disc derived callus cultures of *Chlorophytum borivilianum* L. *Ann. Plant Physiol.*, **13**(1):79-83.

Pudake, R.N. and Dhumale, DB. (2003). *In vitro* multiplication of *Chlorophytum borivilianum* sant. and fern. *J. Maharashtra agric. Univ.*, **28**(3):265-267.

Purohit, S.D., Dave, A. and Kukda, G. (1994). Micropropagation of safed musli (*Chlorophytum borivilianum*) a rare. Indian medicinal herb. *Pant Cell Tissue & Organ Culture,* **39**(1):93-96

Ram, B. and Pandey, S.T. (2004). Safed musli: Emerging as a miracle crop in India. *Indian Farmers Digest,* **37**: 20-21.

Singh, Aparval, Singh, Saudan, Patra, D.D., Singh, Man, Arya, S.J.K. and Khanuja, S.P.S. (2004). Cultivation and processing technologies of Safed musli (*Chlorophytum borivilianum). J. Medicinal & Aromatic Plant Sci.*, **26** (1):70-76.

Thorpe, T.A. (1980). Organogenesis in vitro: structural physiological and biochemical aspects: *Internat. Rev. Cytol. (Suppl.)*, **11**:71-112.

Wagle, L.M., Gladfelter, H.J. and Phillips, G.C. (1987). *De novo* shoot organogenesis of *Pinus eldarica* Medw. *in vitro* II. Macro and micro photographic evidence of *de novo* regeneration. *Plant Cell Reports*, **6**:167-171.

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