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Adventitious shoot differentiation from cultured stem disc, shoot bud and inflorescence explants of *Chlorophytum borivilianum* L.

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SUMMARY

Stem disc, shoot bud and inflorescence explants of *Chlorophytum borivilianum* Santapau and Fernandes (Safed musli), an important medicinal plant, were cultured on different MS media having different concentrations and combinations of auxins (IAA, NAA and IBA) and cytokinins (BAP and KIN). The different explants behaved differently on different media for the establishment of aseptic culture, their swelling and differentiation of shoots from them. The best explant and the best media for different responses were identified. Further, the development of roots from the base of *in vitro* developed shoots and acclimatization of plantlets resulted in development of an efficient protocol of the micropropagation of this important medicinal plant.

Key words : *Chlorophytum borivilianum*, Tissue culture, Shoot differentiation

Chlorophytum borivilianum L. Sontapau and Fernandes, a medicinal plant, commonly known as Safed musli because it yields milky white tubers on processing that contain saponins , responsible for its medicinal properties. Safed musli is one of the most important drug in Indian systems of medicine namely Ayurveda, Unani and Siddha due to its aphrodisiac and sex tonic properties. It is an integral part of more than 100 Ayurvedic formulations (Singh *et al.*, 2004). The natural availability of the plant is continuously decreasing due to its heavy demand and it faces extinction. To save the plant in its natural habitat, its cultivation is essential. Initiation and expansion of Safed musli cultivation will require substantial amount of quality propagules. Safed musli (*Chlorophytum borivilianum* L.) is generally propagated by seeds as well as vegetative propagules. Seed propagation has not become popular because of poor seed germination (Bordia *et al.*,1995) inferior quality of tuber in comparison to vegetatively propagated plants and seed plants taking longer period for maturity. Vegetative propagation through stem disc is better than seed propagation, but the method is costly and labour intensive.

Tissue culture or *in vitro* technique provides an alternative vegetative propagation method known as micropropagation. Micropropagation can lead to production of very large number of plants in relatively short time and space from a single mother plant. They are normally disease free, genetically uniform and show higher yield. To maintain genetic uniformity in micropropagated plants, they are multiplied through enhanced axillary branch formation. However, the plant of *Chlorophytum borivilianum* lacks a true stem and associated axillary branches. Therefore, the next best way of micropropagating these plants is through adventitious shoot differentiation or caulogenesis. The different explants can be exploited in culture to induce caulogenesis for the micropropagation of *Chlorophytum borivilianum* L.

MATERIALS AND METHODS

Stem disc, shoot bud and inflorescence of *Chlorophytum borivilianum* were used as explants for tissue culture experiment. 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% mercuric chloride solution for 5 to 15 minutes. The sterile explants were inoculated on different MS media having different concentrations and combinations of auxins (IAA, NAA and IBA) and cytokinins (BAP and KIN) under laminar flow. The cultures were incubated at 25±2^{0}C under continuous fluorescent light of 1 K lux.

RESULTS AND DISCUSSION

The results obtained from the present investigation are presented below:

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Selection of explant:

Selection of explants is an important aspect for the success of tissue culture and depended upon the objective of the experiment. Nodal stem and shoot apex are the two most favored explants for enhanced axillary bud formation for the micropropagation of plant. Stem disc is equivalent to nodal stem in case of *Chlorophytum borivilianum*. These stem discs on germination give rise to shoot buds. Therefore, both the stem discs and shoot buds have large portion of juvenile and meristematic tissues which make them more responsive in tissue culture (Joshi *et al.,* 1999; Pudake and Dhumale, 2003; Purohit *et al.,* 1994). Inflorescence, as explant gives response when other explants fail, because it contains a heterogeneous mixture of many tissues and cells. In case of *Chlorophytum borivilianum*, they showed less problem of contamination compared to the most used explant stem disc. Their morphogenetic potential were also good as found in many plants (Coumano- Gilles *et al.,* 1981; Zhong, 1989 and Zhong, 1990). They have good potential for caulogenesis also (Cuenca *et al.,* 1999).

Inflorescence has been used for tissue culture study and caulogenesis of *Chlorophytum borivilianum* (Sharma and Mohan, 2006a; Sharma and Mohan, 2006b; Samantaray *et al.,* 2009). Thus stem disc, shoot bud and inflorescence were selected as explant for caulogenesis in *Chlorophytum borivilianum.*

Selection of medium:

Selection of appropriate medium is the most important factor for the success of tissue culture experiment (Murashige and Skoog, 1962). Murashige and Skoog medium was used as the basal medium, because it is the most commonly used medium (Patel *et al*., 2001) . The three selected explants stem disc, shoot bud and inflorescence were cultured on different MS media having different combinations and concentrations of auxins (IAA, NAA and IBA) and cytokinins (KIN and BAP). The cultured stem disc differentiated adventitious shoot on twelve media (M_1-M_{12}), while shoot buds differentiated adventitious shoots on seven media namely M_3 , M_4 M_5, M_7, M_8, M_{10} and M_{12}. The cultured inflorescence could show adventitious shoot formation only on four media namely M_4, M_5, M_8 and M_{11}.

Establishment and swelling of explant:

The establishment of aseptic culture of all the three explants was followed by their swelling. Swelling was more prominent at the basal region compared to the apical region in case of cultured stem discs. It was reverse in case of inflorescence, where apical regions showed more swelling compared to the basal region. The entire explant swelled in case of shoot buds. Cultured stem discs had more dry matter compared to the other explants, which resulted in their basal regions imbibing more water from the medium and thus showing more swelling. Further, the concentration of phytohormones was also high in their basal region, which often resulted in more cell division leading to more swelling. The cultured inflorescence had immature flower buds with large number of growing regions in the apical portion with a capability of more growth, which is reflected by more swelling in upper region of the explant. The cultured shoot bud, which is generally a hydrated structure showed less but uniform swelling from its entire surface.

Swelling took place after 4 to 7 days of culture in case of stem disc. The highest frequency of swelled stem discs was observed on medium M_7 (95.23%) and the lowest on medium M_{10} (86.36%) (Table 1). In shoot bud, swelling took place after 2 to 3 days of the culture. The frequency of swelling was the maximum on medium M_{12} (60.00%) and the minimum on medium M_4 (42.85%) (Table 2). In inflorescence, swelling took place after 2 to 6 days of culture. Swelling was observed both in floral buds and axis of the inflorescence. The frequency of swelled inflorescence explant was found in between 40-50% (Table 3)

Caulogenesis:

The differentiation of shoots, called caulogenesis, was the first organogenesis observed from the cultured explants of *Chlorophytum borivilianum* L. The differentiation of shoots always occurred directly on the explant. Adventitious shoots were differentiated from the cultured stem disc on twelve media, from cultured shoot bud on seven media and from cultured inflorescence on four media. Shoot differentiation always took place directly from the cultured explant mainly from the swelled region. Shoots were differentiated at earliest (after 6 to 8 days) from cultured shoot buds, followed by stem disc (after 15 to 25 days) and inflorescence (after 20 to 26 days), respectively. Medium M_5 supported shoot differentiation the earliest, while the medium M_8 showed the latest shoot differentiation.

The frequency of shoot differentiation from cultured stem disc ranged from 86.95% on medium M_5 to 45.45% on medium M_9. The mean number of differentiated shoots per culture ranged from 9.88 on medium$_{11}$ to 4.3 on medium M_9 (Table 1). For the cultured shoot buds also the medium M_5 showed the best shoot differentiation with a frequency of 91.3 per cent and 11.47, the mean number of differentiated shoots per culture. The minimum shoot

Table 1 : Stem disc culture of *Chlorophytum borivilianum* L. on MS media supplemented with different concentrations of phytohormones

Media	Response				
	Swelling	Caulogenesis			
	% culture showing swelling	% culture showing caulogenesis	No. of shoot/culture		Shoot growth
			Mean	Range	
M_1 (MS+2.22 µ M BAP)	90.90	68.18	5.73	5-7	++
M_2 (MS+4.44 µ M BAP)	90.47	71.42	6.33	6-7	++
M_3 (MS+6.66 µM BAP)	90.00	70.00	7.0	6-9	+++
M_4 (MS+8.88 µM BAP)	90.00	80.00	6.85	6-8	+++
M_5 (MS+11.11 µM BAP)	91.30	86.95	9.7	8-11	++++
M_6 (MS+2.68 µM NAA + 2.32 µM KIN)	90.00	55.00	5.36	5-6	+
M_7 (MS+5.37 µM NAA + 4.65 µ M KIN)	95.23	61.90	7.61	7-9	++
M_8 (MS+2.68µM NAA + 4.65 µM KIN)	90.47	57.14	6.58	6-7	++
M_9 (MS+5.37 µM NAA + 2.32 µM KIN)	90.00	45.45	4.3	4-5	+
M_{10} (MS+5.71 µM IAA+4.44 µM BAP + 4.65 µM KIN)	86.36	81.81	7.61	6-9	+++
M_{11} (MS+0.57 µM IAA + 4.44 µM BAP + 4.65 µM KIN)	90.00	85.00	9.88	8-11	++++
M_{12} (MS+2.46 µM IBA)	89.47	68.42	6.38	6-7	++

differentiation with a frequency of 68.1 per cent was found on medium M_7 but for the number of shoots per culture medium M_8 showed the minimum number 5.42 (Table 2). The best frequency of shoot differentiation from cultured inflorescence was found on medium M_8, but the best number of shoots per culture (6.87) was found on medium M_5. The four media on which shoots were formed from the cultured inflorescence showed similar responses (Table 3).

Shoot bud explant was the best followed by stem disc and inflorescence for the differentiation of shoots. Young shoot buds consisted of more juvenile tissues and thus were more responsive for tissue culture responses including caulogenesis (Pudake and Dhumale, 2003; Purohit *et al.*,1994). They also found shoot buds more responsive than other explants for caulogenesis in *Chlorophytum borivilianum*. Inflorescence was the other explant, besides stem disc and shoot bud, that differentiated shoots in culture in *Chlorophytum borivilianum*. The response of inflorescence explant for caulogenesis was lowest and was around 40 per cent. The use of inflorescence explant has shown good results for shoot differentiation and ultimately micropropagation in many plants (Dave *et al.*, 2004). The other advantages of inflorescence explant were non-destruction of mother plant, less contamination and presence of more juvenile tissues. Shoot regeneration was obtained from cultured inflorescence explant. The immature unopened floral buds of the inflorescence explant were converted into vegetative shoots (Sharma and Mohan, 2006a; Samantaray *et al.*, 2009). They also got low frequency of caulogenesis from cultured inflorescence explants as found in the present investigation.

The medium M_5 supported the best shoot differentiation followed by medium M_{11}, medium M_{10} and medium M_4. All these media had either only cytokinin or a higher concentration of cytokinin compared to that of auxin. Pudake and Dhumale (2003) found the best *in*

Table 2 : Shoot bud culture of *Chlorophytum borivilianum* L. on MS media supplemented with different concentrations of phytohormones

Media	Response				
	Swelling	Caulogenesis			
	% culture showing swelling	% culture showing caulogenesis	No. of shoot/culture		Shoot growth
			Mean	Range	
M_3 (MS+6.66 µM BAP)	47.61	76.19	8.37	7-10	+++
M_4 (MS+8.88 µM BAP)	42.85	80.95	9.11	8-10	+++
M_5 (MS+11.11 µM BAP)	52.77	91.30	11.47	10-13	++++
M_7 (MS+5.37 µM NAA + 4.65 µ M KIN)	50.00	68.18	7.86	7-9	++
M_8 (MS+2.68µM NAA + 4.65 µM KIN)	47.36	73.68	5.42	5-6	++
M_{10} (MS+5.71 µM IAA+4.44 µM BAP + 4.65 µM KIN)	50.00	85.00	9.23	8-11	+++
M_{12} (MS+2.46 µM IBA)	60.00	75.00	6.20	5-7	++

Table 3 : Inflorescence culture of *Chlorophytum borivilianum* L. on MS media supplemented with different concentration of BAP, NAA, KIN and IAA

Media	Response				
	Swelling	Caulogenesis			
	% culture showing swelling	% culture showing caulogenesis	No. of shoot/culture Mean	Range	Shoot growth
M_4 (MS+8.88 µM BAP)	40.50	35.00	3.42	3-4	++
M_5 (MS+11.11 µM BAP)	45.45	36.36	6.87	6-8	+++
M_8 (MS+2.68µM NAA + 4.65 µM KIN)	47.82	43.47	4.3	3-6	+++
M_{11} (MS+0.57 µM IAA + 4.44 µM BAP + 4.65 µM KIN)	42.42	33.33	5.5	5-6	+

vitro shoot differentiation on a medium with slightly lower concentration of BAP (2mg/litre) compared to the present observation of the best caulogenesis on medium M_5. However, Purohit *et al. (*1994) got the best caulogenesis on a medium containing higher concentration (22.2 µM) of BAP. All these workers got multiple shoot formation without any callus on these media with higher concentration of BAP as found in present investigation. Good caulogenesis was achieved on MS medium supplemented with only BAP (Sharma and Mohan, 2006a ;Sharma and Mohan 2006b). Similar, to the observation of good caulogenesis on media M_{10} and M_{11}, a combination of both cytokinin BAP and kinetin was found effective for caulogenesis (Purohit *et al.,*1994) . The only difference was the absence of auxin in the medium used by them compared to the present investigation. The best caulogenesis was achieved on MS medium supplemented with kinetin with 2,4-D (Sharma and Mohan, 2006a). The addition of NAA or IAA at lower concentrations along with BAP in the medium improved shoot differentiation (Purohit *et al.,*1994 and Samantaray *et al.,* 2009).

Thus, adventitious shoots were differentiated through caulogenesis in good numbers and good frequencies from the three cultured explants namely stem disc, shoot bud and inflorescence of *Chlorophytum borivilianum*. The subsequent development of roots from their base, which actually resulted on MS medium supplemented with 2.46 µM IBA, resulted in the development of an efficient protocol of the micropropagation of the plant.

REFERENCES

Bordia, P.C., Joshi, A. and Simlot, M.M. (1995). Safed musli, In: *Medicinal and Aromatic Plants* (Eds. K.L. Chadha and R. Gupta). Advances in Horticulture, Malhotra Publishing House, New Delhi, **2**: 429-451.

Coumano- Gilles, MF. Kevers,C., Coumans, M., Ceulemans, E. and Gasper, T. (1981). Vegetative multiplication of sugar beet through *in vitro* culture of inflorescence pieces, *Plant Cell Tissue & Organ. Culture*, **1**:93.

Cuenca, S., Amo–Marco, J.B. and Parra, R. (1999). Micropropagation from inflorescence stems of the Spanish endemic plant *Centcurea Paui* Loscos ex Willk (Compositae), *Plant Cell Reports*, **18**: 674.

Dave, A., Joshi, N. and Purohit, S.D. (2004). *In vitro* propagation of *Chlorophytum borivilianum* L. using encapsulated shoot buds. *European J. Hort. Sci*., **69**(1):37-42.

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.

Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissues cultures. *Physiol. Plant*, **15**:431-497.

Patel, A.A., Patel, S.R., Patel, C.L. and Prajapati, B.S. (2001). Effect of media composition on *in vitro* multiplication of sugarcane varieties. *Indian J. Genet.* , **61**:82-83.

Pierik, R.L.M. (1987). *In vitro Culture of Higher Plants*. Martinus Nijhoff, Dor dreehet, pp. 45-82.

Pudake, R.N. and Dhumale, D.B. (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* L.) a rare. Indian medicinal herb. *Plant Cell Tissue & Organ Culture*, **39**(1):93-96.

Sharma, Urvashi and Mohan, J.S.S. (2006a). *In vitro* clonal propagation of *Chlorophytum borivilianum* Sant and Fernand a rare medicinal herb from immature floral buds along with inflorescence axis. *Indian J. Experimental Biol.*, **44**: 77-82.

Sharma Urvashi and Mohan JSS. 2006b. Reduction of vitrification in *in vitro* raised shoots of *Chlorophytum borivilianum* L. Sant and Fernand, a rare potent medicinal herb. *Indian J. Experimental Biol.*, **44** : 499-505.

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* L.). *J. Medicinal & Aromatic Plant Sci.*, **26**(1):70-76.

Samantaray, Sanghamitra , Kumar, S.V. and Maiti, S. (2009). Direct shoot regeneration from immature inflorescence cultures of *Chlorophytum arundinaceum* and *Chlorophytum borivilianum. Biologia.*, **64**(2): 305-309.

Street, H.E. and Shillito, R.D. (1977). *Nutrient media for plant organ, tissue and cell culture*. In: CRC Handbook in Nutirtion and Food (Ed. Rechcigl M.) CRC Press, Boca Raton, F.L.

Zhong, Z. (1989). Cauliflower tissue culture and its application. *Shanghai agric. Sci. Technol.*, **3**:39.

Zhong, Z. (1990). Using floral stock culture for hybrid seeds production of *Brassica oleracea var italica*, *Acta Agric Shanghai*, **6** : 21.

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