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SOMACLONAL VARIATIONS IN COLOCASIA ESCULENTA (L) SHOTT.

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ABSTRACT

This research was aimed at developing a protocol for in vitro micropropagation of C.esculenta and to study the somaclonal variations occurring in the plant. C.esculenta is consumed as vegetable in all parts of the world and reports of medicinal properties are also available. In this study, the protocol was developed for mericulture of C.esculenta var. Sree Kiran. The plant was regenerated in Murashige-Skoog medium with 2mg/L 2,4-D, 1mg/L TDZ, 800mg/L glutamine and 0.5% activated charcoal with 12 hours photoperiod. The plants were acclimatized in soilrite. After 15 days transferred to greenhouse condition and maintained in pots. The somaclonal variations like changes in plant height, number of leaves per plants, total chlorophyll content were studied in these plants. There was change in the morphological traits and chlorophyll content in the tissue culture developed plants compared to wild type plants.

INTRODUCTION

C.esculenta is a vegetable crop grown for its leaves and corms and is a major staple food in many countries. The plant has got many medicinal values too. It is a galactogue, nerve tonic, hepatic tonic, used to treat diarrhoea in traditional medicinal system (Prajapathi et al., 2014). The plant propagates by vegetative method by axillary buds (Ivancic,1992). The plant get infected by many devastating pathogens like, Dasheen Mosiac Virus, Taro Bacilliform Virus, Colocasia Bobone disease Virus, Cytohabdovirus, fungi like *Pythium, Phytophthora colocasiae, Sclerotium rolfsii, Cladosporium colocasiae,* pests like taro beetle (Akwee et al., 2015, Revill et al., 2005). So, the need of the hour is generate disease free plantlets for cultivation and which can be achieved by in vitro meristem culture.

Plant tissue culture is often referred as most useful method for production of genetically variant novel plants. These genetic variations occurring in tissue culture plants regenerated from somatic cells are referred as somaclonal variations (Chawla, 2002). There are only two recent reports are available about somclonal variation in C. *esculenta* (Krishna et al., 2016; Nurilmala et al., 2017). But, somaclonal variations in Indian varieties of C. *esculenta* are not reported yet. So this study was aimed to study the somaclonal variations *in vitro* regenerated plantlets *C. esculenta* from meristem culture.

MATERIALS AND METHODS

A standard variety of C. *esculenta, Sree Kiran* was procured from CTCRI, thiruvanthapuram, Kerala, India and maintained under greenhouse condition in the institution. The tips (3-5 mm) of the runners arising from the corm were excised and used as explants. The explants were thoroughly washed under running tap water and surface sterilized by series of treatment with disinfectants viz., in tween-20 for ten minutes, 0.1% cetrimide for ten minutes, in 0.5% cefotaxime for 30 minutes, in 2% bavistine for two hours on rotary shaker with gentle agitation, in 70% alcohol for 10 minute and 10% hydrogen peroxide for ten minutes. Each disinfectant treatment is followed by sterile water wash to remove the traces of disinfectants. Finally, the explants were blot dried on sterilized blotting paper. The MS basal medium with different combinations of auxins and cytokinins was prepared, autoclaved and inoculated with surface sterilized explants. The cultures were incubated in a photoperiod of 12 hours at 25^{0}C.

The regenerated plantlets were acclimatized in soilrite for 15 days and transferred to greenhouse condition.

The somaclonal variations in terms of morphology were noted in terms of plant height from base to tip, number leaves per plant, root numbers and total chlorophyll content was determined.

Total chlorophyll content was estimated according to the protocol of Porra et al., 1989; Sumantha et al., 2014. The estimation was performed in triplicates by collecting leaves from three different plants.

RESULT AND DISCUSSION

Among the different combination of plant growth regulators used, the meristems were propagated in MS medium with 2mg/L 2,4-D, 1mg/L TDZ, 800mg/L glutamine, 0.5% activated charcoal. The regeneration frequency of the explant in this medium was up to 85%. The activated charcoal was used in the medium to avoid the tissue browning initiated by the leaching of phenolics due to damage caused to explant tissue (Thomas 2008). L-glutamine serves as organic nitrogen source to promote the regeneration of the explant (Nyman et al., 1987). Shoot formation initiated after 15 days of incubation and by 32nd day roots were also formed in the medium with same composition. The sub-culturing frequency was for every 15 days interval. The completely developed plantlets were acclimatized in soilrite. Up to 90% plants were survived during acclimatization. Bhuiyan and his co-workers (2009) studied the effect of BA and TDZ on clonal propagation of *C.esculenta*, and they obtained similar results to this study, that the medium with 1mg/L TDZ was more efficient in inducing shoot elongation than the medium with BAP. Hossain 2012, reported that the meristem culture in *C.esculenta* propagated well when incubated under dark condition than under light. But the leaves were pale green, smaller and taller when compared to plants grown under light conditions.

The plant height, root numbers, number of leaves in the wild type plant and in vitro propagated plants were determined (table no 1). It was observed that the micropropagated plants were shorter in length than the wild type plants. The micropropagated plants have produced 3 leaves, whereas the wild type plant bears 4 leaves. Micropropagated plants show increased root number and root length than the wild type plant and the former were visibly greener than the latter. There are no reports about somaclonal variations in Indian verities of *C.esculenta* yet. The somaclonal variation induced in the plants may be due usage of synthetic plant growth regulators 2, 4-D and TDZ. Especially, the TDZ is known to induce some morphological abnormalities in plantlets when used as a growth regulator in the medium (Dewir et al., 2018).

Estimation of total chlorophyll content was carried out by extracting the pigments in 80% buffered acetone under dark conditions (table no 2). There was no change in the concentration of chlorophyll a between wild type and micropropagated plants. Whereas, there was considerable increase in the concentration of chlorophyll b and 1.5 fold increase in the total carotenoid content.

Table no 1: Comparison of morphological variations in wild type and *in vitro* micropropagated plants of *C.esculenta.*

Trait	Wild type plant	Micropropagated plants
Plant height in cm	31.4±3.44	24.53 ±6.63
Number of leaves	4±0.0	3±0.0
Number of roots	5±1.2	7±2.1
Length of roots	5±2.67	8±4.6

The results presented as Mean±SD. Mean presented is average of 40 different plants

Table no 2: Total chlorophyll content in wild type and *in vitro* micropropagated plants

Pigment	Wild plants (in µg/mL)	Micropropagated plants (in µg/mL)
Chl a	2.5±0.11	2.8±0.15
Chl b	0.96±0.05	1.8±0.1
Chl a+ Chl b	3.4±0.05	4.4±0.11
Carotenoids	0.68±0.03	1.49±0.04

The results presented as mean±SD. The mean presented are average of three trials

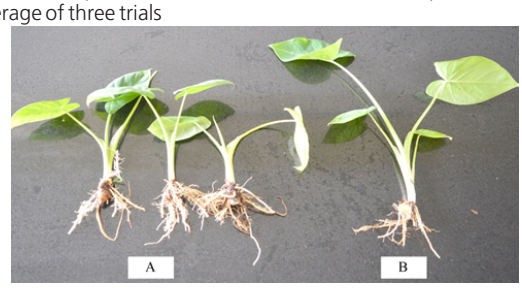

Figure 1: Somaclonal variations- A) In vitro micropropagated plants showing stunted growth, three leaves, increased root number and length, B) Wild type plants showing increased length, 4 leaves, decreased root number and length.

CONCLUSION

Meristem culture provides a unique opportunity to produce disease free plantlets in C.esculenta. Morphological changes observed in micropropagated plantlets may be due to the abiotic stress induced in cultural conditions. Distinctive mutations raised in tissue culture may sometimes result in elite characters in the regenerated plants which cannot be achieved by conventional methods of breeding.

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