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MICROPROPAGATION OF *CUCURBITA MAXIMA* DUCH. THROUGH SHOOT TIP CULTURE

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Abstract

Micropropagation of pumpkin (*Cucurbita maxima* Duch.) was achieved using shoot tip of *in vitro* grown seed derived plants of two cultivars namely, Bikrompuri and Baromasi of Bangladesh. The excised shoot tips were cultured on MS medium containing KIN, BA, NAA at various levels of concentration and combination for shoot induction and proliferation, and best response was found at 3.0 mg/l of BA. Shoots were rooted most effectively in ½ MS medium supplemented with 1.0 mg/l IBA. Bikrompuri was found more responsive than Baromasi for rapid clonal propagation.

Key words: Micropropagation, Direct organogenesis, Shoot tip, Pumpkin

Introduction

Cucurbita genus consists of 13 species, including *C. maxima* Duch. (Pumpkin) is a widely cultivated vegetable in East Asian countries, such as China, Japan and Korea and good sources of carbohydrate, fat, protein, mineral salts and vitamins (Gopalan *et al.* 1982). Many human nutritional deficiencies and diseases commonly encountered in developing countries are preventable by an intelligent and fulfil utilization of vegetables. Therefore, genetic improvements are recommended for vegetable crops including pumpkin. Although conventional breeding methods are most widely used for crop improvement, but there have some potential limitations (Chaudhury 1994). Some of genetic potential is not available due to sexual incompatibility barriers appearing in intergeneric and interspecific levels as observed in several crops (Deakin *et al*. 1971, Niemirowicz-Szczytt and Kubicki 1979, Olmstead 1989). Pumpkin is a cross-pollinated plant. So, it can not produce sufficient hybrid seeds because large scale emasculation is not possible. This plant shows abrupt inbreeding depression after two generation of selfing.

The techniques of plant tissue culture and genetic manipulation are useful to solve those problems (Razdan 1993). Recent developments in biotechnology have opened up several ways for the crop breeding using transformation in which heterologous genes can be introduced into exiting cultivars. In many instances, however, the lack of an efficient regeneration system causes limitations on the use of gene transfer technology for the vegetable crops. An efficient plant regeneration system is, therefore, essential for transformation and propagation as well as for *in vitro* breeding through selection of somaclonal variation and somatic hybridization. In comparison with cucumber (*Cucumis sativus*) (Monohiuddin *et al*. 1997) and watermelon (*Citrullus vulgaris*) (Dong and Jia 1991, Dabauza *et al*. 1997), less attention still has been given to tissue culture of *Cucurbita* species. Therefore, attempts were taken to develop efficient protocol for plant regeneration through direct organogenesis of the studied genus.

Materials and Methods

The materials for the present investigation were comprised of two cultivars of pumpkin such as Bikrompuri and Baromasi. Seeds of these cultivars were collected from “Sufola Biz Vandar”, BRAC centre, Gazipur and

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"Shyamoli Biz Vander" Udoyon Road, Chapai Nawabganj. Shoot tips (2-3 mm) isolated from the seed derived *in vitro* grown plants of these cultivars were used for experimentation. MS medium with different concentrations and combinations of KIN, BA, NAA with 3% sugar was used for shoot multiplication while ½ MS medium with different concentrations of IBA, NAA and IAA were used for root formation. The medium was solidified with 6.2% agar.

Shoot tip were cut and carefully inoculated on the agar gelled semi-solid MS medium (Plate 1 a). All the inoculated culture tubes were incubated in the growth chamber providing proper culture environment. After multiple shoot formation, the plantlets were transferred to rooting media for sufficient root. The culture tubes were checked regularly to observe the morphogenic response. When the regenerated plantlets produced sufficient roots and attained a height of 7-9 cm were transferred in the soil and acclimatized.

Results and Discussion

In the present investigation different concentrations and combinations of cytokinins KIN and BA or a combination of BA and NAA were used to see the response towards multiple shoot formation from shoot tip of two pumpkin cultivars viz. Bikrompuri and Baromasi (Table 1).

In the present study among all the combinations and concentrations, the longest shoots (6.1±0.85 cm) and the highest percentage of shoot formation (90.45%) were observed in treatment with 3.0 mg/l BA after 30 days of culture (Plate 1 b, c, d). However, the highest number of shoots per shoot tip (16.5±0.95) was noted in the media containing 2.0 mg/l BA. Further increasing the concentration of BA did not improve the shoot size and number. Several workers reported similar results in cucumber (Kathal *et al*. 1988; Misra and Bhatnagar 1995) and in *Cucurbita* interspecific hybrid cultivar "Shintoza" (Sarowar *et al*. 2003). Whereas, in case of MS0, the longest shoot (2.5±0.55 cm), number of shoots per shoot tip (2.1±0.12) and highest percentage of shoot formation (10%) were not performed well, thus suggested the necessity of using phytohormone for effective shoot formation and multiplication. Increased concentration of BA reduced shoot length and promoted massive base callus. Khan *et al.* (1994) obtained similar results at 2.0 mg/l BA for *Chrysanthemum morifolium.*

When BA was tested in combination with NAA and KIN result shows that single use of BA is more effective than combination treatment with NAA and KIN for direct organogenesis from shoot tips in *Cucurbita maxima*. Although the combination of 3.0 mg/l BA +0.2 mg/l NAA shows high (80.10%) percentage of shoot formation, but the shoot number (14.1±0.32) and length (5.8±0.75 cm) were not increased. Single use of KIN was also not encouraging. This is further supported in Figs. 1, 2 and 3 when the data from Table 1 was calculated combinedly. This finding is similar to Sarowar *et al*. (2003). The superiority of BA over other cytokinins, auxin and gibberillin for shoot multiplication has been reported by other investigators (Vieitez and Vieitez 1980; Misra and Chaturvedi 1984, Lim-Ho and Kong 1985, Mhatre *et al*. 1985, Arulpragasem and Latiff 1986, Benjamin *et al*. 1987 and Amin *et al*. 1997).

The combined result presented in Fig. 4 calculated from Table 1 further shows that Bikrompuri was more responsive for multiplication of plantlets than Baromasi; thus proven differential genotypic performance in *in vitro* system (Zelcer *et al*. 1984, Capote *et al*. 2000 and EL-Bakry 2002).

Table 1. Shoot multiplication from isolated shoot tips in MS medium contained different concentrations and combinations of phytohormone. In each treatment 10 explants were used. Data were recorded after 21 days of inoculation.

Treatment (mg/l)		Cultivars					
		Bikrompuri			Baromasi		
		Explants induced multiple shoots (%)	Mean No. of shoots/ Culture ±SE	Mean length of longest shoot (cm) ±SE	Explants induced multiple shoots (%)	Mean No. of shoots/ Culture ±SE	Mean length of longest shoot (cm) ±SE
MS0 (control)		10.00	2.1± 0.12	2.5± 0.55	9.11	2.0± 0.55	2.1± 0.75
KIN	1.0	35.66	5.3 ±0.33	4.7 ±0.27	28.00	3.7 ± 0.05	4.2 ±0.59
	1.5	40.95	6.1 ±0.30	5.0 ±0.82	40.45	5.2 ±0.94	4.8 ±0.33
	2.0	50.37	6.1 ±0.36	4.9 ±0.17	48.42	5.9 ±0.32	4.7 ±0.53
	3.0	48.75	5.9 ±0.35	5.1 ±0.16	42.97	5.5 ±0.23	4.8 ±0.24
	4.0	42.50	5.6 ±0.24	4.9 ±0.31	39.55	5.1 ±0.85	4.1 ±0.61
BA	1.0	66.12	8.9 ±0.64	3.1±0.11	55.45	7.1±0.29	2.8±0.29
	2.0	75.25	16.5±0.95	5.2±0.11	67.54	13.1±0.64	4.9±0.12
	3.0	90.45	14.1±0.65	6.1±0.85	85.75	11.2±0.64	5.8±0.22
	4.0	80.58	12.8±0.29	4.8±0.12	75.36	8.5±0.51	4.2±0.12
	5.0	70.64	10.9±0.14	2.2±0.11	65.45	9.1±0.29	2.0±0.07
BA+KIN	1.0+2.0	45.15	6.9±0.84	4.5±0.54	32.75	6.8±0.58	4.7±0.12
	2.0+2.0	50.33	7.2±0.55	5.1±0.66	45.85	7.4±0.19	5.4±0.64
	3.0+2.0	60.66	6.8±0.33	5.5±0.66	58.10	7.1±0.28	5.6±0.21
	4.0+2.0	48.72	6.1±0.78	5.1±0.32	35.00	6.9±0.14	5.3±0.11
	5.0+2.0	40.25	5.8±0.50	4.5±0.38	30.55	5.9±0.91	4.8±0.25
BA+NAA	1.0+0.2	60.15	8.12±0.35	5.1±0.39	55.12	8.8±0.52	4.9±0.55
	2.0+0.2	70.45	11.0±0.21	5.4±0.15	65.25	10.1±0.11	5.1±0.63
	3.0+0.2	80.10	14.1±0.32	5.8±0.75	75.15	9.7±0.67	5.5±0.32
	4.0+0.2	65.75	10.9±0.31	5.5±0.11	60.10	8.6±0.40	5.1±0.22
	5.0+0.2	59.25	8.5±0.19	5.1±0.05	54.19	7.9±0.33	4.9±0.58

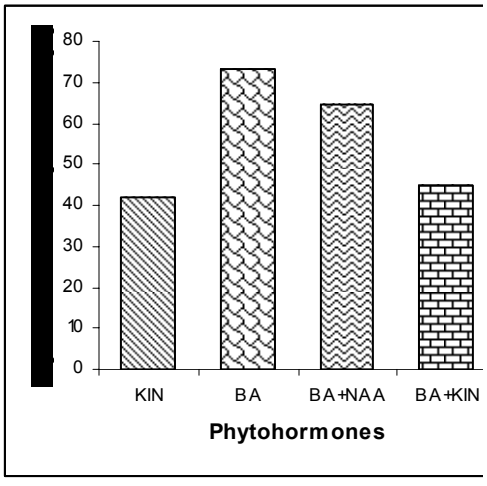

Fig 1. Comparison among different kinds and combinations of phytohormone on multiple shoot initiation (%).

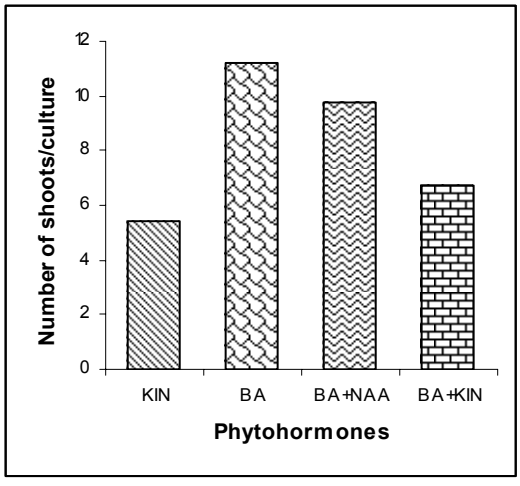

Fig 2. Comparison among different kinds and combinations of phytohormone on number of shoots/culture.

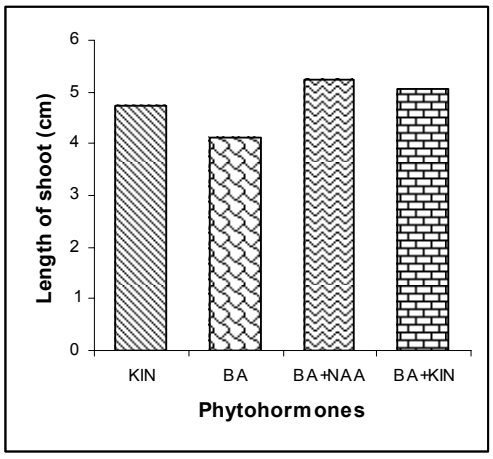

Fig 3. Comparison among different kinds and combinations of phytohormone on length of shoot (cm)

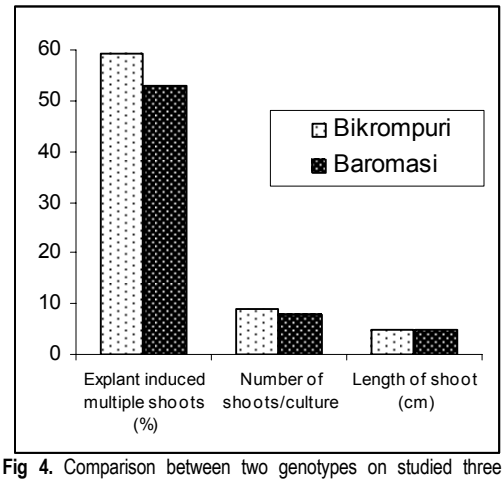

Fig 4. Comparison between two genotypes on studied three shooting parameters.

Elongated shoots (<4 cm) started root initiation within 7-8 days of inoculation when cultured onto ½ MS medium added with different concentrations of NAA, IBA and IAA individually. And distinct variation was observed among the treatments and between the cultivars (Table 2). All the treatments induced roots, but maximum rooting (93.11 %) and vigorous growth of plantlets were observed on medium containing 1.0 mg/l IBA within 12-14 days of transfer (Plate 1e). This was in accord with earlier reports on *Cucumis melo* (Haque *et al.* 1984, Kathal *et al*. 1988, 1994, Singh *et al*. 1990 and Khalekuzzaman *et al*. 2003). However, the highest mean number of roots (11.66±2.05) and highest mean length of longest root (7.75±0.93 cm) was recorded in 2.0 mg/l of IBA. The figures 5, 6 and 7 prepared from combined data of Table 2 show that IBA is more effective than NAA and IAA for root formation and proliferation. Similar to shoot, good response for root formation and proliferation was observed in cultivar Bikrompuri than Baromasi (Fig. 8).

The successful establishment of plantlets in soil (Plate 1 f) suggested that shoot tips could be used for rapid direct organogenesis in *Cucurbita maxima*.

Table 2. Proliferation of adventitious roots from *in-vitro* grown microclones of two pumpkin cultivars in ½ MS medium with various concentrations of IBA, NAA and IAA singly. Data were recorded after 15 days of inoculation.

Treatment		Cultivars					
		Bikrompuri			Baromasi		
(mg/l)		Root formation (%)	Mean no of roots/culture ±SE)	Mean length of root in cm ±SE)	Root formation (%)	Mean no of root per culture ±SE)	Mean length of root in cm ±SE)
½ MS0		5.0	2.50±0.95	0.85±1.15	5.1	1.9±0.85	0.80±1.11
IBA	0.5	58.33	11.12±1.05	7.50±0.64	50.17	10.11±1.04	7.23±0.64
	1.0	93.11	8.66±1.74	6.08±0.33	87.45	7.90±1.12	5.91±0.30
	2.0	76.25	11.66±2.05	7.75±0.93	67.54	10.33±1.95	7.10±0.85
	3.0	66.35	7.12±1.55	7.25±0.74	59.33	6.75±1.23	7.14±0.71
NAA	0.5	50.22	6.95±0.61	5.01±0.25	45.11	5.10±0.57	4.80±0.23
	1.0	70.32	8.11±2.11	6.00±0.78	60.14	7.01±1.25	5.10±0.43
	2.0	50.15	7.5±1.15	5.03±0.54	40.22	6.23±01	4.50±0.32
	3.0	40.45	5.12±0.25	4.80±0.63	30.44	4.30±0.23	3.90±0.12
IAA	0.5	60.13	4.50±0.51	8.50±0.64	57.33	4.10±0. 41	7.91±0.55
	1.0	64.32	5.61±0.67	9.00±0.75	59.60	5.01±0.61	8.10±0.61
	2.0	50.85	4.10±0.04	7.71±0.51	50.33	3.5±0.31	6.70±0.17
	3.0	40.85	3.80±0.31	6.10±0.71	30.35	2.90±0.21	5.50±0.30

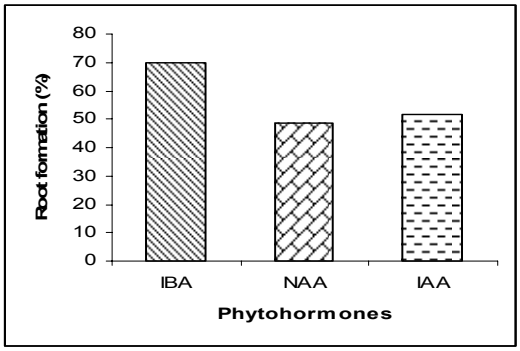

Fig 5. Comparison among different kinds of auxin on multiple root formation (%).

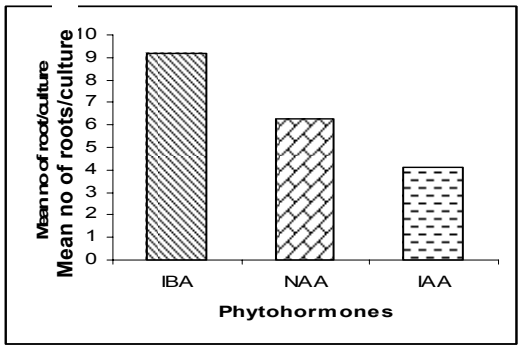

Fig 6. Comparison among different kinds of auxin on number of roots/culture.

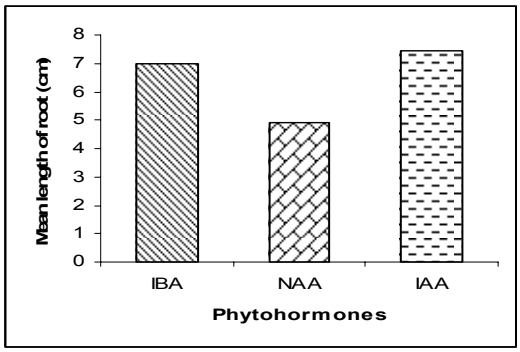

Fig 7. Comparison among different kinds of auxin on length of root (cm)

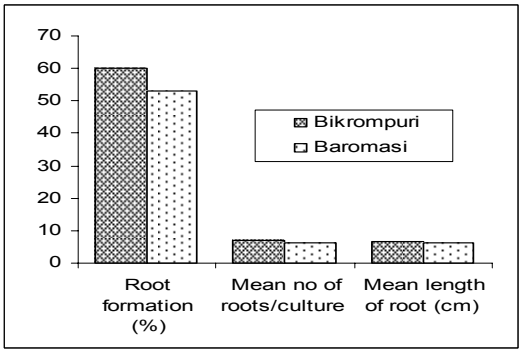

Fig 8. Comparison between two genotypes on studied three rooting parameters.

Plate 1

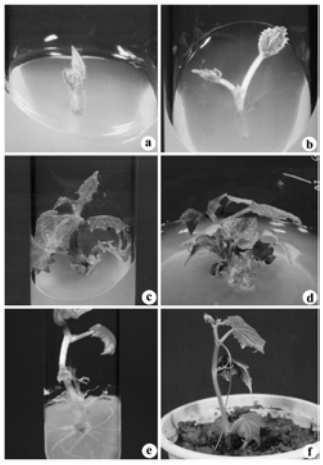

Plate 1. Figures **a-f** showing different stages of culture for multiple shoot formation and acclimatization of plants derived from shoot tip explant in *Cucurbita maxima*. **a.** Inoculation of excised shoot tip. **b.** Development of shoot tip after 4 days of inoculation. **c.** Proliferation of multiple shoots after 15 days of inoculation, **d.** Farther proliferation of multiple shoot after 30 days of inoculation. **e.** Development of rootlets in excised shoot. **f.** Acclimatization of plantlet in soil.

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