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Effective Propagation and Evaluation of Salt Tolerance in *Coleus forskohlii*, an Endangered Herb

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ABSTRACT

Present study describes the result carried out on *Coleus forskohlii* a vegetatively propagating medicinal herb of potential significance in drug industry. When nodal segment and apical shoot tip was used as ex plant on MS media supplemented with α –Naphthalene acetic acid- NAA (1 mg/ml) and 6-Benzylaminopurine - BAP (2 mg/ml) the ex plant propagated effectively after 15 days of incubation at desired temperature and photoperiod of light. The propagation in apical tip(as ex-plant) was more robust compared to nodal segment(as ex plant).Only shooting could be visualized when nodal segment was used as ex plant but, both rooting and shooting could be noticed in apical tip when used as ex plant. Same composition of media (MS, supplemented with BAP (2 mg/L) and NAA(1 mg/L) has been found to propagate both the ex plant. Whereas, the media has influenced both rooting , shooting and multi shooting in the apical tip its effect has been restricted to shooting and multi shooting in the nodal segment. Effect of 0.1% to 0.4% of Nacl on propagation of fresh plant and sub cultured plant has been monitored which suggest inhibitory action of 0.4% of Nacl on both the explants. It appears that BAP and NAA supports growth of sub cultured ex plant up to a concentration of 0.2% of Nacl.

Keywords: *Coleus forskohlii*, propagation influenced by BAP and NAA, Propagation under Nacl stress.

INTRODUCTION

Coleus forskohlii is an important medicinal and perennial herb of the family Lamiaceae. Because of continuous collection of its root from wild sources, the plant has been included in the list of endangered species [5]. The crop has a great potential in future due to the expected increase in demand for forskolin which is widely used in glaucoma, cardiac problems, eczema, asthma, and hypertension and also used in the treatment of certain types of cancers. Its cultivation has however picked up as a crop having economic potential [24]. With the present annual production of about 100 tons from 700 ha in India, cultivation of *C. forskohlii* is picking up in recent

years. Micropropagation technology has been widely applied for the production of a large number of economically important plants including trees [15, 11] horticultural crops [22,8] and medicinal plants [9,12]. *Coleus forskohlii* is a fast growing herb and hence more amenable to get exposed to variety of environmental stress. Its rapid mode of vegetative propagation makes it a suitable candidate for micro propagation. Such work carried out on this plant also testifies this conclusion. Medicinal plants are important as variety of metabolic products synthesized by them have been widely used to reduce human suffering .It has been reported that environmental factors such as changed pH, changed nutrient composition has a direct effect on the synthesis of the plant based metabolites. Besides inducing change in the chemistry of the plant it can also change the pattern of growth of the plant, both *in situ* as well as *ex situ.* Present study is an attempt to assess the role of different concentration of Nacl on the micro propagation of *Coleus forskohlii*. This will open up scope for carrying out further studies on *Coleus forskohlii* when grown under stress condition.

MATERIALS AND METHODS

2.1. Source of ex plant

Field grown plants of *Coleus forskohlii* was used as explants for *in vitro* cultivation. Various parts of the plant such as stem, node of the stem, was used as plant source.

Sterilization

The plant material was first washed in continuous running tap water for 2 hours and then in sterilized distilled water mixed with tween 20. Washing in tween 20 was performed with vigorous shaking to remove surface adhered contaminants. The plant sample was once again washed with sterilized distilled water mixed with Bavistin (01%).The plant sample was then washed three times in sterilized distilled water before transfer to laminar flow chamber for transfer to culture media. The plant sample was further disinfected with 0.1 %(w/v) mercuric chloride ($HgCl_2$) for 1 min followed by thorough rinsing in autoclaved distilled water for at least 7 to 8 times. To avoid bacterial contamination the plant sample was treated with Mox solution (500 mg Mox dissolved in 100 ml of sterilized distilled water). The surface sterilized explants contained, nodal segments having length of 0.5 to 1cm containing single node as well as apical tip of the plant.

2.2. Culture medium and condition

MS medium was used for in-vitro culture of explants [18]. This medium was fortified with 3% sucrose, and 0.8% agar-agar (used to solidify the medium). The pH of the medium was adjusted to 5.8 by adding 1N NaOH / 1N HCl and then autoclaved at121^0 C for 20 minutes. α – Naphthalene acetic acid- NAA (1 mg/ml) and 6-Benzylaminopurine - BAP (2 mg/ml) were used to observe its effect on shoot regeneration and rooting. In order to evaluate the regenerating capability of old tissues and new tissues nodal segments and apical tip were used as ex plant. The cultures were maintained at 25 ± 1°C under 16 h photo-period provided by white fluorescent tubes.

2.2.1. RESULTS AND DISCUSSION

Review of literature [2, 10, 13, 2 0] on micro propagation of *Coleus forskohlii* suggest that this plant can be propagated on MS medium quite easily. There is report of its propagation from every part of the plant but there is scanty report regarding difference in the mode of propagation when nodal segment was used as ex plant when apical tip was used as ex plant.

3.1. Standardizing the technique of propagation

In order to streamline the process of propagation of *Coleus*, the plant was cultivated on MS medium supplemented with α –Naphthalene acetic acid- NAA (1 mg/ml) and 6-Benzylaminopurine - BAP (2 mg/ml).Nodal

segment and apical part of the plant measuring 0.5 cm to 01 cm was used as source of ex plant.

3.1.1. Propagation from nodal segment as ex plant

After ten days of transfer of the ex plant, growth of the plant was observed (Fig 1). After 15 days of growth the ex plant exhibited robust growth (Fig 2) as well as multi shooting (Fig 3).

Fig 1. Nodal segment (After 10 days)

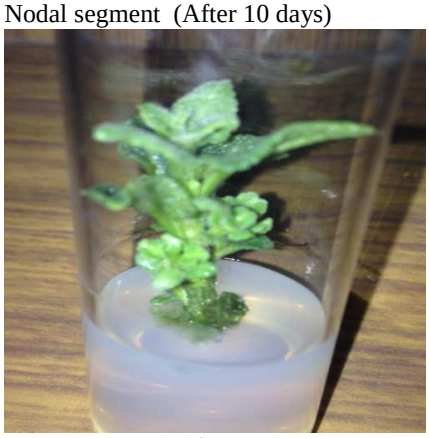


Fig 2. Nodal segment (After 15 days)

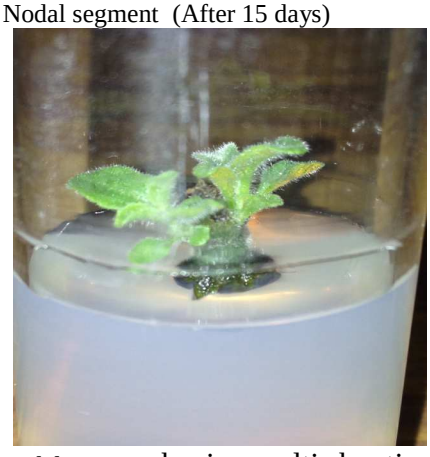


Fig 3-Nodal segment having multi shooting

The growth of the explants on basal media supplemented with α –Naphthalene acetic acid- NAA (1 mg/ml) and 6-Benzylaminopurine - BAP (2 mg/ml) opened up scope to carry out further propagation work .In order to evaluate the role of nodal segment as ex plant and stem tip as ex plant, the tip of the plant was taken as a source of ex plant.

3.1.2 Propagation from apical tip as ex plant

About 0.5 Cm to 01 cm of the freshly cut stem tip was used as source of ex plant. After performing the standard protocol for washing and sterilization the ex plant the plant was was transferred to the media and observed for recording the relevant data. After 15 days of incubation at desired temperature and desired intensity of light the inoculated ex plant turned into a robust growing plant but without root and hence not fit for acclimatization (Fig 4).With lapse of time (25 days), besides change in the pattern of growth of the plant such as plant attained a height of 3 cm (2 cm in the propagated plant from nodal segment) broader leaves (compared to plantlets from the nodal segment) were found. White coloured roots (8-10) were also found to emerge from the lower region of the plant (Fig 5).This report draws two significant conclusions

1. That MS medium supplemented with α – Naphthalene acetic acid- NAA (1 mg/ml) and 6-Benzylaminopurine - BAP (2 mg/ml) can be used both for rooting and shooting. For rooting the period of incubation can be extended 10 days. This also suggests that the response of apical tip taken as ex plant is better than nodal segment taken as ex plant. It appears that growth of nodal segment in this media induces formation of callus but when apical tip is taken as ex plant the same composition of media favours both shoot development as well as profuse rooting. Rooting in *Coleus* has significance as herbal preparation extracted from the root of this plant is used to cure many disease.

2. That MS medium supplemented with α – Naphthalene acetic acid- NAA (1 mg/ml) and 6-Benzylaminopurine - BAP (2 mg/ml) can be used both for propagation of nodal segment as well as apical tip.

Fig 4. Apical Tip

Fig 5. Apical Tip, showing rooting

In yet another set of experiment when apical tip was used as ex plant, robust growth of the plant along with multi shooting (Fig 6) and profuse rooting (Fig 7) was noticed after 20 days of growth. In earlier studies [10] different concentration of 6-benzyl amino purine (1-2 mg/L) and kinetin (1-2 mg/L) was used for propagation of *Coleus*..During the study it was noticed that 6-benzylaminopurine at a concentration of 1.5 mg/L was optimum for shoot regeneration. Kinetin did not induce shoot generation when compared to control culture. In this study *in vitro* generated shoots propagated in different combinations 6-benzyl amino purine and kinetin were further transferred to MS media mixed with different concentration of Indole-3-butyric acid (IBA) and Indole-3-acetic acid (IAA).It was noticed that IBA and IAA did not contribute at all in regeneration of root in the sub cultured ex plant. This conclusion could be drawn from the fact that with the increase in the concentration of growth hormone decrease in the number

Fig 6 . Apical tip with multi shooting

Fig 7. Apical tip showing profuse rooting

Of emerging rootlets takes place. Thus the present method of propagation of *Coleus* ,both in respect of regeneration of shooting and rooting seems to be more useful as it prevents the worker

from cumbersome task of modifying the composition of the medium every time for individual experiment.

On scanning the observations made in published articles it appears that *Coleus* as a medicinal herb has wide application in pharmaceutical industry .This has often been regarded as a cheap alternative of various drugs. Thus a serious thought regarding mass cultivation of the plant is compulsion. Its cultivation under different soil condition as well as weather conditions has to be explored. Keeping this view in mind *Coleus* plant was propagated in different concentration of sodium chloride to evaluate the tolerance of the plant to different concentration of Nacl. This can suggest ways and means to cultivate this plant in soil having low concentration of Nacl. Such soil conditions are common in various parts of India.

4. Micro propagation under salt stress

Salinity stress is a critical environmental constraint to crop productivity especially in arid and semiarid regions. Most of the crop plants react to salinity conditions resulting into decreased yield. The cause of stress can be due to decreased in water potential of the root medium, the toxicity of Na+ and Cl – and also nutrient imbalance by depression in uptake and/or shoot transport[17,7,16]. Toxic accumulation of Na+ and Cl - in the leaves has also been correlated with stomatal closure and reduction of total chlorophyll content in leaves both of which limit the amount of photosynthetic production [21].Cell and tissue culture techniques have been considered as one of the potential approaches for the development of plants with increased tolerance to environmental stresses in general and for salt stress in particular. The successful selection of mutant lines from cultured cells and the regeneration of whole plants from such cells have stimulated many attempts for the development of salt-tolerant plants. This approach has been successfully applied on Potato which is considered moderately salt sensitive compared with other crops [14]. A range of developmental responses in potato is affected by salinity. A small number of potato genotypes have been reported in salinity tolerance under outdoor, greenhouse or *in vitro* conditions and field trials [1, 3, 4, 6, 19] have been successfully employed. Recently [13] have tried to improve plant tolerance to salinity injury through either chemical treatments (plant hormones, minerals, amino acids, quaternary ammonium compounds, polyamines and vitamins) or bio fertilizers treatments(Asymbiotic nitrogen-fixing bacteria, symbiotic nitrogen-fixing bacteria and mycorrhiza) or enhanced a process used naturally by plants to minimize the movement of Na+ to the shoot, using genetic modification to amplify the process, helping plants to do what they already do - but to do it much better.

4.1 Impact of different salt concentration on propagation of *Coleus*

In order to evaluate the effect of different salt concentration (0.1%, 0.2%, 0.3% and0.4%) on propagation of *Coleus* different ex plants were used. In earlier studies carried out in this laboratory at higher concentration of Nacl (0.5%, 1.0%, 1.5%, 2.0%) the ex plant failed to grow at all. The ex plant constituted nodal segment taken from fresh pot growing plant and also freshly propagated ex plant procured from laboratory. This was done with the sole intention to study the effect on a widely growing plant and to compare it with laboratory grown plant.

4.1.1. Effect of different concentration of Nacl on propagation on *Coleus*-when ex plant was taken from sub cultured plant.

Nodal segment from fresh subculture in the range of 0.5 to 01 Cm was taken as ex plant. The segment was washed and sterilized as per the method described. It was then transferred aseptically to the media and observed for the effect in different environmental condition. This has been depicted in Fig-8(a-e)

a

b

c

d

e

Fig 8- a-only BAP+NAA, b- BAP+NAA+0.1% Nacl, c- BAP+NAA+0.2% Nacl, d- BAP+NAA+0.3% Nacl, e- BAP+NAA+0.4% Nacl

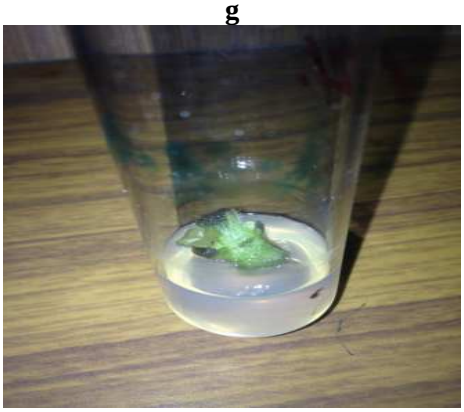
On review of Fig 8, it appears that 0.2% Nacl, when mixed in MS media containing BAP (2 mg/L) and NAA (1 mg/L) favours propagation of *Coleus* ex plant to an unlimited extent. Treatment with 0.1% of Nacl culminated into poor regeneration of the ex plant. Observations were made at regular intervals but after 28 days more apparent results were noticed. There is report [13] that plant hormones improve plant tolerance to salinity injury. Diminished growth of the ex plant was noticed after 28 days of incubation in presence of 0.3% and 0.4% of Nacl. At a concentration of 0.4% greater effect of Nacl was noticed as blackening and arrest of growth was visualized in the ex plant .The study suggests favouring effect of hormonal treatment up to the extent of 0.2% of Nacl

4.1.2. **Effect of different concentration of Nacl on propagation on *Coleus*-when ex plant was taken from fresh plant.**

In another set of experiment Nodal segment collected from potted plant in the range of 0.5 to 01 cm was taken as ex plant. After performing preliminary protocol for washing and sterilization the ex plant was transferred aseptically to the media and observed for the effect in different environmental condition. This has been shown in Fig-9(a-e)

f

g



h

i

j

Fig 9 - f-only BAP+NAA, g- BAP+NAA+0.1% Nacl, h- BAP+NAA+0.2% Nacl.
i- BAP+NAA+0.3% Nacl, j- BAP+NAA+0.4% Nacl

This reveals the inhibitory effect of Nacl on propagation of ex plant of *Coleus* from very beginning. On comparison of growth depicted in Fig 9(f) and Fig 9(g) it becomes apparent that Nacl even at a concentration of 0.1% influences reduced growth of the ex plant. At a concentration of 0.2% and 0.3% of Nacl growth is further reduced and this has been demonstrated in Fig 9(h) and Fig 9(i).The data can support our contention that when sub cultured ex plant was used hormone (BAP and NAA) treatment minimized and supported (Fig 8 c) the effect of 0.2% Nacl but the same could not hold true when potted ex plant was used for propagation (Fig 9 h).

5. CONCLUSIONS

MS media enriched with α –Naphthalene acetic acid- NAA (1 mg/ml) and 6-Benzylaminopurine - BAP (2 mg/ml) has been found to influence propagation of *Coleus ex* plant quite effectively. Same composition of the media has been found to support shooting and rooting in the ex plant. The media also favours multi-shooting in the ex plant. Propagation in apical tip when taken as explant was quicker and greater than nodal segment. Effect of low concentration of Nacl was also monitored which suggest that ex plant taken as fresh culture was more sensitive to the action of Nacl compared to ex plant taken from sub

cultured stock. Growth hormone BAP and NAA has been noticed to have additive role to play in sub cultured ex plant up to a concentration of 0.2% of Nacl. The knowledge derived from present study can be useful in planning propagation under salt stress condition.

REFERENCES

1. Ahmad R, Abdullah Z U N. (1979). Salinity induced changes in the growth and chemical composition of potato. *Pakistan J. Bot.* 11: 103-112.
2 .Asamenew T, Narayanaswamy MP . 2004. *In vitro* shoot multiplication and rooting in *Coleus forskohlii*. Briq., AGRIS Rec-4;126-134.
3. Barnes W C and Peel T C. (1958) .The effect of various levels of salt in irrigation water on vegetable crops. *Proc. Am. Soc.Hort. Sci*. 72: 339-342.
4. Bernstein L , Ayers A D (1951) Salt tolerance of six varieties of green beans. *Proc. Am. Soc. Hort. Sci*. 57: 243-248.
5. Boby BU, Bagyaraj DJ.(2003). Biological control of root rot of *Coleus forskohlii Briq*. using microbial inoculants.*World J. Microbiol. Biotechnol.,* 19: 175-180.
6. Bouaziz E. (1980). Tolerance a la salure de la pomme de terre. *Physiologie Vegetale 162* 18:11-17
7. Chapin E S. (1991).Integrated responses of plants to stress.A centralized system of physiological responses. *Bioscience* 40: 29-31.
8.Costa M. G. C., Alves V. S, Lani E. R. G.,Mosquim P.R., Carvalho C. R. and Otoni W. C.(2004). Morphogenic gradients of adventitious bud and shoot regeneration in epicotyl explants of citrus. Sci. Hort, 100: 63-74.
9. Dave A, Biolochi G and Purohit S.D. (2003).Scaling-up production and field performance of micro propagated medicinal herb Safed musli (*Chlorophytum borivilianum*). In *vitro* Cell Developmental Biology of Plants, 39: 419-425
10. Dubey P, Gangopadhyay M ,Dewanjee S and Ali N.2011.Establishment of a rapid multiplication protocol for *Coleus forskohlii* Briq and *in vitro* conservation by reduced growth. Indian J of Biotechnology.vol 10 pp 228-231.
11. Feysiaa T, Welander M and Negash L. (2005).Micro propagation of *Hagenia abyssinica*: A multipurpose tree. Plant Cell Tissue Organ Culture, 80:119-127.
12. Guo B, Gao M and Liu CZ. (2007). *In vitro* propagation of an endangered medicinal plant Saussurea involucrate Kar. et Kir.,Plant cell report, 26: 261-265.
13. Hamdia, M.A, Shaddad, M. A. K. (2010). Salt tolerance of crop plants.Journal of stress physiology &biochemistry Vol. 6 No. 3.
14. Maas E V , Hoffman G J (1977) Crop Salt Tolerance - Current Assessment. *Journal of the Irrigation and Drainage Division. Proc. Am. Soc. Civil Engr.* 103: 115-134.
15. Mao AA, Netton A, Fay MF and Caligari PDS.(2000). *In vitro* propagation of *Zusea cubeba* (Lours) Pers:A multipurpose tree. Plant Cell Report, 19: 263-276.
16. Marschner H (1995) Saline soils. In: *Mineral Nutrition of higher plants*. Academic Press, New York. pp. 657-680
17. Munns R , Termaat A. (1986). Whole plant responses to salinity. *Aust. J. Plant. Physiol*. 13: 143-160.
18.]. Murashige T, Skoog F A, Revised medium for rapid growth and bioassays with tobacco tissue cultures, Physiol Plant,15, 1962, 473-497.
19. Paliwal K V, Yadav B R. (1980).Effect of saline irrigation water on the yield of potato. *Indian J. Agricultural Sci*. 50: 1-33.
20. Reddy S P ,Rodrigues R and Rajasekharan R.(2001).Shoot organogenesis and mass propagation of *Coleus forskohlii*. Briq.,from leaf derived callus., Plant Cell Tissue Organ Cult,66;183-188.
21. Romero-Aranda R , Syvertsen J P. (1996).The influence of foliar applied urea nitrogen and saline solutions on net gas exchange of Citrus leaves. *J Amer. Soc. Hort. Sci*. 121: 501-506
22. Sharma AK, Agnihotri S, Singh S.K, Jain Mand Chaturvedi HC.(2004).*In vitro* cloning of male and female *Carica papaya* through tips of shot and inflorescence, Indian J. Biotech, 3: 235-240.
23. Sharma N,Chandel KPS and Srivastava V K.(1991).*In vitro* propagation of *Coleus forskohlii*. Briq., a threatened medicinal plant,Plant cell Rep,10;67-90.
24. Vishwakarma RA, Tyagi BR, Ahmed B and Hussain A (1988).Variation in forskorin content in the roots of *Coleus forskohlii*. *Planta Med.,* 54(5): 471-472.