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RESEARCH ARTICLE LIFE SCIENCE

IN VITRO CLONAL PROPAGATION OF ONION (*ALLIUM CEPA* L.) THROUGH SHOOT TIP CULTURE

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Abstract: The aim of the present investigation was to standardize the *in vitro* shoot tip culture technique considering various culture aspects for direct plant formation of two onion (*Allium cepa* L.) varieties 'Indian' and 'Taherpuri'. About 1.5-2.2 mm shoot tips were collected from bulbs and were sterilized with 0.1% $HgCl_2$ in various duration. Then they were cultured on MS medium supplemented with various hormonal concentrations and different media composition for primary establishment. BAP 1.5 mg l^{-1} + NAA1.0 mg l^{-1} was found to be the best formulation for primary establishment of shoot tips. Primarily established shoot tips were further cultured for shoot multiplication, and 2ip 2.0 mg l^{-1} + NAA 0.5 mg l^{-1} was found the most effective for shoot multiplication. The highest percentage of shootlet produced root on MS+2.0 mg l^{-1} IBA for both the cultivars (Taherpuri 90% and Indian 80%). The plantlets were transferred to small pots containing soil : sand 1:1 and kept under shade and covered with perforated polythene sheet. After proper hardening the plantlets were transferred to field.

Key words: Onion, cytokinin, auxin, shoot tip, plantlet

Introduction

Onion (*Allium cepa* L.) is the oldest among the cultivated spices. It is widely distributed throughout the temperate northern hemisphere of the globe. There are 80 species mostly in the western states of the USA, extending to Mexico and Guatemala. Onion is regarded as a long-day plant and bulbing varies with variety and ranges from 12 hours for very early type to 15 hours for late type. Onion is one of the most common and important spice crops in Bangladesh. It is used in almost all food preparations and as an integral part of Bangladeshi diet (Hossain and Islam, 1994). Onion is also used as preservative and medicine (Vohra *et al*., 1994). Since onion is propagated vegetatively, it is infected with viruses and other pathogens, which cause a decline in onion production. Micropropagation using shoot tips is the most common application for rapid production of disease free planting material. Shoot multiplication cycle is very short (2-6 weeks) and multiplication can be carried out throughout the year irrespective of season.

Onion is a cross-pollinated crop and sufficient hybrid seeds cannot be produced because large-scale emasculation is not feasible due to smallness of flower. So, conventional breeding method has some limitations in onion improvement. Through Tissue culture techniques, successful maintenance and multiplication of male-sterile lines can be done and hybrid seed production would be facilitated. The onion cultivars of Bangladesh are poor yielding. Introduction of high yielding exotic cultivars can play a vital role in increasing yield. To meet up the onion demand of ever increasing population, *in vitro* propagation system using shoot tip is one of the methods for production of disease free plant of the high yielding exotic varieties within limited space and time. Therefore, the experiment was undertaken considering the objectives: (i)

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standardization of duration of treatment with $HgCl_2$ and doses of growth hormones for primary culture establishment of two onion varieties (ii) optimization of growth regulators for rapid shoot multiplication (iii) selection of suitable concentration of auxins for efficient rooting *in vitro*.

Materials and Methods

About 20-30 mm diameter bulbs were isolated from two field grown varieties of *A. cepa* viz. Indian (exotic) and Taherpuri (local). At first roots, leaves or shoots were removed from the onion. After that the outer dry scales and hard basal portion of the bulbs were removed. The bulbs were then treated with tween-20 followed by washing with distilled water. Then the flashy scale leaves were removed from the bulbs and subsequently sterilized with various concentrations of $HgCl_2$ (0.01-1.5%) for different duration (1-15 min.) followed by rinsing 5-6 times with sterile distilled water to remove $HgCl_2$. The sterilized bulbs were cut and the shoot tip of about 1.5-2.2 mm in size were separated using binocular microscope and then inoculated aseptically onto culture establishment media. Initial culture establishment media consisted of full strength MS basal salts supplemented with various levels (0.1, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 mg l^{-1}) of different cytokinins viz. kinitine, 2ip and BAP. For shoot multiplication MS media fortified with different concentration levels of NAA viz. 0.1, 0.2, 0.3, 0.4 and 0.5 mg l^{-1} in combination with (i) Kinetin 1.5 mg l^{-1}, (ii) 2ip 2 mg l^{-1} and (iii) BAP 1.5 mg l^{-1}. For root induction , MS medium was supplemented with NAA (0.01, 0.05, 0.1, 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 mg l^{-1}) and IBA with same concentration level as NAA.

Inoculated shoots were transferred immediately under light (2000-3000 lux) provided by 40 watt white cool fluorescence tubes. The temperature and humidity of the culture room were 25±2ºC and 65%, respectively. The photoperiod was maintained as 14 hours light and 10 hours dark. For root induction the same condition was also maintained. Visual observation of culture was made every week. Data on shoot proliferation and root induction were used to calculate the percentage of cultures responding per treatment. For each treatment 10 explants were used for shoot proliferation and root induction. All the treatments were repeated thrice. Data were analyzed as means ± SE according to Mian and Mian (1984).

Results

Effect of different strength and duration of time of $HgCl_2$: In the present investigation it was noticed that most of the explants treated with 0.1% $HgCl_2$ for 5 minutes were contamination free. Whereas, with the same concentration level, when the treatment duration was lowered below 5 minutes maximum explants got infected with fungus. If the treatment is prolonged more than 5 minutes duration the percentage of survival was also decreased and when the explants were treated with 0.1% $HgCl_2$ for 13-15 min tissue damage occurred (data not shown).

Effect of different kinds and concentrations of cytokinin on primary establishment of shoot in culture: Result of the effect of different kinds of cytokinin on shoot establishment in culture showed that the response of cultured shoot tip was different with different kinds and concentrations of cytokinins. The shoot tips responded best on the medium, containing BAP 1.5 mg l^{-1} for both the onion varieties (Fig. 1). Almost similar results were observed on the medium containing 2ip 2.0 mg l^{-1} and kinetin 1.5 mg l^{-1} (Table 1).

Table 1. Effect of cytokinins on establishment of shoot on MS medium.

Cytokinins (mg l^{-1})	Primary response of explants (Frequency of response (%)	
	Taherpuri	Indian
0.1	30	40
0.5	40	50
1.0	50	60
1.5	70	70
2.0	40	50
2.5	30	40
3.0	20	30
0.1	30	30
0.5	40	40
1.0	50	50
1.5	70	70
2.0	85	80

2.5	60	40
3.0	40	30
0.1	40	30
0.5	50	40
1.0	60	60
1.5	90	80
2.0	50	50
2.5	40	40
3.0	30	30

Multiplication of shoots from primary established shoot tip culture: The best results of onion shoot multiplications and elongations were showed by MS medium fortified with 2ip and NAA (Table 2). Here, the highest shoot multiplication (10.5±0.489 for Taherpuri and 7.3±0.326 for Indian variety) were found at 2.0 mg l^{-1} 2ip + 0.5 mg l^{-1} NAA (Fig. 2). On the other hand the highest shoot length development (13.32±0.52 cm for Taherpuri and 9.27±0.669 cm for Indian variety) were found at 2.0 mg l^{-1} 2ip + 0.2 mg l^{-1} NAA (Table 2).

Table 2. Shoot multiplication of onion on MS medium containing different cytokinins with different concentrations of auxin (NAA).

Phyto-hormones (mg l^{-1})	Average number of shoot per explant		Mean shoot length (cm)	
	Taherpuri	Indian	Taherpuri	Indian
1.5 KIN +NAA				
+0.1	5.1±0.378	5.1±0.314	5.74±0.2	5.5±0.177
+0.2	5.8±0.29	4.24±0.2	5.34±0.16	5.43±0.238
+0.3	6.6±0.26	5.6±0.163	5.94±0.133	5.89±0.134
+0.4	5.5±0.23	5.4±0.211	4.96±0.079	5.09±0.172
+0.5	7±0.298	6±0.33	8.01±0.34	7.15±0.316
2-2ip+NAA				
+0.1	7.1±0.249	5.2±0.433	13.4±0.33	5.2±0.258
+0.2	7.5±0.372	6.9±0.378	13.32±0.52	9.27±0.669
+0.3	8.2±0.489	6.2±0.33	13.07±0.556	8.97±0.897
+0.4	8.8±0.32	6.6±0.221	11.01±0.86	8.04±0.912
+0.5	10.5±0.489	7.3±0.326	7.52±0.26	4.22±0.325
1.5 BAP+NAA				
+0.1	4.8±0.416	4.8±0.249	6.61±0.339	6.05±0.216
+0.2	5.4±0.22	4.6±0.163	6.44±0.191	6.09±0.114
+0.3	5.6±0.306	5.3±0.3	5.86±0.209	5.38±0.37
+0.4	5.4±0.221	5.4±0.26	5.39±0.34	4.64±0.146
+0.5	6.6±0.339	6.09±0.114	5.96±0.21	5.18±0.12

Table 3. Induction of adventitious roots on *in vitro* grown micro-clones of onion in MS medium with various concentrations of NAA and IBA

Phyto-hormones (mg l^{-1})	Frequency of root induction (%)		Mean length of longest root per explant (cm)		Mean no. of roots per explant	
	Taherpuri	Indian	Taherpuri	Indian	Taherpuri	Indian
NAA						
0.01	32	27	4.63±0.104	4.29±0.244	4.5±0.37	4.2±0.83
0.05	40	39	4.5±0.198	5.25±0.2	6±0.258	5.5±0.34
0.1	67	51	7.15±0.12	6.92±0.199	8.1±0.481	7.5±0.5
0.5	95	80	8.69±0.128	8.34±0.157	10±0.73	9.4±0.618
1.0	61	65	7.22±0.133	7.04±0.161	8.3±0.495	7.9±0.84
2.0	56	44	6.48±0.183	6.56±0.187	7.5±0.34	7.5±0.372
3.0	45	38	6.36±0.256	6.14±0.33	6.2±0.32	5.9±0.31
4.0	33	20	5.65±0.248	4.5±0.103	4.7±0.33	5±0.21
5.0	25	22	4.32±0.204	3.52±0.21	4.4±0.45	3.8±0.326
IBA						
0.01	43	42	6.47±0.21	6.41±0.24	7.1±0.314	6.5±0.4
0.05	47	47	7.41±0.19	7.57±0.2	8.6±0.3	7.7±0.36
0.1	61	56	7.86±0.27	7.76±0.28	4.7±0.353	8.2±0.416
0.5	67	65	7.89±0.369	8.23±0.329	9.3±0.495	8.7±0.495
1.0	68	50	8.28±0.665	11.2±0.79	8.3±0.9	7±0.447
2.0	99	92	14.4±0.66	14.47±0.49	14.2±0.742	15. ±10.64
3.0	71	77	9.05±0.417	9.05±0.478	9.3±0.422	9.4±0.702
4.0	60	50	8.82±0.265	8.17±0.34	8.3±0.448	7.4±0.45
5.0	47	56	7.21±0.34	8.78±0.44	8.1±0.64	8.3±0.538

Induction of roots on in vitro regenerated shoots: For adventitious root induction *in vitro* regenerated shoot were cultured on MS medium fortified with different concentrations of NAA and IBA. In case of IBA, at 2 mg l^{-1} it produce root in 99 % inoculated shoot in Taherpuri and 92% in Indian variety (Fig. 3). In the same concentration of IBA the average length and number of root per inoculated shoot were also observed. For Taherpuri the length and number of root were 14.4± 0.66 cm and 14.2±0.742, respectively. Moreover, in Indian variety the average length and number of root were 14.47±0.49 cm and 15±0.64, respectively.

In case of NAA fortified MS media, 0.5 mg l^{-1} showed better performance and it produce root in 95% inoculated shoot in Taherpuri variety and 80% in Indian variety. In this concentration longest root and root number were observed in Taherpuri variety and they were 8.69±0.128 cm and 10±0.73, respectively (Table 3).

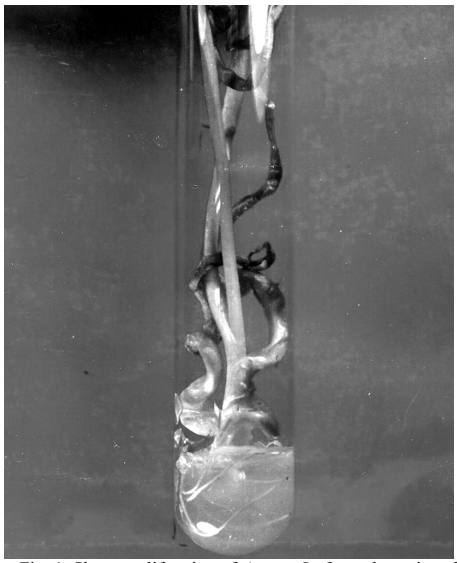

Fig. 1. Shoot proliferation of *A. cepa* L. from shoot tip culture on MS medium supplemented with 1.5 mg l^{-1} BAP

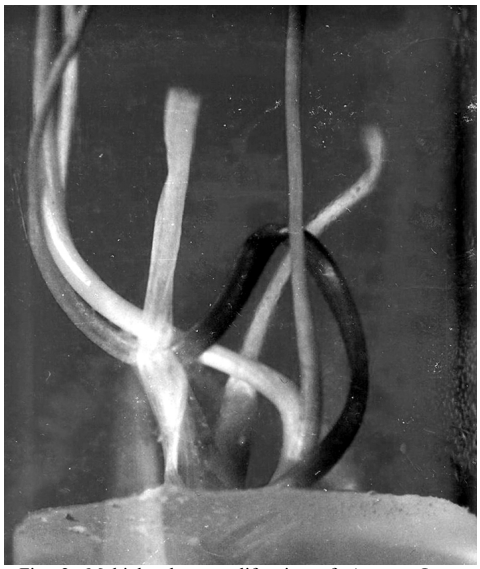

Fig. 2. Multiple shoot proliferation of *A. cepa* L. on media supplemented with 2.0 mg l^{-1} 2ip + 0.5 mg l^{-1} NAA.

Fig. 3. Hairyroot developed of *A. cepa* L. on media containing 2 mg l^{-1} IBA

Fig. 4. Shoot tip derived plant (*A. cepa* L.) in soil

Discussion

Different types of sterilizing agents with different concentrations are used to avoid contamination in tissue culture. The duration of sterilization is also different with different workers. There are many reporters on using $HgCl_2$ and ethyl alcohol as surface sterilizing agent. Faizum (1999) reported that 0.1% $HgCl_2$ for 8 minutes was suitable for surface sterilization.

Dustan and Short (1978) reported that BAP 1.5 mg l^{-1} was superior for culture response, these results are in agreement with the present findings on primary establishment of shoot in culture.

Hussey (1978) reported the number of adventitious shoots produced from leaf base varied from 10-20 on media containing BAP or 2ip within NAA at 0.12 or 0.5 mg l^{-1}. On the other hand, Roksana (1998) observed that high percentage of shoot multiplication (80%) in garlic can be developed using media containing 1.0 mg l^{-1} 2ip + 0.5 mg l^{-1} NAA. The suitability of BAP, NAA and Kin for shoot multiplication was observed by Nagasawa and Finer (1988), Shibli *et al.* (1999) and Suh and Park (1986).

Begum *et al*. (2000) found that 0.1-1.0 mg l^{-1} NAA to be the best for root induction in shoot tip culture of *Ocimum sanctum* L. In this experiment IBA showed superiority over NAA for induction of adventitious root both in Taherpuri and Indian varieties.

Conclusion

In the present study it was observed that *in vitro* shoot tip culture is an efficient technique for the successful clonal propagation of onion (*A. cepa* L.). To perform this technique effectively, 0.1% $HgCl_2$ for 5 minutes can be used as disinfectant. 1.5 mg l^{-1} BAP, combination of 2-ip and NAA and 2.0 mg l^{-1} IBA can be used as growth regulators for primary establishment of shoots, multiplication of primary established shoots and induction of roots, respectively.

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CORRIGENDUM

The article entitled 'Information technology enabled business process re-engineering: the right startup for effective knowledge management system' published in Volume 6 No. 1&2, 2005 was erroneously printed with single author (Mehedi Hasan Md. Hefzur Rahman). It was written by two authors- Mehedi Hasan Md. Hefzur Rahman and Md. Mizanur Rahman. The first author was the corresponding author. Correct citation of the said article will be as follows:

Rahman, M.H.M.H. and Rahman, M.M. 2005. Information technology enabled business process re-engineering: the right startup for effective knowledge management system. *Khulna University Studies*, 6(1&2): 79-86, 2005.

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