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Short communication

In vitro propagation of short day onion

R. Passi, A.S. Dhatt*, J.S. Sandhu, A.S. Sidhu and M. Kaur**
Department of Vegetable Crops
Punjab Agricultural University, Ludhiana 141 004

Onion (*Allium cepa* L.) is multiplied through seed and sets or vegetatively. The multiplication by seed and sets requires two-year cycle under north Indian conditions. Completing one seed cycle, bulbs are produced through seedlings from November to May, stored for five months and replanted in November to harvest the seed in next May. Handling of bulk quantity bulbs (>25 q ha^{-1}) for 5 months, storage losses (15-40%), higher market prices of bulbs at the time of replanting (> Rs. 1,500 q^{-1}) and risk of diseases particularly purple blotch, downy mildew and stempyllium blight to seed crop are the major limiting factors in onion (Anjaneyulu *et al*., 1). Genetic contamination by out crossing, inbreeding depression and poor longevity of seed are the other problems with seed multiplication.

Standardization of *in vitro* micropropagation in onion is highly desired for bulking and maintenance of healthy selected genotypes. The successful maintenance and multiplication of male sterile lines for use in hybrid seed production would also be facilitated by this procedure. Successful multiplication for a single cycle of regeneration has been reported (Hussey, 3). In fact, no controlled embryo or shoot production has been obtained from long-term tissue culture in onion (Novak *et al*., 9). The plantlet dormancy and decrease in regenerative ability or multiplication rate are the limiting factor (Hussey and Falavigna, 4). This communication describes the protocol for *in vitro* propagation for mass multiplication of onion.

The field grown onion bulbs of variety Agifound Dark Red (ADR) were used in the present study. After removing outer leaves, explants were thoroughly wiped with cotton swab dipped in 70% ethanol followed by washing in running tap water, Teepol™ and distilled water. Surface sterilization was carried out under aseptic conditions involving initial dipping in 70% ethanol (30 sec.) followed by treatment with 0.5% Bavistin™ (15 min.) and finally with 0.1% mercuric chloride (10 min.). Each step follows 3-4 times rinsing with sterile water. After surface sterilization, basal plate explants (Fig.1A) were cultured in the test tubes and, then in glass jars. Murashige and Skoog (8) basal media having 3.0% sucrose and 0.8% agar was supplemented with different levels of BAP (1.0, 2.0, 3.0, 3.5, 4.0 and 5.0 mg/l) and NAA (0.1, 0.2 and 0.5 mg/l) at pH 5.8 (Table 1). Antibiotic Omnatex™ (Cefotaxime) was also added to the medium aseptically after autoclaving to check systemic bacterial contamination. Cultures were incubated at 25±2°C under16/8 h photoperiod (illuminated with 40 W white fluorescent tubes) followed by dark period.

The sprouts obtained from established explants were gradually separated and sub-cultured after 30 days on MS medium supplemented with different concentrations and combinations of cytokinins (BAP @ 1.0, 2.0 and 3.0 mg/l and kinetin @ 0.5 mg/l) and auxin (NAA @ 0.5 mg/l) for *in vitro* shoot multiplication (Table 2). The average number of shoots derived at the end of each sub-culture out of a single propagule was reformed as multiplication fold. The multiple shoots clumps were separated into individual shoot and cultured on the half-strength MS medium supplemented with combinations of auxins IBA (0.5, 1.0, 1.5 and 2.0 mgl^{-1}) and NAA (0.5 mgl^{-1}) as given in Table 3. Plants with freshly developed roots were thoroughly washed in running tap water and kept on half-strength MS medium (without sugar and vitamins) in open glass jars and plastic trays under high light intensity for 4-5 days in the incubation room for elongation of roots and hardening of plants. Fully-grown hardened plants were transferred to polythene bags containing field soil and farmyard manure and kept in glasshouse for 10-12 weeks at 30±2 ^{0}C and 80±3% RH. For *in vitro* establishment 30 explants were used for each treatment, while for *in vitro* shoot multiplication and rooting 20 explants per treatment were used. All experiments were replicated five times and analysed as per complete randomized block design (Snedecor and Cochran, 11).

The effects of growth hormones on shoot regeneration are presented in Table 1. It was highest on MS medium containing 3.5 mgl^{-1} BAP + 0.5 mgl^{-1} NAA (55.52%), followed by 4.0 mgl^{-1} BAP + 0.5 mgl^{-1} NAA (55.39%) and lowest (23.37%) with no use of growth hormones. It was observed that low concentration of auxin (0.1 mgl^{-1} NAA) gave poor response for shoot establishment, whereas, high

*Corresponding author's E-mail: ajmerdhatt@gmail.com
**School of Agricultural Biotechnology, PAU, Ludhiana 141004

Table 1. Effect of growth regulators on *in vitro* shoot initiation in onion.

Treatment (mg l^{-1})		Culture establishment (%)
BAP	NAA	
0.0	0.0	23.37 ± 1.43
1.0	0.1	26.23 ± 1.36
2.0	0.2	36.44 ± 1.56
3.0	0.5	44.71 ± 1.57
3.5	0.5	55.52 ± 1.50
4.0	0.5	55.39 ± 1.78
5.0	0.5	49.25 ± 1.85

Table 2. Effect of growth regulators on *in vitro* shoot multiplication in onion.

Treatment (mg l^{-1})			Shoot multiplication (%)
BAP	kinetin	NAA	
0.0	0.0	0.0	0.00
1.0	0.0	0.5	56.64 ± 6.36
2.0	0.0	0.5	72.32 ± 1.43
3.0	0.0	0.5	67.99 ± 1.14
4.0	0.0	0.5	61.87 ± 1.63
2.0	0.5	0.0	46.44 ± 10.41
3.0	0.5	0.0	54.22 ± 3.29
4.0	0.5	0.0	49.73 ± 8.24

Table 3. Effect of growth regulators on *in vitro* rooting in onion.

Treatment (mg^{-1})		Rooting (%)
IBA	NAA	
0.0	0.0	32.13 ± 1.61
0.5	0.0	63.21 ± 1.44
1.0	0.5	87.37 ± 1.35
1.5	0.5	72.41 ± 1.67
2.0	0.5	55.24 ± 1.51

concentration of cytokinins (5.0 mgl^{-1} BAP) suppressed the growth and low concentration developed coiled and morphologically abnormal shoots. The fortification of MS basal medium with BAP @ 3.5 mgl^{-1} + NAA @ 0.5 mgl^{-1} found to be the best option for shoot initiation in onion variety ADR (Fig.1B). This is in agreement with the findings that supplementation of BAP and NAA in the media proliferated the regeneration response in onion (Hussey, 3; Pike and Yoo, 10).

The results of *in vitro* shoot multiplication are given in Table 2. The sprouts obtained from established explants were gradually separated and subcultured on MS medium with different concentrations and combinations of cytokinin and auxin after 30 days. Although, there was no multiplication of propagules on MS basal alone; however addition of growth hormones gave 46.44 to 72.32% increase. It was highest, when MS basal medium was fortified with 2.0 mgl^{-1} BAP + 0.5 mgl^{-1} NAA (Fig.1C and D). Whereas, maximum shoot multiplication with 3.0 mgl^{-1} BAP + 0.5 mgl^{-1} kinetin combinations was 54.22%. It was also observed that established shoots became morphologically abnormal when left for 60 days on the same medium without the subculture, or subcultured after 30 days

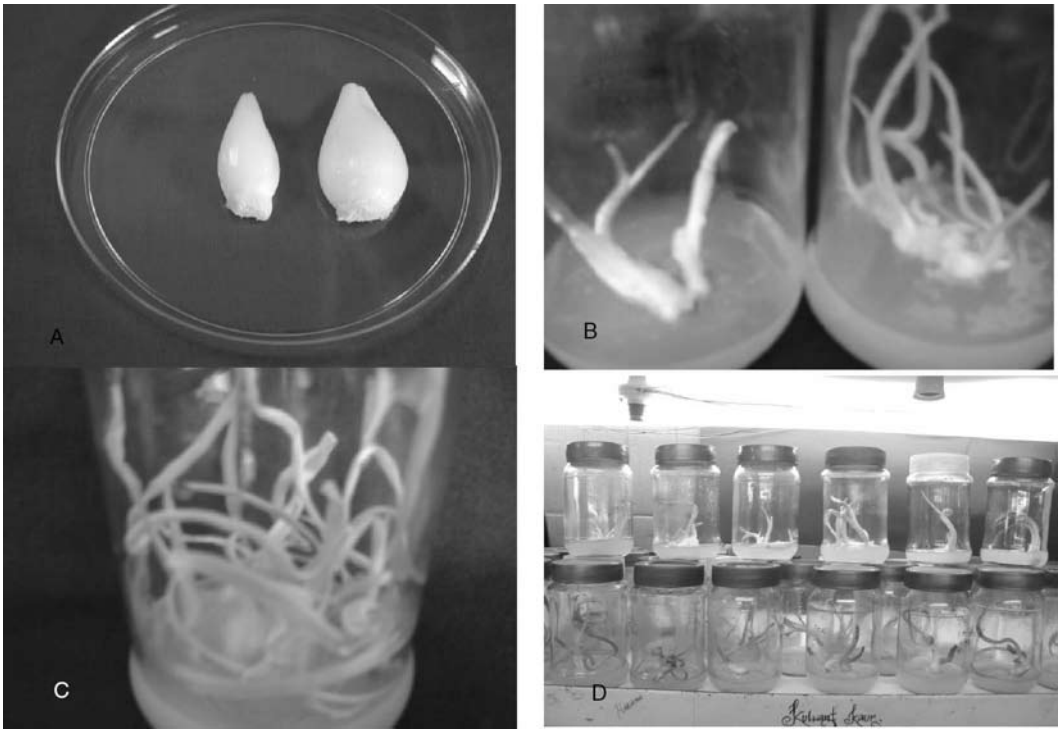

Fig. 1: A. Explant used for multiplication; B. Shoot initiation in onion on MS basal medium with BAP @ 3.5 mgl^{-1} + NAA @ 0.5 mgl^{-1}; C. Shoot multiplication on MS medium fortified with 2.0 mgl^{-1} BAP + 0.5 mgl^{-1} NAA; and D. Multiplied onion plants in bulk.

onto a medium with the same concentration of growth regulators. The effect of growth regulators on percent shoot multiplication per explant was significant. BAP increased the number of shoots and NAA number of roots. However, combination of both stimulated shoot growth and suppressed the root elongation. The higher concentration of BAP in shoot initiation media gave carry over effect on shoot multiplication by giving more number of small sized shoots. The differential response of BAP and NAA on shoot multiplication of different genotypes has also been reported in onion earlier (Gems and Martinovitch, 2; Hussey, 3; Kahane *et al*., 5; Kamastaityte and Stanys, 6; Khar *et al*., 7).

In vitro induction of roots on the excised shoots is very important in mass multiplication through micropropagation. The results of multiple shoots clumps separated into individual shoots and cultured on the half-strength MS medium supplemented with different combinations of auxins are given in Table 3. Rooting on MS medium alone was 32.13% and it was increased to 63.21% with the addition of 0.5 mgl^{-1} IBA and, enhanced to 87.37% with 1.0 mgl^{-1} IBA + 0.5 mgl^{-1} NAA. However, further addition of IBA (1.5 and 2.0 mgl^{-1}) along with NAA dropped the rooting efficiency.

Tissue culture generated plants are usually fragile and tender. The direct transferring of these plants shows poor field establishment. Therefore, fully grown *in vitro* rooted plantlets were removed from culture vessels and thoroughly washed in running tap water. Firstly, plants were hardened *in vitro* for five days in the incubation room by placing on moist cotton in half-strength MS salts and then on moist cotton. Subsequently, transferred to soil in incubation room having high humidity and finally to soil in polythene bags kept in the glasshouse. The direct micropropagation could produce thousands of onion plants of elite genotypes of economic importance in few months from a single basal plate. It also avoids the conventional constraints and intervening callus phase risks for clonal fidelity.

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