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IN VITRO REGENERATION OF TOSSA JUTE THROUGH CALLUS CULTURE

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ABSTRACT

This research work has been done on the creation of desired variants through somaclonal variation by producing callus tissue from the *in vitro* regeneration of jute (*Corchrous olitorius* L. Var O-9897). The work was carried out in 2024 at the tissue culture lab of the Cytogenetics Department, Bangladesh Jute Research Institute. Sterile seeds were grown in semisolid agar media without using growth regulators. After 5 to 6 days healthy and sterile plantlets were found (germination above 95%). Cotyledonary shoot tip was used as an explant for *in vitro* germinated seedlings. After two weeks of cotyledonary shoot tip culture,friable and small size (2.2-4.2 mm) callus growth was found in a medium containing (BAP 2.5 + IAA 0.5), (BAP 0.2 + IAA 1.0); (NAA 0.75+BAP 3.5 + IAA 0.75)and only BAP 2.0with 3% sugar, 1% agar and p^H was adjusted at 5.8. Cultures were placed in a growth room with 26 ± 2^0C under 3000 lumens of fluorescent tubes with 16h photoperiod. Fresh callus was subculture on different concentration and combination of hormone treatments (11 types) for shoot regeneration. MS medium without hormone (control) and with hormone BAP 2.5 + IAA 0.5 and BAP 2.0 gave shoot initiation successfully.

Key words: Tossa jute, callus, hormone, shoot tip and somaclonal variation

Introduction

Subsequently Jute the 'Golden fibre' of Bangladesh has been considered as the major source of foreign exchange earnings of the country (Islam and Ali, 2017). Bangladesh is now the second largest producer of jute and produces 42% of the world's jute, at 1.33 million tonnes. India produces around 2 million tonnes (about 55% world production). China comes third with 45,000 tonnes. However, Bangladesh ranks at the top in jute exports (MoFA, 2022). It exports 285 types of jute products (MoFA, 2022; FAO, 2023). National average yield is increased from 1.59 to 1.98 tons per hectare. Jute is still contributing about 4% GDP to the national economy and earns about 5% of foreign exchange as well (Islam and Ali, 2017). Low natural genetic diversity is a serious limiting factor for academic progress and agronomic improvement of crops like tossa jute (*Corchorus olitorius* L.) which is an economically important bast fibre crop (Choudhary, 2018). One of major constraints to improve jute is the non-availability of modern varieties with improve plant types including resistance to diseases. Chances for availability of new genotypes of tossa jute with diseases resistance are very remote unless new technique like genetic transformation is launched to create variability (Huda *et al*., 2007). Application of tissue culture techniques for rapid multiplication and inducing variation in plant materials has added a new dimension in recent years. The primary requirement for successful gene transfer techniques is a robust, reproducible and efficient in vitro regeneration protocol. However, jute tends to be more resistant to regeneration and transformation than other crops. Despite this challenge, numerous studies have been conducted on the 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, potential exploitation of these methods for improvement of jute (*Corchorus olitorius* L.) which is a very important fiber yielding commercial crop remains almost untapped so far. The standardization of the method of *in vitro* regeneration of jute plants may ultimately lead to successful inter specific hybridization which has been a long cherished goal of the jute breeders. (Ghosh and Chatterjee, 2009).But now a day's human is aware of his health during use of synthetic. High quality and high yield of jute may be achieved by biotechnological approaches. Induction of somaclonal variation through callus culture may also provide some quality plants. Use of mutation during tissue culture method may bring heterogeneity, which may be a mean of producing high quality and high yield jute.

Materials and Methods

Seeds of tossa jute (Variety O-9897) were surface sterilization by using firstly detergent powder in running tap water for 10 minutes. After washed seed were taken carefully in laminar flow cabinet and pour all tap water from the seeds. Freshly prepared 0.1% mercuric chloride solution was used as a second seed sterilized chemical for 5 minute. Than rinsed the seeds by sterile distilled water in two to three times for 5 minutes under laminar air flow cabinet. The seeds were then aseptically placed on semisolid agar medium (MS) without any hormone use. The seeds germinated within 2-3 days. The cotyledonary leaf with tip was used as a expant from 5-7 days old seedlings and were cut into small pieces. Each piece was then placed aseptically on 10 ml semi-solid nutrient medium. Murashige and Skoog's (1962) media with various combinations of different auxins (NAA and IAA) and cytokines (BAP) were used for explant culture. The p^H of the media was adjusted to 5.8, solidified with 1% agar (Bacteriological grade, BDH) and sterilized for 15 minutes at 15 PSI pressure. The cultures were grown at 26±1°C with at 55-60% relative humidity under Philips Fluorescent day light tubes emitting 3000 lumen for 16 hours light and 8 hours dark period. The callus tissues were sub cultured at intervals of 2-3 weeks into fresh media.

Results and Discussion

In vitro preparation of explant: A popular cultivated tossa jute variety O-9897 seeds were used for explant preparation. After 5-6 days old seedlings were used as an explant (Fig 1). All live seeds (above 95%) were germinated in MS medium. cotyledonary shoot tip was collected from healthy plantlets and culture on MS medium with use of different hormone combination for callus production. Hossain *et al.,* (2024) found that maximum seed germination rate (97%) was recorded from cotton supported liquid MS medium in tossa jute (variety O-9897).

Callus induction: To develop and establish a reproducible *in vitro* regeneration protocol for high yield jute variety through somaclonal variation, explants were cultured (Fig. 2) on MS medium containing Cytokinin (BAP) & Auxin (NAA, IAA) for callus initiation. After 15 days of explant culture friable and small size (2.2-4.2 mm) callus growth was found (Fig. 3) in a medium containing (BAP 2.5 + IAA 0.5), (BAP 0.2 + IAA 1.0),(NAA 0.75+BAP 3.5 + IAA 0.75) and BAP 2.0 (out of 20 different hormone concentration and combinations) with 3% sugar, 1% agar and p^H was adjusted at 5.8 (Table 1 and Fig. 4). And after 24 days callus induction was stopped. Ghosh and Chatterjee (2009) reported that the cut ends of the explants initiated callusing within 10-20 days and subsequently the entire explants were gradually involved in callusing within 25-30 days in tossa jute. After 7–10 days of culture, the swelling of the explants was observed and the callus size was attained about 5–10 cm within 14 days (Hossain *et al.,* 2024).

Regeneration of shoot: Fresh callus was subculture for shoot regeneration medium containing hormone (Fig. 5). After four weeks BAP 2.5 + IAA 0.5; BAP 2.0 and control gave shoot successfully (Table 2). Hossain *et al.,* (2024) observed that after 8–10 days later shoots were developed from calli.

Fig 1: Sterile plantlets for explant

Fig 2: Explant culture on MS medium for callus development

Fig 3: Callus of variety O-9897 after 15 days

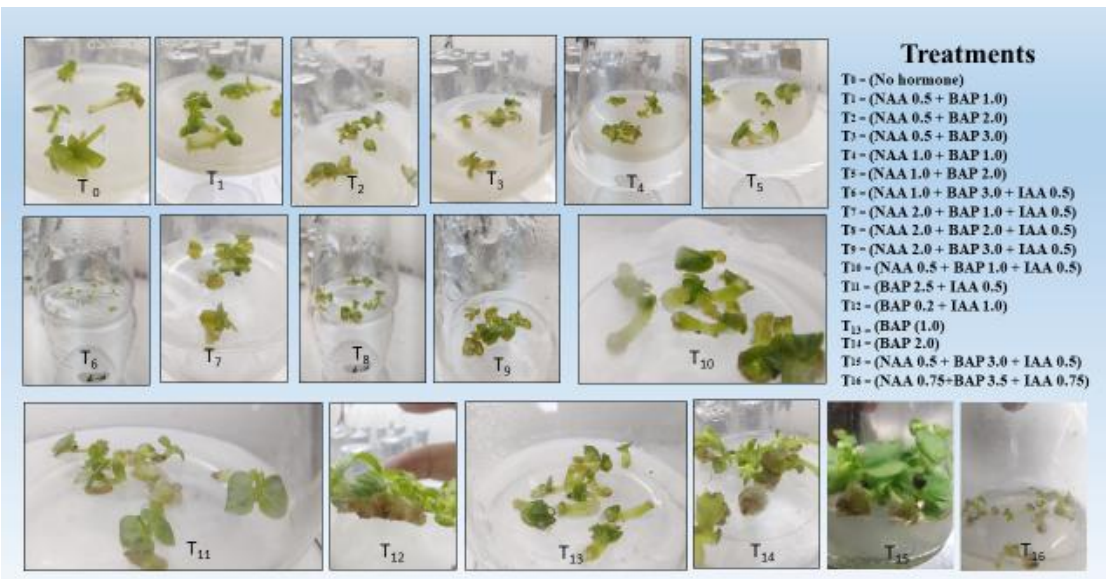

Fig 4: Callus from different treatments (after 2 weeks)

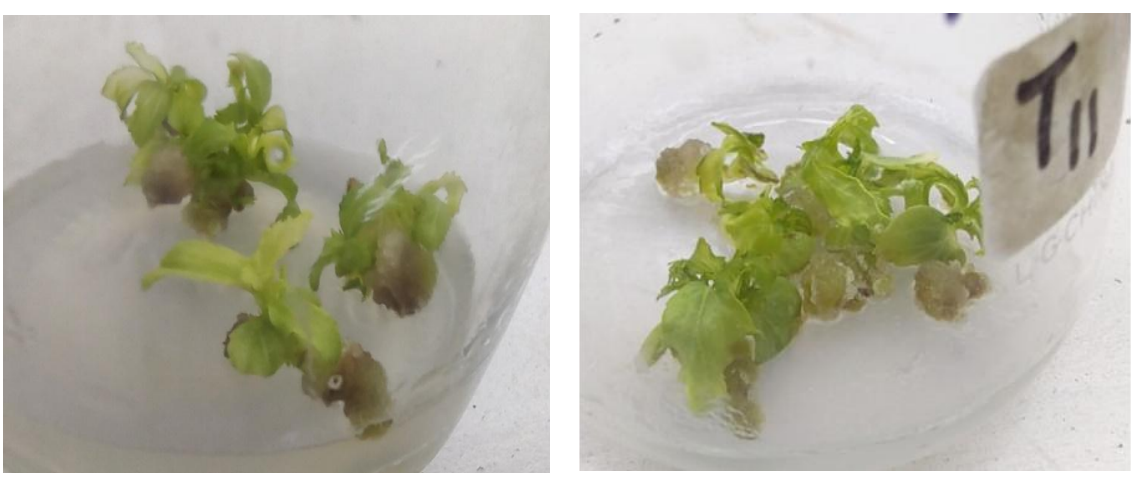

Fig 5: Shoot initiation from callus on MS medium with BAP 2.5 + IAA 0.5 (T_{11}) after 30 days

Table 1. Effect of growth regulators on callus induction from shoot tip explants

MS with hormone treatment	Days to callus initiation	Days to callus induction	Size of Callus (mm)	Weight of callus (g)	Color And Type
No hormone (Control)	-	-	-	-	-
NAA 0.5 + BAP 1.0	-	-	-	-	-
NAA 0.5 + BAP 2.0	-	-	-	-	-
NAA 0.5 + BAP 3.0	-	-	-	-	-
NAA 1.0 + BAP 1.0	-	-	-	-	-
NAA 1.0 + BAP 2.0	-	-	-	-	-
NAA 1.0 + BAP 3.0 + IAA 0.5	-	-	-	-	-
NAA 2.0 + BAP 1.0 + IAA 0.5	-	-	-	-	-
NAA 2.0 + BAP 2.0 + IAA 0.5	-	-	-	-	-
NAA 2.0 + BAP 3.0 + IAA 0.5	-	-	-	-	-
NAA 0.5 + BAP 1.0 + IAA 0.5	-	-	-	-	-
BAP 2.5 + IAA 0.5	14.2	24.3	3.4	0.10-0.20	Green, friable
BAP 0.2 + IAA 1.0	14.6	24.8	2.2	0.01-0.10	Green, friable
BAP 1.0	-	-	-	-	-
BAP 2.0	12.2	20.4	4.2	0.20-0.30	Green, friable
NAA 0.5 + BAP 3.0 + IAA 0.5	15.0	23.4	3.0	0.01-0.10	Green, friable
NAA 0.75+BAP 3.5 + IAA 0.75	15.8	22.2	2.2	0.10-0.20	Green, friable
BAP 3.0 + IAA 0.5	-	-	-	-	-
BAP 2.5+IAA 0.5+OM abstract	-	-	-	-	-
BAP 2.0 + OM abstract	-	-	-	-	-

Table 2: Effect of growth regulators on shoot regeneration from callus

MS media with hormone	Days to Shoot initiation	Size of shoot	Number of shoots
BAP 2.5 + IAA 0.5	30	1.0-1.50 cm	1-2
BAP 0.2 + IAA 1.0	-	-	-
BAP 2.0	32	1.0-1.50 cm	1-2
BAP 2.5+IAA 0.5+OM abstract	-	-	-
BAP 2.0 + OM abstract	-	-	-
IBA 0.5 + BAP 0.2			
IAA 0.5 + BAP 2.5	-	-	-
NAA 0.5 + BAP 2.5	-	-	-
OM 100ml/l (Potato + Banana)	-	-	-
OM 100ml/l (Coconut water)	-	-	-
No hormone (Control)	26	0.5-1.0	1-2

Conclusion

It was urgent need to develop a reproducible protocol for jute tissue culture. The present study was concluded that, the genotypes O-9897 of *C. olitorius* in MS media supplemented with 2.0 mg/L BAP could be used for callus induction and shoot regeneration. Its need to develop rooting medium for successful plant regeneration also need to acclimatization of plantlets.

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