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IN VITRO REGENERATION OF CAPE GOOSEBERRY (*PHYSALIS PERUVIANA* L.) THROUGH NODAL SEGMENT

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ABSTRACT
A Protocol for *in-vitro* propagation of Cape gooseberry (*Physalis peruviana* L.) was standardized using nodal segments and internodal segments. Nodal segments (1-2cm) having atleast one axillary bud was cultured on Murashige and Skoog (MS) medium supplemented with BAP and IBA. Nodal explant was found better than internodal explants. The plant growth regulators BAP (2.5 mg/L) and IBA (0.05 mg/L) were judged to be best for shoot proliferation on the basis of sprouting percentage, number of shoots, shoots length and number of leaves. The regenerated shoots from nodal segments were excised aseptically and transferred to the rooting medium supplemented with IBA (0.05mg/L) and was found superior for days to root initiation, number of roots and root length. Addition of activated charcoal at 450 mg/L resulted in 100 per cent browning free culture.

INTRODUCTION

Cape gooseberry (*Physalis peruviana* L.) is a tropical fruit from the solanaceae family, which comprises many agriculturally important crops including potato, tomato, pepper, eggplant and tobacco (Knapp, 2001). The cape gooseberry fruit contains high levels of vitamin A, C and B-complex as well as compounds of anti-inflammatory and antioxidant properties (Ramadan, 2011). Epidemiological studies indicate that increased consumption of fruit of cape gooseberry could lead to lower risk of chronic degenerative diseases (Reddy *et al.,* 2010).

The fruit is sweet and have good content of iron, phosphorus and fiber, often used in making jellies, jams, juices and ice creams (Muniz *et al*., 2014). Polyphenols in the fruit demonstrate anti-inflammatory and antioxidant properties (Franco *et al*., 2007). Given its value, the plant is increasingly becoming an important crop in functional food production and represents an emerging market of growing economic importance (Ramadan and Morsel, 2007).

Cape gooseberry is conventionally propagated through seed. However, there are many problems associated with seed propagation such as poor germination of seed, variability in vigour, seedling growth coupled with susceptibility to number of diseases. Micro propagation is preferred over conventional method of propagation owing to its faster multiplication rate, uniformity in planting materials and production of disease free materials. There are a limited number of in vitro regeneration studies concerning the micro propagation of *P. peruviana* (Rodrigues *et al.,* 2013b), not containing information about the synergistic effects of plant growth regulator (PGR) combinations on growth parameters. The use of tissue culture methods for the selection of the best cultivars for agricultural practices and clonal propagation might be conducive for micro propagation, especially when taking into account high consumption due to value-added natural compounds, limited plant production, and requirements of manpower, transport, and storage (Rodrigues *et al.,* 2013a). Moreover, there are few reports available till date on micro-propagation of cape gooseberry. Hence, it is need of the hour to develop proper protocol for in-*vitro* propagation of cape gooseberry.

MATERIALS AND METHODS

Explant viz; nodal and intermodal segments (1-2 cm) were collected from two month old greenhouse plants and the experiment was conducted in the Plant Tissue Culture Laboratory, Department of Horticulture, Institute of Agricultural Sciences, Banaras Hindu University during 2012-2014.

Explants were thoroughly washed under running tap water for 15-20 minutes. The explants were treated with Bavistin (100mg/100ml) + Streptocycline (20-40mg/100ml) for 10 minutes in rotator shaker. This facilitates proper action of sterilant chemicals on explants taken.The explants were then washed with double distilled water (DDW) and transferred to laminar air flow chamber (LAFC) which was pre-sterilized with UV light for 40 minutes. The explants were then surface

sterilized by dipping in NaOCl (1%) for different periods. After that they were immersed in 70% ethanol for 30 seconds and washed 3-4 times with double distilled water. . These sterilants are also toxic to the plant tissues, hence proper concentration of sterilants, duration of exposing the explant to the various sterilants, the sequences of using these sterilants has to be standardized to minimize explant injury and achieve better survival (CPRI, 1992). A cut was given to basal as well as upper portion of sterile explants to remove undesired/dead portion and washed with double distilled water for 3-4 times. Sterilization of growth media and instruments were done by autoclaving at 121°C at 15 psi for 20 minutes.MS medium (Murashige and Skoog 1962) was used in all treatments with agar 8g/L as gelling agent and sucrose at 30g/L along with growth regulators and other additives. The pH of the medium was adjusted to 5.8.

The explants were inoculated to MS medium supplemented with combination of 6-benzyl amino purine (BAP) along with Indole-3-butryric acid (IBA). Later for induction of rooting *in-vitro* proliferated shoots were transferred to half MS medium supplemented with different concentrations of IBA and activated charcoal. All the cultures were maintained under the photoperiod of 16/8 hours light and dark cycle at 25 ± 2°C.

Rooted plantlets were transferred to *ex-vitro* condition for acclimatization. They were removed from agar medium and washed in shallow tray containing sterile water to remove adhering agar. They were then transferred to pot containing sterile sand, soil and FYM (1:1:1). The sterilized pots were kept under plant growth chamber with punched polythene covering at top of the plants. The plants were placer under this condition for about 10-15 days. Then, pots were kept in polyhouse for 2 weeks for hardening. Thereafter the hardened plants were transferred to the field.

The experimental data were subjected to analysis of variance using completely randomized design (CRD). Four replications were taken for each treatment.

RESULTS AND DISCUSSION

The growth and development aspect of plant , influences by the phytohormone cytokinin at different level including a prominent role in the regulation of cell proliferation (Schaller *et al.*, 2014). Within nine days after culture initiation, new leaves started forming from the nodal segment (Fig. 1.a.). Cytokinin positively regulates cell division and also serves a key role in establishing organization within shoot stem cell centers (Schaller *et al.*, 2014). Activated charcoal is an essential component of plant tissue culture media. It is a strong adsorbent that can absorb toxic substances (Zhou, *et al.*, 2010).

Browning decreased significantly with increasing

Table 1: Effect of growth regulators on shoot proliferation

Concentration of growth regulators (mg/L) (BAP+IBA)	Sprouted shoot (%)	Number of shoots	Shoot length (cm)	No. of leaves
T_1 (0.0+0.0)	0.00 (0.00*)	0.00	0.00	0.00
T_2 (0.5+0.01)	53.00 (46.70*)	1.50	1.45	2.75
T_3 (1.0+0.02)	61.00 (51.33*)	2.25	2.25	3.00
T_4 (1.5+0.03)	72.37 (58.27*)	2.75	2.75	4.75
T_5 (2.0+0.04)	79.33 (62.94*)	3.50	3.45	6.25
T_6 (2.5+0.05)	86.25 (68.26*)	4.75	4.87	8.25
T_7 (3.0+0.06)	81.50 (64.52*)	3.25	4.12	7.50
T_8 (3.5+0.07)	76.26 (60.83*)	2.50	3.75	6.50
T_9 (4.0+0.08)	59.37 (50.38*)	2.00	3.50	5.25
T_{10} (4.5+0.09)	51.0 (45.55*)	1.50	1.97	4.75
S.E	1.267	0.532	0.338	0.524
C.D at 0.05	2.599	1.092	0.694	1.076

*values in parentheses are arcsine transform

Table 2: Effect of growth regulators on root proliferation

Concentration of growth regulator (mg/L)(IBA)	Days to root initiation	Rooting (%)	Rootlength (cm)	Number of roots
T_1 (0.00)	0.00	0.00 (0.00*)	0.00	0.00
T_2 (0.01)	38.75	22.75(28.47*)	1.86	1.39
T_3 (0.02)	35.00	33.15 (35.14*)	2.08	2.12
T_4 (0.03)	32.50	51.82 (46.02*)	2.32	2.98
T_5 (0.04)	28.25	76.40 (63.04*)	2.54	3.12
T_6 (0.05)	24.50	80.07 (63.46*)	3.24	3.84
T_7 (0.06)	26.00	75.12 (60.05*)	1.79	1.06
T_8 (0.07)	27.75	68.25(55.68*)	2.12	1.53
T_9 (0.08)	31.00	62.97 (52.49*)	2.46	2.34
T_{10} (0.09)	33.25	56.05 (48.45*)	2.74	3.26
T_{11} (0.10)	35.50	40.00 (39.21*)	3.11	3.48
S.E.d	0.577	3.207	0.291	0.504
C.D at 0.05	1.185	6.553	0.594	1.029

*values in parentheses are arcsine transform

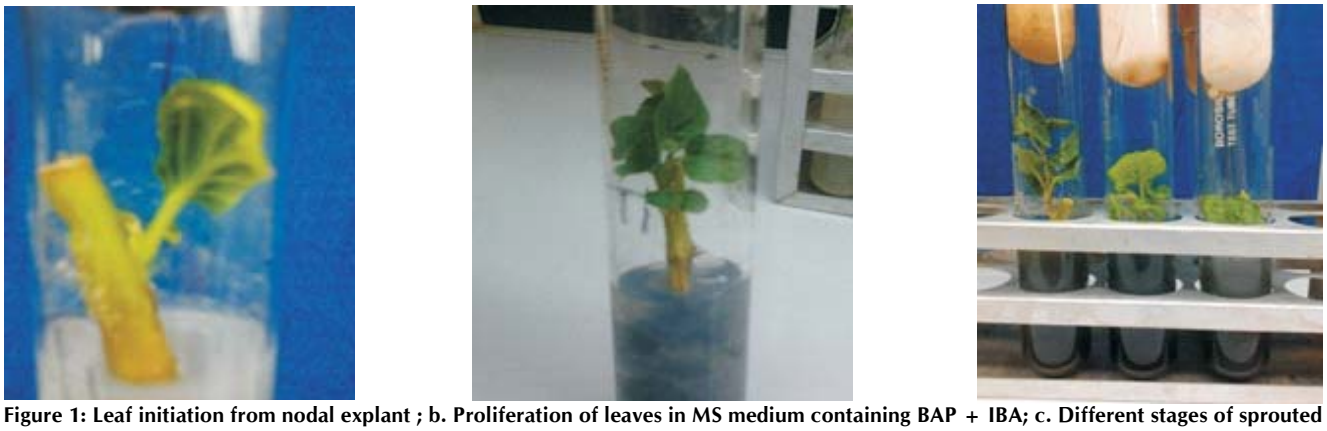

Figure 1: Leaf initiation from nodal explant ; b. Proliferation of leaves in MS medium containing BAP + IBA; c. Different stages of sprouted shoot

Figure 2: a. Root initiation after 24 days in MS medium containing IBA; b. Rooted plantlet after washing in DDW; c. Tissue cultured plant of capegooseberry in pot after secondary hardening

concentration of activated charcoal and no browning was recorded when medium was supplemented with activated charcoal at 450 mg/L. BAP (2.5 mg/L) in association with IBA (0.05 mg/L) gave best results exhibiting maximum sprouted shoots (86.26%), number of shoots (4.75), shoots length (4.87cm), number of leaves (8.25) per explant as compared to the rest of the treatments. Cytokinins concentration (BAP) increased the number of shoot up to certain optimal level and then decrease with further increase in cytokinins concentration (Table 1). These results corroborate the findings of (Molla *et al.,* 2011).

Higher concentration of BAP had inhibitory effect on sprouting of nodal segments and addition of IBA in medium helped to nullify the effect of higher cytokinin concentration. The type and the concentration of the growth regulator in the medium were found to be important factor for multiple shoot induction (Bhat *et al.,* 2013, Pawar *et al.,* 2013, Pawar *et al.,* 2012,).

The phytohormones auxin are essential for the control of diverse aspects of cell proliferation and differentiation processes in plants (Suzaki *et al.,* 2013). As for as the impact of IBA and NAA on rooting is concerned, IBA was found to be better for root induction in shorter period of time than NAA. It is due to the reason that IBA is photo insensitive and is not affected by IAA oxidase or other enzymes and hence persists for longer periods (Yu *et al.,* 2000). There was no rooting on MS basal medium without IBA.

The data in (Table 2) reveal that different concentrations of IBA influenced *in-vitro* rooting potential of cape gooseberry microshoots. IBA (0.05mg/L) proved to be best for days to root initiation (24.50), root length (5.82 cm), number of roots (10.25) and rooting percent (80.07). The results of present investigation corroborate the findings of Osman *et al,* (2010). The complete plantlets with well developed roots were transferred to plastic pots filled with mixture of sand, soil and FYM (1:1:1) before transferring to field directly.

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