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Plant regeneration from callus derived from *Solanum laciniatum* mesophyll protoplasts

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Abstract. Protoplasts were isolated enzymatically from leaf mesophyll tissue of a medicinally important plant *Solanum laciniatum* Ait. and were cultured in agarified Murashige and Skoog's (MS) medium, supplemented with 2,4-dichlorophenoxyacetic acid (1 mg/1) and kinetin (1 mg/l). Callus tissue was obtained from protoplasts within one month. Plant regeneration from the protoplast derived callus tissue was achieved in MS medium supplemented with 2.25 mg/l Benzyladenine and 0.2 mg/l Indole butyric acid.

Key words: Medicinal plant; Protoplast culture; Regeneration; *Solanum*.

Introduction

The important medicinal plant *Solanum laciniatum* Ait. has been used as a commercial source of the steroidal alkaloid Solasodine which occurs as a glycoside in a mixture with other such alkaloidal glycosides and is found in the leaves and fruits. Solasodine is the nitrogen analogue of diosgenin and is equally acceptable as a raw material for diosgenin (Chatterjee, 1978). Corticosteroids and antifertility drugs may also be synthesized from Solasodine. The plant grows widely in lower altitude of Eastern Himalaya (India).

Tissue culture of this important medicinal plant has been done previously by some workers (Davis and Dale, 1979; Chandler and Dodds, 1983). In leaf derived callus cultures of *S. laciniatum*, Solasodine concentration increased up to 15% by the manipulation of macronutrients in the culture medium (Chandler and Dodds, 1983). Considering the potential usefulness of tissue culture methodology in this material, the present investigation has been undertaken to regenerate intact plants from isolated leaf mesophyll protoplasts of *S. laciniatum* with a view to use this technique for the future improvement of this material.

Materials and Methods

The plant and seeds were collected from the different parts of the Eastern Himalayas (3000 ft. - 6000 ft.). The plants were then grown in our glass house at 24°C.

Conditioning of Plant Material

The young fifth and sixth leaves from the shoot tip of field grown *Solanum laciniatum* Ait. were surface sterilized in 10% commercial calcium hypochlorite solution for 10 min and then washed three times in sterile distilled water. Under sterile conditions, 1 cm^2 leaf segments were cut and placed onto solidified Murashige and Skoog's (MS) medium (1962) supplemented with 1.7 mg/1 2-isopentyladenine (2i PA). The explants were incubated in light (16 h, 3000 lux) at 22°C for regeneration of plantlets directly from leaf explant.

Isolation and Culture of Leaf Mesophyll Protoplasts

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Leaves from two months old conditioned plants were used as explant for protoplast isolation. The peeling of epidermis from the leaf in this material is not very easy. So under sterile condition, the leaf lamina was cut into thin strips with a fine scalpel. These thin leaf segments were immediately placed in liquid cell and protoplast washing medium with 13% mannitol (CPW 13M) for 1 h. The CPW contains KH_2PO_4 27.2 mg/l, KNO_3 101.0 mg/l, $CaCl_2 \cdot 2H_2O$ 1480.0 mg/l, $MgSO_4 \cdot 7H_2O$ 246.0 mg/l, KI 0.16 mg/l, $CuSO_4 \cdot 5H_2O$ 0.025 mg/l at pH 5.8. CPW 13M was then replaced by filter sterilized enzyme solution containing 3% Meicelase and 0.5% Macerozyme in CPW 13M. After overnight incubation (about 14 h) the enzyme solution was gently replaced by CPW 13M without disturbing the digested leaves. The digested leaves were then squeezed and the whole suspension was then passed through 100 μ and 60 μ stainless steel mesh filter to remove larger debris. The filtrate was centrifuged at 100 g for 10 min. The supernatant was pipetted off and the pellet was resuspended in CPW with 21% sucrose (CPW 21S). It was again centrifuged at 200 g for 5 min. The viable and debris free intact protoplasts formed a dark green thick band at the top surface of CPW 21S medium. The green band containing protoplasts was taken out carefully and was washed twice in CPW 13M. Finally, the protoplasts were suspended in 10 ml liquid MS medium containing 1 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D), 1 mg/l Kinetin (Kn), 1% sucrose and 10% mannitol (10M).

Different densities of protoplasts such as 1×10^4, 5×10^4 and 1×10^5/ml were plated in solid MS 10M medium containing 2,4-D (1 mg/l), Kn (1 mg/l) and 1% sucrose. The cultures were then incubated at 25°C with an illumination of about 1000 lux (16 h photoperiod). The plating efficiency (PE) was noted after 21 days. The plating efficiency of protoplasts derived from tissue sulture derived plants was found to be 40%, whereas in case of field grown plants it was 25%. Hence, this method of conditioning of the plant has a great potential value.

Shoot Initiation from Protoplast Derived Callus

For the initiation of shoots from the callus of protoplast origin (p-callus) three cytokinins [2 -isopentyladenine (2i PA), 6-Benzyladenine (BA) and zeatin] and three auxins [Indole butyric acid (IBA), Indole acetic acid (IAA) and α-napthaleneacetic acid (NAA)] were used in a number of combinations.

Results and Discussion

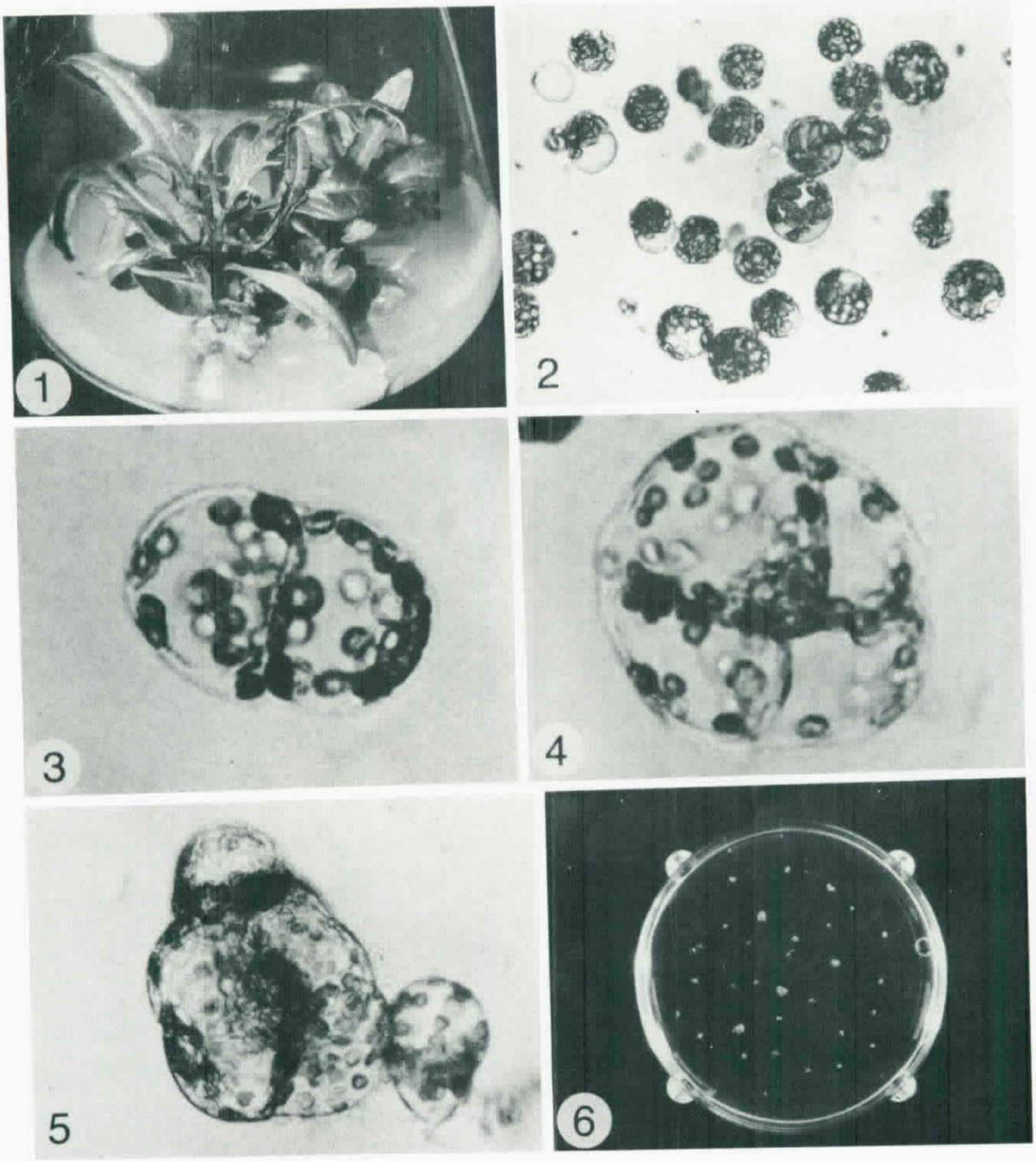
From the leaf segments of field grown plants in MS medium containing 2i PA (1.5 mg/l), numerous shoot buds were developed after 2-3 weeks of incubation. The shoot buds developed into plantlets (Fig. 1) within five weeks. The individual plantlets were transferred aseptically in hormone free MS medium and were maintained as stock source of explant for protoplast culture.

The yield of isolated protoplasts (Fig. 2) from leaf mesophyll tissue of *S. laciniatum* was 5×10^5 protoplasts/ml. The first division of protoplast (Fig. 3) was observed after three days of plating. The second division (Fig. 4) was noted after 5-6 days. The third division (Fig. 5) occurred after 11 days and a multicellular colony was formed at 16-18 days. The plating efficiency noted after 21 days was 50-60%. Of all the cell densities tried, 5×10^4 protoplasts/ml showed best growth. The isolation of protoplast in *S. laciniatum* was reported by Serraf *et al.* (1988) which was cited in Roest and Gilissen (1989). But it is noted that the reproducibility of the protoplast is more when cultured from tissue culture derived plants growing in the test tube. Serraf *et al.* (1988) noted only the production of shoot buds in the calli.

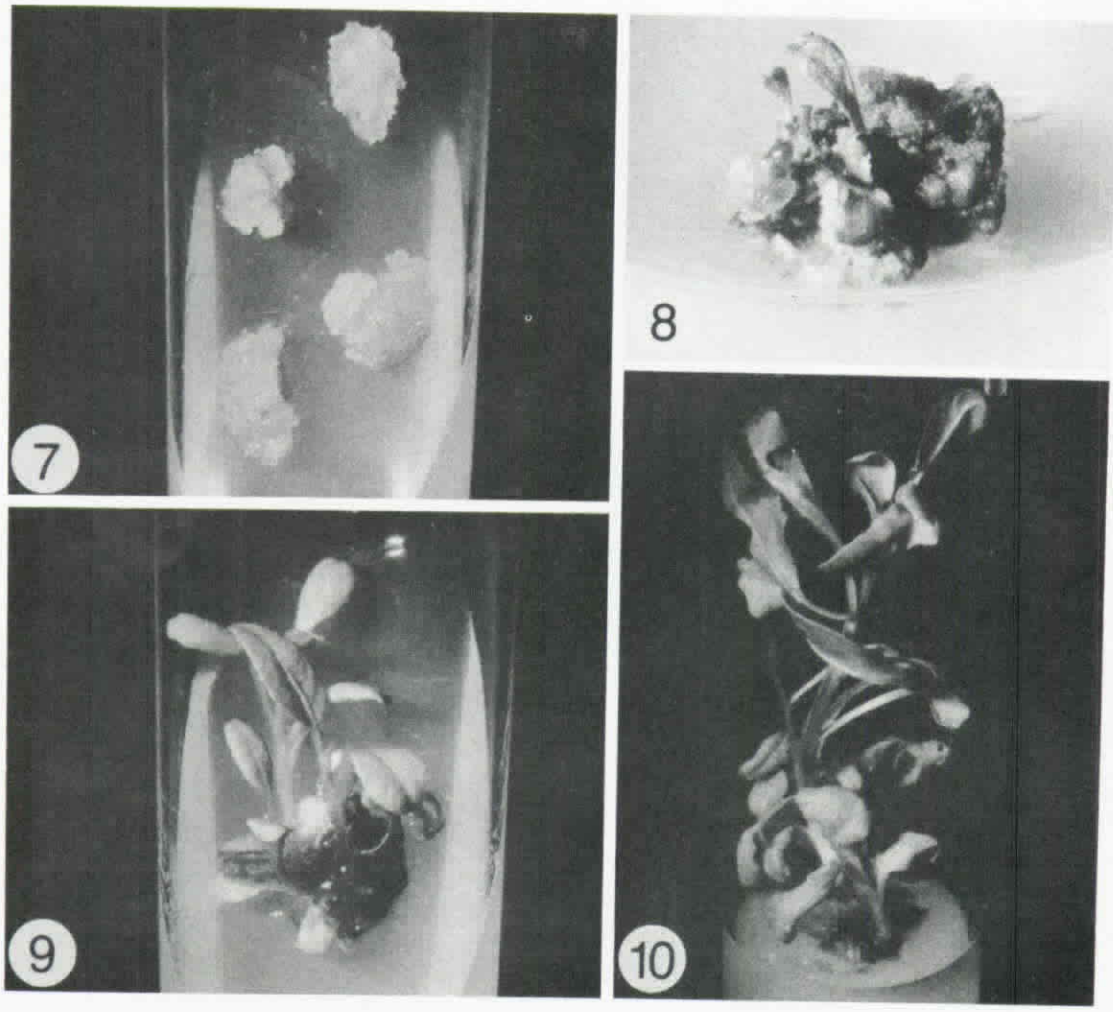
Numerous small pin-head shaped white calli (Fig. 6) were found to develop within one month. These calli were subcultured in the same nutrient medium without mannitol and the amount of sucrose was increased from 1% to 3%. In this medium, calli grew rapidly. In the second subculture after 4 weeks callus mass (Fig. 7) was transferred to the shoot initiating media.

After 15 days in this media, minute green dots appeared on the surface of the callus tissue. These green dots ultimately gave rise to shoot buds (Fig. 8) and subsequently shoots (Figs. 9 and 10) were formed.

Shoot initiating media containing high concentration of cytokinins (2.25 mg/l BA or 2 mg/l 2i PA or 2 mg/l zeatin) alone and in combination with low concentration of IBA (0.2 mg/l) or NAA (0.2 mg/l) supported shoot initiation in p-calli. The combination of auxin and kinetin or only cytokinin initiated shoots in the calli while auxin alone did not show any shoot initiation. MS medium supplemented with BA (2.25 mg/l) and IBA (0.2 mg/l) was found to be suitable for the ini-



Figs. 1–6. Fig. 1. Plantlets from leaf segments. Fig. 2. Protoplasts isolated from mesophyll tissue (×2500). Figs. 3–5. First second and third divisions of protoplast, respectively (×4000). Fig. 6. Small pin head shaped calli from protoplasts.



Figs. 7–10. Fig. 7. Masses of calli after second subculture. Fig. 8. Shoot buds from calli. Fig. 9. Development of shoots. Fig. 10. Mature plantlets.

tiation of shoots.

Regenerated shoots were transformed to root forming plantlets by incubating the shootlets in hormone free medium under light conditions for 4–6 weeks.

The results show that callus can be initiated in MS medium containing 1 mg/l of 2,4-D and Kinetin. But for reproducible regeneration of plantlets, 2,4-D should be omitted from the media after the initiation of calli. In the present investigation it has been noted that either cytokinin alone or in combination with auxins are essential for the initiation of shoots. Another interesting finding is that 2,4-D should be replaced by IAA or NAA in the media to obtain shoots in the calli. Again, the leaves from tissue culture derived plants showed more regeneration (50%) than from field grown plants (27%). Similar change has also been reported by Mroginski and Kartha (1981) in shoot initiation of calli in *Stylosanthes quianensis*. Both hormones should be omitted for root production.

This reproducible method of protoplast isolation, culture and plant regeneration of *Solanum laciniatum* can be used for inducing high reproducibility in other plants.

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鳥茄 (*Solanum laciniatum*) 葉肉細胞原生質體之培養與植株再生

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利用酵素處理，自重要藥用植物鳥茄 (*Solanum laciniatum*) 的葉肉組織分離出原生質體，並將之培養於添加 2,4-二苯氧醋酸 (1 mg/l) 和活動素 (1 mg/l) 的 MS 固體培養基上。由原生質體起源之癒傷組織在一個月內形成。此癒傷組織可在添加苯腺嘌呤 (2.25 mg/l) 和吲哚丁酸 (0.2 mg/l) 的 MS 培養基上獲得再生植株。