J. Agrofor. Environ. 7 (1): 41-44, 2013 ISSN 1995-6983

In Vitro regeneration of *Corchorus capsularis*

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Abstract: The experiment was conducted during May to December 2008 in the Biotechnology Laboratory of the department of Biotechnology, Bangladesh Agricultural University, Mymensingh to observe the callus induction, regeneration potentiality and to establish a suitable in vitro plantlet regeneration protocol of *Corchorus capsularis*. MS medium supplemented with different concentrations of phytohormone and their 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. CVL-1, CVE-3 and BJC-7370. The highest callus induction (96.43%) was observed in CVE-3 in the MS media supplemented with 2.5 mg/L BAP + 0.5 mg/L IAA. Genotype CVE-3 in MS media supplemented with 2.5 mg/L BAP + 0.5 mg/L IAA produced the highest percentage of shoot regenerants (87.50%) followed by CVL-1 (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 CVE-3 (75.00%). The *in vitro* regenerated plantlets from the genotypes CVE-3 and CVL-1 were established in the field successfully. Therefore, genotypes CVE-3 of *C. capsularis* in MS media supplemented with 2.5 mg/L BAP + 0.5 mg/L IAA for callus induction and shoot regeneration and ½ MS + 0.6 mg/L IBA for root formation would be used for further research work.
Key words: *Regeneration, Phytohormone, Corchorus capsularis*.

Introduction

Jute 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. as *tossa* jute (2n=14). It is one of the important cash crops of Bangladesh 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 with the range of 18000 million dollars (FAO, 2005). In addition, with the launching of global campaign for environmental awareness international opinion is being created on 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 insect-pest attack, poor soil fertility, photo-insensitivity and abiotic stresses like drought, flood, low temperature etc. are detrimental to this crop.

To maintain a sustainable improvement in jute productivity under less favourable environment can only be achieved with a constant flow of new genetic materials (Aggarwal, 2000). Conventional breeding techniques are lengthy processes whereas the techniques of plant tissue culture have been developed as a powerful tool for crop improvement (Carlson, 1975; Razdan and Cocking, 1981and Larkin and Scoweroft, 1982).

Biotechnological approach techniques are important to explore improved varieties of crops but the pre-requisite for the genetic transformation in any crop is to establish an efficient regeneration system from explants to produce mature fertile plants. 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. capsularis*.

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. capsularis* viz. CVL-1, CVE-3 and BJC-7370.

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 concentration 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 concentration 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% cow dung was used for transplanting of plantlets from culture vessel to pot.

Culture Techniques

i) Axenic culture: Twenty five sterilized *C. capsularis* seeds were placed into sterilized seed germination medium in each vial. The culture was then incubated in dark till the germination of seeds and then transferred to 16 hours light for normal seedling growth. Seven days old seedlings were used as source of explants.

ii) Explant culture: Cotyledons with attached petiole were cut off from the seedlings and incubated to culture vial. Seven explants from each genotype were inoculated in each culture vial. The culture vials containing the explants were placed under fluorescent light in a room with controlled temperature (22 ± 2°C) using 16 hours photoperiod.

iii) Subculture of the callus for shoot regeneration: Six calli of 20-25 mm in diameter from each genotype were used in this step in MS media containing different concentration and combination of IAA and BAP. The sub-cultured vials were incubated at 22 ± 2°C with 16 hours photoperiod.

iv) Subculture of the regenerated shoot for root initiation: Shoot regenerated five calli of each genotype were cultured in vials with freshly prepared root induction medium to form root. The vials were again incubated at 22 ± 2°C with 16 hours photoperiod.

v) Preparation of pot and transplantation: The plantlets of 5-7 cm in length having enough shoot and root system were taken out from the vials and then transplanted to pots containing of garden soil, sand and cow dung. 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 seems 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. Per cent callus induction = {(Number of explants induced callus) ÷ (Number of explants incubated)} × 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. Percent shoot regeneration = {(Number of calli with plantlets) ÷ (Number of inoculated calli)} × 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 formula. Per cent plant establishment = {(Number of established plantlets) ÷ (Total number of plantlets)} × 100.

Results and Discussion

Callus Induction: Plantlet regeneration from the cotyledons with attached petiole via unorganized calli was the ultimate goal of this study and callus induction is the first step of organogenesis and subsequent plantlet regeneration. To achieve this goal, seven explants from the each genotype were cultured on MS media supplemented with different concentrations and combination of phytohormones. The cumulative effect of genotype and phytohormone concentration showed significant effect on callus formation of *C. capsularis* (Table 1).

Table 1. Effect of genotypes and phytohormone concentration on callus induction of *C. capsularis*

Genotypes	Conc. of BAP (mg/L) + 0.5 mg/L IAA	No. explants showing callus	Percentage of callus induction	Days to callus induction
CVL-1	1.5	4.50de	64.29de	7.25bcd
	2.5	5.75b	82.14b	6.50d
	3.5	5.25bcd	75.00bcd	7.25bdc
	4.5	4.50de	64.29de	7.75ab
CVE-3	1.5	5.25bdc	75.00bcd	7.25bcd
	2.5	6.75a	96.43a	5.50e
	3.5	5.50bc	78.57bc	6.75cd
	4.5	4.75cde	67.86cde	7.50abc
BJC-7370	1.5	4.00e	57.14e	8.25a
	2.5	5.75b	82.14b	6.75cd
	3.5	5.25bcd	75.00bcd	7.50abc
	4.5	4.25e	60.71e	8.25a
LSD		0.7170	10.24	0.7561
CV (%)		9.76	9.76	7.31

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

Table 2. Effect of genotypes and BAP concentration shoot regeneration of *C. capsularis*

Genotypes	Conc. of BAP (mg/L) + 0.5 mg/L IAA	No. explants showing shoot regeneration	% of shoot regeneration	Days to shoot regeneration
CVL-1	1.5	3.25de	54.17de	14.75bc
	2.5	4.50ab	75.00ab	13.00c
	3.5	3.75bcd	62.50bcd	13.50bc
	4.5	2.75e	45.83e	15.50abc
CVE-3	1.5	3.75bcd	62.50bcd	14.25bc
	2.5	5.25a	87.50a	8.75d
	3.5	4.50ab	75.00ab	13.50bc
	4.5	3.75bcd	62.50bcd	14.50bc
BJC-7370	1.5	2.75e	45.83e	16.25ab
	2.5	4.25bc	70.83bc	13.75bc
	3.5	3.50cde	58.33cde	14.25bc
	4.5	2.75e	45.83e	17.75a
LSD		0.7465	12.44	2.544
CV (%)		13.96	13.96	12.54

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

The highest percentage of calli (96.43%) was obtained from the genotype CVE-3 (Plate 1) cultured on MS medium supplemented with 2.5 mg/L BAP+ 0.5 mg/L IAA followed by BJC-7370 (Plate 2) and CVL-1 at the same concentration level of BAP and IAA on MS medium. Performance of CVE-3 at 2.5 mg/L BAP + 0.5

mg/L IAA supplemented MS medium in response to days required for callusing was the best. The minimum number of days required for callusing (5.50) was recorded in CVE-3 cultured on MS medium supplemented with 2.5 mg/L BAP+ 0.5 mg/L IAA and BJC-7370 required maximum days (8.25) 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. capsularis*.

Shoot Regeneration: Six calli from each genotype were induced to MS media supplemented with same phytohormone 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, number of shoot regeneration per vial and days required for shooting (Table 2).

Table 3. Effect of genotypes and IBA concentration root formation C. capsularis

Genotypes	Conc. of IBA (mg/L)	No. root initiation	Root initiation (%)	Days to rooting
CVL-1	0.2	2.25	45.00	13.25a
	0.4	2.50	50.00	12.25ab
	0.6	3.25	65.00	9.50c
	0.8	3.25	65.00	10.50c
CVE-3	0.2	2.75	55.00	12.25ab
	0.4	3.25	65.00	12.00ab
	0.6	3.75	75.00	8.25d
	0.8	3.25	65.00	9.75c
BJC-7370	0.2	2.25	45.00	12.25ab
	0.4	3.25	65.00	11.75b
	0.6	3.50	70.00	9.75c
	0.8	2.50	50.00	13.25a
LSD		0.7656	8.618	1.140
CV (%)		17.47	17.47	7.08

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

Plate-1 Plate-2 Plate-3

Plate-4 Plate-5 Plate-6

Plate 1-8: (1) Callus initiation from cotyledon of the genotype CVE-3 on MS + 2.5 mg/L BAP + 0.5 mg/L IAA, **(2)** Callus initiation from cotyledon of the genotype BJC-7370 on MS + 2.5 mg/L BAP + 0.5 mg/L IAA, **(3)** Shoot regeneration from cotyledon derived callus of the genotype CVE-3 on MS + 2.5 mg/L BAP + 0.5 mg/L IAA, **(4)** Root regeneration from cotyledon derived callus of the genotype CVE-3 on MS + 2.5 mg/L BAP + 0.5 mg/L IAA, **(5)** Transplanted cotyledon with attached petiole derived plant of CVE-3, **(6)** Established plant of CVE-3 in soil

Numbers of explants showing shoot regeneration (5.52) and percentage of shoot regeneration (87.50%) was the highest in MS + 2.5 mg/L BAP+ 0.5 mg/L IAA with CVE-3 (Plate 3) followed by CVL-1(75.00%) at the same combination of hormones. The lowest percentage of shoot regeneration was obtained from the genotypes CVL-1 and BJC-7370 when cultured on MS + 4.5 mg/L BAP + 0.5 mg/L IAA (45.83%). Number of calli showing shoot per vial was highest on CVE-3 (3.50) and BJC-7370 (2.75) on MS + 2.5 mg/L BAP + 0.5 mg/L IAA medium followed by CVE-3 × MS + 3.5mg/L BAP + 0.5 mg/L IAA (2.50) and BJC-7370 × MS + 3.5 mg/L BAP + 0.5 mg/L IAA (2.25). Similar findings were also obtained by Bari (2006). Khatun (2001) and Paul (2003) suggested that low concentration of IAA enhance shoot regeneration in *C. capsularis.* The genotype CVE-3 took the minimum days (8.75) for initiation of shoot on MS medium supplemented with 2.5 mg/L BAP + 0.5 mg/L IAA preceded by CVL-1 × MS + 2.5 mg/L BAP + 0.5 mg/L IAA (13.00), CVE-3 × MS + 3.5 mg/L BAP+ 0.5 mg/L IAA (13.50) and CVL-1 × MS + 3.5 mg/L BAP + 0.5 mg/L IAA (13.50). The genotypes BJC-7370 took the maximum time (17.75) for shoot initiation on MS + 4.5 mg/L BAP + 0.5 mg/L IAA.

Six calli from each genotype were induced to MS media supplemented with same phytohormone 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, number of shoot regeneration per vial and days required for shooting (Table 2).

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Root Formation: Shoot regenerated calli were then induced to half-strength MS media supplemented with different concentrations of IBA to develop root. Combined effect of genotypes and IBA concentrations on number of root formation and per cent root formation were found insignificant but significant variation observed in days to root initiation (Table 3). The highest root formation (75%) and the minimum days for root initiation (8.25) were recorded from the genotype CVE-3 on half-strength MS media supplemented with 0.6 mg/L IBA (Plate 4) followed by BJC-7370 × ½MS + 0.6 mg/L IBA (70%, 9.75), CVL-1 × ½MS + 0.6 mg/L IBA (65%, 9.50) and CVE-3 × ½MS + 0.8 mg/L IBA (65%, 9.75). The maximum days required to rooting were recorded in CVL-1 × ½ MS + 0.2 mg/L IBA (13.25) and BJC-7370 × ½MS + 0.6 mg/L IBA (13.25).

Transplantation and establishment of plantlets: After sufficient development, plantlets were taken out from the culture vials and excess agar around the root was washed off to prevent microbial infection. Five plantlets of each of the genotypes were transplanted in plastic pots into a growth chamber with controlled environment for proper hardening (Plate 5). The survival rate of the transplanted plantlets was 80%. The plantlets after their transplantation in the soil were subsequently watered with Hoagland's solution. Gradually, the plantlets were adapted to the soil in uncontrolled environment (Plate 6).

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