Plant Tissue Cult. & Biotech. **17**(1): 11-18, 2007 (June)

PTC&B

In vitro Regeneration in Three Varieties of White jute (*Corchorus capsularis* L.)

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Key words: Regeneration, White jute, *Corchorus capsularis*

Abstract

Healthy multiple shoot regeneration was observed from petiole-attached cotyledon (PC) explants of CVL-1 on MS containing 0.2 mg/l BAP and 1.0 mg/l IAA. On the other hand, the best response for multiple shoot regeneration in CVE-3 was obtained when the same explants were cultured on MS with 2.5 mg/l BAP and 0.5 mg/l NAA. However, the same explant of D-154 was found to show less responsive compared to other two varieties to produce multiple shoots. Cotyledonary nodal explants of all the three varieties were found to produce maximum number of multiple shoots on MS supplemented with 0.2 mg/l BAP and 1.0 mg/l IAA. Best root induction was observed at the base of the *in vitro* regenerated shoots on half the strength of MS supplemented with 0.3 mg/l IBA. The *in vitro* grown plantlets were successfully transplanted into soil. They grew up to maturity, flowered and fruited like the control plants.

Introduction

Jute is commonly known as the golden fiber of Bangladesh and still considered as the principal cash crop of the country. Next to cotton, it is the world's second most popularly cultivated fiber crop on a global scale (Kundu 1956, Kirby 1963). More than 90% of the total world productions of the raw jute fibers are obtained from Indo-Bangladesh subcontinent (Hossain et al. 1994). Unfortunately, jute is steadily losing its market in the face of its competition with synthetic fibers. In the backdrop of this situation, production of high yielding and better quality jute varieties is urgently felt to improve the present condition facing this natural fiber crop.

There are over 100 species of the genus *Corchorus*, of which only two, namely, *C. capsularis* L. and *C. olitorius* L. are cultivated as fiber crop commercially. The crop has considerable commercial importance due to its diversified value-added industrial products. However, the crop demands immediate attention of plant breeders. Conventional breeding methods for the development of better quality of jute fibers have proved to be of limited success due to their narrow genetic base and because of the presence of strong sexual incompatibility

barrier between the two cultivated species (Islam and Rashid 1960, Patel and Datta 1960, Swaminathan et al. 1961, Sarker and Hoque 1992, 1994). Although the tissue culture technique has created new possibilities for the improvement of various crop plants its contribution seems to be very limited in the production of disease resistant plants as well as plants of better agronomic characters. Under these circumstances, the improvement of this plant can be achieved through the application of modern biotechnological techniques such as plant genetic transformation. This technology has been considered as a powerful pre-breeding method that can provide a solution to certain constraints that limit crop production and can be used to widen the genetic base of a crop by incorporating specific genes for desirable characters.

The most important pre-requisite of gene transfer technique is a robust, reproducible and efficient *in vitro* regeneration protocol. But, jute is generally recalcitrant in their response to regeneration and transformation compared to other crops. There are a number of reports on *in vitro* regeneration of jute (Islam et al. 1982, Rahman et al. 1985, Das et al. 1986, Ahmed et al. 1989, Saha and Sen 1992, Seraj et al. 1992, Khatun et al. 1993, Hossain et al. 1994, Abbas et al. 1997, Saha et al. 1999). However, the regeneration protocols described in various reports in the past need to be improved further in view of their reproducibility. It was with this background, attempts have been made to establish an efficient *in vitro* plant regeneration system from different explants of *C. capsularis* which may be applied to obtain jute transgenics of desired traits using *Agrobacterium*-mediated or other powerful techniques such as particle gun bombardment.

Materials and Methods

Seeds of the three cultivated varieties, namely CVL-1, CVE-3 and D-154 of white jute (*Corchorus capsularis* L.) collected from Bangladesh Jute Research Institute, Sher-e-Bangla Nagar, Dhaka were used in the present investigation.

Various explants used for *in vitro* regeneration were collected from *in vitro* raised seedlings. For this purpose seeds were washed with detergent under running tap water followed by rinsing in 70% alcohol for 30 seconds and with 0.1% $HgCl_2$ solution for 25 to 30 mins. The seeds were then washed five times with sterilized distilled water. The surface sterilized seeds were soaked overnight in sterile distilled water or inoculated into sterile water soaked filter papers and water-agar medium for supporting seed germination and seedling development. Seed germination was found to be better on water-agar medium than sterile water soaked filter paper. Cotyledon was found to enlarge in size during germination on sterile water soaked filter paper. Such enlarged cotyledons were also used as explants for regeneration. Various explants such as, petiole-attached cotyledons (PC), cotyledonary nodes, mature embryos with or without cotyle-

dons were used for *in vitro* regeneration. PC and cotyledonary nodes were collected from five to six days old seedlings. Mature embryo and embryo with cotyledon explants were isolated from overnight soaked seeds.

MS supplemented with different concentrations and combinations of BAP (0.2 to 1.0 mg/l), IAA (0.5 to 1.0 mg/l), NAA (0.5 to 2.5 mg/l) and Kn (0.25 to 0.5 mg/l) were used for the induction and development of multiple shoots from different explants of varieties CVL-1, CVE-3 and D-154. All media contained 3% sucrose and 0.8% agar with pH 5.8, adjusted before autoclaving. For rooting 3 - 4 cm long regenerated shoots were separated and cultured on freshly prepared rooting medium containing full and half strength of MS with different combinations and concentrations of IBA and NAA. All cultures were maintained under 16 h photoperiod at 25 ± 2˚C. Following the development of sufficient roots, 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 fruiting.

Results and Discussion

The *in vitro* regeneration of shoots using various explants from three jute cultivars was investigated and the number of shoots obtained from different explants are shown in Table 1. Multiple shoot regeneration was found best from PC explants when they were cultured on MS containing BAP, IAA and NAA. Differential responses were obtained in different varieties. MS supplemented with 0.2 mg/l BAP and 1.0 mg/l IAA proved to be the best for multiple shoot regeneration in CVL-1 and D-154. On the other hand, CVE-3 showed best response on MS with 2.5 mg/l BAP and 0.5 mg/l NAA. No remarkable variation was observed in shoot regeneration in case of PC as well as in cotyledonary node explants. However, cotyledonary node explants produced a lower number of multiple shoots compared to those obtained from PC. It was further observed that the mature embryos with or without cotyledons were found not to be suitable as explants for multiple shoot regeneration. However, almost all of the mature embryo explants with or without cotyledons produced single shoots.

The mean number of shoots per explant from PC of CVL-1 was 6.8. However, the rate of multiplication of shoots was low in case of the CVE-3 (2.2/explant) and D-154 (2.8/explant). Initiation of shoots were observed within one week of inoculation. Stereomicroscopic view of initiation of shoots from PC in CVL-1 is shown in Fig. 1. Shoots regenerated in this medium (MS with 0.2 ml/l BAP and 1.0 mg/l IAA) from PC were healthy, green and produced a large number of leaves (Fig. 2). This hormonal combination (0.2 mg/l BAP and 1.0 mg/l IAA) proved to be the most effective in case of PC explants in terms of multiple shoot development. Fully developed multiple shoots were found to

achieve on this medium within six weeks (Fig. 3). Similar response towards multiple shoot regeneration was observed by some previous workers when they used 0.2 mg/l BAP and 1.0 mg/l IAA in variety D-154 and Tri-cap-2. (Khalekuzzaman et al. 2000 and Nahar et al. 2003).

Table 1. The number of shoots obtained from different explants cultured on MS containing different hormonal supplements.

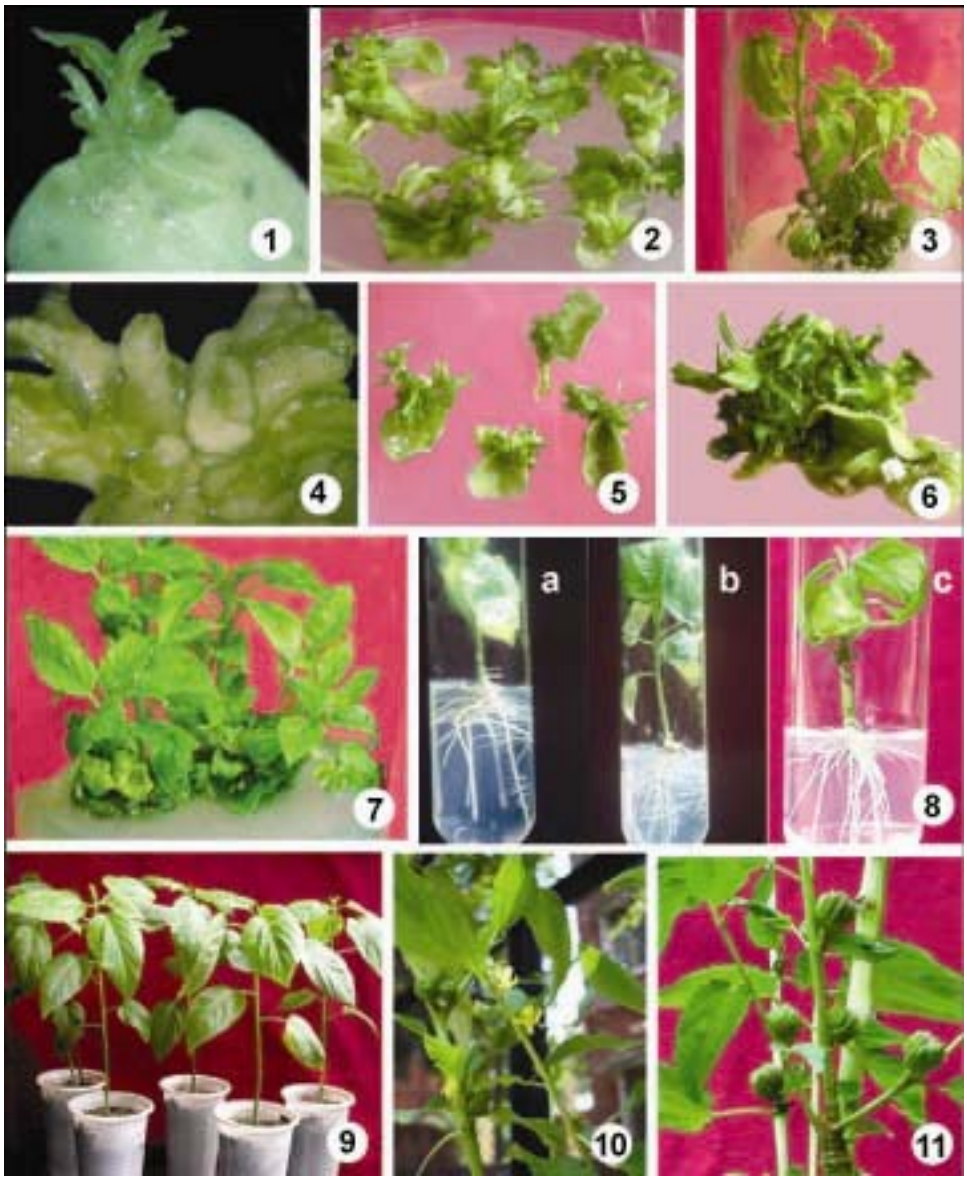
Explants	Hormonal combinations (mg/l)			Mean No. of shoots/ explant of varieties			Mean length of shoots after 30 days
	BAP	IAA	NAA	CVL-1	CVE-3	D-154	
PC	0.2	1.0	-	6.8	2.2	2.8	2.5
	2.5	-	0.5	-	12.5	-	3.1
CN	0.2	1.0	-	5.1	2.0	2.1	2.6
	2.5	-	0.5	-	4.1	-	2.5
ME	0.2	1.0	-	1.0	1.0	1.0	2.6
	2.5	-	0.5	1.0	1.0	1.0	2.5
MEC	0.2	1.0	-	1.0	1.0	1.0	2.5
	2.5	-	0.5	1.0	1.0	1.0	2.4

PC = Petiole-attached cotyledon. CN = Cotyledonary nodes. ME = Mature embryos. MEC = Mature embryos with cotyledon.

The above concentrations of BAP and IAA were used to see their effects on shoot regeneration from PC and cotyledonary nodal explants of D-154 and CVE-3. In case of CVE-3, mean number of shoots/explant was 2.2 and 2.0 in PC and cotyledonary node respectively. On the other hand, in D-154 the mean number of shoots per explant was 2.8 and 2.1, respectively. It was observed that the initiation of shoots from PC and cotyledonary node explants took seven to fifteen days (Table 2).

The shoots initially regenerated on MS with 0.2 mg/l BAP and 1.0 mg/l IAA in all the varieties were subcultured on MS with 0.2 mg/l BAP for their proliferation. The subcultured shoots incresed three-fourfolds in all the variety. However, the rate of shoot multiplication was low in all the varieties.

In variety CVE-3 multiple shoot regeneration from PC explants was also tried on MS supplemented with BAP and NAA. Maximum number of shoots (8 - 15/explant) from this explant was obtained on MS supplemented with 2.5 mg/l BAP and 0.5 mg/l NAA (Table 2). In this hormonal combination 97% of the explants responded towards shoot initiation and it took seven to ten days on this medium (Fig. 4). Multiple shoots were regenerated from this induced shoot buds (Fig. 5). Regenerated shoots were subcultured on MS with 0.2 mg/l BAP and 1.0 mg/l IAA for multiplication and elongation. A mean number 12.5 of elongated shoots per explant was observed on this medium (Figs. 6, 7). In case of variety CVE-3 this medium has been proved to be the best for shoot regeneration and proliferation from petiole-attached cotyledonary explants. Available literatures indicate that there is no report on the *in vitro* regeneration of shoots in the variety CVE-3.



Figs. 1-11: Regeneration in three varieties of jute namely, CVL-1, CVE-3 and D-154. 1. PC explant from CVL-1 showing the initiation of shoots (×15). 2. Multiple shoots developed from PC of CVL-1. 3. Same as Fig. 2 but showing the fully developed multiple shoots. 4. Initiation of multiple shoot buds from PC of CVE-3 (x 18). 5. Shoot initiation from PC of CVE-3. 6. Proliferation of multiple shoots from PC of CVE-3. 7. Fully developed multiple shoots of CVE-3. 8. Formation of roots from excised shoots of CVL-1 (a), D-154 (b) and CVE-3 (c) on half strength of MS with 0.3 mg/l IBA. 9. Plantlets of CVE-3 in soil. 10. Mature plant of CVE-3 with flowers and fruits. 11. Developed fruits of CVE-3.

For the induction of roots, 3 - 4 cm long shoots were excised and cultured on both full and half strength of MS with various concentrations of IBA and NAA. All three varieties showed similar responses to the rooting media. The highest number of roots was observed in half strength of MS with 0.3 mg/l IBA (Fig. 8). The number of roots was 10 - 15 per shoot. Cent per cent rooting response was observed in this medium and it took some five - seven days for the initiation of

Table 2. Effect of different combinations of hormone in MS on multiple shoot formation from PC explants of CVL-1, CVE-3 and D-154.

Variety	Hormonal supplements (mg/l)			% of responsive explants	Days required for shoots initiation	Mean No. of shoots/ explant	Mean length of shoots (cm) after 30 days
	BAP	IAA	NAA				
CVL-1	0.2	0.5	-	76.25	8-15	2.5	2.8
	0.5	0.5	-	73.34	8-15	2.0	2.9
	1.0	0.5	-	68.19	8-15	3.5	2.3
	0.2	1.0	-	95.72	8-15	6.8	2.5
	0.5	1.0	-	72.96	8-15	3.2	3.0
	1.0	1.0	-	80.13	8-15	2.5	2.2
CVE-3	0.5	-	0.25	81.2	7-12	2.5	1.25
	1.0	-	0.25	85.3	7-12	3.5	1.5
	1.25	-	0.25	90.6	7-12	4.0	2.2
	2.0	-	0.5	91.3	7-12	3.6	1.7
	2.5	-	0.5	96.5	7-12	12.5	2.8
	2.5	-	1.0	90.1	8-15	2.5	2.2
D-154	0.2	0.5	-	80.5	8-15	1.5	2.2
	0.5	0.5	-	85.25	8-15	1.3	2.9
	1.0	0.5	-	78.33	8-15	1.0	2.3
	0.2	1.0	-	94.0	8-15	2.0	2.5
	0.5	1.0	-	88.0	8-15	1.2	2.1
	1.0	1.0	-	83.1	8-15	1.0	2.2

roots. The mean number of roots was higher in half strength of MS than the full strength of MS. Ahmed et al. (1989), Das et al. (1986), Seraj et al. (1992) and Khalekuzzaman et al. (2000) reported this combination as suitable for *in vitro* root induction. Development of roots from the base of the regenerated shoots for all the three varieties was uniform. After sufficient development of roots, plantlets of CVL-1, CVE-3 and D-154 were successfully transplanted into small plastic pots containing soil (Fig. 9). Following proper acclimation the plantlets were transferred to the field. These plants flowered within four months after transplantation (Fig. 10) and set fruits (Fig. 11). Pods in mature plants were normal with well-developed seeds which were almost identical to those of the original seeds.

Results of the present investigation showed that it is possible to regenerate large number of multiple shoots from the PC explants on MS with 0.2 ml/l BAP and 1.0 mg/l IAA from CVL-1 and D-154. On the other hand, 2.5 mg/l BAP with 0.5 mg/l NAA for CVE-3. Root induction in all the three varieties were also identical on half strength MS containing 0.3 mg/l IBA. The regeneration protocol

Table 3. Effect of IBA and NAA in full and half strength MS medium for root induction in CVL-1, CVE-3 and D-154.

Medium	Hormonal supplements (mg/l)		% of responsive shoots forming roots	Days to root induction	No. of roots /shoot
	IBA	NAA			
MS	0.2	-	70	7-8	2-3
	0.3	-	90	5-7	5-7
	0.5	-	50	7-8	2-4
	-	0.2	65	5-7	2-3
	-	0.3	80	5-7	3-5
	-	0.5	55	7-10	2-4
Half strength of MS	0.2	-	90	5-7	7-10
	0.3	-	100	5-7	10-15
	0.5	-	80	8-10	6-8
	-	0.2	65	5-8	8-9
	-	0.3	75	5-7	10-12
	-	0.5	40	8-10	5-8

developed here has been proved reproducible and more or less genotype independent. This protocol may be effectively used for the improvement of different jute cultivars through *Agrobacterium*-mediated or any other suitable methods of genetic transformation.

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