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In vitro Regeneration of Cultivars of *Colocasia esculenta* (L.) Schott

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ABSTRACT

Background and Aim: *Colocasia esculenta, a member of Araceae is one of the major tuber crops grown widely throughout the Pacific islands, Asia and Africa. Corms are good source of starch and known for their medicinal value. Since traditional propagation is time consuming and labour intensive, in vitro technique is the alternative strategy to overcome these limitations. Further, in vitro technology is well established as one of the viable method to conserve the elite germplasms. Hence, an attempt is made in the present investigation to multiply the three elite cultivars of Colocasia esculenta by employing in vitro techniques.*

Materials and Methods: *Standard tissue culture techniques were followed to raise the cultures. Segments of corms with buds of three elite cultivars- Muktakeshi, Telia and Sree Reshmi, procured from Central Tuber Crops Research Institute, India, were cultured on MS and L2 media supplemented with different concentrations and combinations of growth regulators. Cultures were maintained under controlled conditions of light and temperature.*

Results: *Both direct and indirect organogenesis were observed from the cultures. M S media supplemented with NAA (10.74µM) and BAP (17.76µM) was found to be more suitable for proliferation of shoot buds from explants without the formation of callus. Whereas, L2 fortified with 2, 4-D (9.04µM) and Kin (6.97µM) has induced profuse callus and subsequent regeneration of shoots. Thus, obtained shoots from both direct and indirect pathways were rooted on the same media. They were acclimatized in a potting mixture of sand and cocopeat in the ratio of 1:1. Nearly 94% of the regenerated shoots were survived after transferring them to field.*

Key words: *Colocasia esculenta, Cultivars, Callus, Micropropagation, Conservation.*

INTRODUCTION

Colocasia esculenta (L.) Schott, commonly called as Taro, is one of the oldest cultivated tuber crops grown for its edible corms and leaves[1]. It is an important tropical and subtropical crop, used as a staple or subsistence food by millions of people throughout South Pacific Islands, Asia and Africa[2].

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According to Food and Agriculture organization of United Nations, the corms, leaves and petioles are used As vegetables and are known for their important sources of carbohydrates, proteins, minerals and vitamins[3]. Since, corms are important source of carbohydrates as an energy source, they are used as staple food in tropical and subtropical countries[4]. Apart from its nutritive value, it is one of the important medicinal plants used to cure several ailments[5]. Root extracts of taro is used to treat rheumatism and acne while leaf extract is useful to stop bleeding at wound sites. It is also used in neutralizing the snake poison[6].

Taro is traditionally propagated through small tubers or parts of old ones and is time consuming[7]. Further taro corms and cormels have very short storage life under natural conditions[8]. In addition to this, taro is highly susceptible to a wide range of pathogens and pests resulting in depletion of elite germplasms[9]. Under these circumstances, unconventional propagation techniques such as *in vitro* propagation can be employed to multiply and to conserve elite germplasms. Micropropagation is an accomplished method to propagate many economically important crops, which improves the production with defined growth media containing appropriate growth regulators[10]. The multiplication of taro by tissue culture has several advantages such as rapid means for multiplying clones and provides a phytosanitary method for producing disease-free plants through the culture of meristems. There are a few reports on obtaining multiple shoots from meristem, leaf and axillary bud cultures of various cultivars of taro [11,12,13]. The three elite cultivars- Muktakeshi, Telia and Sree Reshmi,which are known for Pest resistance, drought resistance and reduced levels of acridity respectively, are selected for the present study. Since each cultivar require different culture requirements to express their morphogenetic potential, an attempt has been made to compare the response of these three selected cultivars by employing tissue culture techniques.

MATERIAL AND METHODS

Explant Source:

The tubers of Muktakeshi and Telia were procured from Central Tuber Crops Research Institute, Bhuvaneshwar, India, while the tubers of Sree Reshmi were procured from Central Tuber Crops Research Institute, Thiruvananthapuram, India (Fig.1). Few of these tubers were stored at room temperature for culture purpose and the rest were planted for further tuber multiplication.

Culture Conditions:

The tubers were washed thoroughly and surface sterilized with Tween – 20, a detergent, for about 30 min and rinsed under running tap water. Tuber segments with buds were used as explants. The explants were then washed with Bevastin, a fungicide for 30 min and washed thoroughly under running tap water. The surface sterilized explants were then transferred to sterile laminar air flow chamber, wherein it was treated with 0.1% (w/v) streptomycin for about 3 min and rinsed thrice with sterile distilled water. Later it was immersed in 0.1% (w/v) mercuric chloride for 2 min and again rinsed thoroughly with sterile water. These sterilized explants were used for inoculation. Two different culture media, Murashige and Skoog's (MS)[14] and Phillip's and Collins (L2)[15] media composed of sucrose (3%) and agar (0.8%) were prepared. The pH of the media was adjusted to 5.6. Both the media were supplemented with various growth regulators either alone or in combinations. The cultures were regularly subcultured and maintained at 23±2°C under 16:8 light and dark regime. Cultures were illuminated with white fluorescent tubes of intensity 25µmol m^{-2} s^{-1}.

Acclimatization:

For acclimatization, plantlets were removed from culture bottles and washed thoroughly to remove agar. The regenerated plants were then transplanted on potting mixture of sand and cocopeat in the ratio of 1:1.After 3 weeks, 94% of the regenerated plants were hardened and successfully acclimatized to the natural conditions.

Statistical Analysis:

All the experiments were carried out in triplicates. The percentage of growth response for each cultivar of *Colocasia esculenta* were subjected to Analysis of Variance and a post Hoc test using Duncan's Multiple Range Test analysis at $p \leq 0.05$.

RESULTS AND DISCUSSION

Rapid multiplication of medicinal plants without any interference of seasons is an added advantage of *in vitro* multiplication[13.] The success of plant regeneration is highly influenced by the culture medium and the type of growth hormones used to raise the cultivars. Murashige and Skoog's (MS)[14] and Phillips and Collins(L2)[15]media were used in the present study. Explants cultured on both the basal media did not show any response even after 60 days of culture. However, both direct and indirect organogenesis was observed from the cultures of three cultivars selected depending on the type of the media and the combination of growth regulators.

The following general observation refers to all the three cultivars selected for the study. However, the specific observations were mentioned wherever it is needed.

Direct Organogenesis:

Proliferation of multiple shoots were observed when the explants were cultured on MS supplemented with either NAA /Kin/ BAP/2, 4-D. However, the time taken for the induction of multiple shoots from the explants varies with the type of hormone added to the culture medium. Presence of BAP has promoted the direct organogenesis within the four weeks of culture, while NAA, Kin and 2,4-D has delayed the organogenesis for nearly 60 days of culture. Among the three cultivars selected for the present study, Muktakeshi and Sree Reshmi showed maximum morphogenetic potential when cultured on MS+BAP (13.32µM) (Table 1). Ngetich et al., [16] have reported the proliferation of multiple shoots from the meristem culture of three varieties of *Colocasia esculenta* on MS supplemented with either BAP or NAA. However, Chand et al.,[17] have succeeded in obtaining multiple shoots from meristem cultures of cv. Niue on modified MS supplemented with TDZ. They have noticed vigorous growth of multiple shoots on TDZ supplemented medium rather than BA. On the contrary, Vaurasi and Kant[18] have found that BAP in the medium induce better growth than TDZ in taro varieties while examining the effects of three salinity levels under the influence of BAP and TDZ on growth and development of *in vitro* grown plants. However, in the present study, MS media supplemented with combination of auxin and cytokinin induced maximum number of multiple shoots from the cultures compared to single hormone. Synergistic effect of auxin and cytokinins on the induction of multiple shoots is well documented in many taxa[19]. The highest number of multiple shoots was recorded on MS+NAA(10.74µM) + BAP (17.76µM) in cv. Sree Reshmi(Fig. 2A)and then subcultured to NAA (2.68µM) + BAP (6.66µM) + GA_3 (2.88µM) (Fig. 2B) for shoot elongation and further multiplication. This is in conformity with the observations of Hossain et al.,[11] in *Colocasia esculenta* cv. Antiquorum L. and Tejavathi and Niranjan[20] in *Celastrus paniculatus*. Addition of GA3 along with auxins and cytokinins generally improves elongation of shoots[21,22].

Indirect organogenesis:

In the present study, greenish yellow callus was obtained when explants were cultured on MS supplemented with 2,4-D and Kin. However, profuse callus formation was observed from the cultures when grown on L2 medium fortified with 2, 4-D (9.04 µM) + Kin (6.97 µM) (Fig- 2C) and (Table 2). L2 medium proved to be superior than MS in terms of induction of callus and shoot regeneration from the explants of all the three cultivars. L2 medium was particularly formulated to initiate callus and cell suspension cultures of Red Cloves and other legumes[15]. L2 medium is different from MS medium in not having nicotinic acid and with reduced quantity of ammonium salts. 2,4-D is known for callus induction from the explants when supplemented with various media either

alone or in combinations[23]. However, Hossain et al.,[11] had noticed the proliferation of shoots from the meristem of *Colocasia esculenta* cv. Antiquorum on 2,4-D + Kin without the callus formation. Reddy et al.,[22] have noted L2 medium along with Kin and IAA was most suitable for shoot bud proliferation from axillary bud cultivars of *Asclepias curassavica*. The effect of Kin on induction of multiple shoots in cultures can be attributed to its specific mechanism of action in preventing auxin synthesis[24]. Shirin et al.,[25] have reported that Kin +NAA in MS was found to be best for shoot regeneration from the internode and leaf derived calli than BAP +NAA in potato cultivars. However, Yam et al., [13] have recorded callusing and plantlet regeneration on MS containing NAA and BA in *Colocasia esculenta* var. *esculenta*.

Rooting and Acclimatization:

The regenerated shoots obtained through both direct and indirect pathways were rooted on the same media after 10 days of shoot regeneration in the present study (Fig. 2E). Regenerated shoots of *Colocasia esculenta* cv. Niue rooted on the same medium after third subculture to TDZ or BA[17]. In *Colocasia esculenta* cv. Antiquorum, profuse rooting was observed on the same regenerating medium (MS+NAA+BAP) itself[11]. However, Ngetich et al., [16] have obtained rooting of the regenerated shoots of three varieties of *Colocasia esculenta* on transferring to NAA supplemented medium. Nearly 94% of survival was recorded in the present study (Fig. 2F).

Table 1: Effect of different concentrations of growth hormones on multiple shoot proliferation from nodal explants of *Colocasia esculenta* on MS medium

Media+ Growth regulators in (µM)	Shoot number in Different cultivars		
	Muktakeshi	**Telia**	**Sree Reshmi**
MS+ NAA(21.48µM)	1.8±0.68^{h}	1.3±0.51^{hi}	1.9±0.42^{i}
MS+ NAA(26.85µM)	1.4±0.38^{j}	1.2±0.31j	1.9±0.21^{ij}
MS+ BAP(13.32µM)	**2.5±0.05^{d}**	**1.9±0.61^{d}**	**2.8±0.08^{d}**
MS+ BAP(22.20µM)	2.1±0.05^{e}	1.6±0.24^{e}	2.3±0.18^{f}
MS+ 2,4-D(22.62µM)	1.9±0.24^{g}	1.3±0.58h	2.0±0.18^{h}
MS+ Kin(18.58µM)	1.6±0.16^{i}	1.4±0.28^{g}	2.1±0.21^{g}
MS+NAA(10.74µM)+BAP(6.66µM)	3.2±0.17^{c}	2.6±0.28^{b}	3.3±0.16^{c}
MS+NAA(10.74µM)+BAP(17.76µM)	3.5±1.02^{b}	2.5±0.13^{c}	4.8±1.03^{b}
MS+NAA(2.68µM)+BAP(6.66µM)+GA3(2.88µM)	**5.9±0.32^{a}**	**3.9±0.42^{a}**	**7.2±0.05^{a}**
MS+2,4-D(6.78µM)+Kin(13.94µM)	2.0±0.00^{f}	1.5±1.06^{f}	2.5±0.06^{e}

Data represented by mean±SE of three replicates. Mean followed by letter are significantly different (p≤0.05) using Duncan's multiple range test.

Table 2: Effect of different concentrations of growth hormones on multiple shoot regeneration from callus of nodal explants of *Colocasia esculenta* on L2 medium.

Media+ Growth regulators in (µM)	Shoot number in Different cultivars		
	Muktakeshi	**Telia**	**Sree Reshmi**
L2+ 2,4-D(22.62µM)	1.9±0.24^{c}	1.3±0.58^{d}	2.0±0.18^{d}
L2+ Kin(18.58µM)	1.6±0.16^{d}	1.4±0.28c	2.1±0.21^{c}
L2+2,4-D(6.78µM) +Kin(13.94µM)	2.6±1.2^{b}	1.9±0.86^{b}	2.4±0.23^{b}
L2+2,4-D(9.04µM) +Kin(6.97µM)	**4.0±0.00^{a}**	**3.5±1.06^{a}**	**3.5±0.0^{a}**

Data represented by mean±SE of three replicates. Mean followed by letter are significantly different (p≤0.05) using Duncan's multiple range test.

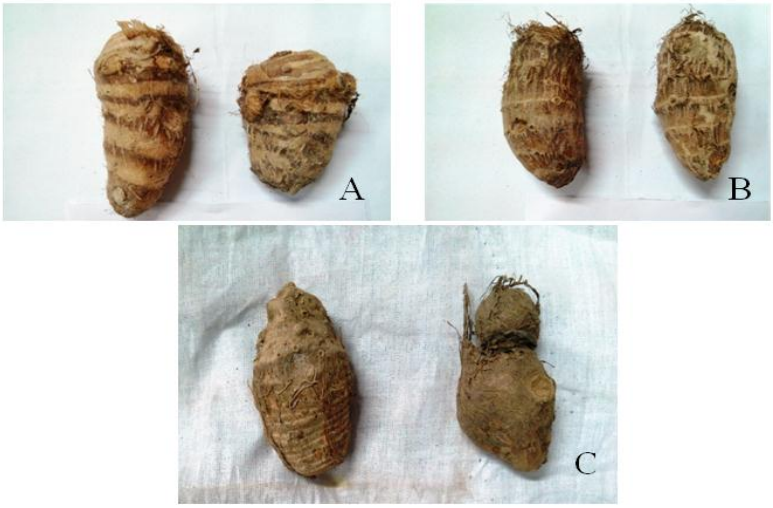

Fig 1: Freshly harvested tubers of three cultivars of *Colocasia esculenta*

A. cv. *Muktakeshi*
B. cv. *Telia*
C. cv. *Sree Reshmi*

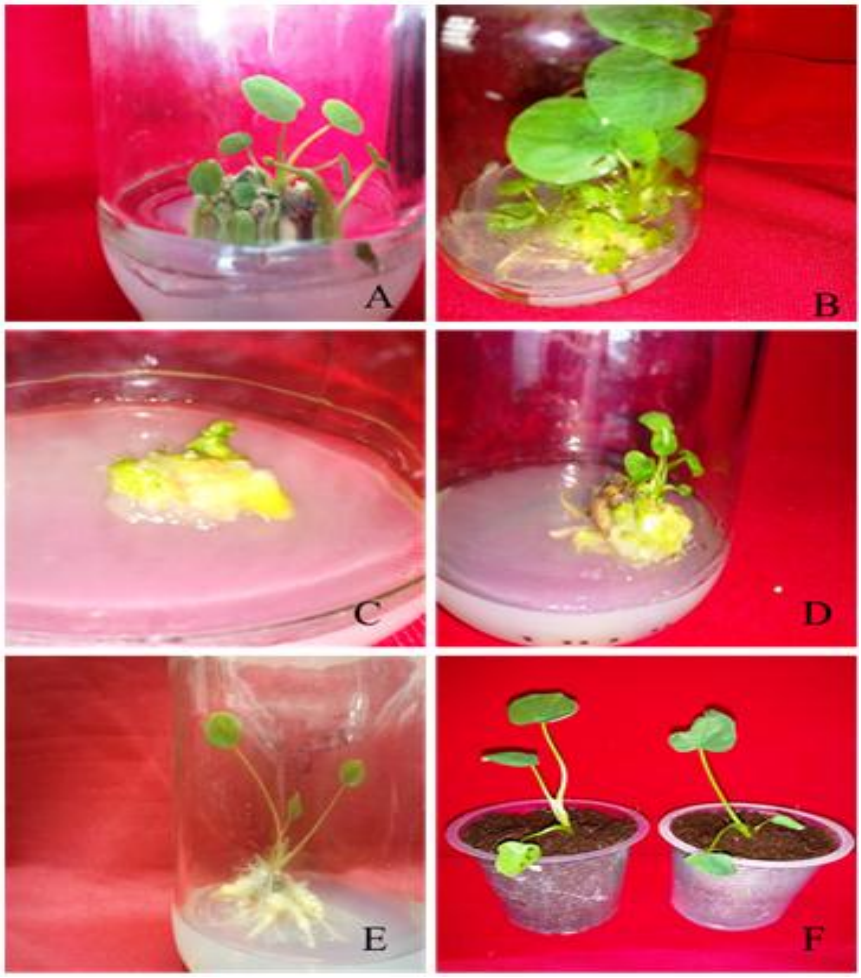

Fig 2: Multiple shoot regeneration from the cultures of *Colocasia esculenta*

A. Direct proliferation of shoots from the explant on MS+NAA(10.74µM) +BAP(17.76µM).
B. Multiple shoots on MS+NAA (2.68µM) +BAP(6.66µM) +GA3(2.88µM) without callus formation.
C. Greenish- Yellow friable callus formation on L2 +2,4-D(9.04µM) + Kin (6.97µM).
D. Induction of multiple shoots from the callus on L2 +2,4-D(9.04µM) + Kin (6.97µM).
E. Profuse rooting from the basal region of regenerated shoot on MS+NAA(10.74µM) + BAP (17.76µM).
F. Potted plant.

CONCLUSION

From the aforesaid data, it can be concluded that cv. Muktakeshi and Sree Reshmi have responded better to the culture condition, than cv. Telia in terms of induction of multiple shoots. Among the media used, MS is suitable for direct multiplication whereas L2 induces callus and subsequent regeneration of shoots from the callus. The present study is a step forward for understanding the tissue culture requirements for the different cultivars of *Colocasia esculenta*.

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