Ontogenesis of \textit{in vitro} shoot bud proliferation in \textit{Solanum sarrachoides} Sendt.

N BANERJEE, S MUKHOPADHYAY and A K SHARMA  
Centre of Advanced Study in Cell and Chromosome Research, Department of Botany,  
University of Calcutta, 35, Ballygunge Circular Road, Calcutta 700 019, India  
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Abstract. \textit{In vitro} direct induction of multiple shoot buds of \textit{Solanum sarrachoides} Sendt. was obtained from leaf segments using Murashige and Skoog’s basal medium supplemented with 6-\textgamma-\textgamma-(dimethylallylamino) purine (1.5–2.5 mg l$^{-1}$). Maximum shoot bud proliferation was observed in the medium containing 2 mg l$^{-1}$ and better growth of plants with well defined roots, leaves, flowers and fruits in the subsequent stages of development. Histological examination at different stages of development confirmed that the multiple buds produced on excised leaf segments arise adventitiously through the formation of numerous tiny protuberances. The complete sequence of events leading to multiple bud formation based on histological studies has been presented.

Keywords. \textit{Solanum sarrachoides} Sendt.; histogenesis; micropropagation; organogenesis

1. Introduction

\textit{Solanum sarrachoides} Sendt., an annual weedy herbaceous plant, is widely distributed in south America and north western parts of the United States. It synthesizes the alkaloid solasodine, used for commercial production of steroid pharmaceuticals (Mann 1978; Schilling 1984). Unlike other solanaceous species seed setting is inadequate in this species. Therefore, for multiplication of this newly introduced species which might serve as an alternative source of solasodine in this country, it would be worthwhile to attempt clonal propagation using \textit{in vitro} techniques.

Although a considerable amount of research on direct regeneration of plantlets from leaf segments has been done (Zenkteller 1972; Coleman and Greyson 1977; Bhatt et al 1979), reports on \textit{in vitro} propagation of \textit{S. sarrachoides} either through organogenesis or embryogenesis are meagre (Banerjee et al 1985).

The present communication reports direct regeneration of plants through the formation of adventitious shoot buds on excised leaf segments of \textit{S. sarrachoides}. Detailed histological study of the sequence of events leading to the generation of multiple shoot buds on the excised leaf segments are presented.

2. Materials and methods

\textit{S. sarrachoides} plants were kindly provided by the Department of Biology, West Washington University, USA. The plants were maintained in the glasshouse at Calcutta.

Excised leaf segments were used for \textit{in vitro} studies. Leaves were first surface disinfected with a 0.1% HgCl$_2$ solution for 10 min and washed 3–4 times in sterile distilled water. Leaves were then cut into 1–2 cm$^2$ pieces for use as explants.
The explants were cultured on MS basal medium (Murashige and Skoog 1962) containing 3% sucrose, 0-0% agar. The pH was adjusted to 5-8 prior to autoclaving at 120°C for 15 min. For optimum growth and plantlet regeneration, the basal medium was supplemented with different concentrations and combinations of various auxins and cytokinins.

All the cultures were incubated at 22 ± 2°C under a 16 h photoperiod of 2500 lux light intensity. Four to six weeks after culture initiation, the regenerated shoots were transferred to a medium containing 2 mg l⁻¹ IAA.

For histological analysis, suitable tissue pieces with tiny shoot buds at different stages of development were fixed in FAA (90 ml of 70% ethanol, 5 ml of formaldehyde and 5 ml of glacial acetic acid) and dehydrated gradually in a tertiary-butyl-alcohol series. The tissue was then embedded in paraplast and microtome sections 12 μm thick were cut. Sections were stained in tannic acid-ferric chloride and safranin (Jensen 1962) and made permanent with euparol.

3. Results and discussion

3.1 Micropropagation through adventitious bud formation

After 10–15 days the leaf segment explants in the MS basal medium supplemented with 2iP (1.5–2.5 mg l⁻¹) turned slightly brown. By 3–4 weeks, these explants showed swelling and produced a large number of green nodular protuberances on and around the mid-rib region; particularly near the parts partially submerged in the medium (figure 1). After further culture (6–8 weeks) in the same medium, vegetative shoot buds developed on the upper part of the protuberances. At this stage, rapid multiplication of shoot buds by meristem proliferation was observed with concomitant suppression of shoot elongation as well as leaf expansion (figure 2). For each concentration of 2iP 10 culture tubes were maintained, each inoculated with an excised leaf segment. The average number of shoot buds ± SE per explant was calculated from 5 replicate tubes (table 1). Shoot and leaf development were observed when the small shoot buds were mechanically separated and cultured on the MS medium containing IAA (2 mg l⁻¹). In this medium, shoots gradually developed extensive root systems and often developed flower buds after the shoots reached a minimum height of 3–4 cm (figure 3). Of the regenerated rooted plantlets, 30 healthy plantlets were selected and transplanted in soil to test their survival. Out of 30 plants, 22 (73%) survived.

Among the other hormonal combinations tried in the present investigation, profuse callusing was noted from the leaf segments after 6–8 weeks in the medium containing NAA (2 mg l⁻¹) and BAP (1 mg l⁻¹). The calli grew well and became friable when transferred to a medium containing IAA (1.8 mg l⁻¹) and BAP (2.25 mg l⁻¹). The callus tissue failed to regenerate shoots.

The present study reveals that to obtain adventitious regeneration of plants from excised leaf segments, 2iP (2 mg l⁻¹) is most effective for induction of shoot primordia in S. sarrachoides. In Solanum curtilobum Malliku, a similar response to 2iP was reported (Westcott et al 1979). In Solanum nigrum, S. khasianum and S. dulcamara, BAP was used as the sole hormone with or without IAA to induce shoot buds (Zenkteller 1972; Bhatt et al 1979).
In vitro propagation of S. sarrachoides

Figures 1-3. 1. Development of small nodular protuberances from leaf segments. 2. Regeneration of multiple shoot buds. 3. A complete plant showing well defined roots, leaves and flowers.

Table 1. Average number of shoot buds per explant regenerated in response to different levels of 2iP.

<table>
<thead>
<tr>
<th>2iP (mg l⁻¹)</th>
<th>Mean number of shoot buds explant ± SE</th>
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<tbody>
<tr>
<td>1.5</td>
<td>600 ± 0.45</td>
</tr>
<tr>
<td>2.0</td>
<td>790 ± 1.00</td>
</tr>
<tr>
<td>2.5</td>
<td>470 ± 1.73</td>
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In the present investigation, the initial shoot buds appeared on the globular protrusions originating very near the tissue medium interface. It has been postulated that the physiological gradient of materials in the tissue determines the loci at which the meristemoids are initiated (Ross and Thorpe 1973). The present findings are consistent with this hypothesis since the initial meristematic centres developed in tissue regions closest to the morphogenetic substances in the medium.

3.2 Origin and development of multiple shoot buds

Earlier studies (Thorpe and Murashige 1970; Ross et al 1973) had indicated that the key histogenic events leading to shoot formation involved 2–3 week-old cultures. Therefore, the sequence of events occurring during direct regeneration of multiple shoot buds from leaf segments were studied from 3-, 6- and 8-week-old tissues in
On the basis of histomorphological observations, the entire process could be divided into 4 stages. In two weeks old culture, there was merely a mass of

![Figure 4. Histological representation of the origin and sequential development of adventitious shoot buds. A. Formation of meristematic cell layer in the periphery of protuberance (× 100). B. Appearance of ridges and lobes of compact meristematic cells (× 125). C. Development of shoot meristems from each of the meristematic lobe (× 100). D and E. Further development of shoot meristem showing leaf primordia, vascular strands and glandular hairs (× 100). F. Single shoot bud with well differentiated leaves (× 125). (S. Shoot apex; L. leaf primordia; H. hair; V. vascular strands).]
In vitro propagation of *S. sarrachoides*

parenchyma cells without any sign of cellular differentiation. The earliest histological event that could be related to shoot formation was the appearance of small nodular protuberances after 2–3 weeks on the lower surface of the explants. These nodular structures histologically revealed parenchymatous cell masses with no vascular differentiation.

In the first stage of development, the formation of relatively uniform meristematic cell files was noted throughout the periphery of each protuberance (figure 4A). These bands of meristematic cells were formed as a result of periclinal division and elongation of the cells. The cells of this region were typically small, tightly packed with dense cytoplasm having large nuclei, and with one or two conspicuous nucleoli. In comparison, most of the cells of the underlying inner zone were large and parenchymatous. The presence of large nuclei with one or two nucleoli in the densely cytoplasmic meristematic cells probably implies a high metabolic activity of cells in this region.

The second stage in the sequence of shoot bud proliferation was the appearance of ridges and lobes of compact meristematic cells on the upper surface of the meristematic zone of each nodule leading to an undulated appearance (figure 4B). In the third stage, small protuberances appeared on the undulated surface of the meristematic zone, each of which ultimately gave rise to a shoot primordium (figure 4C). Differentiation of short filamentous glandular hairs could be detected on these newly formed shoot primordia. Lastly, at the fourth stage, there was clear development of leaf primordia on the flanks of each shoot primordium (figure 4D, E). Thus a series of shoot buds differentiated on the surface of each of these meristematic protuberance. At this stage, distinct vascular strands were visible at the base of each primordium connecting the primordium with the rest of the parenchymatous inner tissue (figure 4F).

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