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# Plant Regeneration from the Cotyledonds of Tossa Jute (*Corchorus olitorius* L.)

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**Abstract:** Plant regeneration from the cotyledons of *C. olitorius* has recently been optimized at Bangladesh Jute Research Institute (BJRI). One of the major constraints of getting plant regeneration from the explants of *C. olitorius* was the production of healthy seedlings *in vitro*. An efficient seed germination system and healthy seedling production have been established at BJRI. Seeds of different varieties (O4, O9897, OM1 and O72) of *C. olitorius* germinated on both agar supported hormone free MS medium and cotton supported hormone free liquid MS medium. The percentage of seeds germinated on cotton supported medium was found to be much higher than seeds germinated on agar supported medium. This system ensured maximum of an average of 98% seed germination. Whereas, in case of agar-supported medium an average of 46% seeds could be germinated with very slow growth. The seedlings grown on cotton supported medium were found to be much more healthily than seedlings grown on agar supported medium. Plant regeneration was obtained from the cotyledonary petioles of the varieties (O4, O9897, OM1 and O72) of *C. olitorius* on MS agar solidified medium supplemented by IAA 0.5 and BAP 3.0 mg $l^{-1}$. The efficiency of plant regeneration from the cotton supported seedlings was found to be as good as the agar supported seedlings. The plantlets produced roots on hormone free MS medium readily. After transfer to soil the plants grew into maturity and produced fruits. No morphological changes were noticed.

**Key words:** *Corchorus olitorius*, shoot regeneration, cotyledons, genotypes, hormones

## Introduction

Jute constitutes a major world crop, which is particularly important to the economies of Bangladesh, where the bulk of the world's supplies are grown. The two cultivated species of jute (*Corchorus olitorius* L. and *C. capsularis* L.) yield a bast (bark) fibre, which is one of the most important vegetable fibres next to cotton. As fibre of *C. olitorius* (tossa jute) has more economic value than *C. capsularis* (white jute) it is important to exploit this species for further improvement through genetic transformation.

One of the major constraints to improve jute varieties, in particular, *C. olitorius* (tossa jute) varieties is the non-availability of modern varieties with improved plant types including resistance to diseases. New genotypes including disease resistance characters are, therefore, very much

needed to be introduced in the field of jute breeding. The chances for availability of new genotypes of tossa jute with disease resistance are very remote unless new technique like genetic transformation is launched to create variability.

Several researchers have applied ***in vitro*** techniques for the improvement of jute (Islam, 1981; Rahman ***et al.***, 1985; Ahmed ***et al.***, 1989; Seraj ***et al.***, 1992; Islam ***et al.***, 1992; Khatun ***et al.***, 1993; Saha ***et al.***, 1999 and Naher ***et al.***, 2003). However, no report has been found on efficient and repeatable plant regeneration from the explants of *C. **olitorius***, which could be exploited, for gene insertion protocol. Several attempts have been made using various growth hormones in different combinations and concentrations ***in vitro*** to obtain plant regeneration from different explant sources e.g. hypocotyl segments, cotyledon segments and root segments of *C. **olitorius*** (Khatun, 1993). However, plant regeneration from any of these explant sources of *C. **olitorius*** was found not successful. Only calli and roots were observed from all of these sources. Ghosh and Chatterjee (1990) reported plant regeneration from hypocotyl-derived callus tissue from *C. **olitorius*** on MS medium. However, this plant regeneration system has been found not efficient enough for further exploitation.

Plant regeneration from the cotyledonary petioles of *C. **capsularis*** has been reported earlier (Khatun ***et al.***, 1993). The cut ends of the cotyledonary petioles of ***Brassica*** (Moloney ***et al.***, 1989) and jute *C. **capsularis*** (Khatun, 1993) and has been used earlier for gene insertion through co-cultivation with ***Agrobacterium*** vectors. The cotyledonary petioles of *C. **capsularis*** have been found highly susceptible to ***Agrobacterium***-mediated gene transfer (Khatun, 1990) and also displayed a high plant regeneration rate, often with numerous shoot production per explant (Khatun, 1993; Hossain, 1999). Genetic transformation has been initiated earlier in *C. **olitorius*** using ***Agrobacterium rhizogenes*** vectors (Khatun, 1990) where hypocotyl and cotyledon segments have been used. Vigorous hairy root production was observed from ***Agrobacterium rhizogenes*** infected hypocotyls and cotyledons of *C. **olitorius***. Later plant regeneration system from the cotyledonary petioles of *C. **olitorius*** has been developed and this technique is being used for co-cultivation with ***Agrobacterium tumifaciens*** vectors (Khatun, 2000).

With the aim to develop a protocol for gene insertion in tossa jute through ***Agrobacterium*** vectors, the cotyledons (with their petioles attached) of *C. **olitorius*** varieties have been used as this species has got more commercial importance. Initially, plant regeneration from the explants of *C. **olitorius*** was found to be very difficult in culture conditions (Khatun, 1993). In this study, a protocol for plant regeneration from the explants of *C. **olitorius*** is demonstrated which might be exploited in future for genetic transformation.

## Materials and Methods

### Germination of seeds

Seeds of *C. **olitorius*** L. (vars. O4, O9897, OM1 and O72) were surface sterilized by immersion in 0.1% (w/v) Mercuric Chloride for 20 min, followed by 6 washes with sterile deionized water. Seeds were placed on the surface of 50 ml aliquots of hormone free agar-solidified (0.8%, w/v) MS basal medium (Murashige and Skoog, 1962) contained in 100 ml capacity of conical flasks. In another set of experiment, surgical cotton (1 g approx. in each flask) was used instead of agar

in association with MS basal liquid medium to obtain optimum seedling production. Surgical cotton was placed at the bottom of 100 ml flasks. Each flask contained 20 ml of hormone free MS liquid medium. Cultures were placed in a growth room with 28°C under 1.0 $Wm^{-2}$ of daylight fluorescent tubes with 12 h photoperiod.

**Culture of cotyledons with attached petioles**

The cotyledons (with attached petioles) of 7 day old ***in vitro*** grown seedlings of *C. **olitorius*** (vars. O4, O9897, OM1 and O72) were excised and cultured on MS agar (0.8%, w/v) solidified medium with 3% (w/v) sucrose and pH 5.8 with the supplement of IAA 0.5 mg $l^{-1}$ and with BAP (3.0 mg $l^{-1}$). Explants were cultured in 250 ml conical flasks and maintained at 28°C under a 1.0 $Wm^{-2}$ of daylight fluorescent tubes with 12 h day length. The experiments were repeated 3 times with 3 replications. The results were scored 6 weeks after culture.

Excised single shoots (1.5-2.5 cm in length) were transferred to hormone free MS medium for root production and maintained under the same culture conditions as above. For transfer, sterilized dairy soil (Savar Dairy) and sand mixture was used following the methods demonstrated by Naher ***et al.*** (2003).

**Statistical analysis**

All data were analyzed by using the analysis of variance (ANOVA) method of Minitab Statistical Package, Version-13. Test of differences between means were made at 5% probability level when a significant F value was obtained for varietal effect. A two sample T-test was also performed on the data to compare agar-supported and cotton-supported media. 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.

Data for germination percentage were transformed following Gomez and Gomez (1984).

**Results**

**Germination of jute seeds and production of seedlings *in vitro***

Seeds of *C. **olitorius*** varieties (O4, O9897, OM1 and O72) germinated on both clinical cotton and agar supported MS medium. Seed germination on agar solidified MS medium was found to be very low (38.66 to 46.00%). A dramatic result was obtained for seed germinated on cotton supported liquid MS medium, which was found to be very high (83.32 to 98.00%) compared to agar solidified medium (Table 1). Cotton supported liquid medium also ensured very healthy seedling production. Varietal differences for seed germination were also noticed for *C. **olitorius*** (Table 1).

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

| Medium support used | Varieties used for seed germination | | | | |
|---|---|---|---|---|---|
| Agar/cotton | O9897 | OM1 | O4 | O72 | LSD |
| Germination % in agar supported medium** | 42.66 | 43.33 | 38.66 | 46.00 | NS |
| Germination % on cotton supported medium | 93.33 | 92.67 | 83.33 | 96.67 | 5.52* |
| Germination on cotton (Transformed data) | 9.66 | 9.62 | 9.12 | 9.83 | 0.29 |

** Data transformation was not required for seed germination on agar supported medium.
LSD = least significant difference, *= 5% probability levels, NS= Not significant

Table 2: Percentage of shoot regeneration from the cotyledons (with attached petioles) of different varieties of *C. olitorius* using MS basal medium supplemented by IAA and BAP

| Varieties | Number of cotyledons cultured | Percentage cotyledons producing shoots | Average number of shoots produced cotyledon$^{-1}$ |
|---|---|---|---|
| O4 | 108 | 54.33±1.52 | 6.33±1.15 |
| O9897 | 100 | 59.33±2.08 | 7.33±1.52 |
| OM1 | 110 | 49.66±3.51 | 5.00±1.00 |
| O72 | 112 | 59.00±3.52 | 6.33±1.52 |

The LSD for percentage of cotyledon producing shoot = 5.27 **, Average number of shoots produced cotyledon$^{-1}$ is non Significant

When seed germination on agar supported medium and cotton supported medium were compared by performing a two sampled t-test it was found that germination percentage in cotton supported medium was significantly higher than that of agar supported medium ($P<0.001$). In the case of agar supported medium, the differences of seed germination percentages among the varieties were found to be very low and not significant. Whereas, in the case of the cotton supported medium, the overall differences for germination percentage among the varieties were found significant ($P<0.05$). The best germination percentage was shown by variety O72 (96.67%) and it was significantly higher than O4. On the other hand, variety O4 showed the poorest percentage of germination (83.33%), which was found significantly lower than the varieties O4, O9897 and O72. When the data were transformed into square root values it was observed that the over all differences within the treatment means were also significant ($P<0.05$). However, the difference between the vars. O4 and 09897 was significant and difference between OM1 and 072 was not significant.

**Plant regeneration from cotyledonary petioles of *C. olitorius***

Plant regeneration was obtained from the cotyledonary petioles of ***C. olitorius*** (vars. O9897, OM1, O72 and O4). The cotyledons of all varieties producing shoots from the cut ends of the cotyledonary petioles within 3 weeks after culture on agar solidified MS medium containing IAA (0.5 mg l$^{-1}$) and BAP (0.3 mg l$^{-1}$). The best ***in vitro*** response for percentage cotyledons producing shoots was from var. O9897 (59.33%). The highest average number of shoots cotyledon$^{-1}$ (7.33) was also produced by var. O9897. The overall differences among the varieties and treatment means were significant ($P<0.05$). The differences were significantly higher than variety OM1 but the difference are not significant than the var. O4.

**Rooting of plantlets and transfer into soil**

The regenerated plantlets of all varieties of *C. olitorius* started to produce roots on hormone free agar solidified MS medium within a week. Root production was found to be a less troubled field. Plantlets of all the varieties successfully transferred to soil, grew well into maturity and produced fruits. No morphological changes were noticed.

**Discussion**

Seeds of the varieties of *C. olitorius* (vars. O9897, O4, OM1, O72) germinated on both agar and cotton supported MS medium. The varieties ensured 83.66 to 98.00% seed germination on cotton supported MS liquid medium, whereas, for agar solidified MS medium, seed germination was as low as 38.66 to 46.00% for the same varieties. The differences in percentage of seed germinated between agar supported medium and cotton supported liquid MS medium was found to be statistically significant. Similar finding was reported for seed germination of white jute (*C. capsularis*) varieties (Naher *et al.*, 2003). Though the result of seed germination of *C. olitorius* varieties was found to be more marked than *C. capsularis*, both of the species responded better in cotton supported liquid medium. The seedlings of *C. olitorius* varieties grown on cotton supported liquid MS medium was found to be much more healthily than the seedlings grown on agar supported MS medium. Similar response was reported for *C. capsularis* (Naher *et al.*, 2003). This result could be a valuable addition for tissue culture system as cotton supported seed germination system was comparatively cheaper than agar supported system. Moreover, medium consumption is lesser (i.e. 20 ml flask$^{-1}$) for cotton supported system, whereas, for agar supported system, medium requirement was 50 ml flask$^{-1}$.

Root production was readily obtained from different explants and callus of tossa jute *C. olitorius* and similar result was obtained from the cotyledons of white jute (*C. capsularis*) (Khatun, 1993; Naher *et al.*, 2003). Recently, vigorous root production was reported from the cotyledonary petioles of kenaf (*Hibiscus cannabinus* var. HC-2) at various concentrations of auxins and cytokinins used (Khatun *et al.*, 2003). The present finding also shows similarity with the findings of Srivatanakul *et al.* (2000). They reported that rooting of regenerated kenaf shoots was not found to be difficult to achieve, even, with the presence of high levels of TDZ, which usually act as a potent cytokinin. They have reported that TDZ inhibited root production in woody plant tissue culture but not inhibited root production for kenaf explant culture. Root production was also reported in mesta (Khatun *et al.*, 2001-2002). All of these species e.g. jute, kenaf and mesta are fibre producing plants. From this result, it may be concluded that there might be a relationship between root production and fibre producing plants. These species might contain high level of auxins that are favorable for root production.

Shoot regeneration was observed from the cotyledons of *C. olitorius* in the presence of high level of BAP (3.0 mg l$^{-1}$). This result is comparable with shoot regeneration from cotyledons of other fiber producing plants like kenaf, where, plant regeneration was obtained with BAP (3.0 mg l$^{-1}$ and 5.0 mg l$^{-1}$) and from *C. capsularis* BAP (2.0 v mg l$^{-1}$). Two species of jute (*C. capsularis* and *C. olitorius*) and kenaf responded similarly for hormone combination like IAA and BAP.

In this study, differences were noticed among the 4 accessions (O9897, OM1, O4, O72) of *C. olitorius* 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) and Naher *et al.* (2003) for plant regeneration from 10 accessions of *C. capsularis* cotyledons. Naher *et al.* (2003) demonstrated that the differences were noticed among the 10 accessions of *C. capsularis* in percentage of cotyledons producing shoots and also in number of shoots produced by cotyledon$^{-1}$. All these reports were in agreement with the results of the present investigation on jute for varietal differences.

Plant regeneration from different explants of *C. olitorius* and *C. capsularis* have been tried and reported by Khatun (1993). A detail study of cotyledon segments, hypocotyl segments and root segments have been conducted for morphogenic responses of jute by using various combinations and concentrations of auxins and cytokinins. Plant regeneration was not obtained from any of the explants including the cotyledons without petioles attached. The present finding also indicates that the cotyledons of *C. olitorius* will respond to plant regeneration provided the petioles remain attached and that makes tossa jute (*C. olitorius*) comparable to apple (Kouider *et al.*, 1984); *Brassica* spp. (Sharma *et al.*, 1991); white jute *C. capsularis* (Khatun *et al.*, 1993) and kenaf (Khatun *et al.*, 2003). All these species similarly required an attached petioles for their cotyledons to undergo morphogenesis. Like wise, cultured leaves of *Echeveria elegans* require an attached petiole for plant regeneration (Raju and Mann, 1970).

In conclusion, the experiments reported in this paper demonstrate a dramatic result for seed germination from the varieties of *C. olitorius* on clinical cotton supported liquid MS medium which is very high compared to agar solidified medium. This system also ensured very healthy seedling production. This result could be a valuable addition for tissue culture system as cotton supported seed germination system is comparatively cheaper than agar supported system. A repeatable plant regeneration protocol has been established from the cotyledons (with attached petioles) of *C. olitorius* in BJRI Laboratory. This experiment is still being continued to increase the average number of shoot regeneration cotyledon$^{-1}$ and also to improve the percentage of cotyledons producing shoots. This plant regeneration system can be used for genetic transformation in *C. olitorius*.

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