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Influence of Genotypes on Plant Regeneration from Cotyledons of *Corchorus capsularis* L.

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Abstract: The present study was conducted on influence of genotypes on plant regeneration from cotyledons of ***Corchorus capsularis*** L. Multiple shoots were produced at the cut end of the petioles of each cotyledon of ***C. capsularis*** varieties on MS agar solidified medium supplemented by IAA and BAP. The results indicated that percentage of shoot regeneration from the cotyledon explants were found dependent on the genotypes used. Variation was observed among the varieties in number of cotyledons produced shoots and in number of shoots produced by each cotyledon. The highest number of shoots produced by each cotyledon and the highest number of cotyledons responded for shoot regeneration was recorded from Tri-cap-2. However, jute varieties have shown differences in ***in vitro*** responses and the differences were statistically significant. The results showed that the regenerated plants from all varieties were rooted ***in vitro*** and successfully transferred in pots containing soil. The plants grew well into maturity and produced seeds.

Key words: *Corchorus capsularis*, cotyledons, shoot regeneration, genotypes

Introduction

Jute is an important fibre crop particularly important for Bangladesh where the jute sector accounts nearly 30% of its national export earning. In recent years, jute has regained its importance compared to synthetic fibers due to its biodegradable quality. Conventional methods for the development of better quality of jute fibers have been proved to be of limited success. Tissue culture followed by genetic engineering techniques is a powerful tool to improve this crop. Thus, any improvements in this area of biotechnology will strengthen the hands of breeders to reach their goal quicker then hitherto possible. Various workers have applied ***in vitro*** techniques for the improvement of jute (Islam, 1981; Rahman ***et al.***, 1985; Ahmed ***et al.***, 1989; Saha and Sen 1992; Seraj ***et al.***, 1992; Islam ***et al.***, 1992; Khatun ***et al.***, 1993; Saha ***et al.***, 1999).

Establishment of an efficient plant regeneration system from the explants of ***C. capsularis*** was the prerequisite for the introduction of genetic transformation for this crop. Plant

regeneration system have been reported from the excised ***in vitro*** grown cotyledons (with attached petioles) of *C. capsularis* on MS medium supplemented by IAA and BAP (Khatun ***et al.***, 1993; Saha ***et al.***, 1999).

However, limited number of varieties/accessions of *C. capsularis* have been used and the success rate was not up to the mark. The objective of the this research was to find out the most efficient variety in which regeneration rate would be high. Keeping this view in mind, investigation was carried out to observe the suitable genotype(s) for efficient plant regeneration in *C. capsularis*.

Materials and Methods

The present experiment was performed in the Biotechnological Laboratory of Bangladesh Jute Research Institute (BJRI) during the period of 1999-2001.

Seed germination *in vitro*

Healthy seedling production was found one of the major criteria for plant regeneration from jute explants and thereby to be successful in genetic transformation. To improve the situation experiments for healthy seed germination from the varieties of *C. capsularis* has been repeated several times using clinical cotton-based seed germination system. For this purpose, seeds of *C. capsularis* (Accessions CVL-1, D-154, CVE-3, CC-45, BJC-83, BJC-718, BJC-2142, BJC-7370, Tri-cap 1 and 2) were surface sterilized by immersing in absolute alcohol for 1 min and then in 0.1% Mercuric chloride for 20 min. Seeds were thoroughly washed with autoclaved distilled water for 6 times. The sterilized seeds were transferred in a 100 ml conical flasks containing 50 ml of hormone free MS (Murashige and Skoog, 1962) agar (Sigma, UK, 0.8%, w/v) solidified medium. The cultures were placed in a growth room with 28°C temperature under 1.0 Wm^{-2} of daylight fluorescent tubes with 12 h photoperiod.

In another set of experiment, clinical cotton was used instead of agar as a supporting material for seed germination. As percentage of seed germination from *C. olitorius* (Jute) was increased almost by 2 times in the Biotechnological Laboratory of Bangladesh Jute Research Laboratory using cotton-based liquid medium seeds of *C. capsularis* (vars. D-154, CVE-3, CVL-1 and Acc. Tri-cap-2) was also tested on cotton to see the differences of seed germination. Clinical cotton (1 g approx.) was placed at the bottom of 100 ml flasks and each flask contained 20 ml of hormone free MS liquid medium. Seeds were placed on clinical cotton in association with MS medium. Cultures were placed in a growth room with 28°C temperature under 1.0 Wm^{-2} of daylight fluorescent tubes with 12 h photoperiod.

Culture of cotyledons with attached petioles for plant regeneration

The cotyledons (with attached petioles) were excised from ***in vitro*** grown 10 accessions of *C. capsularis* (e.g. CVL-1, D-154, CVE-3, CC-45, BJC-83, BJC-718, BJC-2142, BJC-7370, Tri-cap-1 and 2). The excised cotyledons were cultured in 250 ml of conical flasks containing 60

ml of agar-solidified MS medium supplemented by IAA (0.5 mgl^{-1}; Sigma, UK) and BAP (2 mgl^{-1}; Sigma, UK). Seven cotyledons were placed in each flask. The cultures were maintained at 28°C temperature under 1 Wm^{-2} of daylight fluorescent tubes with 12 h photoperiod. Each experiment was repeated 3 times with 3 replications. The regenerated plantlets were subcultured on hormone free on agar solidified MS medium without hormone for rooting and then transferred into pots in glass house.

Transfer of regenerated plantlets into soil

More than 1200 plantlets were produced of which 380 plantlets were transferred in pots to see their morphological changes. As pit soil was not available, dairy soil (Savar Dairy) (70%) was mixed with commercial sand (30%). The mixture was sterilized before use. The idea of mixture was to make soil pours for good aeration. Plastic pots (6, 7 cm height) with a small whole at the bottom were used for transfer purposes. The plantlets were washed with sterilized tap water to remove agar and then transferred into 9 cm plastic pots. Pots were placed on 9 cm Petri-dishes containing 20 ml of water. The plantlets were then covered with a cellophane paper bag and placed in a well-ventilated place at room temperature (27-30°C). After one week, 2 wholes were made in each bag to allow some fresh air. In the second week, more wholes were made in the bags and during the third week the bags were removed. During the fourth week, the plants were finally transferred to 30 cm pots (two plants in each pot) containing dairy soil and other fertilizers. Survival rate of the plants were recorded.

Statistical analysis

All data were analyzed using analysis of variance (ANOVA) method of Minitab Statistical Package, Version-13. Test of differences between means were made at the 5% probability level when a significant F value was obtained for varietal effect. Different varietal means were compared by calculating a Least Significant Difference (LSD) as follows:

$LSD = v(2EMS)/n \times t(0.05)\,df$, where

EMS = error mean square

n = number of replication (3)

t = 0.05

df = values from the t distribution tables at 5% probability level and appropriate error degrees of freedom

The germination percentage data were transformed following the formula described by Gomez and Gomez (1984).

Results

Germination of seeds

Seeds were initially germinated from all varieties of *C. capsularis* (e.g. CVL-1, D-154, CVE-3, CC-45, BJC-83, BJC-718, BJC-2142, BJC-7370, Tri-cap-1 and 2) MS agar solidified medium. Later seeds were germinated on cotton and agar supported MS medium for comparative study. Seeds were germinated on both clinical cotton and agar supported MS medium. For seed germination on agar supported medium (Table 1), CVL-1 performed better (89.33%) than other three varieties (var. CVE-3, D-154, Tri-cap-2) and D-154 showed the poorest performance (77.33%). Percentage of seed germination for CVL-1 was found significantly higher than D-154. However, overall differences among the treatment means were found significant ($P<0.05$). When the data were transformed into square root values it was observed that the over all differences within the treatment means were also significant ($P<0.05$). In the case of cotton supported medium, var. Tri-cap 2 showed the highest percentage of germination (97.33%) and D-154 again showed the poorest performance (89.00%). The overall differences for germination percentage among the varieties were significant ($P<0.05$). When the data were transformed into square root values the overall differences within the variety was found also significant. The seedlings of *C. capsularis* varieties grown on surgical cotton-supported liquid MS medium was found to be much more healthier than the seedlings grown on agar supported MS medium.

Differences were observed for seed germination on agar and cotton supported medium.

Table 1: Optimization of seed germination from the cultivars of *C. capsularis* on hormone free agar and clinical cotton supported MS medium

Media support used	Varieties used for seed germination					
Agar/cotton	CVL-1	CVE-3	D-154	Tri-cap-2	SED	LSD
Germination (%) in agar medium	89.33	85.00	77.33	86.33	2.79	6.44*
Germination in agar (Transformed data)	9.45	9.22	8.79	9.31	0.02	0.06*
Germination (%) in cotton medium	94.67	92.33	89.00	97.33	1.53	3.52**
Germination in cotton medium (Transformed data)	9.73	9.61	9.43	9.83	0.095	0.22*

SED = differences of standard error between means, LSD = least significant difference,
* = 5% probability levels, ** = 1% probability levels

In cotton supported medium, germination percentage were found higher than agar supported medium for most of the varieties. These differences were found significant for CVE-3, D-154 and Tri-cap-2. However, the differences for CVL-1 was found significant for cotton and agar supported medium. When a two Sampled ***t***-test was performed on the whole data it was found highly significant as $P < 0.001$.

Plant Regeneration

Two weeks after culture, calli were observed at the cut end of the petioles of the cotyledons of all varieties (vars. CVE-3, CVL-1, D-154 and Tri-cap-2) of ***C. capsularis***. A large number of shoots produced at the cut ends of the petioles of all varieties used (Table 2). The highest percentage of cotyledons responded for shoot regeneration was observed from Tri-cap-2 of 91.0%. This was found significantly higher than all varieties. On the other hand, Tri-cap-1 produced the lowest number of shoots (43.33), which was also found significantly lower than all varieties used. The overall differences for among the varieties were found highly significant ($P<0.001$). However, difference in percentage of cotyledons regenerated shoots was found non-significant among the varieties Tri-cap-1, BJC-2142 and BJC-7370.

The overall differences in number of shoot production cotyledon^{-1} among the varieties were found significant ($P<0.05$). The highest number of shoots produced by each cotyledon was recorded from Tri-cap-2, which makes the average of 32.00 shoots cotyledon^{-1}. This was found significantly higher than all varieties used except var. CVE-3. The lowest number of shoots cotyledon^{-1} was produced by BJC-2142 was 14. This was found significantly lower than CVL- 1, CVE- 3, Tri-cap 2, BJC- 83 and BJC-718.

Table 2: Influence of genotypes of ***C. capsularis*** on plant regeneration from the cotyledons with attached petioles using MS basal medium with IAA and BAP

Varieties	No. of Cotyledons Cultured	Cotyledons producing shoots (%)	Number of shoots produced cotyledon^{-1}
CVL-1	150	67.66±3.79	20.00±2.65
CVE-3	150	89.66±2.52	26.66±2.08
D-154	150	64.00±3.61	16.00±1.73
CC-45	150	57.33±3.51	18.00±2.65
Tri-cap-1	150	43.33±4.04	15.00±2.00
Tri-cap-2	150	91.00±3.61	32.00±2.00
BJC-83	150	68.00±4.58	27.00±2.00
BJC-718	150	85.33±3.22	26.00±3.61
BJC-2142	150	47.66±4.51	14.00±2.65
BJC-7370	150	46.00±2.65	15.00±2.00

The LSD for percentage of cotyledons producing shoot = 6.23***, The LSD for average number of shoots produced by each cotyledon = 4.03***, *** = 0.1% probability levels

The plants grew well into maturity and produced fruits. No morphological changes were noticed. Out of 380, hundred plantlets transferred to the soil 307 plants survived (81%), grew well into maturity and produced fruits. Morphological difference was not found.

Discussion

Seeds of the varieties of *C. capsularis* (vars. CVL-1, CVE-3, D-154, Tri-cap-2) germinated both on agar and cotton supported MS medium of which 77.33 to 89.33% seeds germinated on agar-supported medium and 89.00 to 97.33% in cotton-supported medium. The differences in percentage of seed germinated between agar-supported medium and cotton-supported liquid MS medium was found to be statistically significant. Similar result was reported for seed germination of *C. olitorius* varieties (Khatun, 2001-2002). Though the results of seed germination of *C. olitorius* varieties reported was more marked than *C. capsularis*, both of the species responded better in cotton supported medium. In the case of *C. olitorius* seeds of the varieties ensured 83.66 to 98.00% germination whereas, for solidified medium seed germination was as low as 38.66 to 46.00% for the same varieties (Khatun *et al.*, 2001-2002). The seedlings of *C. capsularis* varieties grown on surgical cotton-supported liquid MS medium was found to be much more healthier than the seedlings grown on agar supported MS medium. Similar response was reported for *C. olitorius*. This result could be a valuable addition for tissue culture system as cotton supported seed germination system was comparatively cheaper than agar-based system as agar was very expensive commercially; whereas, price of cotton is negligible compared to research grade agar. Research grade agar 500 g will cost Taka 4000.00 (approx.) where as, one Kg surgical cotton cost only Taka 60.00 in the local market. This system had been established in BJRI Biotechnological Laboratory for regular practice and being used regularly in this Lab for the seedling production of jute, kenaf and mesta.

Root production was readily obtained from the explants of all varieties of *C. capsularis*. Similar report was obtained from the explants of *C. olitorius* (Khatun, 1993) kenaf and mesta (Khatun *et al.*, 2001-2002). All of these species were producing fibre. From this result was concluded that there might be a relationship between root production and fibre production.

In this study, differences were noticed among the 10 accessions (CVL-1, D-154, CVE-3, CC-45, BJC-83, BJC-718, BJC-2142, BJC-7370, Tri-cap-1 and 2) of *C. capsularis* in percentage of cotyledons producing shoots and also in number of shoots produced by cotyledon^{-1}. Similar observation was reported earlier by Khatun *et al.* (1993) for plant regeneration from *C. capsularis* cotyledons. They had demonstrated that var. C-134 could not respond to plant regeneration in the absence of surfactant when C-134 and D-154 were used. Both of the varieties regenerated in the presence of surfactant. Varietal differences for plant regeneration ability was also reported for other crops e.g. barley, mungbean, maize and sorghum (Koinuma *et al.*,1990; Mendoza and Futsuhara, 1990; Taniguchi *et al.*, 1991; Rao *et al.*, 1992). Koinuma *et al.* (1990)

reported that difference in embryoid or callus formation among the genotypes of maize (***Zea mays L.***) were significant at the 1% level. According to Mendoza and Futsuhara (1990) plant regeneration ability varied with genotypes in mungbean (***Vigna radiata***). Varietal differences were also reported by Taniguchi ***et al.***, (1991) in the ability of callus formation and plant regeneration from mature embryo in barley. Rao ***et al.*** (1992) reported that plant regeneration was high in grain sorghum and sweet sorghum and it ranged from 20 to 87% depending on the genotypes. All these reports were in agreement with the results of the present investigation on jute (***C. capsularis***) for varietal differences.

Finally, it may be concluded that differences noticed among the varieties of ***C. capsularis*** responded for plant regeneration and number of shoot production^{-1} explant were found statistically significant. Varietal differences were also noticed for seed germination in cotton and agar supported MS liquid medium. Cotton supported MS liquid medium was found to be a cheaper and efficient addition for seed germination of jute germplasms.

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