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IN VITRO REGENERATION OF PUMPKIN (*CUCURBITA MAXIMA*) THROUGH SHOOT APICAL MERISTEM

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Abstract

Context: Pumpkin (*Cucurbita maxima*) is very important vegetable crop. It is damaged by several types of diseases, especially viral diseases which reduce the total yield of pumpkin. This is very difficult problem and such type of problem can be overcome by meristem culture.

Objectives: To develop suitable protocols for indirect regeneration of pumpkin plant through shoot apical meristem.

Materials and Methods: Meristems were isolated from 15-21 days *in vivo* grown plants by collecting shoot tips and were prepared (0.2-0.5 mm) under 4X zoom stereo-microscope remaining two leaves primordia. Inoculation of explants was made singly per culture vessel in semisolid MS fortified with different concentrations and combinations of cytokinin and auxin.

Results: Among different concentrations, 1.0 mg/l BAP showed best response for callus induction. Calli were sub cultured for shoot formation in MS containing BAP singly or combination with GA_3 and 1.5 mg/l BAP + 0.1 mg/l GA_3 gave better result. *In vitro* regenerated shoots were rooted well in ½ strength MS with 0.5 mg/l IBA and micro plants were acclimatized successfully in natural condition.

Conclusion: Indirect regeneration of pumpkin plants through shoot apical meristem has been established.

Keywords: Growth regulators, *in vitro* regeneration, Pumpkin, *Cucurbita maxima*.

Introduction

Cucurbitaceae is the largest vegetable family in the world and pumpkin (*Cucurbita maxima*) is very important vegetable of this family. Its fruit as well as leaf and stem are very delicious. Fruit contains 90% fresh water, low amount of fat and rich source of Vit-A. There has been recent interest in edible pumpkin seed, particularly in genotypes with the hull-less trait (Loy 1990). Seed oil used as nerve tonic and anthelmintic (Chopra *et al.* 1956). Pumpkin is also a good source of secondary metabolites; especially saponins have been found (Schultes 1990). It is widely cultivated crop in our country and propagated by seed. This plant is affected by different types of diseases, especially viral diseases such as cucumber mosaic virus, papaya ringspot virus, watermelon mosaic virus and zucchini yellow mosaic virus; which reduce the yield. So, to produce disease free plant, virus elimination is a pre-requisite for successful pumpkin production. Meristem culture is a unique technique to produce pathogen free plants including viroides, mycoplasma, bacteria and fungi (La Motte and Lersten 1972, Walkey 1978, Kartha 1984, Pierik 1989, Bhojwani and Razdan 1996).

Some reasons help meristem to free from virus such as lack of vascular system, absence of plasmodesmata in meristematic tip, quick cell division, competition between synthesis of nucleoproteins for cellular division and viral replication and presence of inhibitor substances. In pumpkin, meristem is very effective part for direct and indirect regeneration of virus free plants. Therefore, an investigation was under taken to develop a suitable protocol for indirect regeneration of plant through shoot apical meristem.

Materials and Methods

The study was carried out at DNA and Chromosome Research Laboratory, Department of Genetic Engineering and Biotechnology, Rajshahi University. Meristems were isolated from 15-21 days *in vivo* grown

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plants. Shoot tips were cut carefully with the help of forceps and dissecting blade then tips were taken into a conical flask and thoroughly washed under running tap water for 30 mins to remove loose contaminates attached to explants. Then these explants were washed with distilled water containing 1% savlon (v/v) and 4 drops of Tween-80 for 20 mins to remove gummy substances. That was followed by successive 3 washing with distilled water to make the material free from savlon. Subsequently the materials were transferred to running laminar air flow hood and sterilized by 0.05% $HgCl_2$ for different periods of (1-10 mins) time to ensure contamination and tissue killing free culture. To remove every trace of the strident, the materials were then washed at least 6 times with sterile distilled water.

Meristems were prepared (0.2-0.5 mm) under 4X zoom stereo-microscope. Microscope and other materials were properly sterilized by 70% ethanol before starting isolation. Meristem were prepared by excising leaves remaining two leaves primordia with apical meristem. Inoculation of explant was made singly per culture vessels. During inoculation, special care was taken that the explant must touch the medium equally. Each culture tube containing more or less 15 ml of nutrient medium was supplemented with different concentrations of a single hormone or combination of hormones. In all the cases, explants were inoculated to semi solid MS (Murashige and Skoog 1962) medium for callus, shoot and root induction with different concentrations and combinations of cytokinin and auxin. The inoculated culture vessels were incubated in a growth chamber containing special culture environment. First the cultures were taken in dark chamber for two days then transferred in the growth chamber illuminated by 40 watts white fluorescent tubes fitted at a distance of 30-40 cm from the culture shelves. The cultures were maintained at 25 ± 2°C under the warm fluorescent light intensity varied from 2000-3000 lux. After ten weeks, the well rooted plantlets were taken out very carefully from test tubes and roots were washed gently and transplanted to small pot containing sterilized soil. They were covered with transparent polythene bags to maintain high humidity and kept in the growth chamber for seven days. Within seven days the plantlets began to form new leaves and resumed fresh growth.

Results

Meristems were cultured on MS supplemented with different concentrations and combinations of BAP and NAA for callus formation and their responses are presented in Table 1. Among different concentrations, 1.0 mg/l BAP singly showed better result than other concentrations and combinations of BAP with NAA and 80% explants responded.

Table 1. Effects of BAP and NAA on callus formation from meristem.

Used growth regulators	% of explants produced callus	Callus type
BAP		
0.5	30	CG
1.0	80	CG
1.5	55	CG
2.0	40	CG
2.5	40	CG
BAP + NAA		
0.5 + 0.5	40	LG
1.0+ 0.5	70	LB
1.0 + 1.0	80	LB
1.5 + 1.0	70	LB
1.5 + 1.5	55	LB
0.5 + 0.5	40	LG

Table 2. Effects of BAP and GA_3 on regeneration from callus.

Used growth regulators	% of callus produced shoots	Mean no of shoots after days	
		14	21
BAP			
0.5	-	-	-
1.0	40	5.2	7.5
1.5	50	7.0	9.2
2.0	30	6.3	8.2
2.5	30	3.0	4.2
BAP + GA3			
0.5 + 0.1	10	2.0	3.2
1.0 + 0.1	40	5.7	6.5
1.5 + 0.1	70	11.2	15.4
2.0 + 0.2	40	6.5	7.0
2.5 + 0.2	30	3.4	3.5

Regeneration of callus was performed using different concentrations and combinations of BAP and GA_3. Among these combination, potential regeneration was found from 1.5 mg/l BAP + 0.1 mg/l GA_3 and the mean

number of shoots per culture was 15.4 ± 0.12 after four weeks of subculture (Table 2). Regenerated shoots were transferred to a rooting medium supplemented with different types of auxin (NAA and IBA). Effective root formation was found using 0.5 mg/l IBA and IBA gave better response than NAA (Table 3). Plantlets were successfully acclimated with natural condition (Figs. A-F).

Table 3. Effect of different concentrations of IBA and NAA on root induction from regenerated shoot.

Media composition half MS with growth regulators (mg/l)	Morphogenic response			
	Mean no. of roots per culture after days		Mean length of the longest root after days in cm	
	15	21	15	21
IBA				
0.5	8.1	18.2	3.5	8.3
1.0	5.2	12.4	2.2	5.8
1.5	4.20	5.5	2.1	4.6
2.0	4.20	5.0	1.5	4.8
2.5	4.25	4.5	1.5	4.5
NAA				
0.5	2.5	3.1	1.5	2.1
1.0	4.5	8.3	2.3	3.5
1.5	4.0	7.3	2.3	3.1
2.0	3.5	3.6	2.1	2.5

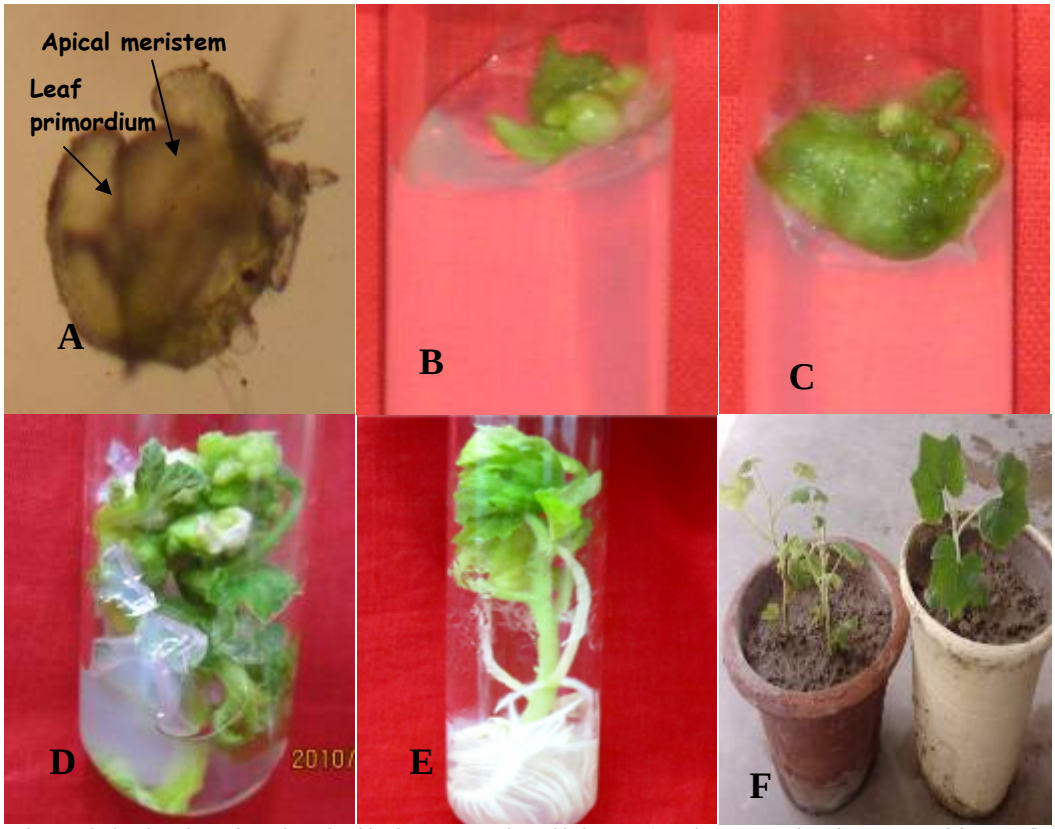

Fig 1. A- isolated meristem from shoot tip of *in vivo* grown 15 days old plant, B&C-meristem was cultured on MS containing 1 mg/l BAP and D-regeneration from callus, cultured on MS containing 1.5 mg/l BAP + 0.1 mg/l GA_3, E-root induction in regenerated shoot after 15 days of culture and F-establishment of regenerated plant on soil.

Discussion

Shoot apical meristem is a unique part of plant capable of quickly cell division and regeneration. In this investigation, different sizes of meristems (0.2-0.5 mm) were isolated and cultured on solidified MS medium for callus induction and plant regeneration. Ahmad *et al*. (2010) isolated meristems of different size (0.3-0.5) of *Cucurbita pepo* L. for production of virus free plantlets. Very small size meristems produced green compact callus but callus formation rate is very slow, on the other hand large size meristems showed high rate of callus formation but calli were light green and comparatively friable and did not show effective regeneration. In summer squash (*C. pepo*) the highest frequency of callus induction was observed in MS with 2.5 mg /l 2, 4-D in both cultivar (Bulum and Rumbo) and hypcotyl was more responsible than epicotyl (Pal *et al*. 2007).

Many workers reported on meristem culture for direct regeneration in many plants. In *C. pepo,* Ahmad (2010) reported that the combination of Kin with GA_3 was found to be most effective for establishment of meristem culture. Another result was found by Pink and Walkey (1984) in the same plant. But Huda and Sikdar (2006) reported on meristem culture of bitter gourd using Kin with GA_3. GA_3 alone or in combination with cytokinin. IBA gave better response than NAA in root formation which was also observed by Haque *et al.* (2008) in Pumpkin.

Conclusions

Hormonal differences and simple rapid *in vitro* regeneration protocol of *L. cylindrica* and *L. acutangula* have been established which will help in conservation and propagation of these two important species.

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