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IN VITRO CLONAL MULTIPLICATION AND TUBERIZATION OF *SOLANUM TUBEROSUM*

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Clonal propagation, multiplication and tuberization of *Solanum tuberosum* L. cv. Chandramukhi were studied *in vitro* through axillary shoot culture on MS (1962) medium with IAA/2-ip or hormone free medium (establishment medium). Shoots were transferred after 21 days to another medium with NAA or IAA (proliferation medium). Subculturing of the grown up axillary shoots was carried out at biweekly interval on fresh proliferation medium. *In vitro* multiplied axillary shoots on transfer to MS medium with BAP and high sucrose (tuberization medium) responded positively with the formation of miniature tubers within 25-30 days after inoculation when incubated at low night temperature of 12-15°C and day temperature of 22-25°C under 1000 lux intensity of fluorescent light for 8-9 h.

Key words: Clonal multiplication, Tuberization, *Solanum tuberosum*.

Tissue culture techniques are attractive tools for the improvement of crop plants. Clonal propagation and multiplication of important plants have been achieved (Anderson. 1980). Potato (*Solanum tuberosum* L.) involves the problem of storage and maintenance of seed tubers. The recent efforts are to produce miniature tubers of desired varieties which can be easily transported to the field for propagation. The idea has been commercially exploited. Much success has been achieved in this direction (Wang and Hu. 1982) but in India no sincere effort has been made. This investigation is an attempt in this direction. Potato cv. Chandramukhi from Kufri which is commonly grown in the plains of Uttar Pradesh are used for clonal propagation and seed tuber production *in vitro*.

MATERIALS AND METHODS

Cultivar Chandramukhi was procured from CPRI Campus at Modipuram (Meerut) in the form of seed potato tubers. The seeds were sown in Petri plates. Shoot tips were harvested from sprouted tubers as they reached about 1 cm in length. Surface disinfection involved washing with Teepol, rinsing in 90% alcohol (30 seconds) and then with 0.05% mercuric chloride for 10-12 min. After washing 5-6 times with double distilled sterilized water, they were inoculated for growth on nutrient medium.

The cultures were incubated in dark at 25±2°C. After 4-5 days, they were transferred to light of 2000 lux intensity. In all tissue culture experiments, MS medium was used and axenic conditions were maintained throughout the experiment. The basal mineral ingradients were supplemented with 100 mg/l meso-inositol, 3% sucrose and combinations of growth regulators. Bacteriological agar 0.6-08% was used for solidification of culture medium. The pH of medium was adjusted to 5.8 prior autoclaving at 1.06 kg/cm^2 pressure for 15-17 min.

Clonal multiplication was attempted via enhanced axillary shoot proliferation, and *in vitro* tuberization. Plantlets regenerated through shoot apex culture on MS solid medium with IAA/2-ip/hormone free medium (establishment medium) were harvested and placed horizontally on fresh MS medium with NAA/IAA (proliferation medium) in 250 ml flasks. Sprouted axillary shoots were harvested after every 15-20 days and subcultured to multiply the clone. Harvested axillary shoots were transferred to MS medium with BAP and high sucrose (tuberization medium). The cultures at this stage were maintained at 10-12°C night temperature and 25°C day temperature in light of 1000 lux intensity.

RESULTS

Shoot apex culture: Shoot tips about 7 mm in length were utilized. These were grown on hormone free medium or IAA (0.5mg/l) or 2-ip (0.5-1.0 mg/l) supplemented medium (Fig. 2). The time required for the excised shoot tips to initiate growth was variable (7-10 days). Rooted plantlets measuring 4-5 cm in length were regenerated in about 3 weeks.

Micropropagation via axillary shoot proliferation: Regenerated plantlets were horizontally layered on surface of MS solid medium + NAA (0.001 mg/l)/ IAA (0.5 mg/l) in 250 ml flasks. Within 3-4 days after

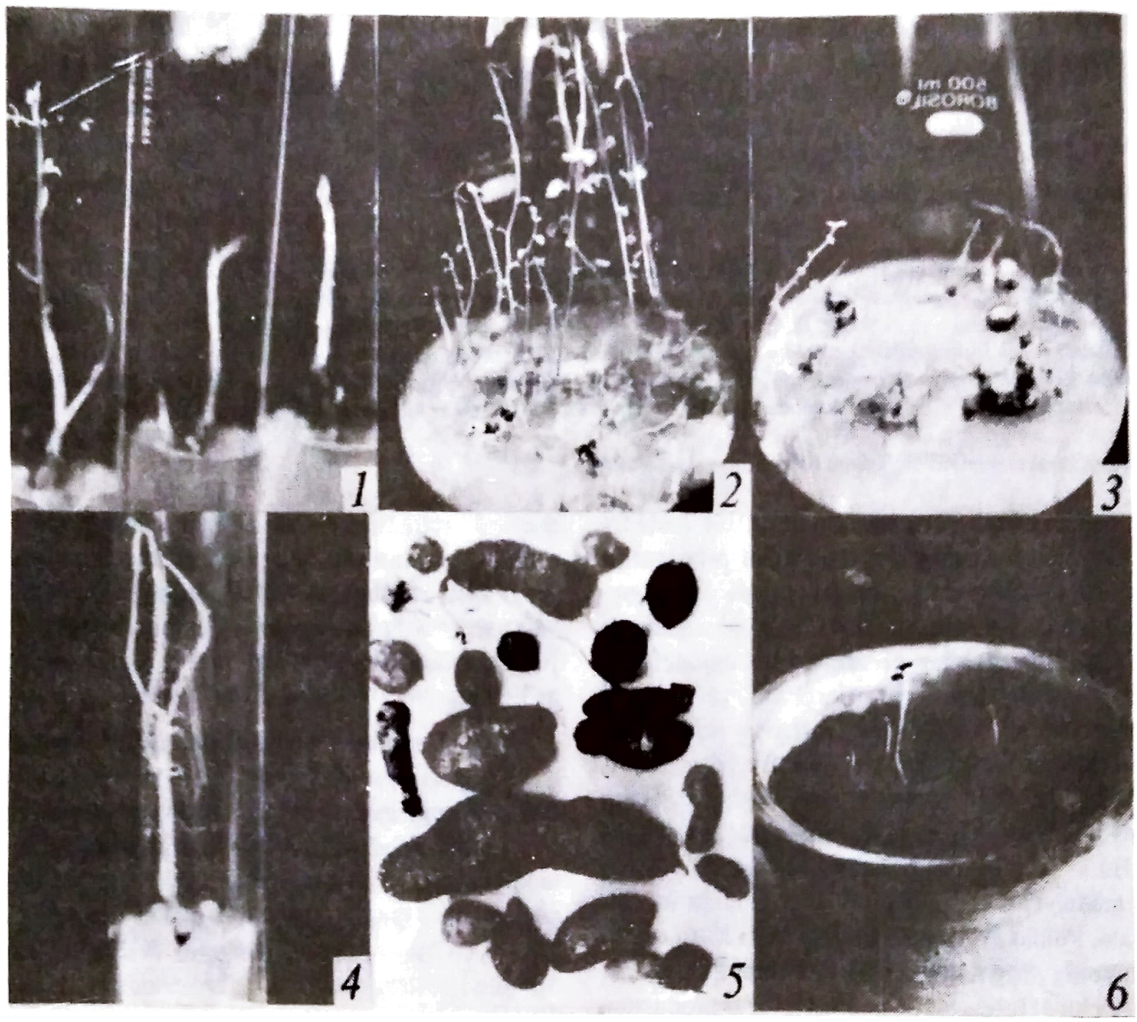

Fig. 1. Growth of shoot apices in BMS medium supplemented with 2-ip (0.5-1.0 mg l^{-1}). Fig. 2. Proliferation of axillary buds on BMS supplemented with NAA (0.001 (mg l^{-1}). Fig. 3. *In vitro* mass tuberization on BMS medium supplemented with BAP (5-8.5 mg l^{-1}) and Sucrose (8%). Fig. 4. *In vitro* sprouting of miniature tuber. Fig. 5. Miniature dormant tubers after harvest. Fig. 6. Sprouting of miniature tubers in soil.

inoculation, axillary buds sprouted and adventitious roots were concomittantly initiated at each node (Fig. 2). Axillary shoots grew rapidly reaching 5-6 cm in about 15-20 days. Adventitious root formation was more profuse on IAA (0.5 mg/l) supplemented medium. The grown up axillary shoots were harvested repeatedly every 15-20 days and subcultured on fresh proliferation medium, thus accomplishing micropropagation.

In Vitro mass tuberization: On tuberization medium (5-8.5 mg/l BAP + 8% sucrose) adventitious root formation coupled with axillary bud elongation was observed 6-7 days after inoculation. After a period of 15-20 days, stolons appeared and showed growth towards the medium. The tip of these stolons started swelling and miniature tubers developed (Fig. 3). Sprouting of miniature tubers occurred *in vitro* in many cases (Fig. 4).

The tubers were harvested after 3 months. On an average, 14-16 miniature tubers were produced per explant (Fig. 5). The size varied between 0.9-5.4 cm in length. Fresh matter weight ranged between 0.2-6.485 gm. Their morphology varied with tubers, spherical to oval elongated ones. Tubers which remained inside the medium were pale in colour, while those exposed to light were green tinged. Each miniature

tuber had 'eyes', the number varied with size of the tuber. When the miniature tubers were field sown, they produced healthy plants (Fig. 6).

DISCUSSION

In shoot tip culture, shoot tips of about 0.7 mm in length were used. Using 0.1 mm long potato meristems, Kassanis and Varma (1967) found that the explant with 1 leaf primordium resulted in a more advanced development than those lacking leaf primordium. The lack of development capacity of apical domes is likely due to its dependence on the subjacent leaf and stem tissues for hormonal resources. The larger the explant, more likely, it is to grow and produce roots, however, explants larger than 0.7 mm long may be virus infected (Mellar and Stace-Smith, 1977).

We noticed that for enhanced axillary shoot proliferation, auxin was essential. Although, in general, it was cytoknin, which overcomes the apical dominance of shoots and to enhance the branching of lateral buds from leaf axils. Use of auxins was found essential to overcome the apical dominance (Lundergun and Janick, 1980). On transfer to tuberization medium, low temperature during dark period coupled with short light period enhanced the induction of tuber. These results are in conformity with those of Chapman (1958).

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