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MICROPROPAGATION OF *SOLANUM VIARUM* DUNAL THROUGH COTYLEDONARY NODE, SHOOT TIP AND NODAL CULTURES

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Cultures of cotyledonary node, shoot apex and nodal segments were raised on Murashige and Skoog's medium supplemented with various auxins and cytokinins. Multiple shoots were initiated in the presence of 6-Benzylaminopurine and kinetin at different concentrations. A large number of plantlets were obtained by rooting these shoots on 1/2 strength Murashige and Skoog's medium supplemented with 3-Indolebutyric acid. After a brief period of acclimatization, these plantlets were successfully transferred to the soil. Cytological analysis of both normal and regenerated plantlets were made.

Keywords: Cotyledonary node; Culture; Micropropagation; Node; Shoot tip; *Solanum viarum.*

Introduction

Solanum viarum Dunal is one of the important medicinal plants cultivated mainly for its berries which contain the glucoalkaloid solasodine. Perusal of literature reveals that most of the *in vitro* studies in the *Solanum* species, which are known to contain the alkaloids, have been restricted to determining the presence and amount of the steroidal alkaloids in the cultures[1-3]. Less attention was paid for either clonal propagation or to induce genetic variation. Hence, the present investigation is an attempt to induce multiple shoots from cotyledonary node, shoot tip and nodal cultures through *in vitro* techniques.

Materials and Methods

Aseptic seedlings were raised on filter paper bridges in test tubes from the surface sterilized seeds. The cotyledonary nodal segments along with cotyledons were obtained from aseptic seedlings, whereas the shoot tips of about 0.2-0.3 cms and nodal segments of about 1 cm were excised from field grown plants. Surface sterilization of field grown explants was done with freshly prepared chlorine water for 15 minutes and followed by 0.1% mercuric chloride for 5 minutes. After each treatment the explants were thoroughly rinsed 3-4 times with sterile distilled water. The explants (both from field grown and aseptic seedlings) were inoculated on MS medium [4] containing 2% sucrose and gelled with 0.8% bacteriological agar. Different growth regulators were supplemented at various concentrations. The pH of the medium was adjusted to 5.6-5.8 before autoclaving at 103.41 kps. for 15 minutes. The cultures were illuminated with white fluorescent light at 1200-1400 lux for 16 hours at 25 ± 2^0C. All the experiments were repeated thrice using 12 replicates per treatment. Number of shoots developed from the cultures in each treatment was recorded after 30 days of culture.

For the study of somatic chromosomes, the root tips were pre-treated with Colchicine (0.5%) for 2 hours and aceto-orcein squashes were prepared following Tjio and Levan technique[5]. For histological studies, bits of cultures were periodically fixed in FAA (Formalin-Acetic Acid-Alcohol) for 24 hours and dehydrated through ethanol-butanol series. Customary paraffin technique was followed and sections were cut at 10-12 µm thickness and stained with Heidenhain's haematoxylin and counterstained with erythrosine.

Results and Discussion

Cotyledonary nodal cultures: When the explants were cultured on basal MS medium, there was no response. But on a medium supplemented with auxins like IAA, IBA, NAA and 2, 4-D callusing of the explants was noticed. A promotory effect of an auxin on inducing callus from different explants of various species is well established[6]. However, multiple shoots were formed from the axils of the cotyledons on MS medium containing BAP (2 mg/1) (Fig.1) or Kin (10 mg/1) (Table1). Similar observations were made by Gulati and Pawan[7] in *Vigna radiata.* In succeeding weeks direct shoot bud formation was observed even from the margins of the cotyledonary leaves (Fig. 2). The multiple shoots thus obtained were periodically subcultured onto fresh medium of similar combinations. The capacity to form multiple shoots was retained even after 8-10 subcultures. During repeated subcultures, the cut ends of the main shoots which remained in the medium after excision of grown up shoots also produced multiple shoots (Fig. 3). At higher concentrations of BAP (10 mg/1) and lower concentrations of Kin (0.2 mg/1), only a few shoot buds were initiated but failed to develop further.

Shoot tip cultures: As in cotyledonary nodal cultures, the shoot tips did not show any response on basal medium. However, in several legumes, the growth of the shoot apex was observed on basal medium[8-10]. When basal medium was supplemented with various auxins at different concentrations the explants showed varied results. IAA (0.5 mg/1) induced little callus formation at the base with poor shoot growth, whereas NAA (0.5 mg/1) resulted in vigorous growth of the shoot tip accompanied by callusing and root initiation at the base. While 2, 4-D (0.5 mg/1) suppressed the shoot tip growth but promoted callusing and induction of thick roots from the callus. However, at higher concentrations of auxins (4 mg/1) shoot tips callused without any growth. The provision of an auxin in the culture medium for the shoot tip growth varies with the species[11-12]. Cytokinins generally stimulate the precocious development of otherwise inhibited axillary meristems. When BAP and Kin were added singly to MS medium, shoot growth was observed depending on the concentrations. BAP at 0.5-2 mg/1 (Fig. 4) and Kin at 2-10 mg/1 induced shoot growth along with multiple shoots with negligible callus at the base (Table 1). This is in contrast to the previous reports made by Chaturvedi and Sinha[13] in *Solanum khasianum* where only callusing has been observed on a medium containing BAP. Smith and Murashinge[12] in *Coleus bluemi* observed that exogenous Kin inhibited the normal development of meristem. However, they stated that extremely low concentrations (0.0003-0.001 mg/1) may be beneficial. Although small quantities of cytokinin may be synthesised by shoots *in vitro*[14], roots are the principle sites for cytokinin biosynthesis. Hence, addition of exogenous cytokinin is essential for shoot tip growth. Even the combination of auxins and cytokinins are favourable for the initiation of multiple shoots and complete shoot formation in several systems[15]. Similar observations were made in the present investigation. It is well established that a proper ratio of cytokinin and auxin is necessary for morphogenesis leading to the development of a complete plant[6].

Nodal cultures: On basal medium, there was no growth of the axillary bud. Though the presence of IAA, IBA and NAA (0.5 mg/1) in

Table 1. Morphogenetic responses of cotyledonary nodes, shoot tips and axillary buds of *Solanum viarum* Dunal to different concentrations of auxins and cytokinins.

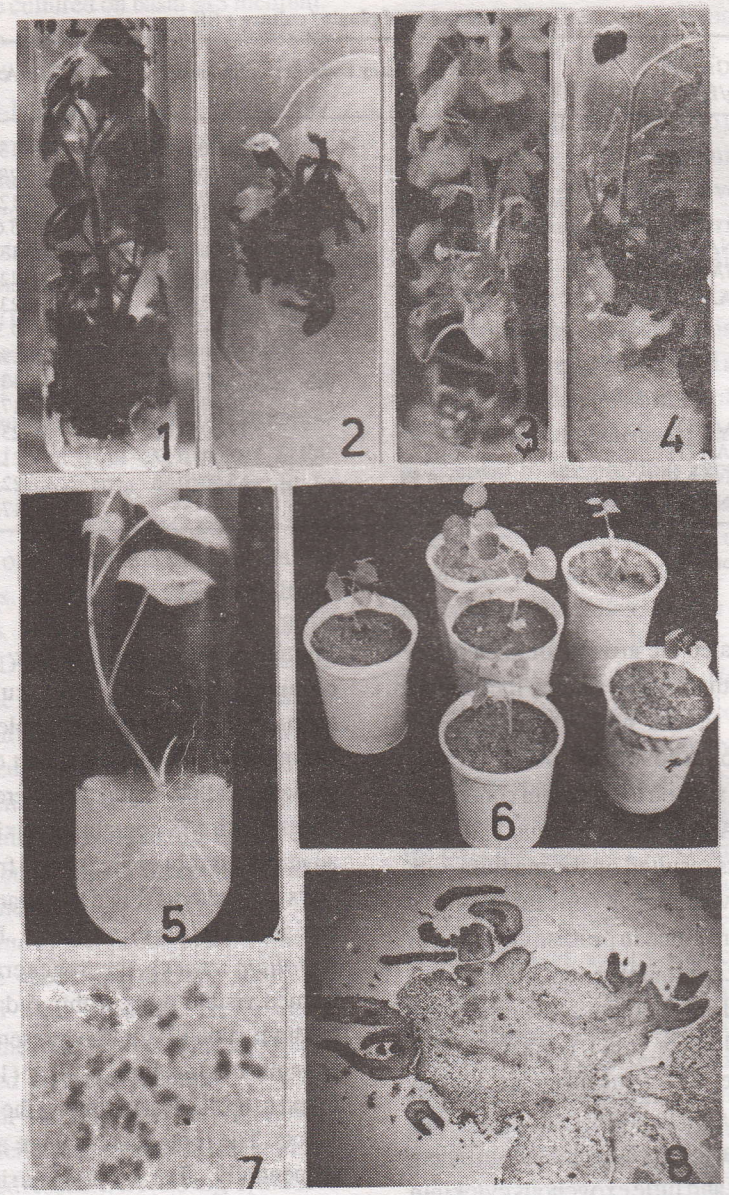
Medium [MS+Growth regulators (mg/1)]	Mean number of plants / culture ± SE		
	Cotyledonary node	Shoot tip	Axillary bud
MS+BAP (0.5)	8.00±0.4	23.00±0.57	13.00±0.66
MS+BAP (2)	28.30±0.46	25.60±0.73	28.00±0.57
MS+BAP (5)	15.66±0.5	12.30±0.50	12.30±0.6
MS+BAP (10)	6.00±0.4	6.49±0.5	5.60±0.5
MS+BAP (1) +NAA (0.1)	5.00±0.4	13.00±0.4	13.60±0.5
MS+BAP (2)+NAA (1)	8.30±0.8	20.33±0.6	22.00±0.57
MS+BAP (2)+IAA (1)	9.00±0.57	21.33±0.49	23.33±0.58
MS+ KIN (0.5)	-	8.30±0.8	9.30±0.5
MS+KIN (2)	9.60±0.6	20.60±0.31	19.60±0.51
MS+KIN (5)	15.00±0.57	21.00±0.66	13.60±0.5
MS+KIN (10)	29.33±0.43	16.00±0.4	17.30±0.6
MS+KIN (2)+IAA (1)	9.33±0.84	19.60±4.68	23.60±0.5
MS+KIN (2)+NAA (1)	6.60±0.3	21.00±0.57	21.33±0.49
MS+KIN (0.1)+NAA (0.1)	7.60±0.58	15.60±0.58	22.00±0.57
MS+KIN (0.1)+NAA (0.1)		-	17.00±0.57

No. of Replications- 3

medium initiated the growth of the axillary bud along with multiple shoots, the amount of callusing at the nodal region varied depending on the auxin used. Scanty callusing was seen on the medium containing IAA and IBA whereas vigorous callusing along the roots resulted on the medium with NAA. However, higher concentrations of all the auxins resulted in profuse callusing of the explants. Large number of multiple shoots were observed on a medium containing either BAP or Kin alone or with IAA/NAA (Table 1). Presence of cytokinins at higher concentrations led to profuse callusing. This may be true that the explants are self sufficient for cytokinins and little exogenous cytokinin may act as a trigger to initiate development. Similar observations were made in the nodal culture of apple root stock by Hicks and Nair[16].

Rooting of regenerated shoots: Shoots thus obtained from the above cultures were inoculated on MS medium supplemented with auxins at lmg/1. The basal part of the shoots tended to callus. When they were cultured on 1/2 strength MS containing auxins at lmg/1, adventitious roots were formed from the basal parts of the shoots (Fig.5) Similar observation was made in *Musa tectius*[17]. However, in *Trifolium repens* the regenerated shoots formed visible roots within 6 days on basal medium alone[18]. In the present work 1/2 strength MS medium + IBA (1 mg/1) was found to be the best in rooting the regenerated shoots. The rooted plants were acclimatized sequentially, first by transferring them to sterile distilled water and then to tap water. Finally they were transferred to soil (Fig.6).

Cytological studies: Somatic metaphase plates of normal plants obtained from germinated seeds as well as regenerated plantlets from



Figs. 1-8: Fig. 1 - Multiple shoots from the axils of the cotyledons on MS + BAP (2 mg/1) ; 2 - Multiple shoots from the margins of the cotyledons on MS + BAP (2 mg/1); 3 - Multiple shoots from cut ends of shoots on MS + BAP (2 mg/1) ; 4 - Growth of shoot tip and multiple shoots on MS + BAP (2 mg /1); 5 - Profuse rooting at the base of the regenerated shoot on 1/2 MS+ IBA (1 mg/1); 6 - Regenerated plants; 7 - Somatic metaphase plate of regenerated plant -2n=24 x 1500; 8 - Initiation of multiple shoots from axillary meristems.

the above cultures showed 24 chromosomes in each (Fig. 7) which conforms to the earlier reports of 2n=24 in normal plants[19]. This observation agrees with the fact that there will not be any change in the genome of the regenerated plants which are raised through clonal propagation[20].

Histological studies: Sections of bits of explants from shoot tip and nodal cultures showed the growth of the axillary buds in addition to the main shoot, thus resulting in multiple shoots. In nodal cultures, apart from the axillary meristems, the lateral meristems are formed all around the nodal region, thus increasing the number of shoot buds (Fig. 8).

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