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# *IN VITRO* MICROPROPAGATION IN TROPICAL SHORT DAY ONION (*ALLIUM CEPA* L.)

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**Abstract**

For *in vitro* micropropagation, short day tropical varieties of onion 'Agrifound Dark Red', 'Punjab Naroya' and 'PRO-6' were exposed to different concentrations and combinations of growth hormones. Pre-sterilization of basal plate of onion in 0.5% solution of bavistin followed by treatment with 0.1% mercuric chloride for 10 min. produced the highest rate of survival of explants (47.9%). Survival was further enhanced to 53.17% with the addition of 750 ppm cefotaxime in MS medium. Varietal differences were observed for *in vitro* establishment, multiplication and root induction. Among various combinations of growth hormones, MS medium supplemented with 4.0 mg/l BAP + 0.5 mg/l NAA, 2 mg/l BAP and 0.5 mg/l NAA and half MS carrying 1.0 mg/l IBA + 0.5 mg/l NAA produced the highest *in vitro* establishment (53.07%), multiplication (64.46%) and rooting (66.37%) respectively, in 'Agrifound Dark Red'.

## Introduction

Onion (*Allium cepa* L.) is multiplied through seed or sets and requires two-years to complete one seed cycle necessitating the dependence on huge resources and the involvement of high risks (Sidhu *et al.* 1992). Poor seed viability, very high out crossing, bulblet formation, dormancy in plantlets, vitrification of tissues and decrease in regenerability for natural vegetative multiplication are some of the limitations in propagation of onion in the open field. Other members of *Allium* family are propagated vegetatively and have high *in vivo* and *in vitro* regeneration potential as reported in garlic (Robledo-Paz *et al*. 2000, Haque *et al*. 2003, Luciani *et al*. 2006) and *Allium wallichii* (Wawrosch *et al.* 2001) and *Allium chinense* (Xu *et al*. 2008). Although, there are reports on *in vitro* callus induction (Martinez *et al*. 2000), shoot regeneration (Khalid *et al*. 2001) and micropropagation (Pike and Yoo 1990, Kahane *et al*. 1992) in long day onion, but little information is available for large scale production of regenerated seedlings. India is the largest producer of short day onion and this crop is grown all over the country in two seasons *viz*. over winter and late summer. Keeping in view the significance of micropropagation for maintenance and multiplication of elite varieties, the present study was planned to study the effect of varieties from both the growing seasons and growth hormones on *in vitro* multiplication of shoots in short day onion.

## Materials and Methods

In this investigation, meristem and basal plate excised from the bulbs of three onion varieties *viz.* 'Punjab Naroya', 'PRO-6' (both of over winter season) and 'Agrifound Dark Red' (late summer season) were used as explants and cultured on Murashige and Skoog (1962) medium 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) maintained at pH 5.8. To check the bacterial contaminations in the cultures, the lukewarm MS media a fter autoclaving was also fortified with 200, 500 and 700 ppm cefotaxime

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(Omnatax$^{TM}$). Both the explants were immersed in 2% teepol solution for 20 min, washed in running tap water for 10 min and thoroughly wiped with a cotton swab. To check fungal infections, explants were also treated with 0.5% bavistin for 15 min and followed by 30 sec treatment with 70% ethanol. Further, the surface sterilization was carried out under aseptic conditions (Laminar Air Flow Cabinet) with 0.1 and 0.2% mercuric chloride and 25 and 50% commercial bleach (chemical composition: sodium hypochlorite 4.0% and sodium hydroxide 1.0%) as per the durations mentioned in Table 1. The explants were washed with sterile water for 3 - 4 times to remove excessive disinfectants. The sterilized explants were cultured on establishment media and incubated at 25 ± 2°C under 16 hrs photoperiod (illuminated with 40 W white fluorescent tubes) followed by 8 hrs dark period. The data were recorded on per cent asepsis and establishment of cultures *in vitro* after four weeks of inoculation. 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 Kn @ 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 subculture out of a single propagule was regarded as the multiplication fold and expressed in per cent. The multiple shoot clumps were separated into individual shoots and cultured on half strength MS medium supplemented with different combinations and concentrations of IBA (0.5, 1.0, 1.5 and 2.0 mg/l) and NAA (0.5 mg/l) as presented in Table 3. For elongation of roots and hardening of plantlets, freshly developed roots were thoroughly washed in running tap water and kept on half strength MS media (without sugar and vitamins) in open glass jars and plastic trays under high light intensity for 4 - 5 days in the incubation room. Fully-grown hardened plants were transferred to polythene bags containing growth media and kept in glass house for 10-12 weeks at 30± 1 $^{0}$C and 80 ± 2% RH.

For *in vitro* establishment, multiplication and rooting, 30 explants were used in each treatment and the cultures were repeated thrice. Data in per cent were converted to arc sine value for analysis of variance (ANOVA). The data were analyzed according to completely randomized design (Snedecor and Cochran 1967) with the help of CPCS-1 software package (Cheema and Singh 1990). Significance of variation among treatments was observed and the results were compared with least square differences.

**Results and Discussion**

The surface sterilization of explants at different concentration of mercuric chloride (0.1 and 0.2%) and commercial bleach (25 and 50%) for variable time intervals are presented in Table 1. Surface sterilization with 0.1% mercuric chloride for 10 min brought highest survival (42.88%) of clean cultures followed by 12 (39.61%) and 8 (25.21%) min durations with same concentration, respectively. The increase in mercuric chloride concentration to 0.2% lowered the survival rate. The maximum survival with commercial bleach was 16.63% only. The effectiveness of mercuric chloride as explant sterilization agent has been reported in onion (Pandey *et al* 1992, Kamstaityte and Stanys 2004) and safed musli (Purohit *et al*. 1994). The concentration and duration of sterilization treatment depend upon the degree of contamination, type and hardiness of explants also. Treatment with 0.5% bavistin before surface sterilization with mercuric chloride further increased the survival per cent (Fig. 1). The elimination of fungal contamination with fungicides was substantiated with the report in onion (Khar *et al*. 2005). The fortification of MS medium with cefotaxime (Omnatax$^{TM}$) was also effective in lessening the bacterial contaminations (Fig. 2). Among various concentrations, the addition of 750 ppm cefotaxime in MS medium augmented the clean cultures to 53.17%. No toxic effect was observed in the range of tested concentrations of cefotaxime. Use of antibiotics to suppress endophytic bacteria has earlier been reported in *Crythanthus* spp. (Moran *et al*. 2002).

Meristem explants cultured on establishment medium (BAP and NAA) showed regeneration within 6 - 8 days of culture initiation, but regenerated shoots started necrosis within 2 - 4 days of establishment and subsequently tissue became dead on all the tested media compositions (Plate 1 A, B and C). Hence, meristem cultures could not be used for further establishment.

**Table 1. Effect of disinfectants on survival of explants (%) in onion.**

| Sterilizing agent | Concentration (%) | Treatment duration (minutes) | Survival (%) |
|---|---|---|---|
| Mercuric chloride | 0.1 | 5 | 14.27 (22.16)* |
| | | 8 | 25.21 (30.12) |
| | | 10 | 42.88 (40.80) |
| | | 12 | 39.61 (38.99) |
| | 0.2 | 5 | 23.67 (29.09) |
| | | 8 | 17.65 (24.81) |
| | | 10 | 13.78 (21.76) |
| Commercial bleach | 25 | 5 | 12.69 (20.81) |
| | | 10 | 16.63 (24.04) |
| | | 15 | 14.26 (22.11) |
| | 50 | 5 | 10.97 (19.32) |
| Control | | 10 | 8.96 (17.39) |
| | - | - | 0.00 (0.00) |
| LSD (p = 0.05) | | | 1.50 |

* Figures in parenthesis are arc sine transformed values.

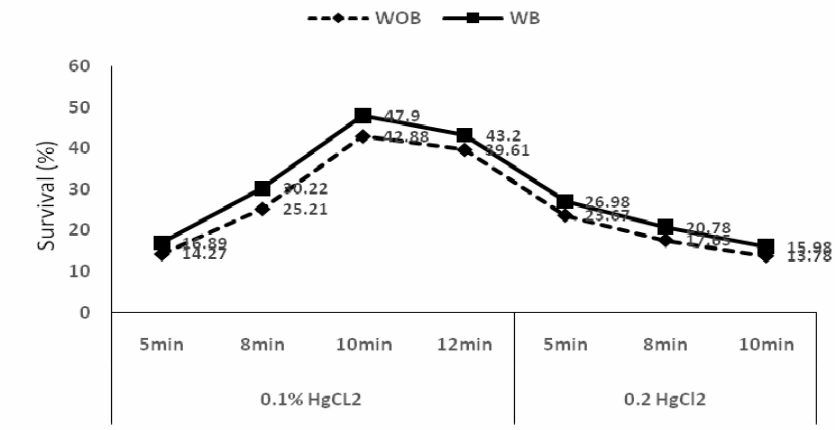


Fig. 1. Effect of pre-sterilization with bavistin on survival (%) of explants.

The basal plates responded to regeneration and shoot multiplication in onion (Plate 1 D, E and F). Significant differences were observed among varieties, media and their interactions for establishment of aseptic cultures (Table 2). The highest proliferation (41.56%) was observed in 'Agrifound Dark Red' followed by 'Punjab Naroya' (37.56%) and 'PRO-6' (35.43%). Among media compositions, the maximum basal plate cultures (53.07%) were established in MS supplemented with 4.0 mg/l BAP + 0.5 mg/l NAA. The interactions between varieties and growth regulators depicted 55.52% establishment in ADR in MS having 3.5 mg/l BAP + 0.5 mg/l NAA.

However, maximum proliferation in Punjab Naroya (55.36%) and PRO-6 (48.46%) was achieved on MS with 4.0 mg/l BAP + 0.5 mg/l NAA. The effectiveness of BAP and NAA supplemented media for proliferation was in accord with the reports in onion (Pike and Yoo 1990) and other monocot bulbous species like Chive (Pandey *et al.* 1992), garlic (Haque *et al.* 1998) and *Crinum macowani* (Slabbert *et al*. 1993).

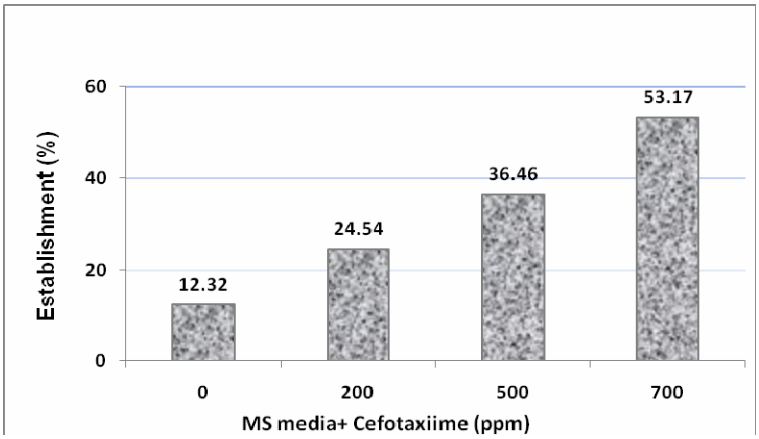


Fig. 2. Effect of cefotaxime on establishment of onion cultures.

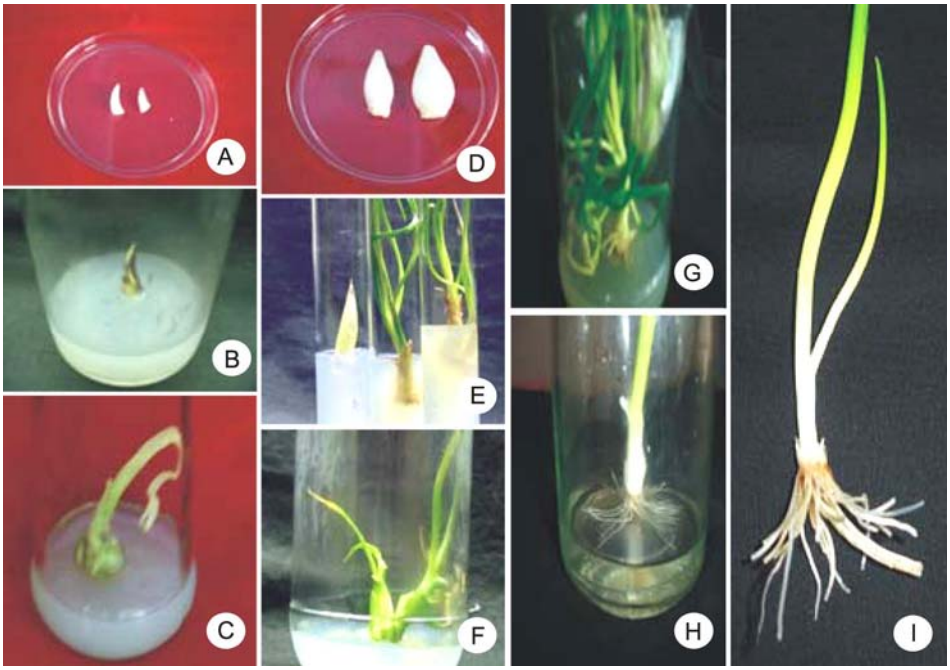


Plate 1. Micropropagation in onion A. Meristem, B. Establishment of meristem C. Necrosis of meristem, D. Basal plate, E. Establishment of basal plate, F. Shoot multiplication from basal plate G. Initiation of roots, H. Proliferation of roots and I. Rooted plantlet.

**Table 2. Interactions of varieties and growth hormones for *in vitro* establishment (%) of basal plates in onion.**

| MS basal + growth hormone (mg/l) | | Varieties | | | Mean |
|---|---|---|---|---|---|
| BAP | NAA | ADR | Punjab Naroya | PRO-6 | |
| - | - | 23.37 (28.29)* | 22.26 (28.13) | 19.98 (26.54) | 21.87 (27.65) |
| 1.0 | 0.1 | 26.23 (30.79) | 25.51 (30.31) | 23.26 (28.81) | 25.00 (29.97) |
| 2.0 | 0.2 | 36.44 (37.12) | 32.45 (34.71) | 31.62 (34.20) | 33.51 (35.34) |
| 3.0 | 0.5 | 44.71 (41.94) | 38.44 (38.30) | 35.70 (36.67) | 39.62 (38.97) |
| 3.5 | 0.5 | 55.52 (48.15) | 43.46 (41.23) | 42.53 (40.69) | 47.17 (43.35) |
| 4.0 | 0.5 | 55.39 (48.08) | 55.36 (48.06) | 48.46 (44.10) | 53.07 (46.75) |
| 5.0 | 0.5 | 49.25 (44.55) | 45.44 (42.36) | 46.49 (42.97) | 47.06 (43.96) |
| Mean | | 41.56 (39.85) | 37.56 (37.58) | 35.43 (36.28) | |
| LSD (p = 0.05): | | Variety = 0.48, Medium = 0.72, Variety × Medium = 1.26 | | | |

* Figures in parenthesis are arc sine transformed values.

The results of adventitious shoots produced from established basal plates gradually separated and sub-cultured on MS medium supplemented with different combinations of cytokinin and auxin are presented in Table 3. All varieties differed significantly for *in vitro* shoot multiplication. Cultivar 'ADR' showed highest shoot multiplication (51.11 %) followed by 'Punjab Naroya' (46.19%) and 'PRO-6' (41.01 %). Among media compositions, MS fortified with 2 mg/l BAP + 0.5 mg/l NAA and 2 mg/l BAP + 0.5 mg/l NAA brought maximum proliferation of shoots (64.46 and 63.41%, respectively) with non-significant differences. Interaction effects depicted maximum *in vitro* shoot multiplication in 'ADR' (72.32%) and 'Punjab Naroya' (66.29%) on MS having 2 mg/l BAP + 0.5 mg/l NAA and in 'PRO-6' (59.75%) on MS supplemented with 3 mg/l BAP + 0.5 mg/l NAA. The proliferation rate may depend upon the degree of cell differentiation in different varieties and their response towards optimal concentration of growth hormones for its stimulation. The differential response of BAP and NAA for shoot multiplication of different varieties has been reported in onion (Gems and Martinovitch 1998, Kamastaityte and Stanys 2004) and other bulbous species like garlic (Ma *et al*. 1994 and Haque *et al.* 1997) and *Allium wallichii* (Wawrosch *et al.* 2001).

**Table 3. Interactions of varieties and growth hormones for *in vitro* shoot multiplication (%) in onion.**

| MS basal + growth hormone (mg/l) | | | Varieties | | | Mean |
|---|---|---|---|---|---|---|
| BAP | Kin. | NAA | ADR | Punjab Naroya | PRO-6 | |
| - | - | - | 0.00 (0.00)* | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) |
| 1.0 | - | 0.5 | 56.64 (48.82) | 50.68 (45.37) | 47.28 (43.42) | 51.53 (45.87) |
| 2.0 | - | 0.5 | 72.32 (58.24) | 66.29 (54.49) | 54.78 (47.73) | 64.46 (53.48) |
| 3.0 | - | 0.5 | 67.99 (55.52) | 62.48 (52.21) | 59.75 (50.63) | 63.41 (52.79) |
| 4.0 | - | 0.5 | 61.87 (51.84) | 57.51 (49.30) | 52.42 (46.37) | 57.27 (49.17) |
| 2.0 | 0.5 | - | 46.44 (42.91) | 40.69 (39.60) | 37.85 (37.91) | 41.66 (40.14) |
| 3.0 | 0.5 | - | 54.22 (47.41) | 48.00 (43.83) | 42.89 (40.89) | 48.37 (44.04) |
| 4.0 | 0.5 | - | 49.43 (44.66) | 43.90 (41.48) | 33.08 (35.08) | 42.14 (40.41) |
| Mean | | | 51.11 (43.68) | 46.19 (40.79) | 41.01 (37.75) | |
| LSD (p = 0.05): | | | Variety = 1.06, Medium = 1.72, Variety × Medium = 2.98 | | | |

* Figures in parenthesis are arc sine transformed values.

*In vitro* root induction, proliferation and development of rooted plant are presented in Plate 1 G, H and I. The varieties, growth hormones and their interactions highlighted significant differences for root induction of micro shoots (Table 4). The highest root induction was observed in 'ADR' (62.07 %) followed by 'Punjab Naroya' (51.37%) and 'PRO-6' (37.00%). The maximum root induction was observed on ½ MS supplemented with 1.0 mg/l IBA + 0.5 mg/l NAA (66.37%). Interactions of varieties and media illustrated highest root induction of micro shoots in 'ADR' (87.37%) on ½ MS with addition of 1.0 mg/l IBA and 0.5 mg/l NAA, followed by 'Punjab Naroya' (75.21 %) on ½ MS with 1.5 mg/l IBA and 0.5 mg/l NAA. The least response was shown in 'PRO-6' (51.54 %) on ½ MS supplemented with 1 mg/l IBA and 0.5 mg/l NAA. In general, low concentration of organic and inorganic salts (½ MS or ¼ MS medium) found better for *in vitro* induction of roots in large number of plant species. The results of the present study are corroborated with the reports of Dian *et al*. (2004) and Khar *et al*. (2005) in welsh onion.

**Table 4. Interactions of varieties and growth hormones for *in vitro* root induction (%) in onion.**

| Half MS basal + growth hormone (mg/l) | | Varieties | | | Mean |
|---|---|---|---|---|---|
| IBA | NAA | ADR | Punjab Naroya | PRO-6 | |
| - | - | 32.13 (34.51)* | 25.25 (30.15) | 17.25 (24.51) | 24.88 (29.72) |
| 0.5 | - | 63.21 (52.64) | 44.4 (41.77) | 34.25 (35.80) | 47.29 (43.40) |
| 1.0 | 0.5 | 87.37 (69.17) | 60.21 (50.87) | 51.54 (45.87) | 66.37 (55.30) |
| 1.5 | 0.5 | 72.41 (58.30) | 75.21 (60.12) | 42.88 (40.89) | 63.50 (53.10) |
| 2.0 | 0.5 | 55.24 (48.00) | 51.64 (45.93) | 39.11 (38.69) | 48.66 (44.21) |
| Mean | | 62.07 (52.52) | 51.34 (45.77) | 37.00 (37.15) | |
| LSD (p = 0.05) | | Variety = 0.56, Medium = 0.72, Variety × Medium = 1.26 | | | |

* Figures in parenthesis are arc sine transformed values.

*In vitro* propagated plantlets were tender and fragile, thereby hardened on moist cotton with half MS salt for five days in incubation room. The hardened plantlets were transferred to growing media and kept in greenhouse at 25 ±1ºC and ± 2% relative humidity, respectively for further growth.

From the present study, it can be concluded that pre-sterilization of basal plate with 0.5% bavistin followed by surface sterilization with 0.1% mercuric chloride for 10 min was most effective to reduce fungal infections in *in vitro* cultures of onion. Addition of 750 ppm cefotaxime in culture media helped in reducing the systemic and bacterial contaminations. Onion varieties and explants had differential response for *in vitro* establishment, multiplication and root induction. Basal plates of onion were the best explants and 'Agrifound Dark Red' was best variety for micro-propagation in onion. Among media combinations, MS media supplemented with 3.5 mg/l BAP + 0.5 mg/l NAA, 2 mg/l BAP and 0.5 mg/l NAA and half MS carrying 1.0 mg/l IBA + 0.5 mg/l NAA were the best for *in vitro* establishment, multiplication and rooting in onion, respectively.

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