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GENETICS AND PLANT BREEDING

In Vitro Propagation and Development of Salt Tolerant Lines of Edible and Medicinal Varieties of *Coleus* sp.

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ABSTRACT

Coleus sp. is one of the most important tuber crops of South East Asia. Pollen grains being sterile in the plant, vegetative propagation are the only method to carry out reproduction. However morphological and genetic variability are relatively absent by normal vegetative reproduction. Thereby tissue culture techniques of micropropagation through axillary shoot proliferation, organogenesis and shoot embryogenesis can improve the genetic constitution of the crops. For axillary shoot proliferation, nodal explants and for organogenesis and callusing leaf explants of *Coleus parviflorus* and *Coleus forskohlii* were used. Furthermore salt tolerant lines were tried to raise using different concentration of NaCl with normal MS media. Morphological and biochemical changes associated with the *in vitro* regenerated types were investigated to maintain the "true to type" propagation. RAPD analysis to find out variation between DNA of both *in vivo* and *in vitro* plants were carried out using OPA 11 and OPB 07 primers. The rate of multiplication was found to be higher in media supplemented with NAA, BA and GA_3. Callusing from leaf explants was found to be very low in media supplemented with only GA_3. Bud break and shoot multiplication was affected by higher concentration of NaCl at 10gm/l and 20gm/l. Evaluation of biochemical constituents revealed no variation in dry matter, starch and sugar content of regenerants and conventionally grown plants. RAPD analysis exhibited no variation in DNA between the *in vivo* and *in vitro* plants.

Highlights

- The rate of multiplication was found to be higher in media supplemented with NAA, BA and GA_3. Callusing from leaf explants was found to be very low in media supplemented with only GA_3. Bud break and shoot multiplication was affected by higher concentration of NaCl at 10gm/l and 20gm/l.
- Evaluation of biochemical constituents revealed no variation in dry matter, starch and sugar content of regenerants and conventionally grown plants. RAPD analysis exhibited no variation in DNA between the *in vivo* and *in vitro* plants.

Keywords: *Coleus* sp, micropropagation, salt tolerant lines

Tropical tuber crops constitute important staple or subsidiary food for about one- fifth of world's population and third most important food crop in the tropical and subtropical regions of the world after cereals and green legumes.

Coleus sp. among the tropical tuber crops are well known for their nutritional, medical and industrial uses. It belongs to the Labiatae family and form rhizomes and tubers. About 150 species of *Coleus* are found of which the most important one are

(a) *Coleus parviflorus*

(b) *Coleus forskohlii*.

Despite a big reservoir of food and energy, tuber crops in general face few biological drawbacks during their propagation by vegetative methods. Further a considerable fraction of the tuber product are found to be susceptible to various pests, insects and nematodes that affects the mortality of the propagules from season to season (Pattnaik and Das 1986; Mohandas 1994; Rajamma *et al*..

1989). Techniques like tissue culture and genetic engineering have tackled the problems associated with conventional propagation, storage and genetic improvement in the crop (Larkin and Scowcroft 1981; Flick 1983; Hahn 1983). Wide collection and utilization of available genetic variability, gene pool development for various characters and its selection are advisable.

(a) Coleus parviflorus: Commonly known as "Chinese potato" or "Koorka", it is an edible tuber having economic and commercial importance. It is extensively grown in Odisha, Kerala and Tamil Nadu and has adapted well in South East Asia (Doraipandian 1973; Hrishi and Mohan 1976).

It is a small herbaceous annual plant with succulent stems and aromatic leaves. Flowers exhibit racemose cymes with green calyx and violet corolla, anther and stigma.

The best season for planting *Coleus* is August-September (Singh and Mandal 1976). Highly irregular meiosis (Ramachandran 1967) and occurance of de synapsis (Vasudevan *et al.* 1967) might have resulted in lack of fertile pollen grains and non availability of genetically variant types in the germplasm (Sreekumari and Abraham, 1985). Induction of variability through conventional breeding is not possible in this crop. Nematode infection also affects its marketability (Pattnaik and Das 1986; Rajamma *et al.* 1989; Mohandas 1994). Tissue culture techniques like micropropagation can reduce the infectious factors to make the crop more productive.

(b) Coleus forskohlii: This species is indigenous to India and is used as a condiment in Ayurvedic medicine. The deterpenoid "Forskohlin" is isolated from its tuber (Bhat *et al.* 1977) and is used for treatment of glaucoma, cardiomyopathy, asthma and certain types of cancer (Shah *et al.* 1980, Desouza *et al.* 1986 and Valdes *et al.* 1987).

It is traditionally propagated by means of vegetative cuttings which is time consuming and provides limited number of propagules. *In vitro* propagation methods offer powerful tools for germplasm conservation and multiplication. Tissue culture methods also help to produce desirable genotypes for mass propagation of disease free propagules and easier maintenance of selected genotypes for breeding programmes.

MATERIALS AND METHODS

Experimental Site: The work was carried out at the tissue culture laboratory and experimental field of "Regional Centre of Central Tuber Crops Research Institute (ICAR)", Bhubaneswar, Odisha.

Source of Explant: For axillary shoot proliferation nodal explants of about 3-5mm of *Coleus parviflorus* and *Coleus forskohlii* were collected from the field. For organogenesis and callusing leaf explants of both the same species were used.

Nutrient Media: The MS basal medium (Murashige and Skoog, 1962) was used throughout the investigation. The media mainly used were freshly prepared, sterilized and slants were prepared for the experiments. All the basal salts and sucrose used were procured from Qualigens Fine Chemicals, India. In addition to the major and minor salts, different organic supplements and growth regulators such as NAA, BA, GA_3, 2,4 D were also added to the media to carry out the experiments related to axillary shoot proliferation, organogenesis and callusing.

For studying the salt stress on the growth of the plants three different types of media namely N_1, N_2 and N_3 were prepared in addition to the basal MS Media. In addition to these Z_{14} and Z_8 media were prepared for studying organogenesis and callusing.

Composition and concentration of 1 litre of Treatment Media

- N_1 Media: Basal MS media + 5gm of NaCl + 1gm of Charcoal.
- N_2 Media: Basal MS media + 10gm of NaCl + 1gm of Charcoal.
- N_3 Media: Basal MS media + 20gm of NaCl + 1gm of Charcoal.
- Z_{14} Media: Basal MS media + 0.5mg of 2, 4D+ 1gm of Charcoal.
- Z_8 Media: Basal MS media + 0.25mg of NAA+ 0.25mg of GA_3 + 0.25mg of BA + 0.25mg of 2,4 D+ 1gm of Charcoal.
- Explant preparation, Inoculation and Incubation:
- For axillary shoot proliferation, nodal explants of about 3-5mm were collected from the field source. Following sterilization they were transferred to MS basal media as well as N_1, N_2 and N_3 media.

- For organogenesis and callusing leaf explants were excised from the shoots obtained from the axillary buds and were inoculated in MS basal media as well as N_1, N_2, N_3, Z_{14} and Z_8 media.
- Three replications per treatment were done and 3-4 explants were inoculated in each tube for micropropagation, organogenesis and callusing. Subculturing was done after 3- 4 weeks.
- All the cultures were kept at 25±2°C under 12 hours' photoperiod and 70-80% relative humidity.

Biochemical Studies

- **Estimation of Dry matter Content:** Freshly harvested tubers were cleaned, sliced into pieces and 10- 20gm of each sample were taken. They were dried in an oven at 70- 80ºC for 48- 72 hours. Oven dried samples were then weighed and the difference was calculated to estimate the dry matter content.
- **Estimation of Starch and Sugar Content:** Starch and Sugar content was measured using titration Potassium Ferricyanide and Fehling's reagent as titrants.

RAPD Analysis

- **Extraction and purification of genomic DNA:** Fresh and young leaf samples of 1.2 gm were collected for isolation of genomic DNA. The method used was SDS Method (Delporta *et al.* 1983) with few modifications.
- **Quantification of DNA:** DNA concentration and purity was measured by using UV- Vis spectrophotometer (UV 1601, Shimadzu, Japan) with TE buffer as blank. The further quantification was accomplished by analyzing the purified DNA on 0.8% agarose gel along with diluted uncut lambda DNA as standard.
- **PCR amplification and RAPD primers.** For RAPD analysis, PCR amplification of 50ng of genomic DNA was carried out using two standard decamer oligonucleotide primers of OPA11 and OPB07. The total number of cycles carried out for amplification was 45 cycles and the PCR products were separated on 1.4% agarose gel.

RESULTS AND DISCUSSION

- **Axillary Shoot Proliferation in *Coleus parviflorus*:** On a plain MS media % explants response was only 22%. Shoot multiplication also occurred at a minimum rate and 14-15 days were required for bud break. However, % explant response increased in Z_8 media as it was found to be as high as 84% and days required for bud break reduced to 8-9 days.
- **Axillary Shoot Proliferation in *Coleus forskohlii*:** On a plain MS media % explants response was only 21%. Shoot multiplication also occurred at a minimum rate and 14-15 days were required for bud break. However, % explant response increased in Z_8 media as it was found to be as high as 83% and days required for bud break reduced to 8- 10 days.
- **Callusing in *Coleus parviflorus*:** Callusing was found to be optimum in basal MS media. However it increased to 80% in Z_8 media. Callusing did not occurred in media supplemented with NaCl.
- **Callusing in *Coleus forskohlii*:** Callusing was found to be optimum in basal MS media. However, it increased to 85% in Z_8 media. Callusing did not occurred in media supplemented with NaCl.
- **Effect of NaCl supplementation:** The % explant response and the days to bud break were not affected when the concentration of NaCl was 5gm/l. At higher dose of 10gm/l and 20gm/l the explants response was reduced in both the species.
- **Axillary Shoot Proliferation:** Influence of growth regulators on axillary shoot proliferation of both *Coleus parviflorus* and Coleus *forskohlii* were more or less the same and in agreement with that of (Nair *et al.* 1994). The nodal explants of both the species exhibited good multiplication response in MS basal medium. The multiplication response was even better in Z_8 media having all the growth regulators in it. However, the growth response was a bit low in Z_{14} media but was higher than in basal MS media.

Table 1: Axillary shoot proliferation in *Coleus parviflorus*

No. of treatments	Growth regulators in mg/l (treatment)	% Explants response	Days to bud break	Mean Shoots
T1	MS (Control)	22.37 ± 1.58	14- 15	1.7 ± 0.56
T2	MS + GA_3 (0.25)	34.26 ± 2.42	10- 14	2.1 ± 0.70
T3	MS+ NAA (0.25) + GA_3 (0.25)+ 2,4D (0.25)+ BA (0.25)	84.62 ± 1.12	8-10	4.8 ± 0.41

Table 2: Axillary shoot proliferation in *Coleus forskohlii*

No. of treatments	Growth regulators in mg/l (treatment)	% Explants response	Days to bud break	Mean Shoots
T1	MS (Control)	21.67 ± 1.55	14- 15	1.5 ± 0.47
T2	MS + GA_3 (0.25)	33.16 ± 2.20	10- 14	2.1 ± 0.65
T3	MS+ NAA (0.25) + GA_3 (0.25)+ 2,4D (0.25)+ BA (0.25)	83.70 ± 1.44	8-10	4.6 ± 0.47

Table 3: Callusing from leaf explant in *Coleus parviflorus*

Number of Treatments	Treatments	% of Callusing
T1	MS (Control)	0
T2	MS + GA_3 (0.25mg/l)	0
T3	MS + 2,4D (0.25mg/l)	52
T4	MS+ NAA (0.25mg/l) + GA_3 (0.25mg/l)+ 2,4D (0.25mg/l)+ BA (0.25mg/l)	80
T5	MS + 5gm/l NaCl	0
T6	MS + 10gm/l NaCl	0
T7	MS + 20gm/l NaCl	0

Table 4: Callusing from leaf explant in *Coleus forskohlii*

Number of Treatments	Treatments	% of Callusing
T1	MS (Control)	Good Callusing
T2	MS + GA_3 (0.25mg/l)	0
T3	MS + 2,4D (0.25mg/l)	70
T4	MS + NAA (0.25mg/l) + GA_3 (0.25mg/l) + 2,4D (0.25mg/l) + BA (0.25mg/l)	85
T5	MS + 5gm/l NaCl	0
T6	MS + 10gm/l NaCl	0
T7	MS + 20gm/l NaCl	0

Table 5: Effect of NaCl supplementation in *Coleus parviflorus*

Number of Treatments	Stress Chemical (Treatment)	% Explant response	Days to Bud Break
T1	MS + 5gm/l NaCl	20 ± 1.6	20- 25
T2	MS + 10gm/l NaCl	15 ± 1.5	25- 30
T3	MS + 20gm/l NaCl	7 ± 2.4	30

Table 6: Effect of NaCl supplementation in *Coleus forskohlii*

Number of Treatments	Stress Chemical (Treatment)	% Explant response	Days to Bud Break
T1	MS + 5gm/l NaCl	22 ± 1.5	20- 25
T2	MS + 10gm/l NaCl	15 ± 1.5	25- 30
T3	MS + 20gm/l NaCl	7 ± 3.1	30- 32

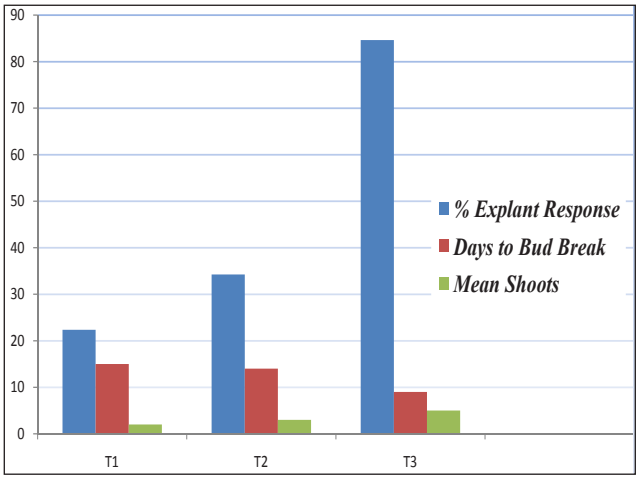

Fig. 1: Axillary Shoot proliferation in *Coleus parviflorus*

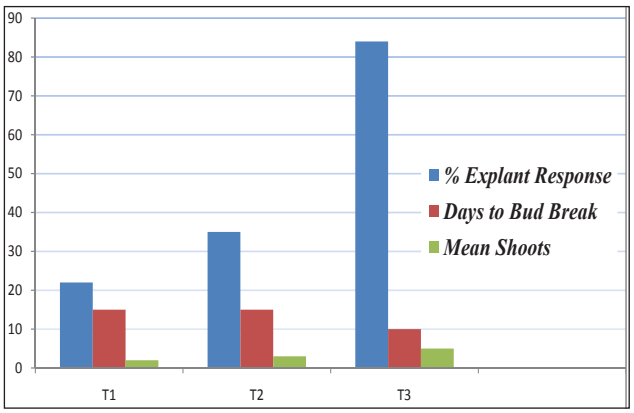

Fig. 2: Axillary Shoot Proliferation in *Coleus forskohlii*

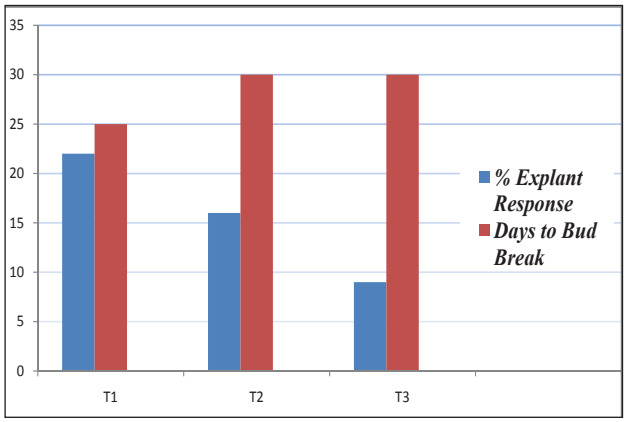

Fig. 3: Effect of NaCl Supplementation in *Coleus parviflorus*

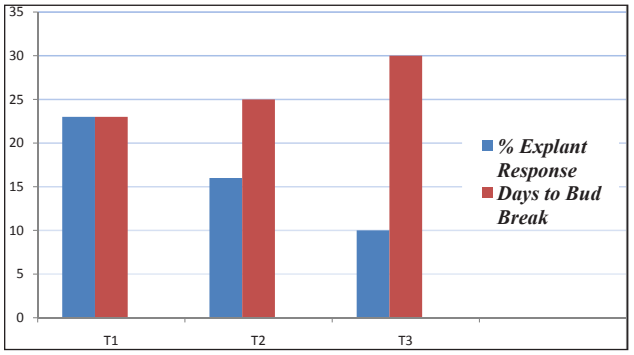

Fig. 4: Effect of NaCl Supplementation in *Coleus forskohlii*

- **Callusing:** There was no callus induction of *Coleus parviflorus* in plain MS media. However, nodal explants of *Coleus forskohlii* exhibited some amount of callus growth in MS media. From this it may be stated that *Coleus forskohlii* contains some inherent factors in the form of hormones or other growth regulators that helped in callus growth even in the media devoid of any growth regulator. However the rate of callusing increased in both the species in Z_8 media. The extent of callusing was influenced by the nature, concentration and combination of callusing media (Jena 2001).
- **Effect of NaCl supplementation:** The stress chemical NaCl influenced the bud break response and shoot multiplication in both the species of *Coleus*. The % explants response and bud break were not affected when the concentration of NaCl was 5gm/l. But in higher dose of 10gm/l and 20gm/l explant response was reduced in both the species which revealed that NaCl has a stress effect on the growth of the plant.
- **Biochemical Studies**: Evaluation of biochemical constituents of both the species revealed no variation in dry matter, starch and sugar content of regenerants and conventionally grown plants. This shows that *in vitro* regeneration had no conspicuous effect in respect of tuber

quality which is essential for "true to type" propagation.

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