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Organogenesis of Diploid *Asparagus racemosus* through Callus Culture

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Development of organized structures from the callus of diploid *Asparagus racemosus* was studied. Roots were formed on MS medium with NAA and zeatin. Shoot buds differentiated in the medium supplemented with IAA and BAP. Histological investigations of organization were made. Cytology of the regenerated plants revealed the diploid constitution. The importance of organogenesis in the propagation of this medicinal plant is pointed out.

Key Words: *Asparagus racemosus*, **Callus culture**, *In vitro* **propagation, Organogenesis**

Introduction

An indigenous medicinal plant of the Liliaceae, *Asparagus racemosus* Willd., is of much interest on account of its sapogenin content which is used as precursor of many pharmacologically active steroids. The plant is perennial and usually propagated by seeds. Although any one plant can be lifted and divided to produce several new plants, but this is a very slow and laborious technique. The present investigation is aimed to develop an effective method for rapid propagation cellular totipotency, an inherent characteristic of plants, may be advantageously employed for the differentiation and regeneration of excised tissues and organs. Regeneration has been recorded for edible European species viz., *A. officinalis* by several authors (Takatori et al. 1968, Wilmar & Hellendoorn 1968, Steward & Mapes 1971, Harada 1973, Bui Dang Ha et al. 1975, Reuther 1977).

Materials and Methods

The plants were collected from a local nursery (Calcutta) and grown in the Experimental Garden of the Department of Botany, University of Calcutta.

Tissue culture: A callus was induced on the shoot segments of *A. racemosus* following the method reported previously (Kar & Sen 1982). Fresh callus after second subculture (30 days for each subculture) as well as the callus

which was eight months old were used as inoculum for differentiation. Pieces of callus weighing 600±30 mg were aseptically inoculated into the culture tubes each containing 20 ml solidified medium of Murashige and Skoog (1962) supplemented with growth hormones (2, 4-D, NAA, IAA, Kn, BAP, Z) to induce organogenesis. *p*H of the medium was adjusted to 5.6–5.8 and autoclaved. The cultures were kept at 22±1°C with 16/8 hr light/dark period. Light of 2000 lux was provided by Philips fluorescent lamps.

Histology: For histological studies the callus was fixed in FAA and passed through tertiary butyl alcohol—paraffin series (Johansen 1940). Microtome sections were cut at a thickness of 12μ. Tannic acid and iron chloride were used for staining (Foster 1934).

Cytology: Root tips and shoot tips of about 35 regenerated plants were treated in saturated solution of para-dichlorobenzene and aesculine mixture (2 : 1) for 3 hr followed by overnight fixation in 1 : 3 acetic ethanol. Aceto-orcein/N HCl was used for staining. Over 100 well-scattered metaphase plates were analysed for studying chromosomes.

Results

A callus was formed in 80–90% of the shoot segments (out of 25 cultures tested and repeated twice) 8–12 days after planting on MS supplemented with 2, 4-D (1ppm) + Kn (1 ppm) (figure 1). The callus turned greenish white, compact and grew slowly when transferred to media suitable for root and shoot differentiation. A histological study of the callus showed meristemoids consisting of small and densely stained cells (figure 2).

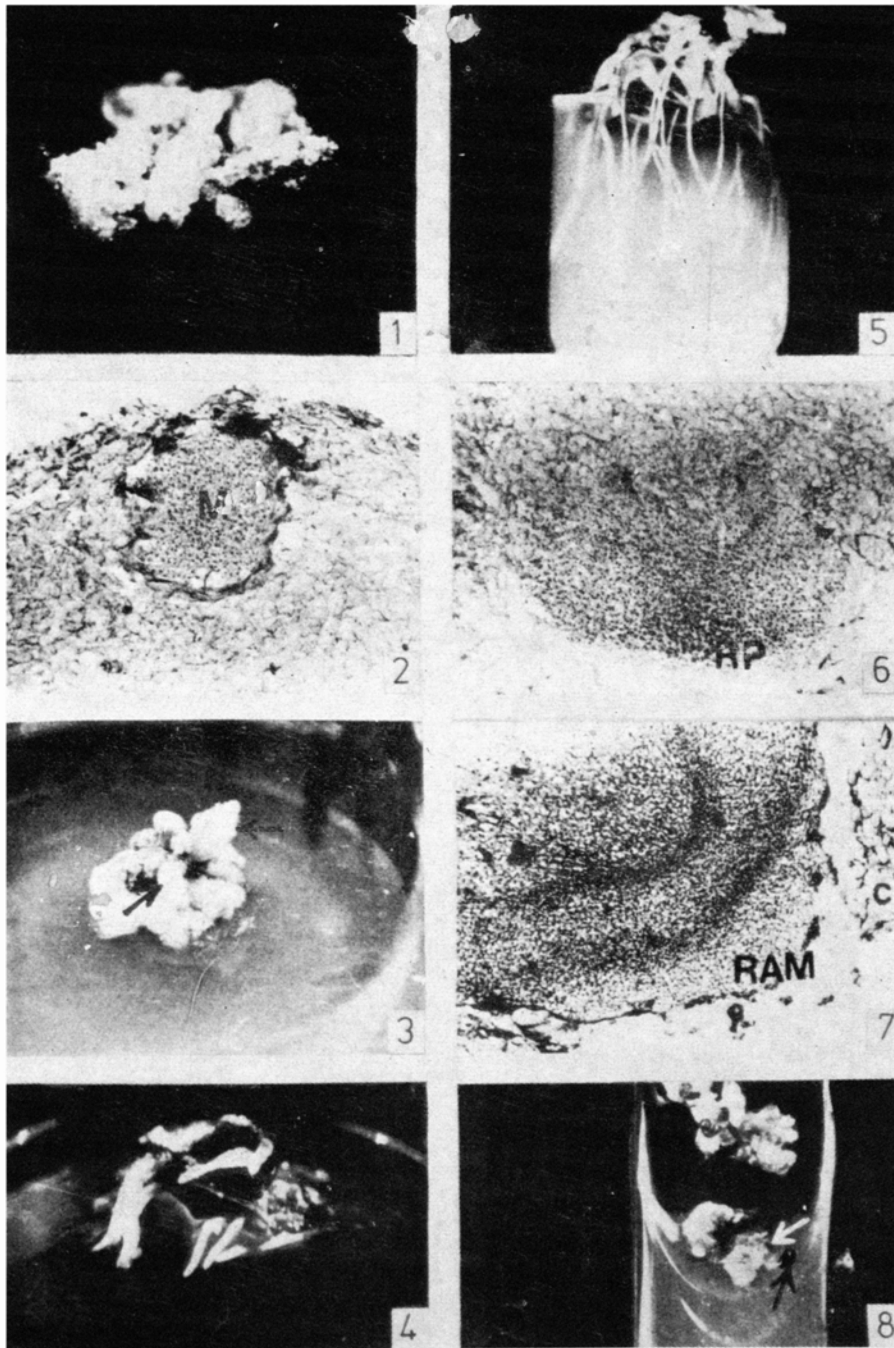
Root formation from callus: Roots were formed after 20–25 days from the upper as well as lower surface of the callus (figures 3,4) on medium containing NAA (0.2 mg/l) + zeatin (0.2 mg/l). Rooting occurred in about 60% of the callus cultures (total number of samples tested was 40), with an average of 6–10 root initials per explant. The latter attained a length of 2–3 cm in two months (figure 5). The roots were white, sometimes green when young but on maturity became slightly brown. Formation of lateral roots was also noted.

Vertical sections of the callus showed that the root primordium originated in the callus tissue as a group of small, densely protoplasmic, meristematic cells which later characteristically organised into root apical meristem (figure 6). Eventually the root primordia emerged from the callus (figure 7).

Shood bud differentiation: After 30–45 days of inoculation green shoot bud formation (figure 8) was noted on the upper surface of the callus. Complete regeneration of plants (figure 9) was recorded in callus on MS + IAA (0.5 mg/l) + BAP (1 mg/l). Each explant produced 4–8 shoot buds which grew up to a length of 4–7 cm within three months (figure 10). To avoid dedifferentiation, transfer of the differentiated callus after 3 months to MS medium with only 0.5 mg/l BAP was necessary. The callus was then transferred to plain MS medium in the next subculture. Gradually roots developed from lower portion of the shoots in this medium. The regenerated plants with normally branched cladodes (figure 11) were morphologically similar to the plants growing in the field.

A histological study showed the development of shoot apical meristems (figure 12) from callus, the basal portions of which were parenchymatous and embedded in the callus. The shoot apical meristem was ensheathed by a pair of young scale leaves (figures 13, 14).

In the eight months old callus cultures the frequency of shoot bud development was rather low and most of the regenerating plants were morphologically abnormal and



Figures 1–8, 1, Callus from shoot segments; **2,** Section of callus showing a meristemoid (M); **3,** Root formation from the upper surface of callus; **4,** Root formation from the lower surface of callus; **5,** Luxuriant growth of roots in a 2-month old callus; **6,** Section of callus showing root primordia (RP); **7,** Emergence of root apical meristem (RAM) through the callus (C); **8,** Shoot bud differentiation on the surface of the callus

Table 1 *Regeneration capacity of two and eight months old callus cultures*

Age of the inoculated callus in months	Total no. of cultures tested	Shoot bud formation	No. of regenerated plants	
			Normal	Abnormal
2	80	36	33	3
8	68	7	1	6

failed to develop into mature plants *in situ* (table 1).

All the regenerated plants that appeared normal were diploid like the original plant (with $2n=20$ chromosomes) as ascertained in all the metaphase plates of root tips (figure 15) and shoot tips (figure 16).

Discussion

The objective of this work was to explore the possibility of rapid propagation of this medicinal plant *in vitro*. Though callus cultures were characterized by a state of variable ploidy (Kar & Sen 1982), all the regenerants showed diploid chromosome complement indicating high potentiality of diploid cells for regeneration as compared to the polyploids. The low percentage of regeneration potentiality in old subcultured callus is probably due to high frequency of polyploidy. Torrey (1966) had also noted that totipotency of polyploid cells is drastically reduced causing a natural exclusion of genetically aberrant plants. We had noted instability of chromosome number and rapid increase in polyploid cells with increasing age of the callus (Kar & Sen 1982). The occurrence of variability in chromosome number in callus tissues grown for prolonged periods was reported by D'Amato (1977). The regeneration of polyploid plants, if possible, from callus tissues and rapid propagation may offer a possibility for augmentation and commercial exploitation of its medicinal principles.

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