In vitro Regeneration of *Corchorus olitorius* var. O-4

Abstract

The purpose of this study was to ascertain the effects of several plant growth regulators on the *in vitro* plant regeneration of the tossa jute plant, including 6-benzylaminopurine (BAP), indole acetic acid (IAA), and indole-3-butyric acid (IBA). However, the cotyledon-attached petioles of *Corchorus olitorius var.* O-4 were used as explants and inoculated in Murashige Skoog (MS) media supplemented with 2.5 mg/L BA + 0.5 mg/L IAA, the greatest number of calluses and shoots were generated. Five leaves were produced by the 2.5 mg/L BA + 0.5 mg/L IAA therapy administered two weeks after induction (WAI). Most roots were regenerated with the IBA treatment at 2.0 mg/L. The greatest percentage of root induction and longest roots were obtained by the treatment 0.6 mg/L IBA. In a shady environment, regenerated plantlets have a survival rate of 68.4%, but in direct sunshine, they have an 87.2% survival rate. Therefore, the results expressed as an efficient method for *Corchorus olitorius var*. O-4 in vitro regeneration has been developed in order to genetically modify the organism.

Keywords: Benzylaminopurine, Indole-3-butyric acid, *In vitro* regeneration.

Introduction

Jute (2n=14) is a dicotyledon plant under the Malvaceae family. Bangladesh is the second producer of jute fibre after India and first exporter in the world. In Bangladesh, jute fibre is known as 'golden fibre'. As it is biodegradable in nature, its fibre is economically and environmentally important. It has diversified use, for instances, biodegradable shopping bag, building material, charcoal production (Jahan *et al*., 2022). But due to global warming, increased population rate and reduced agricultural land, jute production is alarming day by day. As a result, fast growing, high fibre yield, disease and pest resistant jute variety is crying need.

The Bangladesh Jute Research Institute has develped O-4, an enhanced high-yielding tossa jute variety. If the variety is sown early, before March 30th, it will produce flowers. It has been demonstrated that this variety can be grown across the nation in high to medium-high land with loamy to sandy-loamy soils and an effective drainage system. Farmers benefit financially and their lifestyle is improved as a result of high fiber yield, quality, and market price.

Conventional breeding programmes are time consuming. Through genetic transformation of jute, desired variety can be obtained easily. Callus growing plant through tissue culture is one of the ways of tissue culture.

Phytohormones, another name for plant hormones, are signal molecules that are produced in very small amounts within plants. Hormones in plants regulate every facet of growth and development, starting with embryogenesis (Mendez- Hernandez *et al*., 2019), the regulation of organ size, pathogen defense (Shigenaga *et al*., 2016; Burger *et al*., 2019), stress tolerance (Ku *et al*., 2018; Ullah *et al*., 2018) and through to reproductive development (Pierre-Jerome *et al*.,2018). 6-Benzylaminopurine, also known as benzyl adenine, BAP, or BA, is a first-generation synthetic cytokinin that triggers reactions in plants, causing them to produce blooms and increase fruit richness through cell division. IAA has a wide range of actions, including causing cell division and elongation, which have an impact on plant growth and development (Silva *et al*., 2019). On a bigger scale, IAA functions as a signaling molecule that is essential for the growth coordination and organ development of plants. In plant tissue culture, a process

known as micropropagation uses IBA and other auxins to start root production *in vitro* (Bridgen *et al*., 1992). Considering the above facts, the present investigation has been undertaken to find out the performance of different hormones (BAP, IAA and IBA) with the optimum concentration for *in vitro* plantlets regeneration of *Corchorus olitorius* var. O-4.

Materials and Methods

This experiment was carried out in plant tissue culture lab, Molecular Biology Department, Bangladesh Jute Research Institute in March 2022-january 2023. *Corchorus olitorius* var. O-4 (Tossa Jute) seeds were collected from Breeder Seed Department, Genetic Resources and Seed Division, Bangladesh Jute Research Institute, Dhaka 1207.

In this study, experimental material was seed. Seeds were thoroughly cleaned under running water. Trix is soaked in with the explants for ten minutes then repeatedly rinsed with diluted water. In the Laminar Air Flow Cabinet, the remaining tasks were completed (Fig. 1, Plate a). The explants were taken out of that solution after 20 minutes and repeatedly rinsed with distilled water then sterilized for 1 minute with 70% ethanol, followed by 1 minute of distilled water washing. Once more, the explants were sterilized for 10 minutes with a 0.1 % $HgCl_2$. The explants were then cleaned at least three times with double distilled water. Sterilized seeds were then placed in controlled MS medium without hormone for germination (Fig. 1, Plate b.). After 7 days seeds were germinated and cotyledon attached petiole inoculated on liquid MS media (Fig. 1, Plate c.) supplemented with various doses of BAP (1.0, 1.5, and 2.0, 2.5. 3.0 mg/L) and control (0.0 mg/L)) with 0.5 mg/L IAA for callus induction. For shoot induction, of BAP (1.0, 1.5, and 2.0, 2.5. 3.0 mg/L) with 0.5 mg/L IAA. Extracted induced axillary shoots were grown for root induction on four concentrations of IBA (0, 0.5, 1.0, 1.5, and 2.0 mg/L) in an aseptic environment.

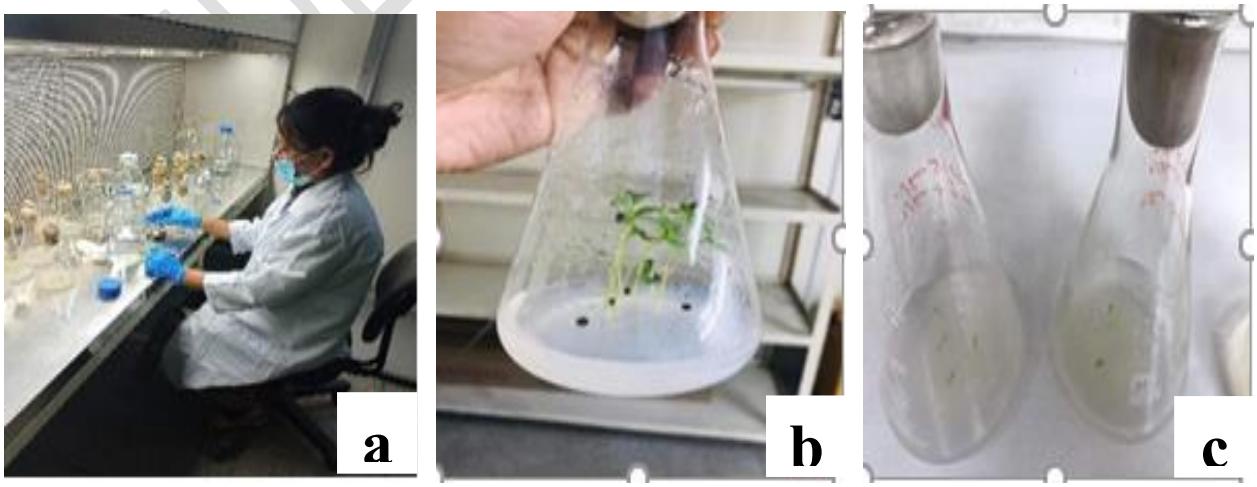

Figure 1. Plate a. Sterilization of Corchorus olitorius O-4 seeds. Plate b. Germination of seeds. Plate c. Cotyledon attached petiole in MS+ 2.5 mg/l BAP+ 0.5 mg/l IAA and Callus initiation in MS+ 2.5 mg/l BAP+ 0.5 mg/l IAA.

The culture vials with fully formed plantlets were moved to normal room temperature after 5 weeks. The rooted plantlets were taken out of the culture vials the following two to three days, and the medium that was still attached to the roots was carefully rinsed away with tap water. Individual plantlets were transplanted into plastic containers filled with a 1:1:1 mixture of soil, sand, and cow dung. For seven days following transplantation, a moist, clear poly bag was placed over the plants and pot to avoid desiccation. The plantlets were housed in a shade house for 12 days to lessen unexpected shock. Plantlets were then moved to the field after 12 days.

Results and Discussions

A series of experiments were conducted for callus, shoot and root induction in *Corchorus olitorius* var. O-4).

Approximately 82.54% (Fig. 4, Plate a) callus response were recorded in MS + 2.5 mg/l BAP + 0.5 mg/l IAA (Table 1). In BAP and IAA supplemented medium 14.32 days were required for callusi initiation (Fig. 4, Plate c) (Table 1). MS+ 1.0 mg/l BAP + 0.5 mg/l IAA produdes lowest number of callus (36.45%) and highest number of days (20.51 days) required for callus induction (Table 1.). According to Ghosh *et al*., 2009, For the induction of callus tissue, MS basal medium supplemented with a combination of vitamins and growth regulators (0.30 mg/L NAA and 0.30 mg/L Kn) was found to be superior than SH (White's medium) medium. This contradicts with the result.

Table 1. Effect of different concentration of BAP and IAA on days to callus initiation and percentage of callus induction

SL No.	Treatment BAP+IAA (mg/L)	Days to callus initiation	Percentage of callus induction
1.	0.0+0.5	0 g	0
2.	1.0+0.5	20.51 a	36.45
3.	1.5+0.5	18.12 bc	57.63
4.	2.0+0.5	14.32 cd	71.12
5.	2.5+0.5	7.54 f	82.54
6.	3.0+0.5	13.74de	69.10
7.	3.5+0.5	20.10 ab	43.38
	CV %	3.74	-

	LSD (0.05)	0.6882	-

Figures in a column followed by no letter(s) differs significantly whereas figures having common letter(s) do not differ significantly from each other as adjusted by DMRT. CV= Coefficient of variation, LSD (0.05) = Least significant difference.

When explants were subcultured on MS+2.5 mg/l BAP+ 0.5 mg/l IAA supplemented medium gives highest number of shoots (85.53%) (Fig. 4, Plate b) and the lowest number of days (9.25 days) were required for shoot initiation. MS+ 3.5 mg/l BAP + 0.5 mg/l IAA produces lowest number of shoots (45.38%) followed by highest days to shoot initiation (Table 2). Mondal *et al*. 2018, described that after 7–10 days of dark incubation, microshoots were similarly induced from epicotyl explants, with the greatest microshoot (2.30 ± 0.15), on MS media supplemented with 0.1 mg/l IAA and 1.0 mg/l Kin. Which is slightly different from our result.

Table 2. Effect of different concentration of BAP and IAA on days to shoot initiation and percentage of shoot induction

SL No.	Treatment BAP+IAA (mg/L)	Days to shoot initiation	Percentage of shoot induction
1.	1.0+0.5	18.5 ab	48.50
2.	1.5+0.5	17.3 bc	54.36
3.	2.0+0.5	14.8 cd	71.12
4.	2.5+0.5	9.25 g	85.53
5.	3.0+0.5	12.7 ef	73.10
6.	3.5+0.5	19.9 a	45.38
	CV %	2.54	-
	LSD (0.05)	0.3313	-

Figures in a column followed by no letter(s) differs significantly whereas figures having common letter(s) do not differ significantly from each other as adjusted by DMRT. CV= Coefficient of variation, LSD (0.05) = Least significant difference.

Shoots were transferred to half strength of MS medium supplemented with different concentrations of IBA for root induction. The excellent rooting (70%) was obtained when shoots cultured on half strength of MS medium with 0.5 mg/l IBA (Fig. 2). Healthy roots were obtained in this medium after 14 days of culture. The lowest percentage (5%) of root was obtained from ½ strength MS medium (controlled) and highest number of days (30 days) is required (Fig. 3). According to Hoque *et al*. 2010, ½ MS+0.6

mg/l IBA in O-9897 had the highest percentage (45%) of shoots and the shortest time (13.75 days) needed to induce shoots. It is almost similar to our result.

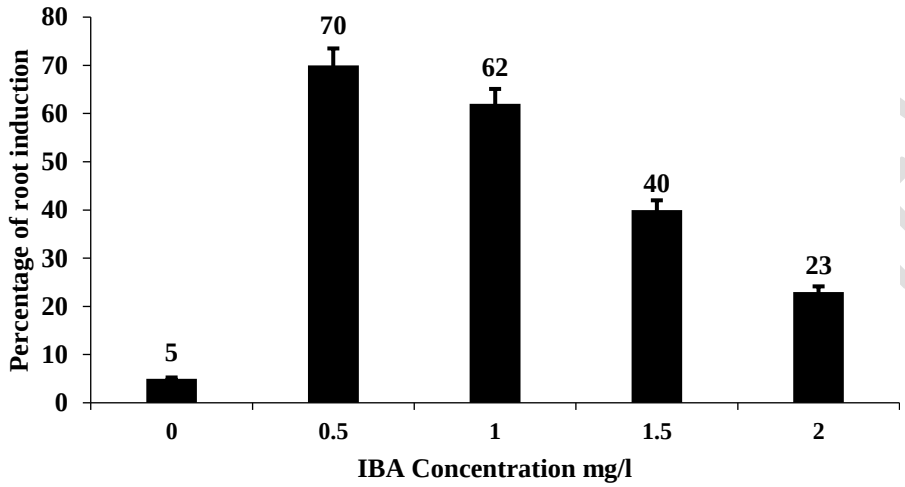

Figure 2. Effect of IBA+ ½ MS strength MS media on percentage of root induction in *Corchorus olitorius* var. O-4

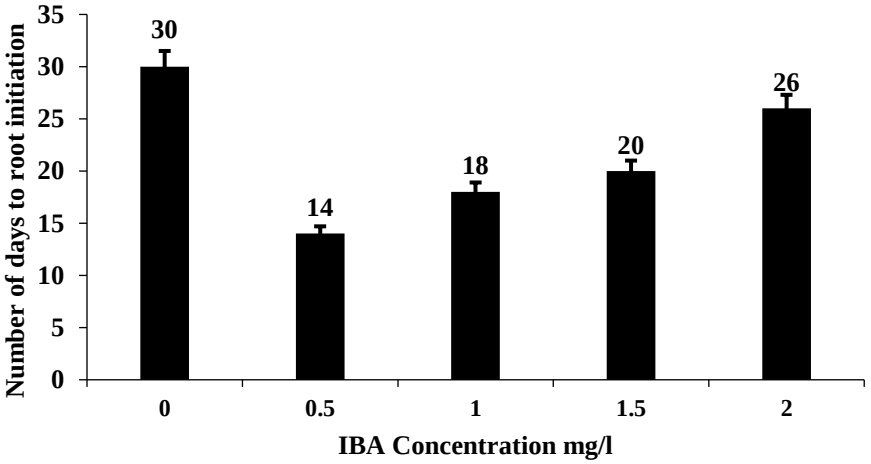

Figure 3. Effect of IBA+ ½ strength MS media on number of days to root initiation in *Corchorus olitorius* var. O-4

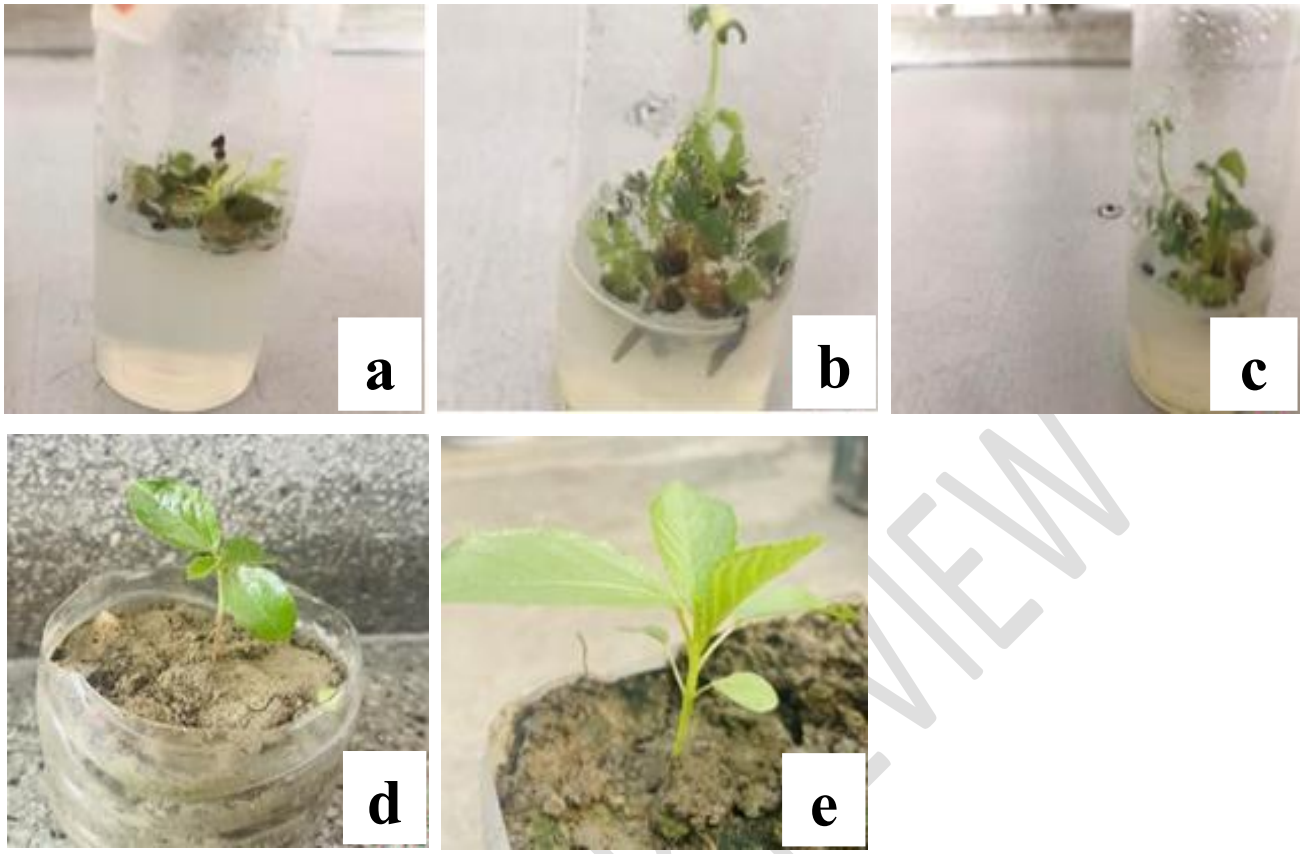

Figure 4. Plate a. Callus growing in MS+ 2.5 mg/l BAP+0.5 mg/l IAA. Plate b. Shoot initiation in MS+ 2.5 mg/l BAP+ 0.5 mg/l IAA. Plate c. Transfer of shoots in ½ MS+0.5 mg/l IBA. Plate d. Acclimatization of plant in shade condition. Plate e. Acclimatization of plant in field condition.

After five weeks of culture, a sizable number of shoots and roots had grown. Without causing any damage to the roots, the plantlets were carefully taken out of the vial. Before being moved to the field settings, plants were acclimated and toughened in the shade house and growth cabinet. When the plants were initially moved, 15 of them survived in shade conditions (75%) (Fig. 4, Plate d). Ultimately, 12 of the 15 plants that were transplanted under normal atmospheric conditions survived, yielding an 80% survival rate (Fig. 4, Plate e). According to Anand and Rao (2000), 75% of plantlets survived in their original state. 90% of plantlets were found to survive in the shadow house's soil.

Conclusion

The present study describes an effective in vitro clonal propagation process for *Corchorus olitorius* var. O-4 that uses cotyledon connected petiole for regeneration and multiplication. This procedure has the potential to be a valuable genetic transformation tool. Additional research can be conducted on the effects of various Tossa Jute genotypes and the micropropagation of Tossa Jute using varying auxin and cytokinine hormone combinations and concentrations.

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