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In vitro regeneration performance of *Corchorus olitorius*

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Abstract

The experiment was conducted during May to December 2008 in the Biotechnology Laboratory of Bangladesh Agricultural University, Mymensingh to observe the callus induction, regeneration potentiality and to establish a suitable *in vitro* plantlet regeneration protocol of *Corchorus olitorius.* MS medium supplemented with different phytohormone concentrations and combinations were used to observe the callus induction, shoot regeneration and root formation ability of the cotyledon with attached petiole derived explant of three genotypes viz. O-9897, O-72 and OM-1. The highest callus induction (92.85%) was observed in O-9897 followed by O-72 (82.14%) in the MS media supplemented with 2.5 mg/L BAP + 0.5 mg/L IAA. Genotype O-9897 in MS media supplemented with 2.5 mg/L BAP + 0.5 mg/L IAA produced the highest percentage of shoot regenerants (83.33%) followed by O-72 (75.00%) in the media supplemented with 2.5 mg/L BAP + 0.5 mg/L IAA. The root formation from regenerants was the best on half-strength of MS media supplemented with 0.6 mg/L IBA in genotype O-9897 (45.00%). The *in vitro* regenerated plantlets from the genotypes O-9897 could be established in the field. Therefore, the genotypes O-9897 of *C. olitorius* in MS media supplemented with 2.5 mg/L BAP + 0.5 mg/L IAA could be used for callus induction and shoot regeneration.

Keywords: Regeneration, Phytohormone, Corchorus olitorius

Introduction

Jute (*Corchorus* sp., 2n=14) is a tropical fibre crop belonging to the family Tiliaceae has high industrial importance. It has two cultivated species: *Corchorus capsularis* L. commonly known as *deshi* jute and *C. olitorius* L. known as *tossa* jute. It is one of the important cash crops of Bangladesh and occupies 5th position among the field crops in respect of cultivated area (BBS, 2008). Bangladesh is not only the second largest producer of jute but also produces the best quality jute and leads the export market (12-13% of foreign exchange) in the world. In 2004 -2005, Bangladesh exported 17.04 lakh bales of raw jute and jute goods and earned about 18,000 million dollars (FAO, 2005). In addition to this, with the launching of global campaign for environmental awareness international opinion is being created in favour of jute for its expanded production and use, as it is biodegradable and friendly to the environment.

Cultivation of jute in Bangladesh is increasingly shifting to less productive land with marginal care due to creating challenges in dealing with new emerging production constraints like pest attack, poor soil fertility, photo-insensitivity and abiotic stresses like drought, flood, low temperature etc. which are detrimental to this crop. To maintain a sustainable improvement in jute productivity under less favorable environment can only be achieved with a constant flow of new genetic materials for above constraints (Aggarwal, 2000).

Conventional breeding techniques are lengthy processes and take longer time to improve a crop variety. The techniques of plant tissue culture have been developed as a powerful tool for crop improvement and received much attention of modern researchers (Carlson, 1975; Razdan and Cocking, 1981and Larkin and Scoweroft, 1982). Biotechnological techniques are important to develop improved varieties of crops. Although, biotechnological research on jute has been initiated in early sixties but output is still very limited. Therefore, the study was taken to optimize the hormonal concentration for *in vitro* regeneration performance of different genotypes of *C. olitorius*

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Materials and Methods

The experiment was conducted during May to December, 2008 in the Biotechnology Laboratory of Department of Biotechnology, Bangladesh Agricultural University, Mymensingh to observe the regeneration potentiality of three genotypes of *C.olitorius* viz.O-9897, O-72 and OM-1.

Culture Media: Half strength MS (Murashige and Skoog, 1962) medium supplemented with clinical cotton or agar was used for seed germination. For callus induction and shoot regeneration, MS media supplemented with a single concentration of IAA (0.5 mg/L) and four concentrations of BAP (1.5, 2.5, 3.5 and 4.5 mg/L) were used in four combinations. Half strength MS media supplemented with four concentrations of IBA (0.2, 0.4, 0.6 and 0.8 mg/L) were used for root initiation. Soil containing 25% garden soil, 50% sand and 25% cowdung was used for transplanting of plantlets from culture vessel to pot.

Culture Techniques

Twenty five sterilized seeds were placed into sterilized seed germination medium in each vial. The culture was then incubated in dark till germination of the seeds and then transferred to 16 hours light for normal seedling growth. Cotyledons with attached petiole of seven days old seedlings were used as explant. Seven explants from each genotype were incubated in culture vial for callus formation. Six calli of 20-25 mm in diameter from each genotype were again cultured in MS media containing different concentrations and combination, of IAA and BAP for shoot regeneration. After shoot regeneration, five calli of each genotype were cultured in vials with freshly prepared root induction medium to form root. The culture vials were incubated at 22 ± 2°C with 16 hours photoperiod for callus formation, shoot regeneration and root development. When the plantlets become 5-7 cm in length having enough root system, they were taken out from the vials and then transplanted to pots containing of garden soil, sand and cowdung. To resist sudden stress, the pots were kept in a growth room for 10-15 days under controlled environment covered with moist polythene. After two to three days the polythene bags were partially removed and completely removed when the complete plantlets were seem to be self-sustainable.

Recording Data

i) Callus initiation

Data on days required for callusing and per cent callus induction were recorded after five to seven days of incubation of explants. The mean value of the data was considered as the days required for callusing. The percentage of callus induction was calculated by the following formula.

$$\text{Per cent callus induction} = \frac{\text{Number of explants induced callus}}{\text{Number of explants incubated}} \times 100$$

ii) Shoot regeneration

The number of shoot proliferated over a number of days were recorded. The mean value of data provided the days required for shoot initiation. The percentage of shoot regeneration was calculated by the following formula.

$$\text{Per cent shoot regeneration} = \frac{\text{Number of calli with plantlets}}{\text{Number of inoculated calli}} \times 100$$

iii) Root formation

Days required for initiation of root from the day of implantation was recorded. The number of roots proliferated over a number of days were recorded. The mean value of the data provided the days required for root initiation. The percentage of established plants was calculated by on the following following formula.

$$\text{Per cent plant establishment} = \frac{\text{Number of established plantlets}}{\text{Total number of plantlets}} \times 100$$

Results and Discussion

Callus induction

Plantlet regeneration from the cotyledons with attached petiole via unorganized calli was the ultimate goal of this study. To achieve this goal, seven explants from the each genotype were cultured on MS media supplemented with different concentrations and combinations of phytohormones. The combined effect of genotype and phytohormone concentration was significant on callus formation of *C. olitorius* (Table 1). The highest percentage of calli (92.85%) was obtained from the genotype O-9897 cultured on MS medium supplemented with 2.5 mg/L BAP+ 0.5 mg/L IAA (Plate-1) followed by O-72 and OM-1 at same concentration of BAP and IAA. Performance of O-9897 was the best at 2.5 mg/L BAP+ 0.5 mg/L IAA in response to days required for callusing. The minimum number of days required for callusing (6.25) was recorded in O-9897 cultured on MS medium supplemented with 2.5 mg/L BAP+ 0.5 mg/L IAA and genotype OM-1 required maximum days (8.50) for callus induction on MS medium supplemented with 4.5 mg/L BAP+ 0.5 mg/L IAA. Khatun (2001) and Paul (2003) obtained similar results in *C. olitorius*.

Table 1. Effect of genotypes and phytohormone concentrations on callus induction of different varieties of *C. olitorius*

Genotypes	Conc. of BAP (mg/L) + 0.5 mg/L IAA	No. explants showing callus	Percentage of callus induction	Days to callus induction
O-9897	1.5	5.50bc	78.57bc	7.75
	2.5	6.50a	92.85a	6.25
	3.5	5.25bcd	75.00bcd	7.00
	4.5	4.50de	64.29de	7.50
O-72	1.5	4.50de	64.29de	7.50
	2.5	5.75ab	82.14ab	6.75
	3.5	5.25bcd	75.00bcd	7.50
	4.5	4.75cde	67.86cde	8.00
OM-1	1.5	4.25e	60.71e	8.25
	2.5	5.75ab	82.14ab	7.00
	3.5	5.50bc	78.57bc	7.75
	4.5	4.25e	60.71e	8.50
CV (%)		10.37	10.37	7.64

Note: Values having common letter are statistically identical and those having different letters are different.

Shoot regeneration

Six calli from each genotype were induced to MS media supplemented with same phytohormones combination as like as callus induction to regenerate shoot. Significant interaction effect of phytohormones and genotypes were observed for number of explants showing shoot, percent of shoot regeneration and days required for shooting (Table 2). Percentage of shoot regeneration was the highest (83.33%) with MS + 2.5 mg/L BAP+ 0.5 mg/L IAA in genotype O-9897 (Plate-2) followed by O-9897 × MS + 3.5 mg/L BAP+ 0.5 mg/L IAA (75.00%) and O-72 × MS + 2.5 mg/L BAP+ 0.5 mg/L IAA (75.00%). In contrast, the lowest percentage of shoot regeneration was obtained from the genotypes OM-1 and O-72 when cultured on MS + 4.5 mg/L BAP + 0.5 mg/L IAA (50.00%). Similar findings were also obtained by Bari (2006). Khatun (2001) and Paul (2003) suggested that, low concentration of IAA enhanced shoot regeneration in *C. capsularis.* In addition, the genotype O-9897 took the minimum days (10.75) for initiation of shoot on MS medium supplemented with 2.5 mg/L BAP+ 0.5 mg/L IAA. The genotypes OM-1 took maximum time in days (17.75) for shoot initiation on MS + 4.5 mg/L BAP + 0.5 mg/L IAA.

Table 2. Effect of genotypes and BAP concentrations on shoot regeneration of different varieties *C. olitorius*

Genotypes	Conc. of BAP (mg/L) + 0.5 mg/L IAA	No. explants showing shoot regeneration	Percentage of shoot regeneration	Days to shoot regeneration
O-9897	1.5	4.25abc	70.83abc	13.50cd
	2.5	5.00a	83.33a	10.75f
	3.5	4.50ab	75.00ab	13.25cd
	4.5	3.75bcde	62.50bcde	13.00de
O-72	1.5	3.50cde	58.33cde	14.25bc
	2.5	4.50ab	75.00ab	12.25e
	3.5	3.75bcde	62.50bcde	13.50bcd
	4.5	3.00e	50.00e	14.50b
OM-1	1.5	3.25de	54.17de	13.50bcd
	2.5	4.00bcd	66.67bcd	13.25cd
	3.5	3.50cde	58.33cde	13.75bcd
	4.5	3.00e	50.00e	17.00a
CV (%)		14.42	14.42	25.87

Note: Values having common letter are statistically identical and those having different letters are different.

Root formation

Shoot regenerated calli were then transferred to half strength MS media supplemented with different concentrations of IBA to develop root. Effect of genotypes and IBA concentrations on number of root formation and per cent root formation were found insignificant but significant variation was observed for days to root initiation (Table 3). The genotype O-9897 on half strength MS media supplemented with 0.6 mg/L IBA showed the best performance by producing the highest root formation (45%) (Plate-3) followed by O-9897 in ½ MS + 0.8 mg/L IBA (40.00%). Whereas, the genotype OM-1 regenerated the lowest number of roots (20.00%) in half strength MS media supplemented with 0.2 mg/L IBA. On the other hand, the minimum days required to rooting were recorded in genotype O-9897 cultured in ½ MS + 0.2 mg/L IBA (13.75) followed by O-72 in ½ MS + 0.6 mg/L IBA (16.25) and OM-1 in ½ MS + 0.6 mg/L IBA (16.25). Genotype OM-1 took the maximum days for root initiation in half strength MS media supplemented with 0.2 mg/L IBA (22.00).

Table 3. Effect of genotypes and IBA concentrations on root formation of different varieties of *C. olitorius*

Genotypes	Conc. of IBA (mg/L)	No. root initiation	Root initiation (%)	Days to rooting
O-9897	0.2	1.25	25.00	20.25bc
	0.4	1.75	35.00	20.00bcd
	0.6	2.25	45.00	13.75g
	0.8	2.00	40.00	17.75e
O-72	0.2	1.25	25.00	21.25ab
	0.4	1.75	35.00	20.25bc
	0.6	1.50	30.00	16.25f
	0.8	1.75	35.00	18.50de
OM-1	0.2	1.00	20.00	22.00a
	0.4	1.50	30.00	19.75bcd
	0.6	1.75	35.00	16.25f
	0.8	1.25	25.00	19.00cde
CV (%)		16.46	16.46	5.26

Note: Values having common letter are statistically identical and those having different letters are different.

Transplantation and establishment of plantlets

After sufficient development of root, plantlets were taken out from the culture vials without damaging roots. Excess agar around the root was washed off by tap water to prevent microbial infection. Five plantlets of each of the genotypes were then transplanted in plastic pots into a growth room with controlled environment for proper hardening. The survival rate of the transplanted plantlets was low (20%). The plantlets after their transplantation in the soil were subsequently watered with Hoagland's solution. As soon as new leaves started to initiate, the plants were watered with ordinary tap water. Gradually, the plantlets were adapted to the soil in uncontrolled environment (Plate 4).

The present study suggests that, the genotypes O-9897 of *C. olitorius* in MS media supplemented with 2.5 mg/L BAP + 0.5 mg/L IAA could be used for callus induction and shoot regeneration.

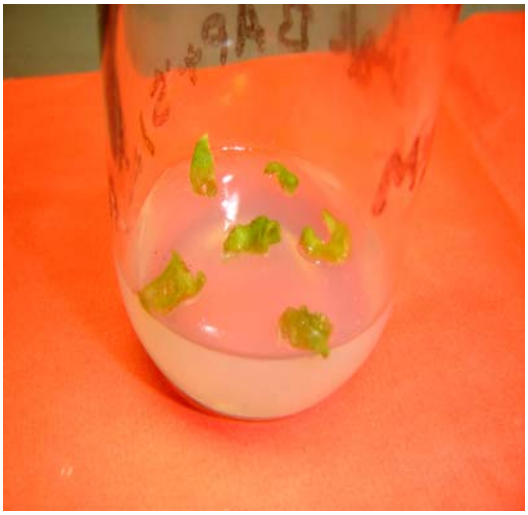

Plate 1. Callus initiation from cotyledon of the genotype O-9897 on MS + 2.5 mg/L BAP + 0.5 mg/L IAA

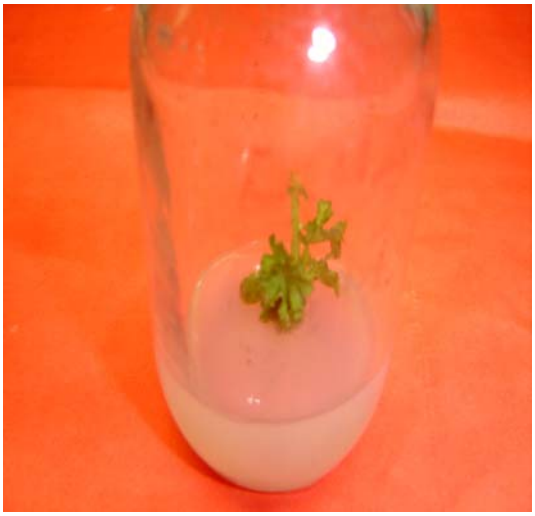

Plate 2. Shoot regeneration from callus of the genotype O-9897 on MS + 2.5 mg/L BAP + 0.5 mg/L IAA

Plate 3. Initiation of roots from regenerated shoot of the genotype O-9897 on ½ MS + 0.6 mg/L IBA

Plate 4. Transplanted regenerated plant of the genotype O-9897

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