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In vitro Plant Regeneration from Seedling-derived Explants of two Cultivars of White Jute (*Corchorus capsularies* L.)

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Abstract

An *in vitro* regeneration system for two varieties of white jute (*Corchorus capsularis* L.) namely, BJC-7370 and BJC-83 was developed. The regeneration protocol was based upon direct organogenesis from seedling-derived explants such as cotyledon with petioles, cotyledonary node. Shoot regeneration was achieved from cotyledon with petiole and cotyledonary node through use of MS supplemented with 0.5 mg/l BAP and 1.0 mg/l IAA for BJC-7370 while for BJC-83 shoot initiation was obtained on 1.25 mg/l BAP and 0.25 mg/l NAA using the same explants. Elongation of shoots was achieved on MS containing 0.2 mg/l BAP for the two said jute varieties. Regenerated excised shoots developed effective *in vitro* root system on half strength of MS supplemented with 0.3 mg/l IBA for both the varieties. The *in vitro* grown plantlets were transferred to soil for acclimation. These plants grew up to maturity, flowered and produced seeds identical to the control plants.

Introduction

Jute is a common term used for both the plant and its fiber. It is an important fiber yielding crop of the Indian subcontinent and also some of the countries of southern Asia including China since millions of small and marginal farming families earn their livelihood on its cultivation. The bast fiber obtained from jute is considered as next to cotton in importance (Kundu 1956, Kirby 1963). The cultivated varieties of jute have been evolved from *Corchorus capsularis* L. (White jute) and *C. olitorius* L. (Tossa jute) through conventional breeding and pure line selection based on their yield and agronomic performances (Ghosh 1983). Jute fiber is biodegradable as well as non-toxic and this crop has considerable commercial importance due to its diversified traditional and non-traditional value-added industrial products.

However, the production and quality of this crop in many countries including Bangladesh are affected by various biotic and abiotic stresses. The

main constraints of jute production are the incidence of fungal diseases as well as infestation by various insects and pests (BBS 2004). Moreover, flood and salinity are the major biotic factors hampering the production and quality of jute fiber. Jute is also gradually losing its market in the competition with synthetic fibers.

Therefore, this crop requires immediate attention of the plant breeders for its improvement. Conventional breeding methods have proved to be limited success towards its improvement due to their narrow genetic base and presence of strong sexual incompatibility between two cultivated species (Patel and Datta 1960, Islam and Rashid 1960, Sarker and Hoque 1994).

Under these circumstances, the improvement of jute can be achieved through the application of modern biotechnological method including genetic transformation. For successful genetic transformation, it is essential to have an efficient plant regeneration protocol from transformed cells or tissue through an *in vitro* culture method. There are a number of reports on *in vitro* regeneration of jute (Islam et al. 1982, Ahmed et al. 1989, Saha and Sen 1992, Seraj et al. 1992, Khatun et al. 1993, Saha et al. 1999, Sarker et. al. 2007). Very few reports are available for the establishment of successful regeneration system applicable for genetic transformation of jute (Hossain et al. 1998, Islam et al.1999, Sarker et al. 2008). Ghosh et al. (2002) developed a protocol of multiple shoot regeneration from transformed explants of *C. capsularis* var. JRC 321. Sarker et al. (2007) reported an in *vitro* regeneration protocol for three varieties of white jute cultivated in Bangladesh, namely CVL-1, CVE-3 and D-154. However, the regeneration protocols described in various reports in the past required to be improved in view of their reproducibility. The present study reports the development of an efficient and reproducible *in vitro* regeneration system for two other varieties of white jute, namely BJC-7370 and BJC-83 cultivated in Bangladesh. Seedling-derived explants from these plants have been utilized for plant regeneration with a view to utilizing them in future transformation experiments.

Materials and Methods

Seeds of two cultivated jute (*Corchorus capsularis* L.) varieties, namely BJC-7370 and BJC-83 collected from BJRI, Sher-e-Bangla Nagar, Dhaka were used in the present investigation. Seedling-derived explants such as, cotyledon with petiole and cotyledonary node were used for *in vitro* regeneration. Preparation of the explants from *in vitro* grown seedlings, as well as the sterilization procedures used in the experiments were carried out following the method described by Sarker et al. (2007).

Experiments were conducted using MS with different concentrations and combinations of BAP, IAA for the induction and development of multiple shoots.

For rooting about 4 cm long regenerated shoots were dissected and placed vertically on rooting medium containing full- and half strength of MS with different concentration of IBA. All media contained 3% sucrose and 0.8% agar or gelrite (Duchefa Biochemie, The Netherlands) with pH 5.8 adjusted before autoclaving. All cultures were maintained in 16 hrs photoperiod at 25 ± 2ºC. When sufficient roots developed, plantlets were transferred to small plastic pots containing sterilized soil. These plantlets were acclimated and then transferred to the field and raised there till their maturity to flowering and fruit set.

Results and Discussion

Emphasis was given to regenerate plantlets in both the varieties using the explants of cotyledon with petiole and cotyledonary node. In case of BJC-7370 different explants were cultured on MS supplemented with various concentrations and combinations of BAP (0.2 to 1.0 mg/l) and IAA (1.0 mg/l) for initiation of shoot. Results of these experiments are shown in Table 1.

Table 1. Effect of different concentrations and combinations of BAP and IAA in MS on shoot initiation of BJC-7370.

Explants	Supplements (mg/l)		No. of explants inoculated	Percentage of explants responded	Days to shoot initiation	Mean No. of shoots/ explant
	BAP	IAA				
CP	0.2	1.0	50	72.30	7 - 14	2.15
	0.3	1.0	50	75.10	7 - 14	2.45
	0.5	1.0	50	93.40	7 - 14	7.1
	0.75	1.0	50	68.50	7 - 14	3.4
	1.0	1.0	50	69.40	7 - 14	3.0
CN	0.2	1.0	50	67.80	7 - 14	3.1
	0.3	1.0	50	72.40	7 - 14	3.3
	0.5	1.0	50	85.50	7 - 14	6.5
	0.75	1.0	50	72.10	7 - 14	3.8
	1.0	1.0	50	65.10	7 - 14	3.5

CP = Cotyledon with petiole, CN = Cotyledonary node.

MS supplemented with 0.5 mg/l BAP and 1.0 mg/l IAA was found to be best for shoot initiation from cotyledonary node and cotyledon with petiole explants (Figs. 2 and 3). The initially regenerated shoots were subcultured on MS supplemented with 0.2 mg/l BAP for multiple shoot regeneration (Fig. 4).

Cotyledon with petiole from BJC-83 was used for shoot initiation. MS supplemented with different concentrations and combinations of BAP (0.5 to 1.25

mg/l) and NAA (0.25 to 1.0 mg/l) were used. Results of these experiments are shown in Table 2.

Table 2. Effect of different concentrations and combinations of BAP and NAA in MS for shoot initiation in case of variety BJC-83.

Explants	Supplements (mg/l)		No. of explants inoculated	Percentage of explants responded	Days to shoot initiation	Mean No. of shoots/ explant
	BAP	NAA				
CP	0.5	0.25	50	60.20	7 – 15	2.1
	0.5	0.50	50	60.00	7 – 15	2.4
	0.5	1.0	50	63.10	7 – 15	2.5
	1.0	0.25	50	82.20	7 – 15	4.5
	1.0	0.50	50	75.10	7 – 15	3.5
	1.0	1.0	50	72.24	7 – 15	3.2
	1.25	0.25	50	94.20	7 – 15	5.8
	1.25	0.50	50	80.20	7 – 15	4.5
	1.25	1.0	50	75.15	7 – 15	3.5

CP= Cotyledon with petiole.

Best response towards the shoot initiation was observed on MS supplemented with 1.25 mg/l BAP and 0.25 mg/l NAA. Initiation of shoots was found to occur within 7 - 15 days. The percentage of responsive explants was 94.20 and the mean number of shoots per explants was 5.8. Initiations of shoot from cotyledon with petiole explants are shown in Fig. 1.

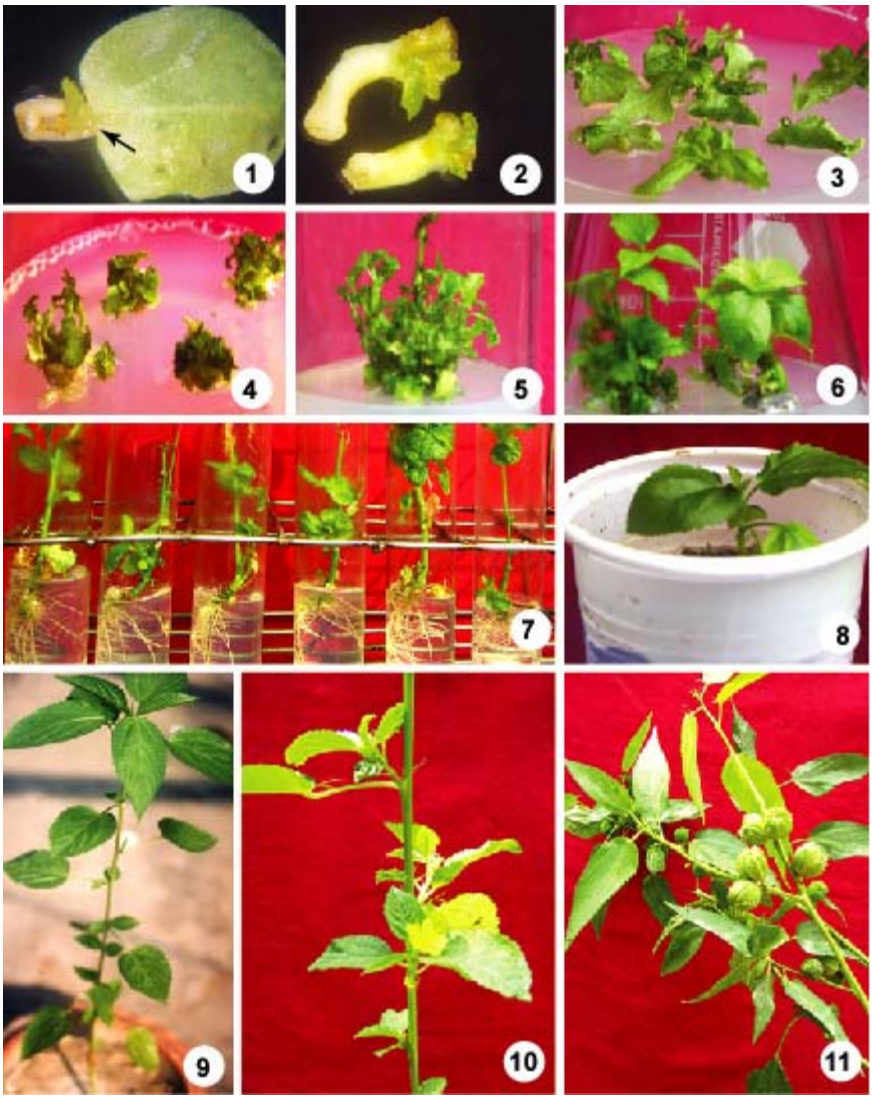
To select the effective medium for shoot multiplication, *in vitro* raised shoots were subcultured on MS supplemented with 0.2 mg/l BAP. In the subculturing medium the number of shoots was found to be increased (Table 3).

Table 3. Multiple shoot formation using 0.2 mg/l BAP in MS following subculturing.

Variety	Explants	No. of subculturing clumps	% of responsive clumps	Mean No. of shoots/subculturing clumps	Mean length (cm) of shoots after 60 days
BJC-7370	CP	25	97.80	10.85	4.5
	CN	25	95.51	9.50	4.1
BJC-83	CP	25	96.35	5.50	4.3

CP = Cotyledon with petiole, CN = Cotyledonary node.

Multiple shoots derived from cotyledon with petiole explants from variety BJC-83 is presented in Fig. 5 and the developing multiple shoots from cotyledonary node in case of variety BJC-7370 is shown in Fig. 6. These developing multiple shoots were found to elongate on the same medium following subsequent culturing.



Figs. 1-13: 1. Stereomicroscopic view of cotyledon with petiole explants from variety BJC-83 showing the initiation of shoots from the regeneration point (arrow) (× 25). 2. Stereomicroscopic view of cotyledonary node explants from variety BJC-7370, showing the initiation of shoots from the regeneration point (× 15). 3. Initiation of shoots from cotyledon with petiole explants of BJC-7370. 4. Shoot proliferation of BJC-7370. 5. Proliferation of shoots of BJC-83. 6. Elongation of shoots of BJC-7370. 7. Development of roots from a number of regenerated shoots of two jute varieties. 8. Regenerated plantlet transferred to soil in small plastic pot. 9. *In vitro* regenerated plants growing in natural condition. 10. Regenerated plant with flowers. 111. Fruits developed on *in vitro* raised plants.

Rooting from the *in vitro* regenerated shoots is an integral part for the development of a complete plantlet. A number of experiments were carried out to induce effective roots at the base of the isolated shoots for all the varieties

studied. Full- and half strength of MS with various concentrations of IBA were used for induction of roots from the *in vitro* grown shoots. The results of this IBA application for root development from regenerated shoots are presented in Table 4.

Highest number of roots was found on the half strength of MS supplemented with 0.3 mg/l IBA. The number of roots was 10 - 12 per shoot. Cent per cent rooting response was observed on this medium (Table 4). Induction of roots required six - seven days from the time of exposure to the rooting medium. The formation of roots on half strength of MS containing 0.3 mg/l IBA for two varieties is shown in Fig. 7. The nature of development of roots from the base of the regenerated shoots for both the varieties was found to be uniform and identical.

Table 4. Effect of IBA in gelrite solidified full and half strength of MS on root induction from regenerated shoots of BJC-7370 and BJC-83 varieties.

Strength of MS	IBA (mg/l)	No. of shoots inoculated for root induction	No. of shoots responded to rooting	Days to root induction	No. of roots/ plantlet
Full	0.1	20	14	7 - 9	2 - 3
	0.2	20	14	7 - 9	2 - 3
	0.3	20	17	6 - 7	3 - 4
	0.5	20	11	7 - 8	2 - 3
	1.0	20	7	7 - 9	2 - 3
Half	0.1	20	16	9 - 10	6 - 7
	0.2	20	17	8 - 9	8 - 9
	0.3	20	20	6 - 7	10 - 12
	0.5	20	15	8 - 10	5 - 6

Following sufficient root formation, plantlets of two jute varieties were transplanted into small plastic pots containing soil for acclimation (Fig. 8). Using this method plantlets were successfully transplanted to soil. The survival rate of the transplanted plantlets was found to be 100%. Plantlets established in soil are shown in Fig. 9. Flowers were found to develop in these regenerated plants after three months of transplantation (Fig. 10). These plants also produced fruits (Fig. 11).

After maturity, seeds from regenerated plants (R_0) were collected and properly dried. Seeds obtained from such fruits were then germinated on sterile water soaked filter paper, where 95% germination of seeds was recorded. The nature of germination obtained from the *in vitro* raised plants was almost identical to those of the control seeds.

The regeneration system developed for *Capsularis*/white jute will contribute to the production of transgenic technologies for the improvement of this important fiber yielding plant.

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