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IN VITRO PROPAGATION OF PUMPKIN AND ASH GOURD THROUGH NODAL SEGMENTS

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Abstract

The present study was undertaken with a view to develop an efficient protocol for *in vitro* multiple shoot formation and subsequent root induction considering various cultural aspects using nodal segments of *Cucurbita maxima* and *Benincasa hispida.* The best hormone for shoot multiplication of pumpkin was BAP (2 mg/l), incase of ash-gourd BAP was 1.5 mg/l. For callus induction BAP+2,4-D was best combination for pumpkin and it was 2.0+0.1 mg/l when in ash gourd BAP+NAA was the best combination. IBA + ½MS medium were used for induction of shoot bud root. In both of pumpkin and ash gourd 1.5 mg/l IBA was found best for induction of roots.

Key words: *In vitro*, MS medium, nodal segments, multiplication, rooting, pumpkin and ash gourd.

Introduction

Pumpkin (*Cucurbita maxima*) and ash gourd (*Benincasa hispida*) are very common and important vegetables in our country. Local name of pumpkin is bilati kumra or mistikumra and that of ash gourd is chalkumra. Seeds of *Cucurbita maxima* have been excavated in Peru and dated 1200 A D but no remains have been count in Mexico and Central America. It has been now spread to most of the world. It contains 90% fresh water and other food values, seed kernel of pumpkin contain 40-50% oil. The oil is prescribed as a nerve tonic. Seeds are also used as anthelmintic (Chopra *et al*. 1956). Mature fruit of pumpkin is the valuable source of vitamin A.

Ash gourd has some medicinal value like- anthelmintic, antiperiodic, aphrodisiac, cancer, demulcent, diuretic, expectorant, febrifuge, laxative Pumpkin and ash-gourd are monoecious plant and are pollinated by bees. Plants are propagated mainly by seeds. But it cannot be easily maintained the genetic stability by seeds propagation. On the other hand they have no conventional vegetative techniques. So, for maintain the genetic stability it needs to develop the plants by special vegetative technique like tissue culture.

The application of tissue culture technique for mass propagation of cucurbitaceous vegetable crops has been well demonstrated (Moreno and Riog 1990, Dong and Jia 1991, Debeaujon and Branchard 1992, Misra and Bhatnagar 1995). Although some success in *in vitro* induction of embryogenesis in pumpkin (Jelaska 1972, 1974) and cucumbers (Novak and Dolezalova 1982, Wehner and Locy 1981). The present paper describes rapid, simple and comparatively efficient shoot regeneration and root induction from nodal segments of pumpkin and ash-gourd and it describes the hormonal differences between two species in different aspects of tissue culture.

Materials and Methods

In both case of pumpkin and ash gourd nodal segments were used as plant material. The segments were cut into pieces carefully with the help of a forcep and dissecting blade. These explants were taken into a conical flask and thoroughly washed under running tap water for 20 minutes to remove loose contaminates attached

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to explants. Then the explants were washed with distilled water containing 1% savlon (v/v) and two drops of tween-80 for 5 minutes to remove gummy substances. This was followed by successively 3 washing with distilled water to make the materials free from savlon. Subsequently the materials were transferred to laminar airflow cabinet. Washed explants were treated by different concentration of $HgCl_2$ at different periods of time. After 7 days of incubation, 80% of the explants were free from contamination and tissue killing when pumpkin explants with 0.03% $HgCl_2$ (w/v) at 3.0 minutes and ash gourd explants with 0.03% $HgCl_2$ (w/v) at 3.5 minutes.

Agar solidifying MS media (1962) containing sucrose 3% and different concentrations and combinations of BAP (0 - 4.5 mg/l), NAA (0.1- 4.5 mg/l), 2,4-D (1.0 -2.5 mg/l) were used for shoot multiplication and callus induction. The pH of the medium was adjusted to 5.7 for pumpkin and 5.6 for ash gourd prior to autoclaving. Initially, the medium (10 ml) was dispensed into test tubes covered with non-absorbent cotton plugs and stream sterilized by autoclaving the medium at 121ºC for 21 minutes at 1.0 Kg/cm^2 pressure. Cultures were incubated at 25 ± 2ºC under the warm fluorescent light intensity varied from 2000-3000 lux.

Rooting of *in vitro* developed shoots of pumpkin and ash gourd was done by growing on half strength MS medium supplemented with IBA (0.5-4.5 mg/l). The newly transferred cultures were kept in dim light for 3 days, and then they were kept in full light as in shooting.

Results and Discussion

The *in vitro* regeneration of shoots of pumpkin and ash gourd using nodal segment explants was investigated and the data on number of shoots obtained from nodal segments are presented in Table 1. Among the different formulations the best formulation for multiple shoot proliferation and higher number of shoots per explants was found in media having 2.0 mg/l BAP in pumpkin but in ash gourd it was 1.5 mg/l BAP.

In vitro grown ash gourd explants gave adventitious shoots elongation on MS + BAP 1 mg/l + IAA 0.5 mg/l (He *et al*. 2006), MS + BAP (1-6 µM) + NAA (0.5 µM) (Dennis Thomas and Sreejesh 2004). Indirect proliferation of shoot of pumpkin, media having 1.5 mg/l BAP gave good result (Bologun *et al*. 2007). In other cucurbitaceous species like *Cucumis sativus*, shoot proliferation was better in 2 mg/l BAP + 1.0 mg/l $AgNO_3$ (Li *et al*. 2008). In pointed gourd a very high rate of shoot regeneration (93.86) was achieved when nodal explants were cultured on MS + 2.0 mg/l BAP + 0.3 mg/l NAA and the female genotype is better than the male genotype (Malek *et al*. 2007). In case of mulberry only BAP showed the best result for shoot multiplication from nodal segments (Zaman *et al*. 1992).

For callus induction different concentration and combinations of different growth regulators were used (Plate 2). Ninety percent of pumpkin explants produced callus when media having 2,4-D 2.0 mg/l + BAP 0.1 mg./l and fresh weight callus was 2.8 gm (average) but in case of ash gourd 80% explants induced callus when media having 2.0 mg/l NAA + 0.1 mg/l BAP and fresh weight of callus 2.90 gm(average). Auxin have been reported to induce callus formation in tissue culture of plant (Tissert 1995). In water melon NAA and IAA promoted excessive callus formation (Compton and Gray 1993). Juvenile tissues in Ash gourd well developed green cotyledons from the grown embryos where isolated and cultured on MS with 2,4-D (1-6 µM) gave good calli (Dennis Thomas and Sreejesh 2004). In summer squash the highest frequency of callus induction was observed in MS medium fortified with 2.5 mg/l 2,4-D and hypocotyl gave more responce than epicotyl (Pal *et al*. 2007).

Different species of Cucurbitaceae have difference in callus induction. The differences might have arisen due to many other factors like- genotypes, medium composition, physical growth factors like- light, temperature, moisture are important for callus induction (Pierik 1975).

For induction of roots in pumpkin and ash gourd, half MS + IBA was used. In both cases 1.5 mg/l IBA was the best hormonal dose but the result was not same. In pumpkin mean number of root per explant after 21 days were 6.5 and mean length of the longest root per explant after 21 days 5.5 cm. but in ash gourd mean number of root was 6.5 and mean length of the longest root 5.8 cm. after 21 days.

Table 1. Effect of different concentration of BAP on shoot multiplication of pumpkin and ash gourd.

Plant	Supplement MS + BAP (mg/l)	% of explants regenerated shoot	Mean no. of shoot after days		Mean length of longest shoot after days (cm)	
			15	21	15	21
Pumpkin	0	2	-	1	-	1.5
	0.5	20	1	1.5	1	1.8
	1.0	30	1	2	1.8	2.5
	1.5	55	2	3.5	2.6	3.6
	2.0	85*	4	6**	3	5.8***
	2.5	70	3	3	3	3.5
	3.0	70	2	3	2	2.7
	4.0	50	1	2	1.7	2.5
	4.5	30	2	2	1.5	2
Ash gourd	0	2	-	1	-	1.5
	0.5	30	1	1.5	2	2.8
	1.0	25	2	2	1.8	2.5
	1.5	90*	2	3.5	2.5	3.5
	2.0	85	3	5**	3	5.8***
	2.5	65	3	3.2	3	3.5
	3.0	70	3	3.3	2.1	2.7
	4.0	60	1	2	1.8	2.5
	4.5	30	2	2.5	1.6	2

*Highest % of explants regenerated shoot, ** Highest mean no. of shoots and *** Highest mean length (cm) of longest shoot after 21 days

Table 2. Effect of different combinations of 2,4-D with BAP on callus induction from nodal segments and shoot tips of pumpkin and ash gourd.

Plant	Media		No. of explants responded (No. inoculated 20)	% of explants induced callus	Fresh weight of callus (gm)
Pumpkin	Supplement MS + (2,4-D + BAP) mg/l				
	Nodal segments	1.0 + 0.1	14	70.00	1.79
		1.5 + 0.1	16	80.00	1.50
		2.0 + 0.1	18*	90.00**	2.80***
		2.5 + 0.2	17	85.00	2.01
	Shoot tips	1.0 + 0.1	15	65.00	1.50
		1.5 + 0.1	14	70.00	1.80
		2.0 + 0.1	14	70.00	1.60
		2.5 + 0.2	17	85.00	2.30
Ash Gourd	Supplement MS + (BAP + NAA) mg/l				
	Nodal segments	0 + 0.1	8	40.00	1.50
		0.1 + 0.5	9	45.00	1.50
		0.1 + 1.0	7	35.00	1.60
		0.1 + 2.0	16*	80.00**	2.90***
		0.2 + 2.5	13	65.00	2.00
		0.2 + 3.0	14	70.00	2.80
		0.3 + 4.5	11	55.00	1.70
	Shoot tips	0 + 0.1	7	35.00	1.70
		0.1 + 0.5	7	35.00	1.75
		0.1 + 1.0	7	35.00	1.82
		0.1 + 2.0	8	40.00	1.90
		0.2 + 2.5	9	45.00	2.20
		0.2 + 3.0	12	60.00	1.50
		0.3 + 4.5	10	50.00	1.48

* Highest no. of explants responded , ** Highest % of explants induced callus, *** Highest average fresh weight of callus

Table 3. Effect of different concentrations of IBA for root induction of pumpkin and ash gourd.

Plant	½ MS + IBA (mg/l)	Root development	Morphogenic response			
			Mean no. of root per explants after days		Mean length of longest root after days (cm)	
			15	21	15	21
Pumpkin	0.5	+	2.2	2.6	3.0	3.5
	1.0	+	3.2	3.6	3.0	3.7
	1.5	+	4.5	6.5*	4.5	5.5**
	2.0	+	4.2	5.2	4.0	4.5
	2.5	+	4.3	5.0	4.2	4.8
	3.0	+	4.3	4.8	4.0	4.2
	3.5	+	4.0	4.5	3.5	3.5
	4.0	+	3.5	3.6	3.0	3.6
	4.5	+	3.0	3.5	3.0	3.2
Ash gourd	0.5	+	2.1	2.5	3.0	3.5
	1.0	+	3.25	3.6	3.5	3.7
	1.5	+	5.2	6.5*	4.5	5.8**
	2.0	+	4.2	5.5	4.1	4.6
	2.5	+	4.2	5.0	4.2	4.8
	3.0	+	4.25	4.5	4.0	4.5
	3.5	+	4.0	4.5	3.6	3.7
	4.0	+	3.5	3.6	3.0	3.6
	0.5	+	2.1	2.5	3.0	3.5

* Mean no. of roots per explants after 21 days (highest), ** Mean length of longest root per explants after 21 days in cm. (highest)

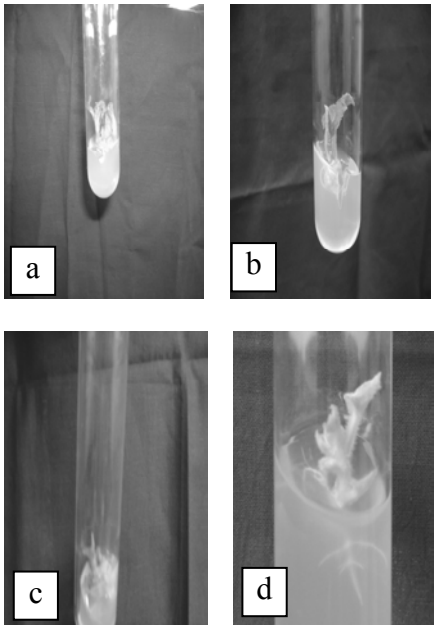

Plate 1 a. Multiple shoots formation from nodal segment of young plants of pumpkin MS media having 2.0 mg/l BAP. b. Multiple shoots formation from nodal segment of young plant in MS medium containing 2.5 mg/l BAP+0.1 mg/l NAA. c. Induction of callus from nodal segment in MS + 2.0mg/l 2,4-D + 0.1 mg/l BAP. d. Induction of roots from media having ½ MS + 1.5 mg/l IBA.

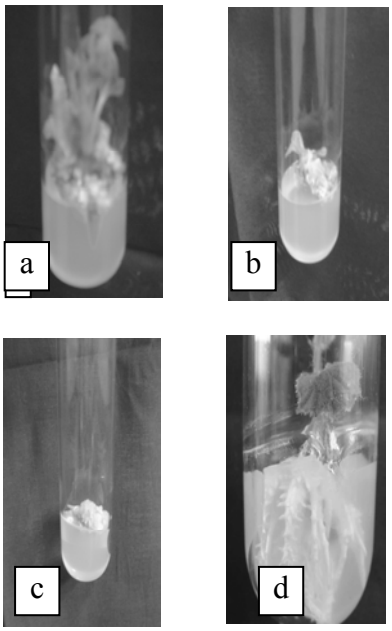

Plate 2 a. Multiple shoots formation from nodal segment of young plants of ash gourd in MS media having 1.5 mg/l BAP. b. Multiple shoots formation from nodal segment of young plant in MS media having 2.0 mg/l BAP+0.1 mg/l NAA. c. Induction of callus from nodal segment in MS + 2.0 mg/l NAA + 0.1 mg/l BAP. d. Induction of roots in media having ½ MS + 1.5 mg/l IBA.

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