



MICROPROPAGATION OF ONION (*ALLIUM CEPA* L.) THROUGH BULBLET FORMATION

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Abstract: Multiple shoot clumps or microclones were collected from two onion (*Allium cepa* L.) varieties for bulblet formation. MS medium supplemented with various concentrations of BAP, Kn, 2ip and NAA were used. The height responded shoot was observed 90% and 80% in Taherpuri and Indian variety, respectively on MS supplemented with 2.0 mg l⁻¹ BAP+0.5 mg l⁻¹ NAA. The best bulblet formation was obtained in MS containing 1.0 mg l⁻¹ 2ip. Finally, the rooted bulblets were hardened and established in soil under natural environment.

Key words: *In vitro*, Onion, Bulblet, Cytokinin, Auxin

Introduction

Onion (*Allium cepa* L.) belongs to Liliaceae and widely distributed throughout the temperate northern hemisphere of the globe. Onion seeds germinate best at 15.5°C, but most satisfactorily between 7° and 27°C. The bulb formation in onion is prompted by long day conditions. Under the short photoperiod, the plants produce new leaves without any bulbs. The length of the day required for bulb formation varies from variety to variety (12 hours for early types and 15 hours for late types).

Onion is rich in minerals like phosphorus, calcium and carbohydrates. It also contains protein and vitamin-C. Onion is one of the most common and important spice crops of Bangladesh (Hossain and Islam, 1994). Onion extracts have antibacterial properties and its juice is used as smelling on histrial fits and faintness. Recent research reports that onion in the diet plays a part in preventing heart disease and other ailments (Augusti, 1990).

The onion cultivars of Bangladesh are poor yielding. With a view, to meeting up the increasing demand of onion it is possible to produce a vast number of bulblets from high yield exotic varieties through micropropagation.

Materials and Methods

MS (Murashig and Skoog, 1962) basal medium supplemented with BAP, Kn, 2ip and NAA at different concentrations and combinations were used. The pH of the medium was adjusted to 5.7 and solidified with 0.7% agar. Test tubes containing media were autoclaved at 121 °C at 15 psi pressure for 15 min. For bulblet formation, the shoot tip clumps were repeatedly subcultured in the same medium. The inoculated microclones were immediately transferred to light (300 lux) provided by white cool fluorescence tubes at 25±2 °C and 65% humidity, respectively. The photoperiod was maintained as 14 hours light and 10 hours dark. Data on

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bulblet formation were used to calculate the per cent of explants responded per culture. In each treatment, ten explants were used. For bulblet formation four-six subcultures were done and for root formation, all treatment were repeated three times. Data were evaluated as means \pm SE according to Miah and Miah (1984).

When the bulblets developed a few leaves and sufficient roots, the cotton plugs of culture tubes were removed and the plantlets were taken out carefully from tubes. The roots were washed gently under running tap water to free the media completely. The plantlets were transplanted in pots, containing a mixture of soil and sand (1:1). The pots were kept under shade. Plantlets were covered with perforated polythene sheet to maintain high humidity. Polythene cover was removed after four to five days of transplantation. The plantlets were successfully acclimatized under *ex vitro* condition through the gradual increasing of duration under sunlight. About 75% of Taherpuri and 60% of Indian plantlets survived under natural environment. When the plantlets established they were transplanted in the field.

Results

For bulblet formation regenerated shoot clumps were repeatedly subcultured on MS supplemented with different concentrations, 1-2 mg l⁻¹ of 2ip, BAP and Kn singly or in combination of 2ip+NAA, BAP+NAA, Kn+NAA. The highest percentage of response of inoculated microclones were observed 80% in Taherpuri and 70% in Indian variety when the medium was supplemented with 2 mg l⁻¹ BAP after sixth subculture (Table 1). The highest number of bulb per culture 5.3 \pm 0.33 and 3.9 \pm 0.23 in Taherpuri and Indian varieties, respectively were also observed in 2 mg l⁻¹ BAP after sixth subculture. On the other hand for both varieties the lowest percentage of shoot response and the lowest number of bulbs per culture were observed in 1.0 mg l⁻¹ Kn (Table 1) after fifth subculture.

Table 1. Effect of cytokinins on bulblet formation in microclones.

Growth regulator	Number of subculture	Days to bulb formation		Microclones response (%)		Mean No. of bulbs / culture	
		Taherpuri	Indian	Taherpuri	Indian	Taherpuri	Indian
Cytokinins (mg l⁻¹)							
2ip							
1.0	6	95	105	70	50	4.3 \pm 0.3	3.1 \pm 0.23
1.5	4	115	145	50	40	3.1 \pm 0.31	2.8 \pm 0.32
2.0	5	126	190	40	30	2.8 \pm 0.249	2.5 \pm 0.16
BAP							
1.0	6	123	143	60	50	4.0 \pm 0.258	3.3 \pm 0.21
1.5	5	102	119	70	60	4.5 \pm 0.23	3.6 \pm 0.26
2.0	6	88	102	80	70	5.3 \pm 0.33	3.9 \pm 0.23
Kn							
1.0	5	145	196	30	20	2.2 \pm 0.21	2.1 \pm 0.179
1.5	6	126	168	70	60	3.8 \pm 0.249	3.3 \pm 0.26
2.0	5	128	132	60	50	2.8 \pm 0.149	2.5 \pm 0.22

Table 2. Effect of the combination of cytokinins and auxin on bulblet formation from *in vitro* established micro-clones.

Growth regulator	Number of subculture	Days to bulb formation		Microclones response (%)		Mean No. of bulbs / culture	
		Taherpuri	Indian	Taherpuri	Indian	Taherpuri	Indian
Cytokinins + auxin (mg l⁻¹)							
2ip 1.0 +NAA							
+0.5	4	84	11	60	70	3.2 \pm 0.249	4.7 \pm 0.3
+1.0	4	120	140	80	70	4.7 \pm 0.3	4.1 \pm 0.23
+1.5	5	126	130	70	60	3.8 \pm 0.249	3.5 \pm 0.268
+2.0	5	120	125	50	40	3.2 \pm 0.249	2.6 \pm 0.22
BAP 2.0 +NAA							
+0.5	4	82	98	90	80	6.4 \pm 0.22	6.1 \pm 0.23
+1.0	4	85	115	80	70	5.9 \pm 0.31	5.1 \pm 0.23
+1.5	5	120	158	70	70	4.6 \pm 0.22	4.0 \pm 0.28
+2.0	4	130	180	60	50	3.9 \pm 0.23	3.2 \pm 0.2
Kn 1.5 + NAA							
+0.5	6	105	160	70	60	4.2 \pm 0.29	4.0 \pm 0.21
+1.0	5	140	180	60	50	3.8 \pm 0.249	3.5 \pm 0.268
+1.5	7	130	128	60	40	3.5 \pm 0.26	2.6 \pm 0.22
+2.0	6	185	190	50	40	2.5 \pm 0.16	2.4 \pm 0.16

The combination of 1 mg l⁻¹, 2 mg l⁻¹ BAP and 1.5 mg l⁻¹ Kn with different concentrations (0.5-2 mg l⁻¹) of NAA were used for bulblet induction. Among the concentrations and combinations the highest frequency of shoots responded were found to be 90% and 80% in Taherpuri and Indian varieties, respectively in the medium with 2.0 mg l⁻¹ BAP + 0.5 mg l⁻¹ NAA after fourth subculture. The highest number of bulbs per culture was also observed 6.4±0.22 in Taherpuri and 6.1±0.23 in Indian variety, respectively in the same media and after same number of subculture (Fig. 1). The second highest frequency of shoots responded 80% in Taherpuri and 70% in Indian variety were found in media containing 1 mg l⁻¹ 2ip + 1 mg l⁻¹ NAA after fourth sub-culture respectively. The second highest number of bulbs per culture was observed 4.7±0.3 in Taherpuri and 4.1±0.23 in Indian variety in the same medium after same number of subculture (Table 2).

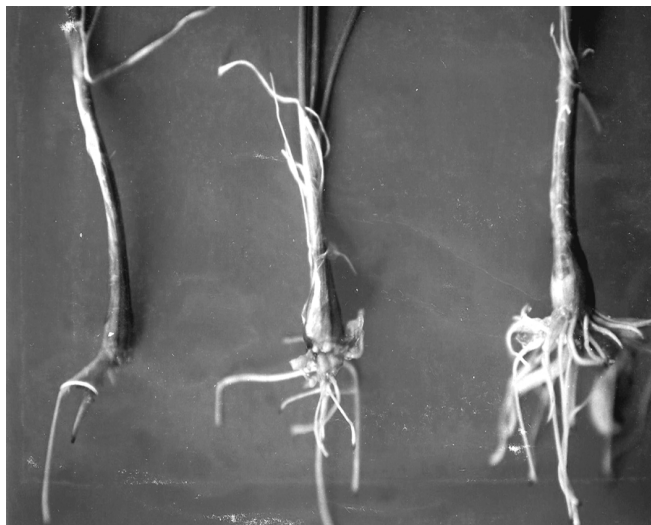


Fig. 1. Bulblet formation from *in vitro* grown microclones.



Fig. 2. Mature plant in field derived from *in vitro* bulblet germination.

Table 3. Effect of different kinds of cytokinins on germination of *in vitro* grown bulblet.

Growth regulators (mg l ⁻¹)	Shoot length (cm)		Root number / culture		Root length / culture (cm)	
	Taherpuri	Indian	Taherpuri	Indian	Taherpuri	Indian
BAP						
0.5	5.23 ± 0.24	4.21 ± 0.21	1.8 ± 0.25	1.2 ± 0.25	0.26 ± 0.005	0.22 ± 0.02
1.0	5.34 ± 0.21	4.34 ± 0.01	1.6 ± 0.16	1.6 ± 0.12	0.38 ± 0.06	0.29 ± 0.06
1.5	6.0 ± 0.16	5.1 ± 0.25	2.0 ± 0.25	1.9 ± 0.25	0.42 ± 0.08	0.4 ± 0.06
2.0	5.19 ± 0.33	4.9 ± 0.31	1.5 ± 0.2	1.3 ± 0.2	0.24 ± 0.04	0.23 ± 0.03
2.5	4.93 ± 0.11	4.0 ± 0.11	1.4 ± 0.16	1.2 ± 0.11	0.21 ± 0.02	0.9 ± 0.01
Kn						
0.5	4.88 ± 0.14	4.6 ± 0.12	-	-	-	-
1.0	4.92 ± 0.19	4.41 ± 0.13	-	-	-	-
1.5	3.98 ± 0.16	3.78 ± 0.22	-	-	-	-
2.0	3.77 ± 0.098	3.61 ± 0.08	-	-	-	-
2.5	3.61 ± 0.22	2.51 ± 0.12	-	-	-	-
2ip						
0.5	5.86 ± 0.08	4.4 ± 0.06	1.5 ± 0.26	1.2 ± 0.121	0.38 ± 0.098	0.31 ± 0.07
1.0	7.1 ± 0.15	7.0 ± 0.21	2.3 ± 0.25	2.1 ± 0.31	0.39 ± 0.24	0.3 ± 0.21
1.5	6.0 ± 0.16	5.6 ± 0.23	3.3 ± 0.36	3.1 ± 0.25	0.49 ± 0.24	0.31 ± 0.14
2.0	5.84 ± 0.64	5.0 ± 0.21	2.4 ± 0.16	2.3 ± 0.36	0.27 ± 0.32	0.23 ± 0.06
2.5	7.4 ± 0.15	4.1 ± 0.17	2.2 ± 0.24	1.1 ± 0.3	0.23 ± 0.41	0.2 ± 0.09

Data were recorded after three weeks of culture.

For germination of *in vitro* grown bulblets of two varieties of onion, MS was supplemented with five different concentrations (0.5-2.5 mg l⁻¹) of BAP, 2ip and Kn. Among different media compositions the highest length (cm) of shoot was observed 7.1±0.15 and 7±0.21 on media supplemented with 1.0 mg l⁻¹ 2ip. Besides, the lowest shoot length of Taherpuri and Indian variety was found 3.61±0.22 and 2.51±0.12 cm, respectively on media having 2.5 mg l⁻¹ Kn (Table 3). The highest numbers of roots were observed 3.3±0.36

and 3.1 ± 0.25 in Taherpuri and Indian variety, respectively in medium containing 1.5 mg l^{-1} 2ip. Alternatively, the lowest number was found 1.4 ± 0.16 and 1.2 ± 0.11 in Taherpuri and Indian variety, respectively in the medium containing 2.5 mg l^{-1} BAP (Table 3). In Taherpuri and Indian variety among different media composition the highest length of roots was observed 0.49 ± 0.24 cm and 0.31 ± 0.14 cm, respectively with 1.5 mg l^{-1} 2ip. On the other hand the lowest length of roots was 0.21 ± 0.02 and 0.9 ± 0.01 cm in Taherpuri and Indian variety, respectively on MS supplemented with 2.5 mg l^{-1} BAP (Table 3).

Discussion

For bulblet formation, it was found that combinations of 2ip / BAP / Kn with NAA had better effect than 2ip, BAP, Kn alone. Roksana (1998) reported that 0.5 mg l^{-1} 2ip + 0.15 mg l^{-1} NAA combination was suitable for bulblet formation in garlic. There are many reports of bulblet formation, which are influenced by a variety of factors (Abo El-Nil, 1977; Bhojwani, 1980; Nagakubo *et al.*, 1993; Seabrook, 1994; Yaseen *et al.*, 1994; Zel *et al.*, 1997). With shoot tips culture of garlic, Nagakubo *et al.* (1993) reported that bulblets were formed after a low temperature treatment of six months followed by cultures on LS medium containing 6-12% sucrose. The percentage of sucrose also influenced the formation of bulblets (Yaseen *et al.*, 1994).

Among 2ip, Kn and BAP, 2ip (1.0 mg l^{-1}) was found to be effective for germination of bulblets. However, in all cases only single shoot was developed with any branch. Roots were developed in all cases during bulblet germination except in the presence of Kn. Roksana (1998) reported that 2ip was found effective than other cytokinins for *in vitro* bulblet germination in garlic. She also observed single shoot germination and absence of root development in presence of Kn. Swamy (1983) reported that the germination of shoot bud and multiple shoots from cotyledon and shoot tip explants of *Capsicum annuum* in MS supplemented with different growth regulators of which IBA was found most effective for regeneration.

Conclusion

In this experiment it is observed that for bulblet formation the combination of 2ip, BAP and Kn with NAA had better effect than 2ip, BAP, Kn alone. On the other hand, among different types of cytokinins 2ip showed the best performance for *in vitro* bulblet germination. The successful establishment of *in vitro* grown bulblets in soil (Fig. 2) shows that the protocol developed in this experiment is efficient for the micropropagation of onion bulblet at a large scale.

References

- Abo El-Nil, M.M. 1977. Organogenesis and embryogenesis in callus culture of garlic (*Allium sativum* L.). *Plant Science Letter*, 9: 259-264.
- Augusti, K.T. 1990. Therapeutic and medicinal values of onion and garlic. In: *Onion and Allied Crops*. Brewster, J.L. and Rabinowitch, H.D. (eds.), Vol. III, Boca Raton, Florida, CRC Press, pp. 93-108.
- Bhojwani, S.S. 1980. In vitro propagation of garlic by shoot proliferation. *Scientia Horticulture*, 13: 47-52.
- Hossain, A.K.M.A. and Islam, J. 1994. Studies of Allium production in Bangladesh, *Acta Horticulture*, 358: 33-36.
- Mian, M.A. and Mian, M.A. 1984. *An Introduction to Statistics*. 4th edition, Ideal Library, Dhaka, Bangladesh, pp. 125-129.
- Murashig, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture, *Plant Physiology*, 15: 437-497.
- Nagakubo, T.A.; Nagasawa, A. and Ohkawa, H. 1993. Micropropagation of garlic through *in vitro* bulblet formation. *Plant Cell Tissue and Organ Culture*, 32: 175-183.
- Roksana, R. 1998. Micropropagation, bulblet formation and somatic embryogenesis in garlic (*Allium sativum* L.). M.Sc. Thesis, Department of Botany University of Rajshahi, Bangladesh.
- Seabrook, J.E.F. 1994. *In vitro* Propagation and bulb formation of garlic. *Canadian Journal of Plant Science*, 74: 155-158.
- Swamy, T.C.N. 1983. Tissue culture and multiplication of chillies (*Capsicum annuum* L.) and Onion (*Allium cepa* L.). *Thesis Abstract*, 9(4): 340-341.
- Yaseen, M.Y.; Splittoesser, W.E. and Litz, R.E. 1994. *In vitro* shoot proliferation and production of seeds from garlic and shallot. *Plant Cell Tissue and Organ Culture*, 37: 243-247.
- Zel, J.; Debelijak, N.; Ueman, R. and Ravinkar, M. 1997. The effect of jasmonic acid, sucrose and darkness on garlic (*Allium sativum* L. cv. Pfeijski, Jesenski) bulb formation *in vitro*. *Cell Division and Biology of Plant*, 33: 231-235.