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Micropropagation of *Chlorophytum borivilianum* to boost its cultivation

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SUMMARY

Chlorophytum borivilianum Santapaw and Fernandes (Safed Musli) is one of the most important plant in Indian systems of medicine due to its aphrodisiac and sex tonic properties. Stem disc, shoot bud, root disc and seed of were cultured on different MS basal media having different concentrations and combinations of auxins (IAA, NAA and IBA) and cytokinins (BAP and KIN) which resulted in differentiation of shoots, roots, formation of callus, regeneration and field transfer of plantlets and ultimately development of micropropagation protocol. The use of micropropagated plants will reduce the cost of planting material and will boost its cultivation.

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

Chlorophytum borivilianum Santapau and Fernandes is one medicinal plant whose natural availability is continuously decreasing due to heavy demand of useful parts. It is commonly known as Safed musli,because it yields milky white tubers on processing. Safed musli is considered as one of the most important drugs in Indian system of medicine namely Ayurveda, Unani and Siddha due to its aphrodisiac and sex tonic properties. For this reason that it is an integral part of more than 100 Ayurvedic formulations (Singh *et al*., 2004; Phurailatpam *et al*., 2009).

Unprecedented increase in the demand of safed musli and a consequent rise in its prices necessitated its systematic cultivation about a decade ago. Safed musli cultivaton has been initiated in many areas of Maharastra,Madhya Pradesh, Chattishgarh, Rajasthan, Uttar Pradesh,Bihar and many other states.The further increase in its cultivation will help in reducing pressure on natural forest resources and ultimately help in saving the plant from being extinct. Expension of Safed musli cultivation will require substaintial amount of quality propagules.Safed musli is generally propagated by seeds as well as vegetative propagules.Seed propagation has not become popular due to the poor seed germination (Bordia *et al.,* 1995).Poor tuber development in the seed raised plant and higher period taken for maturity and harvesting of these plants.Vegetative propagation through stem disc is better then seed propagation, but the method is costly(Phurailatpam *et al.,*2009;Rani *et al.,*2009).

Tissue culture or *in vitro* technique provides an alternative vegetative propagation method known as micropropagation.Micropropagation can lead to production of a 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 more many vigour and higher yield. Tissue cultured medicinal plants have been reported to exhibit higher medicinal value. Micropropagation would also help in ensuring the production of uniform plants, thereby restricting the variation in commercial population and quality of roots, which at present, is a major problem encountered with seed and stem disc propagating material.

MATERIALS AND METHODS

Stem disc, shoot bud, root disc, inflorescence and seeds of *Chlorophytum borivilianum* were used as explants. 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 surface sterilized explants were inoculated on different MS media having different concentrations and combinations of auxins (IAA, NAA, and IBA) and

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cytokinins (BAP and KIN).The culture were incubated at 25±2^{0}C under continuous fluorescent light of 1 k lux. Differentiated shoots from different explants were separated aseptically and each one was subcultured on different media for root development. Tissue culture plantlets with well formed roots were transferred to 1:1 mixture of sterilized sand and FYM and were progressively acclimatized and hardened for the field transfer. The micropropagation protocol was developed.

RESULTS AND DISCUSSION

The experimental findings obtained from the present investigation have been presented in the following heads:

Selection of medium:

Murashige and Skoog(1962) medium was used as basal medium. It was supplemented with different concentrations and combinations of auxins namely NAA, IAA and IBA and cytokinin namely BAP and KIN to develop an array of media. Initially stem disc of *Chlorophytum borivilianum* was cultured on each of these media and based on the response and as per suggestions of previous workers; finally 12 media (M_1-M_{12}) were selected for different tissue culture responses in the experiment (Table 1).

Selection of appropriate medium is the most important factor for the success of tissue culture experiments (Patel *et al.,* 2001).MS medium was used as a basal medium, because it is the most Important basal medium (Pierik, 1987; Street and Shillito, 1977). The MS basal medium was supplemented with different phytohormones. Out of these media some consisted of only cytokinin and were considered favourable for shoot differentiation. Some consisted of only auxin and were considered good for root differentiation. The media with both auxin and cytokinin were supposed to given different type growth and differentiation following the classical concept of Miller and Skoog (1957), who suggested that higher concentration of cytokinin in the medium promoted shoot differentiation, while higher concentration of auxin promoted root differentiation.

Selection of explants:

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, 1977). When true clonal propagation is the objective of *in vitro* micropropagation, explants with a preformed shoot bud, either terminal or axillary is the most desirous. Thus for true micropropagation only shoot bud or nodal stem are taken as explants.

Since, there are no proper stems with nodes and internodes in *Chlorophytum borivilianum* and it is reduced to stem disc, which can be considered as equivalent to nodal stem. Stem disc on germination can form shoot bud, which can also be used as an explants for micropropagation.The other explants root disc; inflorescence and seed were cultured to find out their suitability for adventitious shoot differentiation for micropropagation at enhanced rate. Stem disc,shoot bud(Purohit *et al.,*1994;Gothwal *et al.,*1998;Joshi *et al.,*1999;Suri *et al.,*1999;Dave *et al.,*2004 and Rani *et al.*, 2009) and inflorescence (Sharma and Mohan, 2006a, 2006b and Singh and Goyal, 2008) have been used as explants for micropropagation of *Chlorophytum borivilianum.*

Establishment and swelling of the explants:

The establishment of aseptic culture of all the five

Table 1 : The selected media for tissue culture of *Chlorophytum borivilianum*

Sr. No.	Media name	Composition	Purpose
1.	M_1	MS +2.22 µMBAP	Shoot
2.	M_2	MS +4.44 µMBAP	multiplication
3.	M_3	MS +6.66 µMBAP	
4.	M_4	MS +8.88 µMBAP	
5.	M_5	MS +11.11 µMBAP	
6.	M_6	MS +2.68 µM NAA +2.32 µM KIN	
7.	M_7	MS +5.37 µM NAA +4.65 µM KIN	Callus formation
8.	M_8	MS +2.68 µM NAA +4.65 µM KIN	
9.	M_9	MS +5.37 µM NAA +2.32 µM KIN	
10.	M_{10}	MS +0.57 µM IAA +4.44 µM BAP +4.65 µM KIN	Shoot and callus formation
11.	M_{11}	MS +5.71 µM IAA +4.44 µM BAP +4.65 µM KIN	
12.	M_{12}	MS +2.46 µM IBA	Rooting

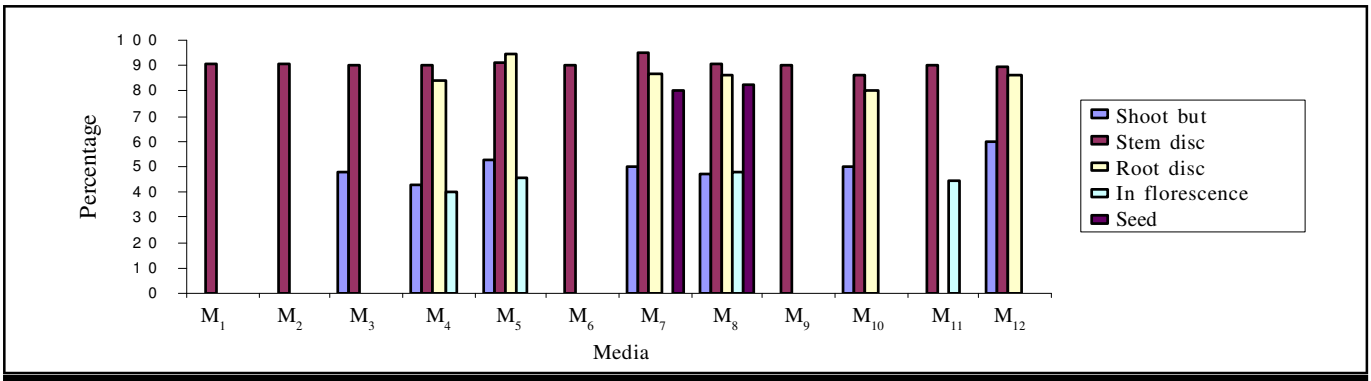

Fig. 1 : Frequency of swelling of explants

explants was followed by their swelling. Swelling was more prominent at the basal region compared to the apical region in the case of cultured stem disc and root disc. The apical region showed more swelling compared to the basal region in case of cultured inflorescence. The entire explant swelled in case of cultured shoot bud and seed. Swelling was more prominent in case of cultured stem disc, root disc and seed. There was no effect of the medium on the process of swelling and all the media showed more or less same swelling response (Fig.1).

Cultured stem discs and root discs had more dry matter compared to the explants, which resulted in the basal regions imbibing more water from the medium and thus showing more swelling. Further, the concentration of phytohormones was also high in their basal regions which often resulted in more cell divisions 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 was reflected by more swelling in the upper region of the explants. Cultured seeds imbibed water from its entire seed coat and as a result showed uniform swelling. Similarly the cultured shoot bud, which is generally a hydrated structure showed less but uniform swelling from the entire surface.

Differentiation of shoots:

Caulogenesis, the differentiation of shoots, was dependent on the medium and the explants in *Chlorophytum borivilianum* tissue culture. The differentiation of shoots always occurred directly on the explants. Medium M_5(MS+ 11.11µM BAP) showed the best response of caulogenesis both for the frequency of shoot differentiation as well as for the number of shoots differentiated per culture. The other media which supported good caulogenesis were,M_{10}(MS + 0.57µM IAA + 4.44µM BAP + 4.65 µM KIN),M_{11}(MS + 5.71µM IAA + 4.44 µM BAP + 4.65 µM KIN),M_4(MS + 8.88 µM BAP),M_3(MS + 6.66 µM BAP) and M_{12}(MS + 2.46 µM IBA)(Fig. 2).All these media had either only cytokinin or a higher concentration of cytokinin compared to that of auxin except medium M_{12},which had only auxin IBA. Differentiation of shoots on a medium with higher concentration of cytokinin was in conformation with the classical concept of hormonal control of organogenesis (Miller and Skoog, 1957).Medium M_5 with 11.11µM BAP showed the best response.

In *Chlorophytum borivilianum* also a slightly lower

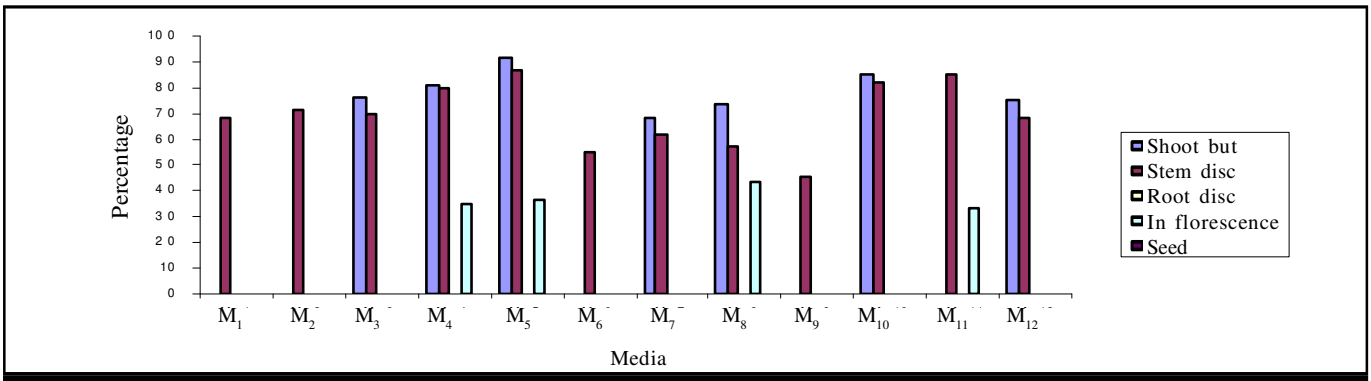

Fig. 2 : Frequency of shoot differentiation

concentration of BAP (2mg/l) was found to be the best for shoot differentiation(Pudake and Dhumale, 2003). Purohit *et al.* (1994) however,could get the best caulogenesis on a medium containing higher concentration of BAP(22.22µM). Singh and Goyal (2008) achieved the formation of the maximum number of shoots on MS medium supplemented with BAP13.32µM and 17.76µM and when the concentrations of BAP was increased and decreased, it resulted in gradual fall in number of shoots per culture from inflorescence of *Chlorophytum borivilianum.*All these workers got multiple shoots formation without any callus formation from the base of the explants on these media with higher concentration of BAP as found in the present investigation.Sharma and Mohan (2006a, 2006b) also got caulogenesis on MS medium supplemented with only BAP.Purohit *et al.* (1994) and Pudake and Dhumale (2003) found a combination of both cytokinin BAP and kinetin being effective for caulogenesis in *Chlorophytum borivilianum.* The only difference was the absence of auxin in the medium used by them compared to the present investigation, where the two cytokinins were used along with an auxin IAA. Sharma and Mohan (2006a) used kinetin with 2,4-D for caulogenesis. Differentiation of shoots on medium M_{12} (MS+2.46µM IBA) was a unique observation as no other worker has reported the same. Only two explants,shoot bud and stem disc ,differentiated shoots when cultured on this medium.The internal cytokinin concentration of these explants may be responsible for the shoot differentiation on this medium,which has a lower concentration of an auxin IBA.

Shoot bud explants 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.Purohit *et al.* (1994) and Pudake and Dhumale(2003) also found shoot buds more responsive than other explants for caulogenesis in *Chlorophytum borivilianum.* Sharma and Mohan (2006 a) and Singh and Goyal (2008) got shoot regeneration from cultured inflorescence explants. The immature unopened floral buds of the inflorescence explants were converted into vegetative shoots. They also got low frequency of caulogenesis from cultured inflorescence explants as found in the present investigation.

Differentiation of roots:

Differentiation of roots or rhizogenesis is an important tissue culture response for full realization of micropropagation, particularly when propagule multiplication is achieved through caulogenesis and not through embryogenesis. Rhizogenesis was observed only on M_{12} medium (MS+2.46µM IBA) among the twelve selected media. Rhizogenesis was only observed from the cultured shoot bud and stem disc. For the frequency of root differentiation, stem disc showed better response than shoot bud (Fig. 3).

IBA is a potent root inducer and has been used for induction of rhizogenesis in many plants during their micropropagation such as some fruits (Barnass *et al.*, 1980) .In *Chlorophytum borivilianum* also, 2.46µM IBA has been found the most potent supplement to the MS medium for inducing rhizogenesis. However,most workers have found a higher concentration of IBA compared to the present investigation, effective in increasing rhizogenesis(Purohit *et al.*,1994; Pudake and Dhumale, 2003; Dave *et al.*, 2004 and Sharma and Mohan, 2006a). Only Suri *et al.* (1999) found a lower concentration of

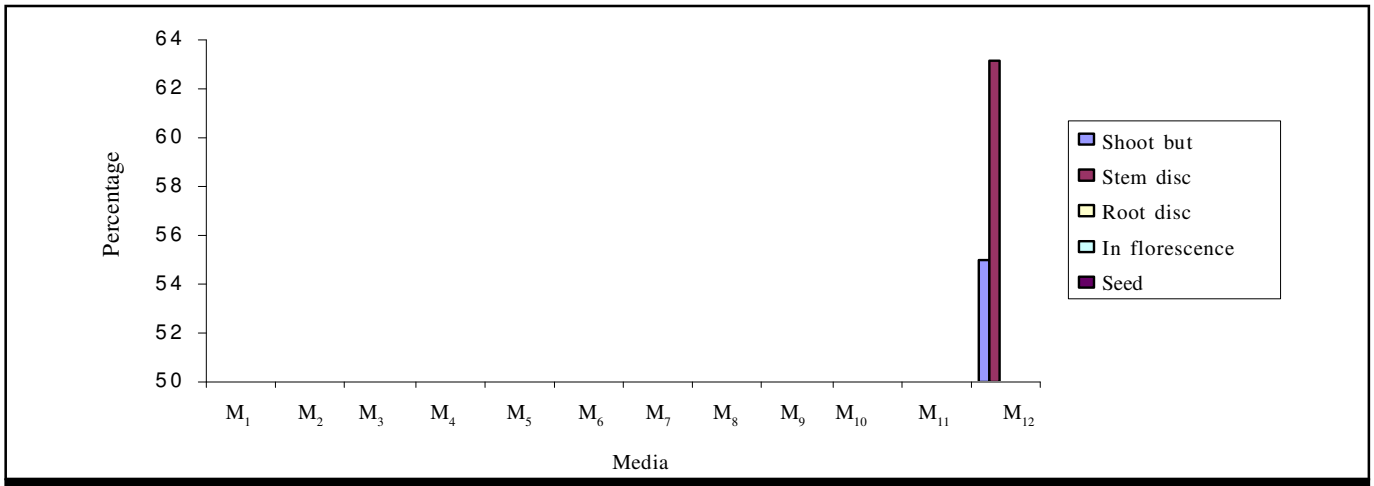

Fig. 3 : Frequency of root differentiation

IBA (0.49µM) suitable for rhizogenesis.Singh and Goyal(2008) suggested that rhizogenesis could be achieved even in absence of phytohormones on MS media with one fourth or half or full salt concentrations as well as half concentration of MS media supplemented with different concentrations of IBA.

Acclimatisation and field transfer:

The cultured shoot buds,both from *in vivo* and *in vitro* developed shoots when cultured on medium M_{12}, resulted in development of roots from their base leading to the development of tissue culture plantlets of *Chlorophytum borivilianum*. These plantlets were transferred into 1:1 sterlized mixture of FYM and sand.These plantlets were progressively acclimatized and hardened for the field transfer. About 80% plantlets servived during acclimatization and field traanfer.

Development a micropropagation protocol:

The tissue culture of *Chlorophytum borivilianum* led to the development a micropropagation protocol.

Shoot bud/ stem disc culture on M_5 and M_{10} media
↓
Differentiation of shoots
↓
Subculture on M_{12}
↓
Differentiation of roots from the base of *in vitro* developed shoot
↓
Acclimatization
↓
Field transfer

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.

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

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.

Gothwal, R.K., Joshi, A., Ganesh, R., Sharma, A. and Sharma, G.S. (1998). Major biochemical constituents in stem-disc derived callus of *Chlorophytum borivilianum* L. *Ann. Plant Physiol.*, **12**(2):181-183.

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.

Miller, C.O. and Skoog, F. (1957). Chemical control of bud formation in tobacco stem segments. *American J. Bot.,* **40** : 768- 773

Murashige, T. and Skoog, F. (1962).A revised medium for rapid growth and bioassays with tobacco tissue 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.

Phurailatpam, A.K., Geetha, K.A., Gajbhiya, N.A., Saravanan, R. and Maiti, Satyabrata (2009). Comparative study of *Chlorophytum borivilianum* Sant and Fernand. and *C. arundinaceum* Baker-Two 'Safed musli' species used as a vital tonics in Indian system of Medicine. *Phytomorphology*, **59**(1&2):79-81.

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*) a rare. Indian medicinal herb. *Pant Cell Tissue & Organ Cult.,* **39**(1):93-96.

Rani, A.S.,Satyakala, M., Kiranmaji, U. and Surya Narayan Murty,U. (2009). Plant regeneration through organogenesis from young leaves of *Chlorophytum borivilianum*. *Indian J. Plant Physiol*., **14**(1):44-49.

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

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

Singh, Rohtas and Goyal, S.C. (2008). Inflorescence rachis segment: A novel explants for rapid micropropagation of *Chlorophytum borivilianum* Sant.et Fernand. *Phytomorphology,* **58**(3&4):187-193.

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.

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.

Suri, S.S., Jain, S., Arora, D.K. and Ranawat, K.G. (1999). *In vitro* high frequency regeneration of plantlets and tuberous root formation in *Chlorophytum borivilianum. Gartenbauwissen schaft*, **64**(3):106-110.

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