Regeneration of plants from hypocotyl derived callus tissue of jute (C*orchorus olitorius* L. var. JRO-632)

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ABSTRACT

The present paper deals with the induction and regeneration of callus tissue from in vitro system of jute (Corchrous olitorius L. Var JRO-632). Hypocotyl of in vitro germinated seedlings was taken as explants. Hypocotyl explants were cultured in both Murashige and Skoog's (1962)(MS) and Schenk and Hildebrand's (1972)(SH) basal media supplemented with different growth regulators in definite concentrations. Callus growth was found better in MS basal medium than SH medium. For morphogenetic study the hypocotyl callus tissues were grown in medium containing different combinations and concentrations of auxins (IAA, NAA, IBA and 2,4-D used separately @ 0.20 mg/L) and cytokinins (BAP and Kinetin used separately 0.15mg/K to 3.2 mg/L, coconut milk 10-35% volume/volume). A simplified method for morphogenesis of calli was standardized. Cytological studies of the regenerated roots from calli as well as from regenerated plants revealed diploid chromosome number while the calli were mixoploid in nature. It was observed that the balance in the concentration of auxin-cytokinin (in initial culture) as well as ploidy level in the cells is most important-factors in controlling differentiation.

KeyWords: Chromosome, hypocotyl, morphogenesis, and regeneration.

Application of tissue culture techniques for rapid multiplication and inducing variation in plant materials has added a new dimension in recent years. However, potential exploitation of these methods for improvement of jute (*Corchorus olitorius* L. and *Corchorus capsularies* 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 interspecific hybridization which has been a long cherished goal of the jute breeders. The object of the present work therefore has been to induce callus, roots and shoots from root cells under cultural condition and to investigate their chromosomal and cellular nature.

MATERIALS AND METHODS

Seeds of jute (*Corchorus olitorius* L. cv. JRO-632) were washed with 0.5% aqueous teepol solution for 15 minutes, surface sterilized by 0.1% mercuric chloride solution for 5 minutes, rinsed 4-5 minutes with autoclaved sterilized distilled water. The seeds were then aseptically placed on 30 ml semi-solid nutrient White's (1963) medium in which no hormone was added. The seeds germinated within 2-3 days. The hypocotyl was dissected from10-16 days old seedlings and were cut into small pieces. Each piece was then placed aseptically on 20 ml semi-solid nutrient medium. Both the Murashige and Skoog's(1962) and Schenk and Hildebrand's(1972) media were tried with various combinations of different auxins(NAA,IAA,IBA and 2,4-D used separately 0.1 mg/L to 0.5 mg/L) and cytokines(BAP,Kinetin used separately 0.1mg/L to 1.0mg/L,coconut milk 10-35% V/V).In other sets either auxins or cytokinins with coconut milk was used. The pH of the media was adjusted to 5.6-5.8,solidified with 0.5% agar-agar (Bacteriological grade, BDH) and sterilized for 15 minutes at 1.05-kg/cm $^{(2)}$ pressure. The cultures were grown at ±1°C with at 55-60% relative humidity under Philips Fluorescent day light tubes emitting 32 x 10 ^{8} μ moles $sec^{-1}m^{-2}$ for 16 hours light and 8 hours dark period. The callus tissues were sub cultured at intervals of 4 weeks into fresh media. Growth index of the callus was calculated on every seventh day.

$$\text{Growth index} = \frac{\text{Final weight-Initial weight}}{\text{Initial weight}}$$

For cytological studies the callus tissue was pretreated in saturated aqueous solution of Para-dichlorobenzene (PDB) for 3 hours at 10-14 ˚C, fixed in Carney's solution (6 Ethanol: 3 Chloroform: 1 Acetic Acid) for 1 hour, hydrolyzed in 1(N) HCL at 60ºC for 10 minutes and stained in 2% propiono-orcein for 4 hours. Histology of the callus tissue was studied from the same squashed preparation for chromosome analysis.

RESULTS AND DISCUSSION

Ms basal medium supplemented with a mixture of vitamins and growth regulators (0.30 mg/L NAA and 0.30mg/L Kn) was found to be better than SH medium for the induction of callus tissue. Swelling of the explants were observed 9-15 days and attained a length of 0.7cm to 0.12 cm by this time. 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. All the culture tubes showed full callusing within this period. The rate of growth of the callus tissue was

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very rapid. The calli were soft, loose and friable. Some cultures also showed root formation from the callus tissue within this period. Rhizogenesis was observed at both the cut ends or any portion of the callus tissue. The nature of the rooting was normal similar to that obtained in *in vivo* condition. The co lour of the callus tissue was yellowish to greenish. It was noted that for rapid production and development of root callus tissue an essential balance of auxins and cytokinins in the culture media was needed. Green nodule formation was found in the callus tissue and these nodules showed the occurrence of Xylogenesis manifested by tracheidal cells. Rhizogenesis was a common feature of this callus tissue. The rootless loose and friable callus with rapid growth was also obtained in the MS basal medium when supplemented with cytokinins or with 10% coconut milk. The growth index showed that in the fourth week of sub culturing growth of the callus was very high.

Table 1: Rate of callus growth of hypocotyl derived callus tissue of jute (*Corchorus olitorius* L. var. JRO-632)

Days in culture	7	14	21	28	35	42	49	56	63	70
Growth index	0.2	0.33	0.86	2.6	3.9	4.7	4.9	5.6	5.9	6.5

Chromosome analysis revealed diploid as well as mixoploid nature and a wide range of chromosomal variation in the 4-5 weeks old callus tissue with predominance of diploid cells. The divisional frequency declined with the increasing age of the callus tissue and there was a positive relationship between age of the callus tissue and the frequency of chromosomal variation.

The hormonal composition of the media was changed for organogenesis from the callus tissue after fourth subculture. NAA was replaced by IBA with Kinetin.Xylogenesis manifested by tracheidal cells was prominent. The chromosome preparation showed the diploid number (2n=14) predominantly during the first subculturing. After first subculture binucleate cells were observed. It was recorded that with increased period of culturing frequency of diploid cells decreased with gradual increase in the number of polyploid cells. It was recorded that the inner regions of the callus which is comparatively older showed high chromosome number as compared to younger superficial part. The occurrence of such irregularities in callus tissue grown for longer period and /or specific nutrients have been reported from time to time by Sunderland (1973) and D'Amato (1977).

Rhizogenesis of the callus tissue was frequently observed with normal rooting having both longitudinal growth as well as lateral branching. When the kinetin concentration was gradually increased keeping the IBA concentration at constant level embryoids formation started in the callus tissue. For shoot regeneration pieces of callus tissue after sixth subculture were placed on MS basal media containing IBA, Kinetin and incubated at the same temperature with 16 hours light daily from a Fluorescent lamp. When MS basal medium was supplemented with 0.30mg/L IBA and 1.65mg Kinetin/L shoot tips were obtained with leaves and roots within 30 days from the greenest part of the callus tissue. In all other combinations the callus tissue formed green nodules only but no shoot tip formation was noticed. Chromosome study from leaf tip of the regenerated plants showed diploid chromosome number (2n =14).

The present investigation, therefore, indicates that for cellular differentiation an optimal level of IBA along with all other ingredients in the media is necessary. There is a regression in cytodifferentiation with lowering of IBA level. The shoots are regenerating from the diploid cells although callus tissue shows the mixoploid nature having both diploid and polyploid cells tissue shows the mixoploid nature having both diploid and polyploid cells. This conforms to earlier findings by De and Roy (1981). The predominantly diploid nature of the roots differmtiated from a missed cell population of the callus with different ploidy levels confirms further the greater potentiality of the diploid cells for regeneration by Banerjee and Sharma (1981).

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