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Research Article

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Determination of apposite plant regeneration protocol for several cucurbits through direct and indirect organogenesis

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ABSTRACT

In the present study, a competent and reproducible practice for the *in vitro* shoot regeneration of *Cucurbita maxima*, *C. pepo* and *Cucumis sativus* was developed from various explants through direct and indirect organogenesis. In *C. maxima*, between cotyledon and leaf segment, cotyledon was found to be most responsive for callus induction in MS medium augmented with 0.5 mg L^{-1} 2,4 dichlorophenoxy acetic acid (2,4-D) plus 100 mg L^{-1} casein hydrolysate and 0.5 mg L^{-1} 2,4-D plus 15% coconut water and for leaf segment it was on MS medium containing 2.5 mg L^{-1} 2,4-D. Comparing the 2 explants it was found that leaf segment was most suitable for callus induction in *C. maxima*. For massive multiplication of *C. pepo* mericlones shoot tip and nodal cutting were used. MS medium containing 3.0 mg L^{-1} 6-benzyl aminopurine plus 0.5 mg L^{-1} gibberellic acid (GA_3) was found most effective for shoot regeneration and 1.0 mg L^{-1} IBA was found most effective for rooting. In this trait cv. Bulum was more responsive than cv. Rumbo. On the other hand, to generate virus free plantlets of *C. sativus*, different concentrations of kinetin were used, and 1.5 mg L^{-1} KIN shown the best performance for primary culture establishment. For shoot multiplication, 1.0 mg L^{-1} BAP and 2.0 mg L^{-1} BAP plus 0.5 mg L^{-1} KIN containing medium shown best result. Subsequently, 2.0 mg L^{-1} BAP plus 0.5 mg L^{-1} KIN was best composition for root induction. Our report demonstrated comprehensive protocols and variability in explants, growth regulator response in shoot regeneration potential of in different cucurbit plants.

KEYWORDS: Callus, cotyledon, *cucurbita*, *in vitro*, regeneration, organogenesis

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INTRODUCTION

Tissue culture techniques can be used for clonal multiplication, *Agrobacterium*-mediated transformation, and *in vitro* conservation of germplasm [1,2]. Species of *Cucurbita* are commercially important. They are being manipulated to produce superior cultivars [3]. The family is genetically diverse and adapted to arid deserts and the temperate zone [4]. Watermelon (*Citrullus lanatus* Thunb. Matsum and Nakai),cucumber (*Cucumis sativus* L.), melon (*C. melo*), pumpkin (*Cucurbita maxima* Duch.), and squash (*Cucurbita* spp.) are the most important cultivated species [5]. Cultivated cucurbits are affected by yield-limiting diseases including those caused by viruses. Control of viruses is necessary for successful commercial cultivation of these species.

In cucurbits breeding and tissue culture have been used to transfer useful traits. The meristem is effective for regeneration of disease-free plants through direct organogenesis [6, 7, 8, 9,10]. *In vitro* manipulation of cucurbits has been used due to a quick response on artificial media and easy accessibility. Members of the Cucurbitaceae are not as recalcitrant as they had seemed to be in the beginning [11,12]. However, regeneration of cucurbits is comparatively low and dependent on the nature of explants [13,14], growth regulator combinations, and physical conditions of culture [15]. The morphogenic potential of the Cucurbitaceae *in vitro* has showed that cucurbit tissues could regenerate via direct and indirect organogenesis with cytokinins [15,16]. Applicability of cytokinin usage for plant regeneration is due to stimulation of cell division, often together with auxins, and release of lateral bud dormancy from inducing shoot-bud proliferation in cuttings and cultures [17,18].

An efficient plant regeneration protocol could help to introduce desired foreign gene(s) into cucurbit genomes.

Commercial application of *in vitro* techniques in cucurbits has been showed and regeneration of plants reported from excised cotyledons [19-26], leaf explants [24,27-29] another culture [30], hypocotyls and cotyledons [31,26], shoot tip meristems [32,33] and nodal segments [34,35]. Somatic embryogenesis can be achieved in several cucurbits [36-38]. This study aimed to standardize efficient protocols for plant regeneration and pathogen-free clonal propagation of cucurbits through direct and indirect organogenesis.

MATERIALS AND METHODS

Plant Material

Cultivars of pumpkin, Bikrompuri and Baromasi; squash, Rumbo and Bulum, and two types of cucumber, slicer (cv. Naogaon Green) and pickler (cv. Green King) were used. Seedwere collected locally. For callus induced regeneration studies in pumpkin, seed were germinated *in vitro*. Cotyledon and young leaf segments were used as the primary explants for callus induction. In squash, multiple shoots were regenerated directly from shoot tips. Shoot tip explants were collected from 25-30 day-old *in vitro* grown seedlings to be used as primary explants. Subsequent shoot tips and nodal segments were collected from meristem derived plants for plantlets propagation.In cucumber, plants were regenerated from shoot apical meristems. Seed were germinated in the field and shoot tip explants collected from 30-45 day old plants and used to isolate meristems.

Culture Medium and Physical Conditions

Full, and half-strength,Murashige and Skoog media, supplemented withconcentrations and combinations of organic additives, and growth regulators, were used for establishment, callus induction, shoot multiplication, and root formation. The medium was adjusted to pH 5.7 using 0.1 N NaOH or 0.1 N HCl before adding agar (0.8%; w/v), and sterilized at 12°C for 20 min at 0.103 MPa. Incubation was at 25±2°C under 16/8 hr light (white light)/dark conditions with a light intensity of 28-30 μmolm^{-2}s^{-1} (cool-white fluorescent lamps, Philips, Shanghai, China).

Surface Sterilization

A solution of 70% (v/v) ethanol and 0.1% (w/v) $HgCl_2$ containing 2-3 drops of Tween-20/100 mL was used tosurface sterilize plant materials.

Culture

For *C. maxima*, MS medium was supplemented with concentrations of dichlorophenoxy acetic acid (2, 4-D), 6-cytokinin (benzyl aminopurine, BAP), kinetin (KIN), and napthaleneacetic acid (NAA), used alone, or in combination with 100 mg L^{-1} casein hydrolysate (CH), or 15% coconut water (CW). After induction, calliwere transferred into fresh medium for further proliferation and maintenance. For indirect organogenesis, embryogenic calli were selected for plant regeneration. Plants were regenerated by transferring calli in to MS medium supplemented with BAP alone, or in combination with KIN, NAA and IBA, for shoot induction and ½MS medium supplemented with NAA or IBA or IAA for root induction.

For *C. pepo*, sterilized seed were placed on agar-solidified MS medium supplemented with 3% (w/v) sucrose for germination. Shoot tips were isolated from apical meristematic areas and inoculated on semi-solid MS medium supplemented with 3% (w/v) sucrose and various concentrations and combinations of BAP, KIN, purine(2iP) and gibberellic acid (GA_3) for multiple shoot proliferation. Multiple shoots were sub-cultured on MS basal medium supplemented with IBA, NAA or IAA for further root induction and proliferation.

For *C. sativus*, meristem isolation and culture was as described [9]. After sterilization of non-living materials, isolated meristem tips were carefully placed on an "M" shaped filter paper bridge in culture tubes (Figure 3A) containing liquid MS medium supplemented with growth regulators for establishment of primary meristems. After 3 to 4 weeks of incubation primary meristems exhibiting morphogenic responses were aseptically removed from culture tubes and transferred to tubes containing MS medium supplemented with 3% (w/v) sucrose, and different concentrations of KIN and BAP for shoot proliferation, or IBA and NAA for root formation.

Acclimatization and data recording

Following regeneration, plantlets were acclimatized as described previously [39]. Data on various growth parameters of in vitro culture were collected according to the previous publication [26].

RESULTS

Plant regeneration from callus in *Cucurbita maxima*

In pumpkin, cv. Bikrompuri and Baromasi, calli were induced from cotyledons and leaf segments of *in vitro* grown plantlets cultured on 2,4-D, KIN, BA, BAP and NAA with CH or CW (Figure1a). The 2,4-D alone was effective for callus induction in pumpkin (Table 1, 2). However, cotyledons were most responsive in MS medium augmented with 0.5 mg L^{-1} 2,4-D+100 mg L^{-1} CH and 0.5 mg L^{-1} 2,4-D+15% CW. Leaf segments were most responsive with 2,4-D and exhibited acceptable results with 0.5 mg L^{-1} 2,4-D+100 mg L^{-1} CH and 0.5 mg L^{-1} 2,4-D+15% CW. 'Bikrompuri' exhibited a better response for callus induction than 'Baromasi' for both types of explants. 'Bikrompuri' exhibited 100% response with 2, 4-D+CH and 2,4-D+CW when cotyledons were used as explants; 'Baromasi' had a lesser response. Presence of CH or CW in the media enhanced white colored callus formation. All leaf segments induced callus in media without CH or CW in 2.5 mg L^{-1} 2, 4-D (Figure 1b). Calli were creamy white and friable. All 'Bikrompuri' leaf explants produced calli when cultured only on 2, 4-D; almost all 'Baromasi' produced calli with the same treatment. (Figure 1c). Use of BAP+NAA and BAP+NAA+CW induced calli at low frequencies.

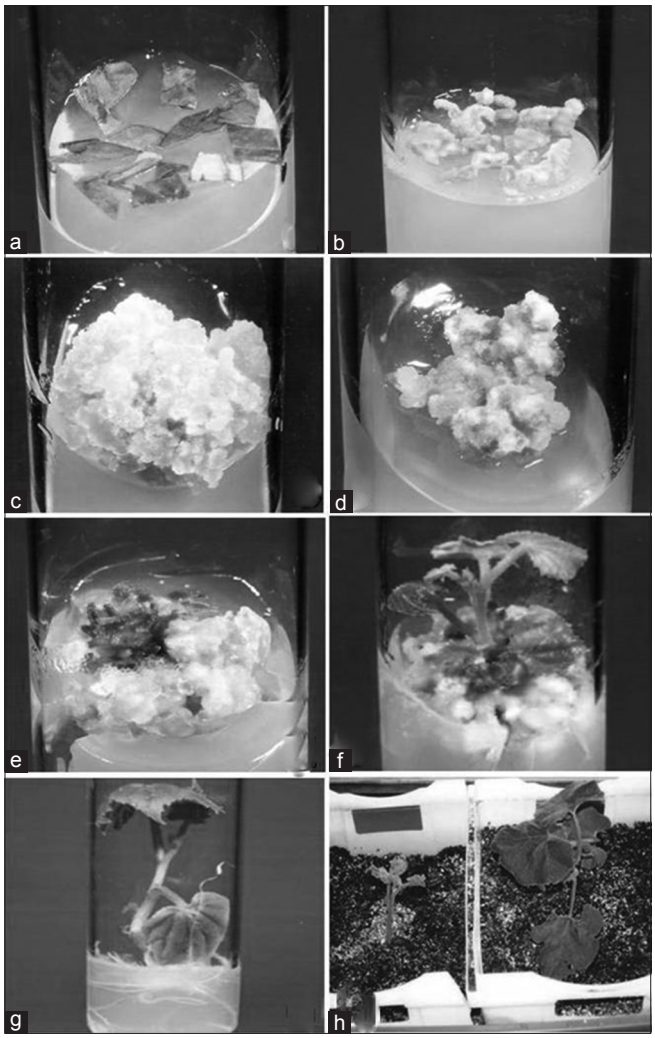

Figure1: Indirect mode of plant regeneration from callus in *Cucurbita maxima*. (a) Inoculation of young leaf segments for callus induction, (b) Callus initiation from leaf segments, (c) Proliferation of callus derived from young leaf segment, (d) Putative embryogenic callus, (e) Callus showing primary initiation of shoot, (F) Plant regeneration from callus, (g) Well rooted regenerated plantlets derived from callus, and (h) Acclimatization of plantlet

Shoot formation in pumpkin cultivars were influenced by concentrations and type of growth regulator (Figure 1e). Among plant growth regulators BAP alone was not useful in shoot regeneration compared with combinations of growth regulators. High regeneration rates were achieved using cytokinin+NAA. The best performance was in MS medium supplemented 1.0 mg L^{-1} BAP+0.2 mg L^{-1} NAA (Figure 1f). For 'Bikrompuri' there was about the same shoot regeneration from cotyledon and leaf explants. There were similar responses from cotyledon and leaf explants for 'Baromasi', which was higher than other growth regulators from cotyledon and leaf explants. For cotyledons there were almost identical numbers of shoots per callus from leaf explants for both cultivars. The fewest number of shoots occurred for KIN+IBA for 'Baromasi' and 'Bikrompuri' (Table 3). For auxins, NAA was better than indolebutryic acid (IBA) and IAA for producing roots from leaf explants, and IBA was better for cotyledons. The best rooting was with½ strength MS medium+0.1 mg L^{-1} NAA from leaf segments than for IBA for cotyledon explants (Table 4). For root induction leaf segment derived shoots responded better than cotyledon derived shoots (Figure 1g).

Table 1: Effect of 2,4-D concentrations, and combination with casein hydrolysate (CH; 100 mg L^{-1}), coconut water (CW; 15%), KIN, BAP, and NAA, in MS medium on callus induction (%) from pumpkin cotyledons. Data recorded 21 days after inoculation. Different letter followed by mean in the same column are statistically significant $p \leq 0.05$

Growth regulator concentration (mg L^{-1})	Concentrations	Mean callus induction (%)	
		Bikrompuri	Baromasi
2,4-D	0.1	32.00^{d}	30.12^{cd}
	0.2	48.00^{g}	45.01^{f}
	0.5	60.50^{i}	56.59^{j}
	1.0	95.75^{p}	94.46^{q}
	1.5	82.01^{n}	75.14^{k}
	2.0	71.39^{l}	68.11^{j}
	2.5	55.09^{h}	51.09^{h}
2,4-D+15% CH	0.02+CH	17.10^{b}	12.39^{a}
	0.05+CH	33.43^{d}	29.03^{c}
	0.1+CH	55.13^{h}	48.30^{g}
	0.5+CH	100.00^{q}	85.11^{o}
	1.0+CH	65.04^{j}	61.91^{i}
	1.5+CH	57.03^{i}	51.47^{h}
2,4-D+100 mg L^{-1}CW	0.02+CW	13.31^{a}	11.01^{a}
	0.05+CW	28.00^{c}	23.31^{b}
	0.1+CW	48.75^{g}	42.11^{f}
	0.5+CW	100.00^{q}	87.40^{p}
	1.0+CW	75.22^{l}	53.01^{h}
	1.5+CW	48.38^{g}	43.11^{f}
2,4-D+KIN	1.0+1.0	30.18^{c}	25.20^{b}
	1.5+0.2	45.20^{f}	40.20^{e}
	1.5+0.5	32.25^{d}	30.30^{c}
	1.5+1.0	28.11^{c}	25.10^{b}
2,4-D+BAP	1.0+0.2	71.91^{l}	70.79^{j}
	1.0+0.5	88.44^{o}	85.25^{o}
	1.0+1.0	62.31^{j}	60.54^{i}
	1.5+0.2	48.65^{g}	45.58^{f}
	1.5+0.5	42.69^{f}	40.68^{e}
	1.5+1.0	38.12^{e}	35.60^{d}
NAA	0.1	30.11^{c}	26.63^{c}
	0.2	42.42^{f}	40.00^{ef}
	0.5	55.85^{h}	50.25^{g}
	1.0	40.18^{e}	35.75^{d}
	1.5	35.11^{d}	32.13^{d}
	2.0	30.01^{c}	27.00^{c}
	3.0	25.12^{b}	24.00^{b}
BAP+NAA	1.0+0.2	43.33^{f}	41.70^{f}
	1.0+0.5	50.00^{g}	48.34^{g}
	2.0+0.2	86.66^{n}	82.04^{no}
	2.0+0.5	80.00^{m}	79.11^{mn}
	3.0+0.2	61.89^{j}	62.00^{j}
	3.0+0.5	38.03^{e}	42.14^{f}
BAP+NAA+15% CW	0.02+0.1+CW	55.01^{h}	50.38^{g}
	0.05+0.1+CW	75.11^{l}	72.37^{m}
	0.1+0.1+CW	90.31^{o}	84.04^{o}
	0.2+0.1+CW	85.20^{n}	76.31^{n}
	0.5+0.1+CW	70.11^{k}	65.04^{j}
	1.0+0.1+CW	49.04^{g}	45.17^{f}

Regeneration of multiple shoot in *Cucurbitapepo*

For *C. pepo* multiple shoot proliferation efficiency of nodal explants was higher than for shoot tip explants, and 3.0 mg L^{-1} BAP+0.5 mg L^{-1} GA_3 was more effective than other treatments

Table 2: Effect of 2.4-D concentrations and combination with casein hydrolysate (CH; 100mgL^{-1}), coconut water (CW; 15%), KIN, BAP, NAA in MS medium on callus induction (%) from leaf segment of pumpkin cultivars. Data recorded 21 days after inoculation. Different letter followed by mean in the same column are statistically significant $p \leq 0.05$

Growth regulator (mg L^{-1})	Concentrations	Mean of callus induction (%)	
		Bikrompuri	Baromasi
2,4-D	1.0	50.25^{e}	48.24^{ef}
	1.5	67.12^{i}	65.15^{i}
	2.0	75.58^{j}	73.18^{jk}
	2.5	100.00^{p}	93.45^{o}
	3.0	90.54^{n}	85.96^{m}
	3.5	65.14^{h}	64.68^{hi}
	4.0	40.51^{c}	38.96^{cd}
2,4-D+100 mg L^{-1}CH	1.0+CH	75.14^{j}	70.85^{j}
	2.0+CH	90.18^{m}	85.21^{m}
	2.5+CH	65.21^{h}	63.51^{i}
	3.0+CH	60.18^{h}	59.10^{h}
	3.5+CH	54.20^{f}	52.25^{h}
	4.0+CH	45.10^{d}	44.18^{e}
2,4-D+15% CW	1.0+CW	75.68^{j}	74.51^{k}
	2.0+CW	90.35^{m}	87.18^{n}
	2.5+CW	79.65^{k}	76.24^{l}
	3.0+CW	65.25^{h}	62.01^{i}
	3.5+CW	52.21^{f}	50.00^{f}
	4.0+CW	44.25^{d}	43.24^{e}
2,4-D+KIN	2.5+0.2	50.54^{e}	48.21^{f}
	2.5+0.5	60.54^{g}	57.45^{gh}
	2.5+1.0	45.21^{d}	45.01^{de}
	3.0+0.2	40.54^{c}	39.24^{cd}
	3.0+0.5	50.12^{e}	49.51^{f}
	3.0+1.0	35.94^{b}	32.15^{c}
2,4-D+BAP	2.5+0.2	70.35^{i}	70.69^{j}
	2.5+0.5	85.64^{l}	84.64^{l}
	2.5+1.0	60.28^{g}	57.69^{h}
	3.0+0.2	55.58^{f}	53.96^{g}
	3.0+0.5	75.64^{j}	75.69^{k}
	3.0+1.0	50.49^{e}	48.87^{f}
NAA	0.1	35.61^{b}	32.61^{c}
	0.2	40.75^{c}	40.10^{d}
	0.5	49.79^{e}	45.15^{e}
	1.0	41.00^{d}	38.10^{d}
	1.5	32.25^{b}	30.10^{b}
	2.0	28.15^{a}	25.00^{a}
BAP+NAA	1.0+0.2	52.08^{f}	49.15^{f}
	1.0+0.5	65.65^{h}	54.65^{g}
	2.0+0.2	72.58^{j}	62.08^{i}
	2.0+0.5	82.40^{o}	78.91^{l}
	3.0+0.2	73.65^{j}	67.54^{ij}
	3.0+0.5	61.54^{h}	55.61^{g}
BAP+NAA+15% CW	0.05+0.1+CW	75.51^{j}	67.54^{i}
	0.1+0.1+CW	89.65^{m}	85.21^{m}
	0.2+0.1+CW	92.45^{n}	90.54^{n}
	0.5+0.1+CW	75.89^{j}	75.28^{k}
	1.0+0.1+CW	70.25^{i}	68.25^{j}
	1.5+0.1+CW	61.54^{h}	55.24^{g}

for proliferation and development of multiple shoots (Figure 2b-d). The highest number of shoots was in the same medium after 42 days of culture. Nodal explants exhibited a better response compared to shoot tip explants. For MS_0 percent shoot formation and numbers of shoot per explants was low. Increased BAP concentration reduced shoot length and promoted massive base callus. The cv. Bulum was more effective for multiplication of

Figure2. Direct multiple shoot regeneration through node culture of *Cucurbitapepo*. (a) Inoculation of isolated nodes on MS semi solid medium, (b) Development of shoot (after 7 days of incubation), (c) Multiple shoot initiation, (d) Development of multiple shoot, (e) Development of roots, and (f) Acclimatization of plantlet

plantlets than 'Rumbo' (Table 5). Percent of shoots developing roots, and length of roots per shoot, were influenced by concentration and type of auxin, 1.0 mg L^{-1} IBA was best for root induction (Figure2e). With increased/decreased concentration of IBA root formation, mean root length and number of roots per shoot decreases for both cultivars. The next suitable auxin was NAA in squash with root induction in medium having 1.5 mg L^{-1} NAA occurring within 35 days. The highest number and length of the longest roots was with 1 mg L^{-1} IBA. The cv. Bulum was more responsive for root induction than 'Rumbo'(Table 6).

Regeneration of multiple shoots in *Cucumis sativus*

Establishment of isolated apical meristems of *C. sativus* was influenced by concentration and combination of KIN, KIN+NAA, KIN+GA_3 and BAP, the 1.5 mg L^{-1} produced better performance (Figure 3b). Most growth response occurred within 8-21 days of incubation for slicer and for the pickler within 10-21 days of incubation (Table 7). The maximum number of shoots was with 1.0 mg L^{-1} BAP medium for both cultivars. The slicer had more shoots, and longer roots and shoots than the pickler (Figure 3c,d). For the slicer the highest number of roots per shoot was with 1.0 mg L^{-1} BAP and 1.0 mg L^{-1}

Table 3: Effect of concentrations and combinations of BAP, BAP+NAA, BAP+IBA, KIN+NAA, and KIN+IBA in MS medium on shoot regeneration from cotyledon and leaf derived callus of 2 pumpkin cultivars. Data recorded 8 weeks after inoculation for regeneration. Different letter followed by mean in the same column are statistically significant $p \leq 0.05$

Growth regulator (mg L^{-1}) concentrations		Cultivar	Shoot regeneration (%)		No. of shoots per callus (±SE)	
			Cotyledon	Leaf	Cotyledon	Leaf
MS_0		Bikrompuri	2.5^{a}	2.6^{a}	0.12^{a}	0.13^{a}
		Baromasi	2.1^{a}	2.3^{a}	0.10^{a}	0.11^{a}
BAP	1.0	Bikrompuri	35.9^{b}	38.1^{bc}	2.9 ± 1.2^{c}	3.0 ± 1.2^{c}
	1.5		42.1^{d}	43.2^{de}	3.0 ± 1.2^{c}	3.1 ± 1.3^{d}
	2.0		52.5^{f}	56.1^{h}	3.2 ± 1.1^{d}	3.4 ± 1.1^{d}
	2.5		63.4^{h}	66.50^{hi}	3.3 ± 1.1^{d}	3.7 ± 1.1^{e}
	3.0		45.9^{d}	48.2^{f}	2.8 ± 1.2^{c}	3.0 ± 1.3^{c}
	3.5		42.6^{d}	45.7^{e}	2.5 ± 1.2^{b}	2.9 ± 1.4^{c}
	1.0	Baromasi	32.1^{b}	32.2^{b}	2.8 ± 0.9^{c}	2.5 ± 0.68^{b}
	1.5		40.9^{c}	43.2^{e}	2.8 ± 1.1^{c}	2.9 ± 1.2^{c}
	2.0		50.8^{e}	52.6^{g}	3.0 ± 1.2^{c}	3.1 ± 1.5^{d}
	2.5		60.2^{g}	$62\ 0.40^{i}$	3.5 ± 1.1^{d}	3.7 ± 0.95^{e}
	3.0		45.9^{d}	47.1^{f}	2.6 ± 0.75^{c}	2.7 ± 0.67^{c}
	3.5		43.9^{d}	46.8^{f}	2.3 ± 0.64^{b}	2.6 ± 0.69^{c}
BAP+NAA	0.5+0.1	Bikrompuri	54.12^{f}	55.12^{fg}	3.9 ± 0.11^{e}	4.05 ± 0.24^{f}
	0.5+0.2		58.22^{g}	60.14^{h}	3.8 ± 0.12^{e}	4.20 ± 0.16^{f}
	0.5+0.5		50.0^{e}	50.14^{ef}	4.1 ± 0.21^{f}	4.10 ± 0.12^{f}
	1.0+0.1		64.47^{i}	65.31^{i}	4.1 ± 0.33^{f}	4.55 ± 0.32^{g}
	1.0+0.2		68.14^{j}	70.54^{j}	5.7 ± 0.14^{i}	5.85 ± 0.18^{i}
	1.0+0.5		48.51^{e}	50.24^{f}	4.1 ± 0.19^{f}	4.05 ± 0.18^{f}
	0.5+0.1	Baromasi	50.36^{e}	54.31^{g}	3.8 ± 0.11^{e}	3.9 ± 0.12^{e}
	0.5+0.2		56.45^{f}	60.10^{h}	3.5 ± 0.35^{d}	4.2 ± 0.31^{f}
	0.5+0.5		48.98^{e}	50.22^{f}	4.0 ± 0.24^{e}	3.8 ± 0.31^{e}
	1.0+0.1		60.24^{g}	63.89^{i}	3.8 ± 0.31^{e}	4.3 ± 0.24^{f}
	1.0+0.2		65.85^{g}	69.11^{j}	4.9 ± 0.33^{g}	5.80 ± 0.33^{i}
	1.0+0.5		$45\ 0.39^{d}$	50.10^{f}	3.5 ± 0.22^{d}	3.8 ± 0.25^{e}
BAP+IBA	0.5+0.1	Bikrompuri	40.11^{c}	40.51^{d}	3.80 ± 0.31^{e}	3.90 ± 0.31^{e}
	0.5+0.2		49.22^{e}	50.12^{f}	3.8 ± 0.22^{e}	4.10 ± 0.25^{f}
	0.5+0.5		32.85^{b}	35.12^{c}	3.60 ± 0.32^{e}	3.75 ± 0.10^{e}
	1.0+0.1		50.33^{e}	50.45^{e}	4.0 ± 0.21^{e}	4.0 ± 0.21^{e}
	1.0+0.2		58.45^{f}	60.85^{h}	4.5 ± 0.21^{f}	4.68 ± 0.23^{g}
	1.0+0.5		42.69^{d}	45.31^{e}	3.20 ± 0.24^{d}	3.28 ± 0.24^{d}
	0.5+0.1	Baromasi	35.75^{b}	38.88^{b}	3.5 ± 0.30^{d}	3.7 ± 0.31^{e}
	0.5+0.2		45.65^{d}	48.55^{ef}	3.7 ± 0.22^{e}	3.9 ± 0.25^{e}
	0.5+0.5		30.95^{b}	32.85^{c}	3.5 ± 0.12^{d}	3.6 ± 0.32^{e}
	1.0+0.1		44.95^{d}	48.85^{f}	3.4 ± 0.23^{d}	3.5 ± 0.20^{d}
	1.0+0.2		55.65^{f}	58.11^{h}	4.2 ± 0.22^{f}	4.5 ± 0.14^{f}
	1.0+0.5		40.25^{c}	40.95^{d}	2.8 ± 0.22^{c}	2.8 ± 0.22^{c}
KIN+NAA	0.5+0.1	Bikrompuri	39.18^{c}	40.14^{d}	3.2 ± 0.54	3.45 ± 0.21^{d}
	0.5+0.2		53.54^{f}	55.45^{g}	3.5 ± 0.21^{d}	3.68 ± 0.22^{e}
	0.5+0.5		40.12^{c}	40.33^{d}	3.1 ± 0.12^{d}	3.30 ± 0.10^{d}
	1.0+0.1		48.31^{e}	50.12^{f}	3.8 ± 0.12^{e}	4.15 ± 0.24^{f}
	1.0+0.2		55.98^{f}	60.45^{h}	4.3 ± 0.51^{f}	4.52 ± 0.25^{g}
	1.0+0.5		42.54^{d}	45.54^{e}	3.80 ± 0.22^{e}	3.85 ± 0.24^{e}
	0.5+0.1	Baromasi	38.11^{c}	38.45^{d}	3.1 ± 0.45^{d}	3.3 ± 0.24^{d}
	0.5+0.2		52.14^{f}	55.11^{f}	3.2 ± 0.22^{d}	3.4 ± 0.23^{d}
	0.5+0.5		35.85^{b}	36.88^{d}	3.0 ± 0.31^{c}	3.2 ± 0.24^{d}
	1.0+0.1		48.33^{e}	50.11^{f}	3.5 ± 0.24^{d}	3.9 ± 0.34^{e}
	1.0+0.2		52.66^{f}	58.66^{h}	4.3 ± 0.33^{f}	4.2 ± 0.44^{f}
	1.0+0.5		41.85^{d}	45.12^{e}	3.45 ± 0.20^{d}	3.6 ± 0.13^{e}

NAA (Figure 3e). The longest shoot was with 1.0 mg L^{-1} BAP and 0.5 mg L^{-1} NAA (Table 8). For the pickler the most roots were with 1.0 mg L^{-1} BAP and 1.0 mg L^{-1} NAA. *In vitro* plants acclimatized in soil pots grew normally (Figure 3f).

DISCUSSION

Members of the Cucurbitaceae are important sources of human food, beverages, medicine, and edible oil. Application of modern biotechnology would be impossible for crop improvement without developing a cell culture system characterized by a high morphogenic potential. Callus-mediated indirect regeneration from explants offers opportunity of genetic transformation as well as somaclonal variation. Mass propagation from shoot tip meristems are of great interest due to being free from disease and clonal fidelity. Regeneration techniques for cucurbits vary with respect to type of explants used and type and concentration of growth

Table 4. Effect of auxin concentration on ½MS medium on adventitious root formation from *in vitro* grown micro-cutting cultured of 2 pumpkin cultivars. Data recorded 4-6 weeks after culture. Different letter followed by mean in the same column are statistically significant $p \leq 0.05$

Growth regulator (mg L^{-1}) concentrations	Cultivar	Root formation (%)		No. of roots (±SE)	
		Cotyledon	Leaf	Cotyledon	Leaf
½ MS_0	Bikrompuri	2.1^{b}	2.5^{b}	0.10^{b}	0.12^{b}
NAA	Baromasi	2.1^{b}	2.3^{b}	0.9^{b}	0.10^{b}
0.05	Bikrompuri	35^{d}	75^{j}	1.50 ± 0.25^{c}	3.45 ± 0.27^{f}
0.10		40^{e}	95^{n}	1.70 ± 0.83^{d}	4.65 ± 0.35^{i}
0.20		45^{f}	80^{k}	1.90 ± 0.87^{d}	2.85 ± 0.25^{e}
0.50		50^{g}	60^{g}	2.46 ± 0.18^{e}	2.40 ± 0.18^{d}
1.00		66^{j}	0^{a}	2.80 ± 0.20^{f}	0^{a}
0.05	Baromasi	32^{d}	72^{j}	1.35 ± 0.22^{c}	3.10 ± 0.21^{f}
0.10		38^{e}	90^{m}	1.60 ± 0.82^{d}	4.00 ± 0.25^{g}
0.20		42^{f}	76^{k}	1.80 ± 0.85^{d}	2.15 ± 0.31^{d}
0.50		47^{g}	57^{g}	1.90 ± 0.65^{d}	2.35 ± 0.13^{d}
1.00		60^{i}	5^{b}	2.50 ± 0.23^{e}	0^{a}
IBA					
0.05	Bikrompuri	45^{g}	60^{g}	2.10 ± 0.14^{e}	3.00 ± 0.27^{e}
0.10		55^{i}	80^{k}	2.55 ± 0.26^{f}	4.10 ± 0.35^{h}
0.20		62^{k}	55^{f}	3.20 ± 0.25^{g}	2.55 ± 0.25^{e}
0.50		75^{k}	45^{d}	3.85 ± 0.35^{h}	0^{a}
1.00		89^{n}	4^{b}	4.2 ± 0.41^{i}	2.5 ± 0.19^{d}
0.05	Baromasi	42^{f}	55^{f}	1.95 ± 0.09^{d}	3.95 ± 0.30^{g}
0.10		53^{h}	75^{j}	2.30 ± 0.23^{e}	2.1 ± 0.23^{d}
0.20		57^{i}	47^{e}	3.00 ± 0.25^{f}	2.15 ± 0.14^{d}
0.50		72^{k}	42^{d}	3.40 ± 0.28^{g}	0^{a}
1.00		81^{m}	0^{a}	3.80 ± 0.35^{h}	0^{a}
IAA					
0.05	Bikrompuri	0^{a}	4^{b}	0^{a}	0^{a}
0.10		29^{c}	45^{d}	2.05 ± 0.35^{e}	2.00 ± 0.24^{c}
0.20		34^{d}	65^{h}	2.20 ± 0.25^{e}	2.20 ± 0.20^{d}
0.50		40^{f}	40^{c}	2.35 ± 0.21^{e}	1.85 ± 0.15^{c}
1.00		52^{h}	5^{b}	2.50 ± 0.20^{e}	0^{a}
0.10	Baromasi	27^{c}	42^{d}	1.90 ± 0.25^{d}	1.95 ± 0.23^{c}
0.20		30^{c}	62^{h}	2.00 ± 0.16^{d}	2.05 ± 0.18^{d}
0.50		34^{d}	38^{c}	2.20 ± 0.31^{e}	1.60 ± 0.14^{c}
1.00		46^{g}	5^{b}	2.45 ± 0.18^{e}	0^{a}

regulators applied even where goals of investigations were similar [27,40,41,42].

Conventionally cucurbits are propagated by seed. Low productivity, disease susceptibility and higher cost of production are constraints faced by growers. Micropropagation techniques can overcome these constraints. It is necessary to develop a suitable protocol for mass clonal propagation of cucurbits. The process of multiplication via direct methods is suitable for low cost micropropagation while maintaining clonal fidelity. Findings from *C. maxima*, *C. pepo* and *C. sativus* demonstrate the possibility of mass propagation of cucurbits through direct organogenesis from meristem; shoot tips, or nodal segment containing pre-existing meristems. Isolation and *in vitro* culture of apical meristems is the method of choice for disease elimination [9].

In the present experiment cucumber meristems were developed into shoots with the highest frequency in 1.5 mg L^{-1} KIN. The slicer had a better response than the pickler. Addition of NAA or GA_3 did not enhance development *in vitro*. In *C. pepo* use of 2.0 mg L^{-1} KIN along with 0.5 mg $L^{-1}GA_3$was most effective for establishment of primary meristems [8] Meristems are difficult to isolate and culture *in vitro*. Considerable numbers of isolated meristems do not survive due to injury during isolation [9]. Culture of shoot tips and nodal segments from healthy plants is an alternative option for high frequency micropropagation. Micropropagation of *C. pepo* from meristem-derived plantlets using shoot tip or nodal segment explants appears to be a useful technique. The highest number of shoots per explants were in 3.0 mg L^{-1} BAP plus 0.5 mg L^{-1} GA_3. The superiority of BAP over other growth regulators has been reported in other cucurbits including an interspecific hybrid [3], and *Cucumis anguria* L. [43]. A combination of KIN, IBA and GA_3 has been reported in *C. pepo* [44]. Thiruvengadam *et al* [45]. demonstrated high frequency of shoot multiplication from shoot tip explants on MS medium supplemented with 2.0 mg L^{-1} BAP+1.0 mg L^{-1} adenine sulphate on *Momordica dioca* L.

For *in vitro* callus induction, 2,4-D is among the most widely used auxin. In this study, successful induction of potentially organogenic calli from cotyledons and leaf explants, and maintenance for further growth, was obtained using this growth regulator. An exogenous supply of growth regulators is recommended to initiate callus from explants [39, 46]. Exogenously supplied auxin, often in combination with cytokinin, are essential for callus induction, but their requirement depends on genotype and endogenous hormone content of explants.

Table 5: Effect of concentration and combinations of growth regulators BAP, NAA, GA_3, 2ip and KIN applied singly, or in combination, on shoot multiplication from shoot tips and nodes excised from meristem derived plantlets of 2 summer squash cultivars. Different letter followed by mean in the same column are statistically significant $p \leq 0.05$

Growth regulator (mg L^{-1})	Multiple shoot induction (%)		No. of shoots per culture (±SE)		Multiple shoot induction (%)		No. of shoots per culture (±SE)	
	Bulum	*Rumbo*	*Bulum*	*Rumbo*	*Bulum*	*Rumbo*	*Bulum*	*Rumbo*
	Shoot tip explants				*Nodal segment*			
MS_0	9.43[a]	8.21[a]	1.94±0.37[a]	1.87±0.49[a]	10.86[a]	9.00[a]	2.11±0.13[a]	2.01±0.57[a]
BAP								
1.0	24.75[b]	14.42[b]	2.70±0.26[b]	2.44±0.63[b]	43.25[f]	38.30[e]	5.71±0.12[d]	3.97±0.43[b]
2.0	59.64[h]	48.77[i]	9.36±0.70[h]	5.83±0.24[e]	69.19[k]	67.70[k]	14.57±0.39[l]	12.20±0.69[k]
3.0	39.27[e]	32.93[f]	6.91±0.43[f]	4.13±0.91[d]	48.41[g]	40.73[e]	8.73±0.17[g]	6.63±0.47[e]
BAP+NAA								
2.0+0.5	45.34[f]	40.45[g]	7.97±0.33[g]	4.25±0.75[d]	60.43[i]	52.68[h]	13.67±0.26[k]	10.30±0.48[i]
3.0+0.5	62.11[i]	56.07[j]	11.63±0.24[j]	8.41±0.19[h]	74.54[l]	63.88[j]	16.72±0.34[m]	12.64±0.37[k]
4.0+0.5	41.97[f]	38.52[g]	7.13±0.56[g]	4.11±0.45[d]	62.13[j]	54.25[h]	14.13±0.55[l]	10.12±0.65[i]
2.0+1.0	42.43[f]	38.59[g]	7.32±0.17[g]	4.12±0.51[d]	58.40[i]	51.19[h]	13.24±0.41[k]	9.80±0.31[h]
3.0+1.0	56.62[h]	42.18[h]	9.42±0.82[h]	5.67±0.47[e]	64.13[j]	57.44[i]	14.33±0.94[l]	11.12±0.43[j]
4.0+1.0	37.39[e]	28.55[e]	6.44±0.21[f]	3.84±0.32[c]	49.55[g]	45.27[f]	9.95±0.50[h]	8.56±0.94[g]
$BAP+GA_3$								
1.0+0.5	33.94[d]	18.78[c]	5.87±0.24[e]	3.01±0.91[c]	58.87[i]	47.26[g]	9.98±0.42[h]	7.87±0.39[f]
2.0+0.5	63.49[i]	57.37[j]	9.85±0.19[h]	8.14±0.83[h]	68.33[k]	53.84[h]	12.72±0.15[j]	9.86±0.55[h]
3.0+0.5	76.35[j]	70.82[k]	13.67±0.43[k]	12.33±0.55[i]	92.51[m]	81.28[l]	20.43±0.29[n]	14.12±0.81[m]
4.0+0.5	59.67[h]	45.26[h]	10.12±0.82[i]	7.12±0.23[g]	74.35[l]	66.94[k]	13.83±0.63[k]	12.76±0.37[k]
5.0+0.5	45.67[f]	30.45[e]	7.17±0.70[g]	4.20±0.45[d]	67.45[k]	54.63[h]	12.87±0.12[j]	8.67±0.48[g]
2ip								
0.5	28.44[c]	21.19[d]	2.75±0.62[b]	2.53±0.50[b]	39.37[e]	34.74[d]	5.76±0.42[d]	5.27±0.43[d]
1.0	45.63[f]	32.54[f]	5.39±0.47[e]	4.18±0.26[d]	55.89[h]	50.63[g]	10.91±0.26[i]	8.63±0.38[g]
2.0	27.59[c]	18.47[c]	3.04±0.91[c]	2.94±0.72[b]	47.62[g]	42.45[f]	6.87±0.52[e]	5.19±0.67[d]
2ip+KIN								
2.0+0.1	44.13[f]	37.29[g]	5.96±0.53[e]	4.08±0.25[d]	41.63[f]	37.72[e]	6.13±0.88[e]	5.96±0.72[d]
1.0+0.5	31.75[d]	25.51[d]	3.44±0.21[c]	3.17±0.94[c]	38.44[e]	36.91[e]	6.11±0.57[e]	5.21±0.62[d]
2.0+0.5	53.48[g]	45.52[h]	7.89±0.31[g]	6.41±0.53[f]	67.74[k]	55.33[h]	13.81±0.18[k]	9.63±0.12[h]
3.0+0.5	38.64[e]	28.35[e]	4.83±0.37[d]	3.80±0.46[c]	45.39[f]	36.79[e]	7.34±0.73[g]	5.52±0.16[d]
2ip+NAA								
1.0+0.5	37.45[e]	27.53[e]	4.33±0.95[d]	4.08±0.27[d]	49.44[g]	41.73[f]	7.24±0.67[f]	6.10±0.41[e]
2.0+0.5	51.27[g]	43.58[h]	6.28±0.35[f]	5.47±0.42[e]	58.12[i]	45.27[f]	9.71±0.49[h]	7.13±0.74[f]
3.0+0.5	39.56[e]	28.31[e]	4.64±0.34[d]	4.23±0.63[d]	40.77[f]	39.89[e]	5.91±0.72[d]	5.87±0.32[d]
KIN								
2.0	32.64[d]	20.39[c]	3.62±0.30[c]	3.41±0.25[c]	37.78[e]	32.17[d]	4.33±0.49[c]	4.17±0.99[c]
3.0	43.57[f]	35.70[f]	4.67±0.17[d]	4.12±0.57[d]	45.43[f]	41.49[f]	5.53±0.97[d]	5.22±0.12[d]
4.0	30.81[c]	18.28[c]	3.37±0.43[c]	3.08±0.64[c]	29.69[c]	23.35[c]	3.94±0.56[b]	3.26±0.75[b]
5.0	21.34[b]	13.57[b]	2.51±0.65[b]	2.42±0.39[b]	20.41[b]	17.11[b]	2.38±0.11[a]	2.19±0.57[a]
KIN+NAA								
1.0+0.5	35.47[d]	26.20[e]	4.33±0.49[d]	4.12±0.54[d]	24.11[b]	19.32[b]	3.62±0.61[b]	2.80±0.41[a]
2.0+0.5	45.69[f]	37.48[g]	5.17±0.23[e]	5.02±0.66[e]	58.17[i]	51.73[h]	6.48±0.67[e]	5.36±0.90[d]
2.5+0.5	40.57[e]	32.74[f]	4.67±0.80[d]	4.33±0.47[d]	42.63[f]	38.61[e]	4.36±0.17[c]	4.06±0.83[c]
3.0+0.5	33.21[c]	25.68[d]	3.83±0.43[c]	3.27±0.30[c]	37.27[e]	32.00[d]	4.33±0.22[c]	3.64±0.65[b]
GA_3								
1.0	28.45[c]	21.51[d]	3.21±0.43[c]	3.10±0.35[c]	32.93[d]	28.45	4.94±0.10[c]	4.73±0.39[c]
2.0	33.26[d]	26.39[e]	4.63±0.17[d]	4.29±0.94[d]	41.27[f]	35.77[d]	5.19±0.23[d]	5.10±0.88[d]
3.0	41.57[e]	38.40[g]	5.54±0.55[e]	5.13±0.27[e]	48.51[g]	42.63[f]	5.71±0.55[d]	5.23±0.47[d]
4.0	30.84[d]	25.64[d]	4.12±0.39[d]	4.02±0.53[d]	39.56[e]	31.12[d]	4.62±0.75[c]	4.19±0.96[c]
5.0	23.41[b]	19.27[c]	2.87±0.62[b]	2.59±0.51[b]	28.79[c]	22.66[c]	4.40±0.14[c]	4.10±0.72[c]

Rao *et al* [47] reported that intermediate levels of auxin and cytokinin in the medium usually promote callus formation. However, 2,4-D seems to be the best auxin for callus induction in plants [48, 49]. The 2,4-D alone was effective for callus induction in pumpkin. Our results are similar to those obtained in *C. pepo* [26]. By contrast, 1.0 mg L^{-1} BAP produced the highest frequency of callus in *C. maxima* from meristem explants [33].

Complex natural additives such as the coconut water, casein hydrolysate are often useful in enhancing callus induction. Coconut contains plant growth hormones normally used in tissue culture [50]. Supplementation with coconut water in tissue culture media enhances callus initiation [51], shootdevelopment [52] and multiplication in combination with synthetic auxins [53]. Total callus induction was obtained with addition of 15% coconut water with 2,4-D from cotyledon explants. Beneficial effects of coconut water have been reported in other crops [54, 55]. In some cases casein hydrolysate caused stimulatory effects on callus induction. Casein hydrolysate, along with2, 4-D, further enhanced callus induction. Our results were similar to those in other crops indicating the response may be universal.

Table 6: Effect of concentration and combinations of auxin hormones on root induction from shoot tips and nodes from excised meristem derived plantlets of 2 summer squash cultivars. Different letter followed by mean in the same column are statistically significant $p \leq 0.05$

Growth regulator (mg L^{-1})	Root formation (%)		Mean length of root (cm)		Roots per shoot	
	Bulum	*Rumbo*	*Bulum*	*Rumbo*	*Bulum*	*Rumbo*
MS_0	5.47[a]	4.22[a]	1.47±0.65[a]	1.10±0.50[a]	2.67±0.43[a]	2.21±0.14[a]
IBA						
0.2	40.25[c]	35.67[c]	8.71±0.29[e]	6.45±0.81[e]	5.17±0.73[c]	4.84±0.34[c]
0.5	68.19[e]	59.41[g]	9.75±0.34[f]	8.46±0.17[f]	9.82±0.17[f]	6.27±0.55[d]
1.0	83.72[f]	76.23[h]	12.44±0.55[h]	10.21±0.32[h]	17.41±0.32[g]	10.53±0.41[g]
2.0	61.55[d]	52.84[f]	10.68±0.26[g]	8.03±0.66[f]	9.22±0.46[f]	6.02±0.23[d]
3.0	39.47[c]	33.63[c]	7.27±0.42[d]	5.54±0.57[d]	4.98±0.28[b]	4.25±0.62[c]
NAA						
0.5	42.63[c]	30.72[c]	5.78±0.63[c]	4.44±0.26[c]	5.16±0.54[c]	4.25±0.25[c]
1.0	61.89[d]	51.27[f]	8.20±0.48[e]	7.30±0.29[e]	7.25±0.60[e]	6.34±0.43[d]
1.5	70.64[e]	62.45[g]	10.64±0.37[g]	9.34±0.65[g]	10.44±0.51[f]	8.43±0.28[f]
2.0	58.47[d]	47.58[e]	8.90±0.72[e]	7.12±0.82[e]	6.57±0.14[d]	6.10±0.29[d]
3.0	46.28[c]	35.88[c]	6.21 ± 0.43[c]	4.48±0.90[c]	5.41±0.34[c]	4.74±0.63[c]
IAA						
0.5	29.74[b]	21.43[b]	4.24 ± 0.60[b]	3.87±0.80[b]	3.49±0.43[a]	3.31±0.56[b]
1.0	45.17[c]	37.12[d]	5.81 ± 0.14[c]	5.54±0.32[d]	5.51±0.82[c]	5.02±0.39[c]
2.0	58.40[d]	51.66[f]	8.47 ± 0.34[e]	7.38±0.66[e]	7.39±0.54[e]	7.14±0.34[e]
3.0	43.62[c]	34.29[c]	6.12 ± 0.43[c]	5.25±0.52[d]	5.26±0.68[c]	4.97±0.76[c]
4.0	25.45[b]	18.88[b]	3.94 ± 0.55[b]	3.27±0.78[b]	3.30±0.95[a]	3.24±0.27[b]

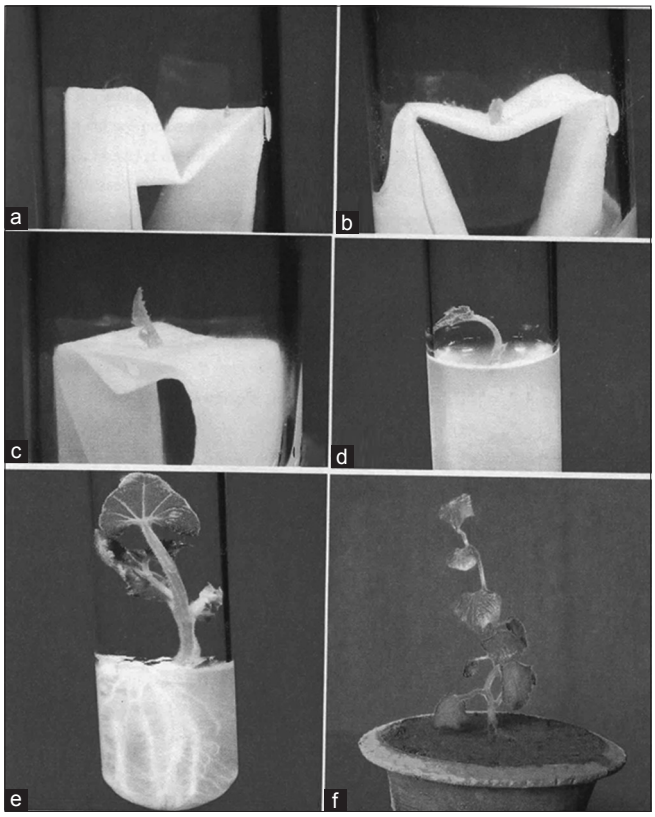

Figure3. Direct shoot regeneration through meristem culture of *Cucumis sativus*. (a) Isolated apical meristem inoculated on paper bridge in liquid medium, (b) Primary shoot initiation on liquid medium, (c) Development of shoot on liquid medium, (d) Development of shoot on semi-solid medium, (e) Root development from meristem derived shoot, and (f) acclimatization of plantlets

Plant regeneration was achieved using cytokinin in combination with IBA or NAA. Skoog [56] determined that organogenesis was controlled by a balance between cytokinin and auxin. Hoque *et al.* [57] found that a combination of 1.5 mg L^{-1} BAP and 0.1 mg L^{-1} NAA was suitable for formation of adventitious multiple shoots in another cucurbita, *M. indica*, indicating the response may be universal for cucurbits. The 1.0 mg L^{-1} BAP+0.2 mg L^{-1} NAA was best for production of multiple shoots from callus in pumpkin. The effect of BAP over KIN in organogenesis has been reported by others [58,59].

In *C. maxima*, although regenerated shoots produced roots simultaneously, it was necessary to culture regenerated shoots in rooting medium. Among auxin types, NAA was best for producing roots. Highest root number observed with 0.1 mg L^{-1} NAA. The findings agree with those observed in *C. lanatus* [60] and cucumber [29]. Further increases in NAA will result in decreased root number.

In *C. pepo*, the auxin IBA was best for root induction. Number and length of roots per shoot were highest as was the highest root induction percentage. Except for 1.0 mg L^{-1} IBA other IBA concentrations resulted in lower root induction. This contradicts with reports on *C. melo* [27] where they observed a low number of shoots developed adventitious roots on basal media supplemented with IAA. There was a good deal of root formation, with a 12 cm mean root length, with 1 mg L^{-1} IBA. This agrees with reports on *C. melo* [22] and *M. charantia* [61] where IBA was the most suitable for rooting. In *C. sativa the* response was different from *C. maxima* and *C. pepo*. Plants obtained *in vitro* were morphologically normal when transferred in soil pots. The protocols described various path of plant regeneration *in vitro* from various explants. These would be useful in applying biotechnological tools for improvement of crops of this family.

Table 7: Effect of concentration and combinations of growth regulators KIN, KIN+NAA, KIN+GA_3 and BAP in MS medium for primary establishment of apical meristems isolated from 30-45 days old-field grown cucumber plants. Different letter followed by mean in the same column are statistically significant $p \leq 0.05$

Growth regulator (mgL^{-1}) concentrations	Slicer		Pickler	
	Days to response	Growth response (%)	Days to response	Growth response (%)
MS0	9-21	30^{a}	10-21	22^{a}
KIN				
0.5	8-21	45^{d}	9-21	38^{c}
1.0	8-21	65^{f}	9-21	59^{e}
1.5	8-21	85^{i}	10-21	79^{g}
KIN+NAA				
1.0+0.5	9-21	75^{g}	10-21	70^{f}
KIN+GA_3				
1.0+0.5	8-21	40^{c}	10-21	40^{c}
BAP				
0.5	7-21	35^{b}	7-21	30^{b}
1.0	8-21	78^{h}	9-21	70^{f}
2.0	9-21	50^{e}	10-21	48^{d}

Table 8: Effect of concentrations and combinations of cytokinin and auxin in MS medium on shoot and root development from primary establishment meristems of 2 cucumber cultivars. Data recorded 21 days after inoculation. Different letter followed by mean in the same column are statistically significant $p \leq 0.05$

Growth regulator (mg L^{-1})	Slicer				Pickler			
	No.of shoots	No.ofroots	Shoot length	Rooting (%)	No.of shoots	No.of roots	Shoot length	Rooting (%)
MS_0								
0	1.12 ± 0.10^{a}	1.92 ± 0.39^{c}	2.35 ± 0.32^{a}	40	1.12 ± 0.10^{a}	1.80 ± 0.27^{b}	2.48 ± 0.14^{a}	50
BAP								
1.0	2.73 ± 0.34^{e}	0.75 ± 0.22^{b}	3.86 ± 0.13^{b}	20	2.57 ± 0.08^{c}	1.12 ± 0.14^{b}	3.75 ± 0.21^{b}	20
1.5	1.92 ± 0.46^{c}	0^{a}	3.69 ± 0.24^{b}	0	2.10 ± 0.19^{b}	0^{a}	3.86 ± 0.22^{b}	0
KIN								
0.5	2.30 ± 0.32^{d}	0.70 ± 0.12^{b}	4.20 ± 0.71^{b}	22	2.10 ± 0.60^{b}	1.00 ± 0.29^{b}	4.90 ± 0.53^{c}	25
1.0	1.21 ± 0.35^{a}	0^{a}	4.5 ± 0.13^{b}	0	1.47 ± 0.16^{a}	0^{a}	5.91 ± 0.42^{d}	0
BAP+NAA								
1.0+0.5	2.10 ± 0.10^{c}	9.13 ± 0.44^{d}	6.50 ± 0.13^{d}	70	2.09 ± 0.12^{b}	7.76 ± 0.15^{c}	5.80 ± 0.70^{d}	80
1.0+1.0	2.30 ± 0.17^{d}	11.80 ± 0.54^{e}	5.80 ± 0.57^{d}	80	2.02 ± 0.41^{b}	10.10 ± 0.29^{d}	5.60 ± 0.35^{d}	76
BAP+IBA								
0.5+0.5	1.19 ± 0.15^{a}	10.72 ± 0.38^{d}	3.22 ± 0.59^{b}	65	1.25 ± 0.15^{a}	9.13 ± 0.63^{c}	2.96 ± 0.19^{a}	59
1.0+1.0	1.50 ± 0.37^{b}	10.90 ± 0.25^{d}	4.47 ± 0.32^{c}	70	1.70 ± 0.43^{a}	9.45 ± 0.45^{c}	4.31 ± 0.12^{c}	65

CONCLUSION

This paper reports various mode of in vitro plant regeneration protocol for three plant species of cucurbita family including *Cucurbita maxima*, *C. pepo* and *Cucumis sativus*. The grow regulator response is variable across species and the explants tested. Such protocol would be useful for various purposes including micropropagation, somaclonal variation and Agrobacterium-mediated genetic transformation.

AUTHOR CONTRIBUTION

MFA, MA and IA designed the experiment. SKR, MSP and FM carried out the experiment. SKR and TT contributed to the analysis and interpretation of the data. MFA and PCM wrote the manuscript. All authors provided critical feedback in preparing the manuscript.

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