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COMPARISON OF THE EFFECTS OF GROWTH REGULATORS ON *IN VITRO* REGENERATION OF RIDGE GOURD AND SPONGE GOURD THROUGH SHOOT

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Abstract

Context: *Luffa cylindrica* and *Luffa acutangula* are highly cross pollinated crops and propagated mainly by seeds. Genetic stability cannot be maintained easily by seed propagation. It can be maintain by developing special vegetative technique through tissue culture.

Objectives: To compare the effects of growth regulators between two species of *Luffa* using shoot tips for develop the rapid, simple and efficient *in vitro* regeneration protocol.

Materials and Methods: Shoot tips used were collected from *in vivo* grown plants. They were excised from plants and surface sterilized by $HgCl_2$ treatment. The isolated tips were cultured on semisolid MS medium supplemented with different concentrations and combinations of different growth regulators.

Results: The highest result of direct shoot multiplication of ridge gourd was observed using 2 mg/l BAP + 0.2 mg/l GA and in case of sponge gourd it was 1.5 mg/l BAP. For callus induction significant result was found using 4 mg/l BAP + 0.2 mg/l NAA in ridge gourd and 3 mg/l BAP + 0.2 mg/l NAA in sponge gourd. Indirect regeneration was performed by subculturing organogenic callus of sponge gourd on MS with 1.5 mg/l BAP + 0.2 mg/l GA_3 and the callus of ridged gourd on MS + 1.5 mg/l BAP + 0.2 mg/l NAA + 0.2 mg/l nicotinic acid. Regenerated shoots of both species were rooted well on MS containing NAA at low concentration.

Conclusion: Hormonal differences and simple rapid *in vitro* regeneration protocol of *L. cylindrica* and *L. acutangula* have been established.

Keywords: Growth regulators, *in vitro* regeneration, ridge gourd, sponge gourd.

Introduction

Luffa cylindrica (L) commonly known as sponge gourd is derived from the cucumber and marrow family raceme and one female flower exists in them. Its fruit, a gourd, is originated from America (Mazali and Alves 2005) and originated in India, where wild types still occur, but has now spread pan tropically to all areas with a high rainfall. Sponge gourd and ridge gourd, *Luffa acutangula,* both are diploid species with 26 chromosomes. *L. cylindrica* is cultivated a cross- pollinated crop (Bal *et al*. 2004). Sponge gourd fibers are composed of 60% cellulose, 30% hemi-cellulose (Mazali and Alves 2005). Edible portion of ridge gourd (fruit) contain 94.2% water, 1.7% fiber and leaves contain different types of vitamins and minerals.

Both plants are propagated mainly by seed, but it cannot easily maintain the genetic stability by seed propagation. On the other hand there are no conventional vegetative techniques. So, to maintain the genetic stability, it needs to develop the plants by special vegetative technique like tissue culture. A good micropropagation protocol can reduce the cost of hybrid seed production which can account for 30% of the total seedling cost. Though sponge gourd and ridge gourd have come from same genus but they show hormonal differences in different cases of tissue culture. Our present investigation describes the hormonal difference between two species using shoot tip explants for development of the rapid, simple and efficient *in vitro* regeneration protocols.

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Materials and Methods

Shoot tips were collected from *in vivo* grown plants. The explants were cut into pieces carefully, kept in a conical flask and thoroughly washed under running tap water for 20 min. to remove loose contaminates. They were then treated with 1% savlon for 5 min. and washed five times with sterile distilled water under aseptic condition. For finally surface sterilization, explants were taken in laminar airflow bench and treated with 0.05% aqueous $HgCl_2$ solution for 2.5 min. The explants were further trimmed to remove excess tissues. Then they were cultured on MS fortified with different concentrations and combinations of BAP (0.5 - 4 mg/l), KIN (0.1 - 0.3 mg/l), NAA (0.1-1.5 mg/l), IBA (0.1- 0.8 mg/l), 2,4-D (0.1-1.5 mg/l), GA_3 (0.1-0.3 mg/l), nicotinic acid (0.2 mg/l) along with 3% sucrose and 0.7% agar. The pH of the medium was adjusted to 5.7 using 0.1 N NaOH or 0.1 N HCl before autoclaving and adding agar. About 10 ml of the medium were taken in each test tube and covered with non-absorbent cotton plug prior to autoclaving at 121°C for 21 min. at 1.5 kg/cm^2 pressure. Cultures were incubated at 25 ± 2°C under the warm fluorescent light intensity varied from 2000-3000 lux.

Results

Among different combinations of growth regulators it was observed that shoot tip explants of sponge gourd gave significant result when MS fortified with 1.5 mg/l BAP and 90% explants produced shoots and the mean number of shoot was 4.8 ± 0.28 (Fig. A). But in case of ridge gourd, MS fortified with 2.0 mg/l BAP + 0.2mg/l GA_3 was the best combination for shoot multiplication, where 90% explants produced shoots (Fig. E). It was observed that MS containing only BAP was better than the combinations of other hormones in sponge gourd but in ridge gourd BAP singly did not show any significant result (Table 1).

For callus induction different concentrations and combinations of different growth regulators were used. In sponge gourd when 3 mg/l BAP + 0.2 mg/l NAA was used, high degree of callus was formed (Fig. B) but in ridge gourd the best combination was 4.0 mg/l BAP + 0.2 mg/l NAA (Fig. F) (Table 2). These two combinations gave light green organogenic calli which were very important for indirect regeneration, where the concentration of cytokinin was higher than auxin.

Indirect shoot regeneration from organogenic callus was observed when different concentrations and combinations of different growth regulators were used (Table 3). Sponge gourd callus produced 100% shoots when 1.5 mg/l BAP + 0.2 mg/l GA_3 was used and mean number of shoots per culture was 3.8 ± 0.28 (Fig C). In ridge gourd 100% callus produced shoots when nicotinic acid (0.2 mg/l) additionally used with BAP (1.5 mg/l) + NAA (0.2 mg/l) (Fig. G). Efficient rooting was achieved in sponge gourd on MS medium supplemented with 0.6 mg/l NAA and 100% microshoots produced root (Fig. D) but in ridge gourd the highest result was 90% when 0.7 mg/l NAA was used (Fig. H) (Table 4). In both cases, IBA did not show any significant result. After 21 days, well developed roots were observed and placed in pots containing sterilized humus soil 80% of which were survived.

Discussion

MS containing only BAP was better than the combinations of other hormones in sponge gourd but in ridge gourd BAP singly did not show any significant result. Haque *et al*. (2008) also reported similar results regarding pumpkin and ash gourd. The fortification of cytokinin for multiple shoot induction at lower concentration has been reported by Kathal *et al*. (1988) and Singh *et al*. (1996). Other species like *Cucumis sativus*, shoot proliferation medium was 2 mg/l BAP + 1.0 mg/l $AgNO_3$ (Li *et al*. 2008). In pointed gourd the highest percentage of shoot regeneration was 93.86 when nodal explants were cultured on MS + 2.0 mg/l BAP + 0.3 mg/l NAA and female genotype is better than the male genotype (Malek *et al*. 2007). Huda and Sikdar (2006) reported that MS fortified with combination of BA, IBA and GA_3 was very good for shoot initiation and elongation from apical meristem of Bitter gourd (*Mormodica charantia* L.). In *Zehneria scabra* (L.f.) for shoot multiplication, high concentration of BAP and IAA was used by Anand and Jeyachandran (2004).

Table 1. Effects of different concentrations and combinations of growth regulators on multiple shoot formation from shoot tips of *Luffa cylindrica* and *Luffa acutangula*

Growth regulators (mg/l)	*L. cylindrica*		*L.acutangula*	
	No of explants producing shoots (out of 10)	Mean no of shoots /culture	No of explants producing shoots (out of 10)	Mena no of shoots /culture
BAP				
0.5	-	-	-	-
1.0	3	2.3 ± 0.07	-	-
1.5	9	4.8 ± 0.282	3	2.6 ± 0.141
2.0	5	2.4 ± 0.01	5	2.4 ± 0.02
BAP+NAA				
1.5+0.1	4	4.25 ± 0.12	5	1.4 ± 0.0
2.0+0.2	2	3.0 ± 0.02	8	2.1 ± 0.08
3.0+0.2	-	-	2	3.0 ± 0.07
4.0+0.1	-	-	-	-
BAP+KIN+GA_3				
1.5+0.3+0.1	4	4.5 ± 0.0	4	2.75 ± 0.05
2.0+0.3+0.3	2	2.3 ± 0.2	1	2.0 ± 0.07
2.0+0.1+0.2	2	2.4 ± 0.17	-	-
BAP+GA_3				
1.5+0.2	2	2.4 ± 0.01	4	2.3 ± 0.08
2.0+0.2	2	1.4 ± 0.06	9	4.6 ± 0.22
2.0+0.3	-	-	7	4.1 ± 0.01
3.0+0.3	-	-	2	1.5 ± 0.01

Table 2. Effects of basal media and phytohormones on induction of callus and characteristics of callus derived from shoot tips of *Luffa cylindrica* and *Luffa acutangula* after 3-4 weeks of culture

Growth regulators (mg/l)	*L. cylindrica*			*L. acutangula*		
	Callus induction	Callus color	Callus texture	Callus induction	Callus color	Callus texture
BAP						
1.0	-	-	-	-	-	-
1.5	+	G	C	-	-	-
BAP + NAA-						
1.5 + 0.1	+	LG	C	+	G	C
2.0 + 0.2	++	LG	C	+	G	C
3.0 + 0.2	+++	LG	CI	++	LG	C
4 + 0.1	++	LG	CI	++	LG	CI
4 + 0.2	++	LG	C	+++	LG	CI
BAP + 2,4-D						
1.5 + 0.1	+	LG	CI	+	B	CI
2.0 + 0.5	++	B	CI	++	B	CI
2.0 + 1.0	++	B	CI	++	B	CI
3.0 + 1.5	++	B	F	+	B	CI
NAA						
0.5	++	LG	CI	++	LG	CI
1.0	++	B	CI	+	B	CI
1.5	+	B	CI	+	LB	CI

G- green, LG- Light green, B- Blue

Table 3. Effects of different concentrations and combinations of growth regulators on multiple shoots regeneration via callus of *Luffa cylindrical and Luffa acutangula*

Growth regulators	*L. cylindrica*			*L. acutangula*		
	No of callus culture	No of callus response	No of shoots/ culture	No of callus culture	No of callus response	No of shoots/ culture
BAP						
1.0	10	-	-	-	-	-
1.5	10	-	-	10	-	-
2.0	10	2	1 ± 0.0	10	-	-
BAP + NAA						
1.0 + 0.1	10	1	2 ± 0.0	10	2	2.5 ± 0.0
1.5 + 2.0	10	2	2 ± 0.0	10	2	2.3 ± 0.01
2.0 + 0.3	10	4	3.5 ± 0.20	10	3	3.0 ± 0.2
BAP +GA_3						
1.5 + 0.2	10	10	3.8 ± 0.28	10	9	4.4 ± 0.02
2 + 0.3	10	4	3.7 ± 0.2	10	7	3.2 ± 0.06
BAP + NAA + Nicotinic acid						
1.5 + 0.2 + 0.2	10	2	2.1 ± 0.23	10	10	4.9 ± 0.02
2.0 + 0.2 + 0.1	10	1	2.3 ± 0.21	10	6	3.4 ± 0.03
2.0 + 0.1 + .01	10	2	2.0 ± 0.20	10	4	2.5 ± 0.2

Table 4. Effects of auxin in semisolid MS media on root formation in *in vitro* regenerated shoots of *L. cylindrica* and *L. acutangula*

Growth regulators		*L. cylindrical*		*L. acutangula*	
Auxin	Amount (mg/l)	% of response	Root formation	% of response	Root formation
IBA	0.1	-	-	-	-
	0.2	-	-	-	-
	0.4	30	+	20	+
	0.5	30	+	30	+
	0.7	50	++	60	++
	0.8	60	+	50	+
NAA	0.1	-	-	-	-
	0.2	20	+	10	+
	0.4	20	+	20	+
	0.5	60	++	50	+
	0.6	100	+++	70	++
	0.7	70	++	90	+++
	0.8	30	+	60	++

Auxins have reported to induce callus formation in tissue culture of plant (Tissert 1995). NAA and IAA promoted excessive callus formation in water melon (Compton and Gray 1993). Halder and Gadgil (1982) was able to produce good callus from cotyledon and embryo axis in squash (*Cucumiss melo*) using only NAA with MS. The best development of callus was observed on medium containing 1 mg/l NAA for *Cucumis metuliferus* (Beharav and Cohen 1994). In summer squash the highest frequency of callus induction was observed using only 2, 4-D (2.5 mg/l) with MS and hypocotyls were more responsible than epicotyls (Pal *et al*. 2007). Different species of cucurbitaceous have differences in callus induction. The differences occur due to many other factors like genotypes, medium composition, physical growth factors like-light, temperature, moisture which are important for callus induction (Pierik 1975).

Lou and Kako (1994) were able to produce somatic embryo and plant regeneration of cucumber. They suggested that cotyledon and first leaf derived calluses produced more embryo than callus from internodes.

Somaclonal variation was often found when plant regenerate from callus. The increase in genetic variation that lead to the improvement was due to the phenomenon of somaclonal variation that is frequently associated with tissue culture (Larkin and Scowcroft 1981). Kathal *et al.* (1994) achieved good result for root induction using only IBA in *C. melo.* Sultana and Bari (2003) reported efficient rooting medium using low concentration (0.1 mg/l) of NAA with ½ MS in watermelon. Anand and Jeyachandran (2004) reported that shoot elongation was simultaneously observed along with root induction using NAA in *Zehneria scabra* (L.f.).

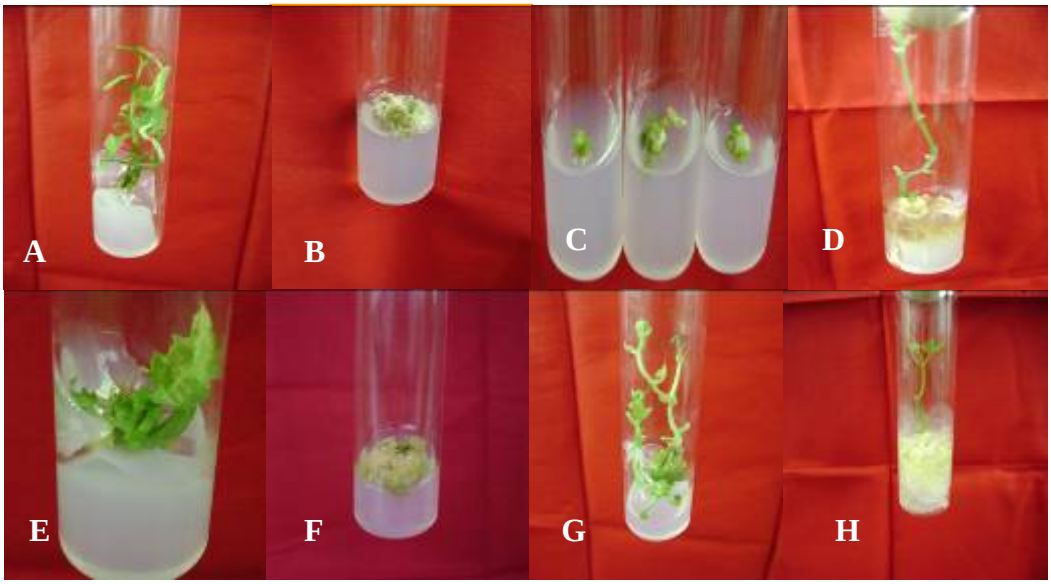

Fig.1. *In vitro* regeneration of *Luffa cylindrica.* A. direct shoots multiplication using 1.5 mg/l BAP. B. light green callus formation using 3.0 mg/l BAP + 0.2 mg/l NAA. C. indirect shoots regeneration using 1.5 mg/l BAP + 0.2 mg/l GA_3. D. root induction using 0.6 mg/l NAA. E. direct shoot multiplication using 2.0 mg/l BAP + 0.2 mg/l GA_3. F. light green callus formation using 4.0 mg/l BAP + 0.2 mg/l NAA. G. indirect shoot regeneration using 1.5 mg/l BAP + 0.2 mg/l NAA + 0.2 mg/l Nicotinic acid. H. root induction using 0.7 mg/l NAA

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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