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Standardization of high frequency regeneration of existing genotypes of Jute (*Corchorus olitorius*) in Bangladesh

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Abstract: Four varieties of *Corchorus olitorius* were used to observe their regeneration potentiality and to establish a suitable *in vitro* plantlet regeneration protocol of jute. Cotyledons were used as explants. MS medium supplemented with different phytohormone concentrations and combinations were used to observe the callus induction ability of the explants. The highest callus induction (88.56%) was observed in O-9897 with the media supplemented with 3 mg/L BAP + 0.5 mg/L IAA. Calli were maintained to get sufficient number of regenerants. The highest percentage of regenerants were found from O-9897 (88.94%) followed by O-72 (85.83%) and OM-1 (72.61%). Among the phytohormone combinations, MS medium supplemented with 3 mg/L BAP + 0.5 mg/L IAA showed the highest shoot regeneration (97.78%). The root formation from regenerants was the best (69.91%) on half strength of MS media supplemented with 0.6 mg/L IBA. The *in vitro* regenerated plantlets from the genotypes O-9897, O-72, OM-1 and O-4 could be established in the field successfully.
Keywords: Standardization, *in vitro* regeneration, Genotypes, Jute (*Corchorus olitorius*), explants, Phytohormone, BAP, IAA and IBA treatment

Introduction

Jute is one of the most important cash crops of Bangladesh. It occupies 5th position after rice, pulses, oil seeds and wheat in respect of cultivated area (BBS, 2005). It is extensively used in the manufacture of different types of packing materials for various agricultural and industrial products. Jute exports constitute a major source of foreign exchange (12-13%) earning in Bangladesh. At present not much success can be achieved for jute production through the conventional breeding methods. Although a number of high yielding varieties of jute have been released from the Bangladesh Jute Research Institute (BJRI) through conventional breeding techniques, these techniques still have some limitations, in particular, time factor. Biotechnology is a recently developed novel approach and therefore, it is very important to explore these techniques for varietal improvement of jute. The chances for availability of new genotype of jute with disease resistance in nature are very remote unless new techniques are launched to create variability. Recent advances in tissue culture and recombinant DNA technology have opened new avenues in transformation of higher plants, which consequently produced many transgenic plants with new genetic properties. The pre-requisite for the genetic transformation in any crop is to establish an efficient regeneration system from explants to produce mature fertile plants. In jute, plant regeneration from the cotyledonary petioles was reported earlier from *Corchorus capsularis* (Khatun *et al.*, 1992) and from cotyledonary petioles of *Corchorus olitorius* (Khatun *et al.*, 2003).

Materials and Methods

Experimental materials

Healthy seeds of four varieties (O-9897, O-72, O-4 and OM-1) of *Corchorus olitorius* L. were used as experimental materials. All the experimental materials were sterilized before use.

Media

The seedlings raised in axenic culture were used as the source of different kinds of explants. The hypocotyls and cotyledonary nodal segments (cotyledons) were used as explants.The explants were first cultured on agar solidified MS (Murashige and Skoog, 1962) medium supplemented with 1.0, 2.0, 3.0, 4.0 and 5.0 mg/L BAP with constant concentration of 0.5 mg/L IAA in each for callus induction and shoot regeneration. When those shoots were about 2-3 cm in length, they were rescued from the vials aseptically inside the laminar air flow cabinet, and were again cultured in vials with freshly prepared root induction medium containing half strength MS medium supplemented with 0.2, 0.4 and 0.6 mg/L IBA for root formation.

Culture technique

Sterilized seeds were placed into sterilized seed germination medium in culture vessels. Twenty five seeds were placed in each vial. The culture was then incubated in dark till the germination of seeds. These were then transferred to 16 hours light for normal seedling growth. Seven days old seedlings were used as source of contamination-free explants. The seedlings raised in axenic culture were used as the source of different kinds of explants. The hypocotyls and cotyledonary nodal segments (cotyledons) were used as explants. Attempts have been taken for the induction of embryogenesis and organogenesis using different explants in MS medium supplemented with different hormones. After the calli attained a size of about 20-25 mm in diameter, they removed aseptically and sub-cultured to other vials, the calli were again cultured on freshly prepared medium containing the same hormonal supplements, for shoot induction from the cells. When shoots were about 2-3 cm in length, they were rescued from the vials aseptically inside the laminar air flow cabinet, and were again cultured in vials with freshly prepared root induction medium to form root.

Results and Discussion

Callus induction

For each treatment, callus induction performances of all the genotypes were observed and the results are shown in the table 1 and 2. At eighth day callus induction started with some varieties, and it required

nine days for some other varieties. The callus induction percentage was highest in O-9897 (88.56%) (Plate-1) followed by OM-1 (85.39%), O-72 (69.50%) and O-4 (68.11%). Khatun *et al*. (2003) obtained the highest callus induction percentage from cotyledon explants in *C. olitorius* on MS medium supplemented with BAP. MS medium supplemented with 3.0 mg/L BAP + 0.5 mg/L IAA performed the best among the treatments (98.61%) followed by 4.0 mg/L BAP + 0.5 mg/L IAA (79.38%) and 2.0 mg/L BAP + 0.5 mg/L IAA (77.50%).

Table 1. Response of genotypes on different characters of callus induction of *Corchorus olitorius*

Genotypes	Percent of callus induction (%)	Days of callus induction
O-9897	88.56	8.27d
O-72	85.39	8.47c
O-4	69.50	8.53b
OM-1	68.11	8.87a

Table 2. Effect of different combinations of phytohormone on callus induction of *C. olitorius*

Phytohormone combinations	Percent of callus induction (%)	Days of callus induction
MS + 1.0 mg/L BAP + 0.5 mg/L IAA	61.53	8.67a
MS + 2.0 mg/L BAP + 0.5 mg/L IAA	77.50	8.42d
MS + 3.0 mg/L BAP + 0.5 mg/L IAA	98.61	8.50c
MS + 4.0 mg/L BAP + 0.5 mg/L IAA	79.38	8.58b
MS + 5.0 mg/L BAP + 0.5 mg/L IAA	72.43	8.50c

Shoot regeneration

Proliferated callus of four varieties of *C. olitorius* were cultured on MS medium supplemented with BAP (1.0, 2.0, 3.0, 4.0, 5.0 mg/L) and constant concentration of IAA (0.5 mg/L) to regenerate shoots. Shoot regeneration was found the highest in O-9897 (88.94%) (Plate-2) followed by O-72 (85.83%), OM-1 (72.61%), and the lowest in O-4 (70.61%) (Table-3). Khatun *et al.* (2003) described similar results in *C. olitorius*. Among the hormone combinations, MS + 3.0 mg/L BAP + 0.5 mg/L IAA showed the highest shoot regeneration (98.61%) followed by MS + 5.0 mg/L BAP + 0.5 mg/L IAA (93.05%) and MS + 1.0 mg/L BAP + 0.5 mg/L IAA (87.22%) (Table-4). Khatun *et al*. (2003) obtained the same results.

Table 3. Response of genotypes on shoot regeneration of *Corchorus olitorius*

Genotype	Percent of shoot regenerated (%)	Days required for shoot regeneration
O-9897	88.94	10.33c
O-72	85.83	10.80a
O-4	72.61	10.80a
OM-1	70.61	10.53b

Table 4. Effect of different combinations of phytohormone on shoot regeneration of . *olitorius*

Phytohormone combinations	Percent of shoot regenerated (%)	Days required for shoot regeneration
MS + 1.0 mg/L BAP + 0.5 mg/L IAA	70.00	10.00c
	83.33	10.33bc
	56.67	11.00b
	87.22	10.33bc
MS + 2.0 mg/L BAP + 0.5 mg/L IAA	66.67	11.00b
	77.78	10.67ab
	61.11	11.33a
	81.11	10.67ab
MS + 3.0 mg/L BAP + 0.5 mg/L IAA	95.28	10.00c
	98.61	11.33a
	98.61	10.33bc
	98.61	10.67ab
MS + 4.0 mg/L BAP + 0.5 mg/L IAA	60.00	10.33bc
	77.78	10.67ab
	65.56	11.00b
	84.72	10.00c
MS + 5.0 mg/L BAP + 0.5 mg/L IAA	71.11	10.33bc
	91.67	11.00b
	71.11	10.33bc
	93.05	11.00b

Root formation

Calli initiated shoot from the cultures of cotyledon as explants of four genotypes of C. *olitorius* were implemented on half strength MS medium supplemented with IBA (0.2, 0.4, 0.6 mg/L) in three combinations. Induction of root from regenerated shoots showed wide variations according to genotypes and different concentrations of IBA in the medium.

Response of the genotypes for root formation

The genotypes O-9897 showed the best performance for number of root regeneration (2.89) and the lowest number of root regeneration was found in O-4 (2.28). Percent root regeneration was highest in O-9897 (68.52%) (Plate-3) and lowest in O-4 (60.91%). The genotypes O-9897 showed the best performance for days to root initiation. It took minimum days (8.222). On the other hand the genotypes O-4 took the highest time for root initiation (10.556) (Table-5). The findings partially supported that of Ahmed *et al.* (1989) who reported that the callus initiate roots on MS medium with 0.3 mg/L IBA. Paul (2003) also agreed these findings.

Table 5. Response of different genotypes on root initiation of *C. olitorius*

Genotypes	Percent of root initiation (%)	Days required for rooting
O-9897	68.52	8.222d
O-72	67.96	8.556c

OM-1	65.19	9.333b
O-4	60.91	10.556a

Plate 1. Callus from cotyledon of the genotype 0- 9897 genotype on MS + 2.0 mg/L BAP + 0.5 mg/L IAA after 15 days of inoculation

Plate 2. Shoot regeneration from cotyledon of the 0- 9897 on MS + 3.0 mg/L BAP + 0.5 mg/L IAA

Plate 3. Initiation of roots from regenerated shoot of genotype 0- 9897 on half MS + 0.6 mg/L IBA

Plate 4. Transplanted cotyledonary node derived plant of 0- 9897 in plastic pot covered with polythene bag kept in net house for hardening

Plate 5. Established plant of 0- 9897 in soil

Effect of phytohormone on root formation

Half strength MS medium supplemented with 0.6 mg/L IBA showed the best performance, by taking minimum days for root formation (8.944), maximum number of root regeneration (2.67) and highest percentage (69.91%) of root regeneration followed by ½ MS + 0.4 mg/L IBA and ½ MS + 0.2 mg/L IBA. The finding partially supported that of Ahmed *et al.* (1989) who reported that the callus initiate roots on MS medium with 0.3 mg/L IBA. Mangal *et al.* (2001) and Cardoza and stewart (2003) explained that half strength MS medium is perfect for root formation. Paul (2003) found that IBA is a suitable phytohormone for root formation.

Table 6. Effect of different concentrations of phytohormone on root initiation of *C. olitorius*

Phytohormone combinations	Percent of root initiation (%)	Days required for rooting
½ MS + 0.2 mg/L IBA	69.82	9.389a
½ MS + 0.4 mg/L IBA	69.63	9.278b
½ MS + 0.6 mg/L IBA	69.91	8.944c

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. The plantlets then transplanted in plastic pots into a growth room with controlled environment for proper hardening (Plate 4). The survival rate of the transplanted plantlets was 75%. 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 (Plate 5).

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