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In Vitro Propagation of Chinese Potato (*Plectranthus rotundifolius* (Poir.) J.K. Morton) Through Axillary Shoot Bud Culture

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Abstract

An efficient and cost effective *in vitro* plant regeneration protocol through single nodal segment culture was achieved in the minor tropical tuber crop, Chinese potato (*Plectranthus rotundifolius* (Poir) J.K. Morton), through axillary shoot bud proliferation in Murashige and Skooge medium augmented with varying concentrations of 6-benzylaminopurine (BAP) and Kinetin (0.5–2.0 mg l^{-1}). BAP at 1 mg l^{-1} was the most effective medium for multiple shoot induction, which gave an average of 38 shoots/node, followed by Kinetin (0.5 mg l^{-1}) inducing 22 shoots/node as compared to the other concentrations of hormones and MS basal medium alone, after 45 days of culture. MS basal medium, though not promoting shoot multiplication, gave higher shoot length with elongated internodes. *In vitro* multiplied shoots transferred to MS medium with 0.5 mg l^{-1} IBA was optimum for healthy rooting. Rooting of the micro shoots also occurred in the Kinetin containing shoot multiplication medium itself from the base and nodes on ageing of the cultures after 85 days of culture. The healthy and vigorous *in vitro* regenerated micro shoots were separated out and were hardened upon transfer to plastic cups with sterile soil and sand. These were successfully acclimatized *ex vitro* in pots with potting mixture under green house conditions for four weeks. The survival rate was 90% and the plants that established in the green house resembled the mother plants in habitat without any morphological variations. This very simple and efficient protocol can be used to mass propagate elite stable clones of this very important aromatic tropical minor tuberous vegetable.

Key words: Chinese potato, 6-benzylaminopurine, Kinetin, axillary shoot bud culture, micropropagation, regeneration, micro shoots, *ex vitro*

Introduction

Plectranthus rotundifolius (Poir.) J.K. Morton commonly called as Chinese potato or Hausa potato, a native of India, is an annual vegetatively propagated herbaceous under-utilized starchy minor tuber crop of the tropical regions of India, Indonesia, Malaysia, Sri Lanka and Africa. It belongs to the family Lamiaceae and has very low genetic variability. It is a monsoon crop of 4-5 months duration with succulent stems and aromatic leaves showing profuse flowering during September-November but with complete sterility due to lack of fertile pollen grains.This crop is grown widely as a vegetable crop in the homestead gardens of Kerala and Tamil Nadu that bears a cluster of heteromorphous aromatic tubers, which makes it likeable as a delicacy among the vegetables. In Kerala, it is commonly called as Koorka or Cheevakizhangu. The composition of the edible tubers are water (75%); carbohydrate (21%); fibre (0.7%); ash (1%); calcium (17 mg $100g^{-1}$); iron (6 mg $100g^{-1}$); thiamine (0.05 mg $100g^{-1}$); riboflavin (0.02 mg $100g^{-1}$); niacin (1 mg $100g^{-1}$); ascorbic acid (1 mg $100g^{-1}$), with arginine, aspartic and glutamic acids as the principal

amino acids in the protein (Kay, 1973). Lack of genetic variability in the crop has greatly affected the improvement. The somaclonal variation has been identified as a potential means of creating genetic variability. Further, the major pest of the crop, the root knot nematode, which perpetuates through the tubers causes great reduction in yield and quality of tubers affecting its marketability. Tissue culture techniques to a great extent could help to overcome the problems associated with conventional propagation, storage and improvement of this vegetatively propagated crop and make it more productive.This paper describes the establishment of a successful method for high frequency plant regeneration of the crop through nodal segment culture.

Materials and Methods

In vitro techniques have the potential of producing very large number of homogenous plants within a short period of time relative to continuous asexual propagation methods. Multiple shoot culture is the most frequently used multiplication technique in *in vitro* plant micropropagation systems as it leads to the production of true to type plants. A suitable nutrient medium is needed for the success of the experimental system of plant tissue culture. In the present experiments, *in vitro* shoot multiplication through nodal segment culture was attempted in solid MS basal medium augmented with cytokinins BAP and Kinetin at different concentrations and with the incorporation of the auxin IBA for rooting in the medium with a view to develop suitable micropropagation protocol in the edible starchy minor tuber crop *Plectranthus rotundifolius*.

Nodal segments excised from the young, healthy and actively growing tuber raised mother plants of the accession, IC469741 (JSR/04-147) collected from Karanthakad in Kasaragod District of Kerala and maintained in pots under green house conditions of National Bureau of Plant Genetic Resources (NBPGR) Regional Station, Thrissur, served as the explant source for the present *in vitro* plant regeneration experiments (Fig.1). The explants were initially washed in running tap water for 30 min and then with aqueous surfactant Labolene 0.1% (w/v) for 15 min to remove the superficial dust particles as well as fungal and bacterial spores and again in tap water followed by repeated rinses in sterile distilled water thrice to remove the adhering detergent and surface contaminants. Further treatments were carried out in the laminar airflow chamber where the explants were surface sterilized with 0.1% (w/v) $HgCl_2$ for 5 min followed by rinsing with sterile double distilled water 3-5 times thoroughly to remove traces of $HgCl_2$. Throughout the experiments, MS media with 3% (w/v) sucrose was used and pH of the media was adjusted to 5.8 and solidified with 0.8% (w/v) agar and dispensed into culture tubes. The cultures were sterilized by autoclaving at 121^0C and 108 Pa for 20 min. The surface sterilized explants were trimmed to 0.5-1.2 cm by removing the cut ends and leaves, and were inoculated into culture tubes containing 15 ml of MS (Murashige and Skoog, 1962) basal solid medium supplemented with myoinositol (100 mg $100g^{-1}$), 3% (w/v) sucrose and individual concentrations of cytokinins BAP and Kinetin (0.5-2.0 mg l^{-1}) for shoot multiplication experiments.

The cultures were then kept in the culture room maintained at a temperature of 25±2°C under 12 h photoperiod at a light intensity of 3000 lux provided by cool-white fluorescent lights at 70-80% RH. In all the experiments, 10 replicates were maintained and the experiments were done using CRD and were monitored periodically and the data on number of shoots/explants and shoot length was recorded. The responding explants were transferred to MS basal media or fresh media either of the same composition or to lower concentrations of the same hormone for further shoot proliferation and elongation. The shoot multiplication rate was 10-20 times higher as compared to the first step. Multiplication could be continued by transferring each of the separated shoot or shoot clumps to the same media or to a medium with lower concentration of BAP or kinetin in jam bottles. The proliferated shoots of 2.0 to 4.5 cm length were excised from cultures and transferred to full strength MS basal medium or medium with 0.25 and 0.5 mg l^{-1} IBA for *in vitro* rooting. The rooting percentage was recorded after 2 weeks of culture. The well developed rooted plantlets were rinsed thoroughly in sterile distilled water to remove the residues and were potted in plastic cups in sterile soil with periodical watering for better rooting and hardening. After 14-21 days, the fully hardened plantlets were transferred to pots with potting mixture, sand and soil (3:1 w/v), under green house conditions for acclimatization and the survival percentage was recorded after 3 weeks.

Results and Discussion

Exploitation of tissue culture for multiplication of desirable germplasm to make it available to the plant breeder is essential. Production of plants from axillary shoots has proved to be the most efficient and reliable method of *in vitro* propagation. Nodal segment culture results in the stimulation of axillary shoot growth by overcoming the dominance of shoot apical meristem by the incorporation of growth regulators into medium (George and Sherrington, 1984). The use of nodal segments as initial explants for the *in vitro* propagation in *Plectranthus rotundifolius* has already been reported by Nair et al. (1994) and Mukherjee et al. (2006) and in *D. alata* and *D. rotundata* (Mantell et al., 1978; Nair and Chandrababu, 1996) which supports the selection of nodal segments as explant for shoot multiplication experiments in this species.

Nodal explants treated with different media formulations resulted in different growth responses (Table1). Effectiveness of MS basal medium for optimum shoot multiplication and rooting in this species was reported (Nair et al., 1994). Cytokinins are known to promote cell division and act as a vital force for bud production and growth. In the present experiments also shoot multiplication was achieved by culturing single nodal segments in MS basal medium supplemented with different concentrations of BAP and Kinetin (0.5-2.0 mg l^{-1}).

The nodal segments when used as explants showed positive responses in shoot characters such as number of shoots per node induced and shoot length in accordance with the hormonal concentrations tried (Table 1). Compared to the nodal segments inoculated onto basal MS, the shoot multiplication rate was very high in medium supplemented with BAP and Kinetin at different levels. Nair et al. (1994) also affirmed the potentiality of higher levels of BAP in promoting direct regeneration from lamina and internodal cuttings in the species. Of the different concentrations of BAP used, 1 mg l^{-1} was the most effective in multiple shoot induction within 45 days of inoculation, which gave an average of 38 shoots ranging from 10-68 (Fig. 2, Table 1) with shoot length ranging from 2.5-13.0 cm followed by Kinetin at 0.5 mg l^{-1} inducing 22 shoots ranging from 10-36 (Fig. 3) with shoot length ranging from 3.4-9.5 cm compared to the other hormonal concentrations tried (Table 1). As the concentration of BAP was increased to 2 mg l^{-1}, the number of shoots induced showed a reduction to 12 per explant and ranged from 3 to 24 (Fig. 4) and shoot length ranging from 3.5 to 8.9 cm suggesting that lower concentration of BAP was best for shoot proliferation in the species. The superiority of BA over other cytokinins for shoot proliferation has been established for plants such as *D. alata* (Mantell et al., 1980) and *D. bulbifera* (Forsyth and Van Staden, 1982). In the case of Kinetin also, as the concentration in the medium was increased to 1 mg l^{-1}, there was reduction in the number of shoots induced to 16 along with the induction of callus (Fig. 5). Higher concentrations of cytokinins, BAP and Kinetin in the medium was found to induce callus (Fig. 6) in the cultures which supports the report on

Table1. Effect of different concentrations of BAP, Kinetin and IBA in MS basal solid medium on shoot multiplication from nodal explants and rooting of the induced shoot buds in Chinese potato

Treatments (mg l^{-1})	Mean number of shoots/explants	Mean shoot length (cm)	Mean regeneration percentage	Callusing	Mean rooting percentage (after 2 weeks)
BAP-0.5	20	13	80	—-	5
BAP-1.0	38	10	95	—-	15
BAP-2.0	12	8.3	89	+	5
Kinetin-0.5	22	8.9	92	+	15
Kinetin-1.0	16	5.5	87	++	15
Kinetin-2.0	10	7.1	90	+++	25
IBA-0.25				+	72
IBA-0.5				+	95
Basal MS	3-4	8.5	90	—-	10
CD (0.05)	8.047	2.263	0.196	—-	4.200

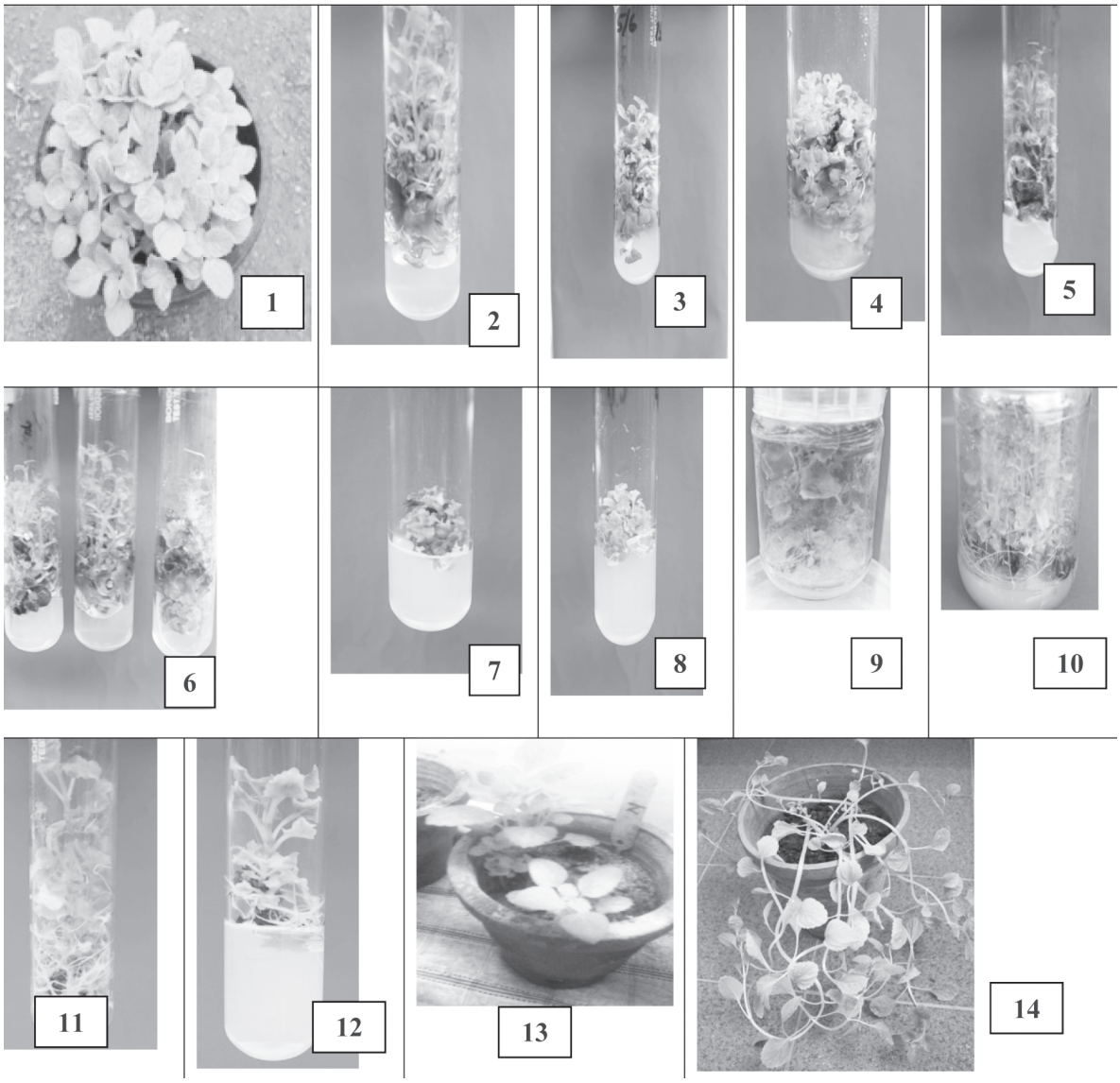

Fig. 1. IC469741 (JSR/04-147), the accession of *Plectranthus rotundifolius* from Karanthakad, Kasaragod District of Kerala, maintained in the field gene bank of NBPGR Regional Station, Thrissur, Kerala, mother plant for the nodal segment explants
Fig. 2. Induction of 38 shoots from single nodal segment explant in MS medium with 1.0 mg l^{-1} BAP
Fig. 3. Induction of 22 shoots from single node in MS medium with 0.5 mg l^{-1} Kinetin
Fig. 4. Reduction in number of shoots to 12 per explant in MS medium with the addition of 2.0 mg l^{-1} of BAP
Fig. 5. Reduction to 16 shoots/explant on increasing the concentration of Kinetin to 1 mg l^{-1} along with callus induction from base
Fig. 6. Reduction in the number of shoots and induction of callus from base in cultures with high concentrations of cytokinins BAP and Kinetin
Fig. 7. Single nodal segment in basal MS solid medium producing only 3-4 shoots/explants
Fig. 8. BAP at 0.5 mg l^{-1} inducing 20 shoots/explant
Fig. 9. Multiplication of induced shoots on transfer to fresh media with low concentration of BAP/Kinetin in jam bottles
Fig. 10. Highest shoot multiplication of induced shoots in medium with kinetin at 0.5 mg l^{-1}
Fig. 11. Vigorous rooting of shoots on transfer to MS basal medium supplemented with 0.5 mg l^{-1} IBA
Fig. 12. Spontaneous rooting in the shoot induction medium itself in cultures containing 2 mg l^{-1} of Kinetin
Fig. 13. Rooted shoots transferred from plastic cups to pots with sterile sand for hardening
Fig. 14. Hardened plants established in pots in the greenhouse

callus induction at higher cytokinin concentrations along with the presence of higher levels of endogenous hormone content in the species. When compared with the check, single nodal segment in basal MS solid medium without any growth hormones, only 3-4 shoots/explants could be induced within the same period (Fig. 7). MS medium free of hormones helping the growth of nodal cultures was reported by Nair et al. (1994). When BAP (0.5 mg l^{-1}) was used, the average number of shoots ranged from 2-32 in 45 days with an average of 20 shoots (Fig. 8, Table 1) and shoot length ranged from 5.9-13.8 cm. Multiplication could be continued by transferring each of the separated shoots/shoot clumps either to the same media or to a medium with lower concentrations of BAP or Kinetin or to basal MS medium without any growth hormones (Fig. 9). The multiplication rate was found to be the highest in medium supplemented with 0.5 mg l^{-1} of Kinetin (Fig. 10). Individual shoots on subculture to MS medium containing IBA (0.25 and 0.5 mg l^{-1}) produced healthy and vigorous roots compared to those transferred to the same shoot initiation medium. Among the two, highest of 95% was obtained in IBA at 0.5 mg l^{-1}containing medium (Fig. 11) followed by 72% in IBA at 0.25 mg l^{-1} in 2 weeks of culture. Spontaneous rooting in the same shoot induction medium was observed in cultures containing Kinetin at all the three concentrations with highest at 2 mg l^{-1} on ageing of cultures along with callus formation (Fig.12). The efficacy of IBA supplemented MS medium in inducing roots from shoot clusters was reported in *Bacopa monnieri* (Sharma et al., 2010), *A. calcarata* (Sudha et al., 2012), *A. officinarum* (Selvakumar et al., 2007) and in *Kaempferia galanga* (Kalpana and Anbazhagan, 2009).

The healthy rooted plants thus obtained were washed well in running tap water to make it free from agar and were separated and transferred to plastic cups with sterile sand for hardening (Fig.13). These plants were transferred to pots with potting mixture in greenhouse and after 4 weeks the survival rate was recorded. Almost 95-100% of the regenerated plants survived and showed vigorous growth without showing any morphological variations (Fig.14). Successful establishment of nodal regenerated plantlets was reported in the species *Plectranthus rotundifolius* by Nair et al. (1994) and Mukherjee et al. (2006). This commercially viable protocol presently developed can be put to use for mass micropropagation for cultivation of this important minor tuberous vegetable.

Conclusion

An efficient and reliable *in vitro* plant regeneration protocol was established in the tropical tuber crop, Chinese potato (*Plectranthus rotundifolius*), through nodal segment culture using Murashige and Skooge medium augmented with varying concentrations of 6-benzylaminopurine (BAP) and Kinetin. BAP 1mg l^{-1} was the most effective in shoot multiplication giving an average of 38 shoots/explant, followed by 0.5 mg l^{-1} Kinetin inducing 22 shoots. *In vitro* shoots transferred to MS medium with 0.5 mg l^{-1} IBA was optimum for healthy rooting. *In vitro* regenerated plantlets were successfully hardened and acclimatized under green house conditions with 90% survival and establishment, resembling the mother plants in their habit, without any morphological variations. The very simple, commercially viable and efficient protocol developed can be put to use for mass multiplication of this very important aromatic minor tuberous vegetable.

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