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CALLUS INDUCTION AND PLANT REGENERATION IN ONION (*Allium cepa* L.) FROM SHOOT TIP EXPLANT

M. H. Rashid[1], M. Khalekuzzaman[2], M. S. Hossain[3], M. S. Rahman[4] and M. F. Hasan[5]

ABSTRACT

An efficient protocol for callus induction and plant regeneration was established from shoot tip explant in onion (*Allium cepa* L.). Shoot tip from field-grown onion bulbs of Taherpuri and Indian cultivars were used as experimental materials in this investigation. Different concentrations of 2, 4-D or NAA were used in MS medium for callus induction. The highest percent (86.6%) of callus induction was 86.6 in Taherpuri and in Indian (80%) cultivar in MS media containing 1.5 mg/l of 2,4-D within fourteen to sixteen days of culture. Different concentrations and combinations of cytokinins (BAP and KIN) and auxins (NAA and IBA) were used for primary establishment of shoot tip. Maximum shoot proliferation was obtained after subculturing the callus in MS medium supplemented with 1.5 mg/l KIN + 1.5 mg/l of BAP within 20 days of callus culture. The highest percentage (100%) of root induction was achieved in Taherpuri cultivar on medium supplemented with 1.0 mg/l of NAA from *in vitro* raised shoots. Regenerated plantlets were successfully transferred on to the natural condition and showed healthy growth.

Key words: Callus induction, plant regeneration, shoot tip, *Allium cepa* L.

INTRODUCTION

Onion (*Allium cepa* L.) is an important spices belongs to the family Liliaceae. Onion is locally known as 'Piyaz'. It is one of the oldest cultivated plant species in the world. People use it as salad and also as spices in cooked food for better taste. This spice contains carbohydrate, vitamins, minerals and protein, and provides most of the trace elements, which can meet the energy requirements of the people living in the developing countries. Bangladesh is producing 142000 metric-tons of onion from 34413 hectares of land annually (BBS, 1997) with yield per hectare is about 4.13 metric-tons. Bangladesh has to depend on import onion from India and Pakistan every year to meet up the demand of our people (Hossain and Islam, 1994). Commercial cultivation of onion is limited mainly in district Faridpur, Rajshahi, Dhaka, Commilla, Mymensingh, Jessore, Rangpur and Pabna (BBS, 1993). Though have some limitation but it had already been proved that *in vitro* clonal propagation is the most efficient and reliable technique for mass propagation of a number of crops specially those are heterozygous and different to propagation vegetatively through conventional method (Litz *et al.*, 1983). There are many reports on micropropagation of several spices and medicinal plants; viz. garlic (Roksana, 1998), different *Allium* species (Kehr and Schaeffer, 1976; Hussey and Hilton, 1978) and other medicinal plants (Sudherson and Hussain, 2002). But there are limited reports available on micropropagation of this valuable spices plant. The present research aimed to establish an efficient reproducible plant regeneration method through callus induction from shoot tips of *Allium cepa* L. Through tissue culture, a huge number of seedlings could be produced which may help to cultivate onion around the year in our country and may be helpful to solve the demand of onion.

MATERIALS AND METHODS

The onion bulbs from two varieties viz. Taherpuri (Local) and Indian (exotic) were collected from Botanical garden experimental field of Rajshahi University, Rajshahi, Bangladesh. They were washed

[1]M.Phil Fellow, Institute of Biological Sciences, University of Rajshahi, [2 & 3] Associate Professor, [4 & 5] MS Student, Dept. of Genetic Engineering and Biotechnology, University of Rajshahi, Rajshahi-6205, Bangladesh.

under running tap water for 20 minutes and treated with 1% tween-80 for 10 minutes followed by repeated washing with autoclaved distilled water. Prior to sterilization the bulbs were dropped in 90% ethyl alcohol for 1 min. Further sterilization was done with 0.1% $HgCl_2$ for 5 minutes followed by repeated washing with autoclaved distilled water to remove traces of $HgCl_2$. The sterilized bulbs were placed on sterile tiles and cut vertically and horizontally with the help of sterilized forcep and scalpel. The apical meristematic areas about 1.5-2.0 mm in size were cut. The excised shoot tips were carefully placed on agar gelled semi-solid MS (Murashige and Skoog, 1962) medium consisting of different concentrations and combinations of auxins (NAA, IBA and IAA) and cytokinins (2ip, KIN and BAP). The pH of the medium was adjusted to 5.8 ± 0.1. The cultures were maintained at 25 ± 2^0 C with light intensity varied from 2000-3000 lux. The photoperiod was maintained generally 16 hours light and 8 hours dark. Regenerated shoot clumps were sub-cultured on MS basal medium supplemented with auxins (NAA and IBA) for further rooting. The well developed plantlets were then rescued very carefully from the tubes and carefully washed out under running tap water. Then the well rooted plantlets transferred to small pots containing sterilized soil and sand mixture in the ratio of 1:1. The pots with plantlets kept in shady place and covered with perforated polythene sheet to prevent sudden desiccation. The soil in pots was sprayed with water everyday to maintain proper humidity. After hardening, the pot was exposed to outer environment, 30-35 days old plantlets were finally transplanted to natural condition, where they eventually developed into mature plants.

RESULTS AND DISCUSSION

Callus induction

For callus induction, shoot tip of onion bulb was cultured on MS media supplemented with different concentrations of 2,4-D (0.01-5.0 mg/l) and NAA (0.01-5.0 mg/l) (Table 1).

Table 1. Effect of various concentrations of 2, 4-D and NAA in MS medium on callus induction from shoot tip.

Growth regulators (mg/l)	Sources of explants					
	Taherpuri			Indian		
	Days to callus initiation	% of culture response (M±SE)	Nature of calli	Days to callus initiation	% of culture response (M±SE)	Nature of calli
2,4-D						
0.01	-	-	-	-	-	-
0.05	-	-	-	-	-	-
0.1	28-30	36.6±0.9	Cr, Fr	30-32	26.6±1.2	Cr, Fr
0.5	20-22	46.6±1.4	Cr, Fr	22-25	33.3±0.8	Cr, Fr
1.0	19-20	53.3±1.2	Cr, Fr	18-20	46.6±1.7	Cr, Fr
1.5	**14-16**	**86.6±1.2**	**Cr, Fr**	**16-18**	**80.0±0.0**	**Cr, Fr**
2.0	17-19	73.3±0.8	Cr, Fr	18-20	56.6±1.8	Cr, Fr
3.0	16-18	66.6±1.5	Cr, Fr	17-19	53.3±1.5	Cr, Fr
4.0	15-17	60.0±0.0	Cr, Fr	17-18	50.0±0.0	Cr, Fr
5.0	-	-	-	-	-	-
NAA						
0.01	-	-	-	-	-	-
0.05	-	-	-	-	-	-
0.1	-	-	-	-	-	-
0.5	-	-	-	-	-	-
1.0	27-30	33.3±0.8	Cr, L	30-35	30.0±0.0	Cr, L
1.5	**22-26**	**40.0±0.0**	**Cr, L**	**25-30**	**36.6±1.5**	**Cr, L**
2.0	25-29	36.6±1.2	Cr, L	28-32	33.3±1.3	Cr, L
3.0	30-32	30.0±0.0	Cr, L	32-35	26.6±1.2	Cr, L
4.0	38-42	26.6±1.3	Cr, L	40-45	23.3±1.7	Cr, L
5.0	-	-	-	-	-	-

Each treatment consisted of 12 replications, Cr = Creamy, L = Loose, Fr = Friable, M=Mean, SE=Standard Error

In case of 2,4-D, MS medium containing of 1.5 mg/l of 2,4-D showed best performance for inducing callus and that was recorded 86.6% for Taherpuri and 80.0% for Indian varieties within 14-16 days and 16-18 days of explants culture, respectively. Among two auxins, 2,4-D was found more effective than NAA in callus induction. In case of NAA, the highest 40.0% (Taherpuri) and 36.6% (Indian) callus formation was observed in 1.5 mg/l of NAA within 22-26 days and 25-30 days of explants culture, respectively. In these treatments, the induced calli were creamy in colour and structurally friable (Fig.1B). Such types of callus induction were also reported by Roksana (1998) in garlic, Kehr and Schaeffer (1976) and Hussey and Hilton (1978) in different *Allium* species.

Shoot regeneration

For shoot regeneration, shoot tip derived calli were aseptically taken out and cut into small pieces and were subcultured on MS medium supplemented with different concentrations and combinations of auxins (KIN and BAP) and cytokinins (2,4-D, IBA and NAA). The highest percentage (90%) of shoot regeneration was for Taherpuri and 83.3% for Indian variety on the medium supplemented with 1.5 mg/l of KIN + 1.5 mg/l of BAP (Fig. 1C). The morphology of the callus remained unchanged after subculture of the explant. After culture on different concentrations and combinations of other growth regulators, the calli increased in size and shown nodular structure shoot produced (Table 2).

Table 2. Effect of different concentrations and combinations of cytokinins and auxins on MS medium for shoot initiation.

Growth regulators (mgl^{-1})	Source of explants					
	Taherpuri			Indian		
	Days to shoot initiation	% of culture response (M±SE)	No. of shoots Callus^{-1} (M±SE)	Days to shoot initiation	% of culture response (M±SE)	No. of shoots/Callus (M±SE)
KIN						
0.1	-	-	-	-	-	-
0.5	18-20	43.3±1.3	1.0±0.0	-	-	-
1.0	16-18	56.6±0.9	1.0±0.0	14-16	56.6±1.4	1.0±0.0
1.5	**18-20**	**73.3±0.8**	**2.3±0.8**	**18-20**	**70.0±0.0**	**2.0±0.0**
2.0	17-19	46.6±1.5	1.0±1.0	16-18	46.6±1.4	1.0±0.0
2.5	16-18	36.6±1.3	1.0±0.0	-	-	-
3.0	-	-	-	-	-	-
KIN+2,4-D						
1.5+0.1	-	-	-	-	-	-
1.5+0.5	13-15	46.6±1.2	2.0±1.0	-	-	-
1.5+1.0	12-14	53.3±1.3	2.0±0.0	14-16	50.0±0.0	1.0±0.0
1.5+1.5	11-13	76.6±0.8	2.3±0.9	15-17	73.3±1.3	2.0±1.0
1.5+2.0	**15-17**	**86.6±0.9**	3.3±0.8	**16-18**	**80.0**±0.0	3.0±0.0
1.5+2.5	14-16	60.0±0.0	2.0±1.0	13-15	40.0±0.0	3.0±1.0
1.5+3.0	15-17	43.3±1.4	1.0±0.0	18-20	33.3±1.2	2.0±0.0
KIN+BAP						
1.5+0.1	-	-	-	-	-	-
1.5+0.5	12-14	53.3±1.3	2.0±1.0	13-19	43.3±1.4	2.0±0.0
1.5+1.0	10-11	66.6±1.6	2.3±1.1	10-12	60.0±0.0	2.0±1.0
1.5+1.5	**19-20**	**90.0±0.0**	4.0±0.0	**19-20**	**83.3±0.9**	3.3±0.9
1.5+2.0	13-15	50.0±0.0	3.0±1.0	14-16	56.6±1.2	3.0±1.0
1.5+2.5	16-18	46.6±1.3	3.0±1.0	16-18	40.0±0.0	2.3±1.2
1.5+3.0	-	-	-	-	-	-
KIN+NAA						
1.5+0.01	-	-	-	-	-	-
1.5+0.05	-	-	-	-	-	-
1.5+1.0	12-14	53.3±1.2	1.0±0.0	14-16	40.0±0.0	1.0±0.0
1.5+1.5	11-13	66.6±1.6	2.0±1.0	10-12	56.6±0.9	2.0±1.0
1.5+2.0	**22-24**	**83.3±0.9**	2.3±0.9	**19-20**	**73.3±1.2**	2.0±0.0
1.5+2.5	24-26	56.6±1.2	1.0±0.0	14-16	56.6±1.3	1.0±0.0
1.5+3.0	-	-	-	-	-	-

In each treatment 10 explants were used, M=Mean, SE=Standard Error

The highest mean number of shoot was 4.0 for Taherpuri and 3.3 for Indian variety on the medium having 1.5 mg l^{-1} of KIN + 1.5 mg l^{-1} of BAP (Fig. D). The highest mean length of shoot was also recorded in the same medium. Such indirect organogenesis was reported in many spices and medicinal plant species including *Abrus precatorious* (Biswas *et al.*, 2007), *Phellodendron amurens* (Azad *et at.*, 2005), *Holostema ada-kodien* (Martin 2002).

Root induction and plantlet acclimatization

Callus derived shoots were cultured in MS medium supplemented with different concentrations of IBA and NAA. The highest percentage of root induction was 100% for Taherpuri and 83.3% for Indian variety on the medium supplemented with 1.0 mg l^{-1} of NAA (Table 3).

Table 3. Effect of IBA and NAA on root induction in callus derived shoots

Growth regulators (mg l^{-1})	Source of explants					
	Taherpuri			Indian		
	% of shoots rooted (M±SE)	No. of roots $shoot^{-1}$ (M±SE)	Root length (M±SE)	% of shoots rooted (M±SE)	No. of roots $shoot^{-1}$ (M±SE)	Root length (cm) (M±SE)
IBA						
0.1	46.6±0.6	5.0±1.0	3.3±1.3	50.0±0.0	2.7±0.6	3.0±1.0
0.2	73.3±1.3	5.9±0.9	4.0±0.0	73.3±1.6	3.8±0.8	3.9±0.9
0.5	93.3±1.4	9.0±1.0	6.6±0.9	90.0±0.0	7.6±0.6	6.0±0.0
1.0	60.0±1.0	7.0±1.0	6.6±0.0	76.6±1.3	5.0±0.9	5.7±0.9
1.5	76.6±0.6	5.6±1.2	5.3±1.3	46.6±0.8	5.0±1.0	4.6±1.4
2.0	56.6±0.6	4.2±0.9	4.6±1.2	43.3±0.9	4.0±0.0	3.6±0.9
3.0	53.3±1.2	4.0±1.0	3.6±0.8	20.0±1.0	3.3±0.0	3.0±0.0
NAA						
0.1	53.3±1.3	3.7±0.6	3.6±0.9	33.3±0.9	3.0±1.0	3.4±0.6
0.2	63.3±1.6	4.8±0.8	4.6±0.6	50.0±1.0	4.9±0.9	4.7±0.7
0.5	76.6±0.6	5.6±0.6	7.0±1.0	73.3±1.4	5.6±1.3	6.0±1.0
1.0	**100.0±0.0**	**10.2±1.2**	**9.0±0.0**	**83.3±0.8**	**9.4 ± 0.9**	**8.3±1.3**
1.5	66.6±1.3	8.0±0.3	7.3±1.4	66.6±1.7	5.7±0.9	5.8±0.8
2.0	46.6±0.6	6.0±1.0	5.6±1.1	46.6±1.5	5.6±1.4	3.8±1.1
3.0	33.3±0.9	5.0±1.0	4.6±1.6	33.3±1.2	4.0±1.0	3.3±1.3

Each treatment derived from 10 explants, M=Mean, SE=Standard Error

The longest root was developed 10.2 cm for Taherpuri and 9.4 cm for Indian variety (Fig. 1E). Earlier reports showed that NAA was found to be better than IBA in P*runus avimmi* and IAA was found to be better than IBA in *P. cerasufera* (Hammerschlag, 1982; Bhojwani, 1980) for root development in garlic. Similar result was also reported by Hasan *et al.* (2008) in *Cassia obtusifolia*. In respect of root induction, our result supported with the previous reports. Shoots with strong and stout root system were acclimatized outside growth chamber for one week and then transferred to earthen pots placed in natural environment containing mixture of sand and soil (1:1). Eighty percent plantlets survived in natural condition. Among two cultivars tested in the present investigation, Therpuri cultivar showed better response compare to Indian cultivar. This Taherpuri cultivar of onion could be used as a suitable explant for large scale micropropagation.

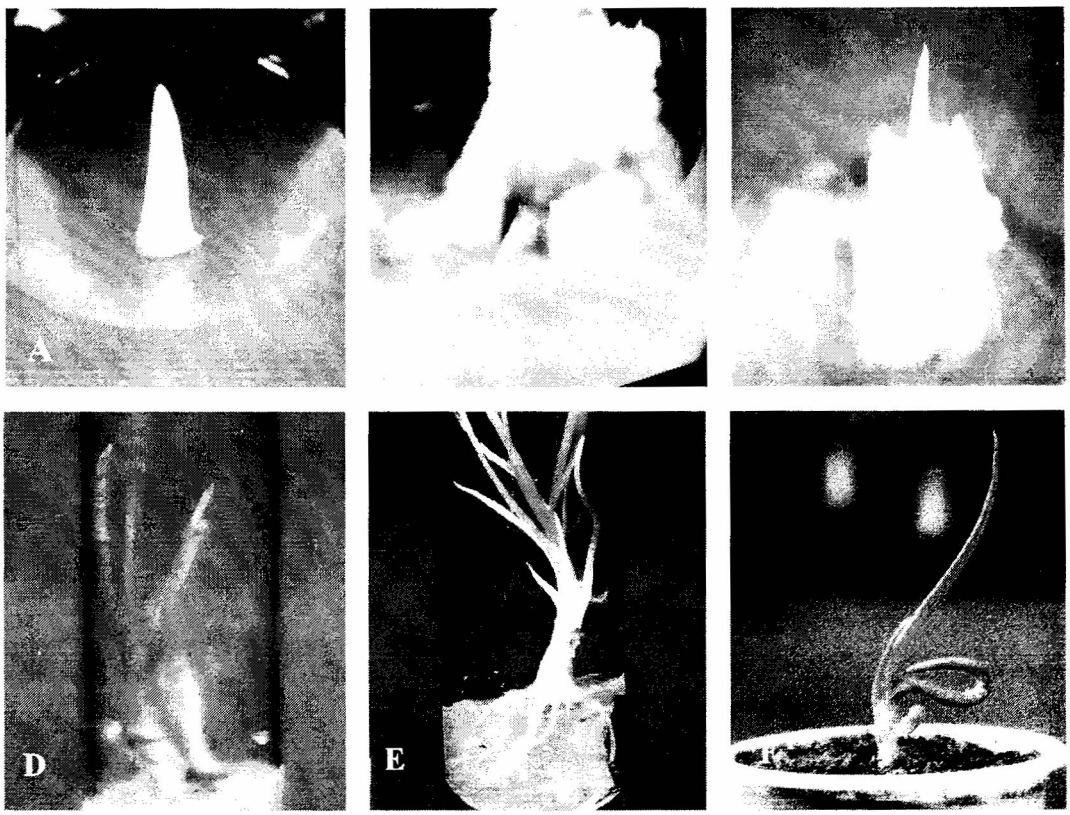

Figure 1. Plant regeneration from shoot tip derived callus

A. Shoot tip cultured on callus induction medium supplemented with MS + 1.5 mg l^{-1} of 2, 4-D
B. Callus induction in MS medium supplemented with 1.5 mg l^{-1} of 2, 4-D
C. initiation of shoot from callus supplemented with 1.5 mg l^{-1} KIN + 1.5 mg l^{-1} of BAP
D. Formation of multiple shoots after sub-culture in same medium
E. Proliferation of roots supplemented with 1.0 mg l^{-1} of NAA
F. Regenerated plant in natural condition.

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