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## Organogenesis in *Saccharum officinarum* L. variety Co 740

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The sterilized explants of sugarcane variety Co 740 dissected under aseptic conditions were cultured onto semi solid modified MS medium and incubated for callusing. Callus pieces were inoculated onto the same medium without 2,4-D for differentiation. The shoots of 6-7 cm height transferred for rooting onto basal half strength MS liquid medium. 4 to 9 roots of 5-8 cm length obtained in 7 to 10 days. Calluses were obtained in 18 days from inoculation, then differentiated into shoots in 22 days. The shoots grew in 40-44 days and subcultured shoots rooted in 7 to 10 days. The complete plants have been regenerated through organogenesis and cane matured in 11 months.

SUGARCANE (*Saccharum officinarum* L.) is a major agricultural crop in tropical and subtropical regions of the world and an important export product in many developing countries. These countries account for 70% of the sugar production from sugarcane. The complete plants of sugarcane variety Co 740 have been regenerated through organogenesis from the callus tissues obtained from very young expanding scaly

leaves. When transferred to soil the regenerated plants grew satisfactorily and looked morphologically normal and canes matured in 11 months.

Being a cash crop, sugarcane has profound influence on the economy of the sugarcane-growing area directly and indirectly. Proper use of seed material with particular reference to newly released and notified varieties of sugarcane has an important vital role in increasing the yields of sugarcane and its industrial products. Rapid multiplication of the identified varieties and large coverage of sugarcane-growing area are possible only through successful transfer of micro-propagated plants to field.

Very young expanding scaly leaves of *Saccharum officinarum* L. variety Co 740 were dissected from the tip shoot of 5-month-old plant. The upper leaves were removed one by one by inserting the scalpel vertically. Care was taken to ensure that no inner young scaly leaves are injured. The material was rinsed with Savlon and surface-sterilized with 70% absolute alcohol and 0.05% mercuric chloride for 10 minutes followed by 3 times washing with autoclaved sterilized water each separately. The initial material thus got ready for dissecting the explants. The explants (0.4 to 0.5 cm<sup>2</sup>) were dissected under aseptic condition in laminar flow transfer cabinet and were cultured onto semisolid modified Murashige and Skoog medium<sup>1-4</sup> containing 100 mg l<sup>-1</sup> myoinositol, 3 mg l<sup>-1</sup> 2, 4-D (dichlorophenoxyacetic acid) and 10% v/v coconut milk. The pH of the medium was adjusted to 5.8. All culture vessels were incubated in dark for 24 h continuously at 26° ± 1°C temperature in BOD incubator for callusing. The callus pieces were inoculated onto the same medium but excluding 2,4-D for differentiation. The culture was kept in fluorescent light for 16h alternated with 8h dark cycle. The shoots of 6-7cm height were transferred for rooting into basal half strength MS liquid medium containing 5mg l<sup>-1</sup> Na<sub>2</sub>Fe EDTA<sup>4</sup>. Roots were obtained in 7 to 10 days after subculturing. There were 4 to 9 roots of 5 to 8 cm length per plant.

The calluses were obtained onto the MS medium described earlier in 18 days from the date of inoculation of the explant. The phytohormone 2,4-D was found very effective in callus formation. The calluses were differentiated into shoots in 22 days onto MS medium containing no 2,4-D. The shoots of 6-7 cm height grew in 40 to 44 days after inoculation. When subcultured, the shoots were rooted in 7 to 10 days. The half strength basal MS liquid medium with 5 mg l<sup>-1</sup> Na<sub>2</sub> Fe EDTA was found effective for rooting. The regenerated plants were hardened successfully in bamboo basket, earthen pots, plastic bags, etc. The medium containing sterilized soil, and vermiculite mixture in 1:1:1 proportion was found best for plants survival. 15 to 20 days were required for hardening. When transferred, the well-rooted plants completed 70-

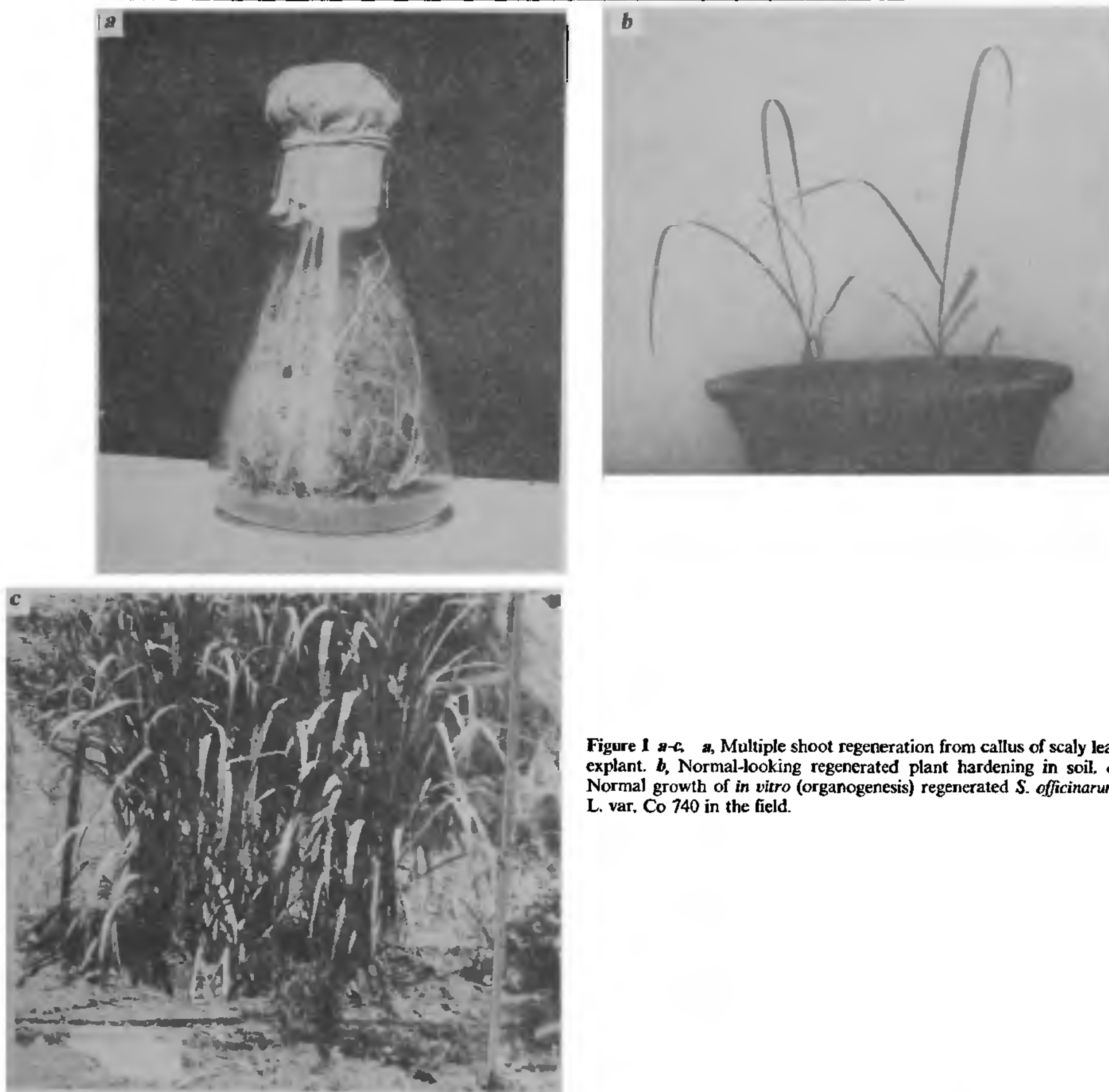


Figure 1 a-c. a, Multiple shoot regeneration from callus of scaly leaf explant. b, Normal-looking regenerated plant hardening in soil. c, Normal growth of *in vitro* (organogenesis) regenerated *S. officinarum* L. var. Co 740 in the field.

day *in vitro* cycle. Plants of 15–20 cm height grew successfully and satisfactorily in the field. The cane materials thus obtained are being used for further field breeding experiments.

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