SOMATIC EMBRYOS IN *ASPARAGUS COOPERI* BAKER

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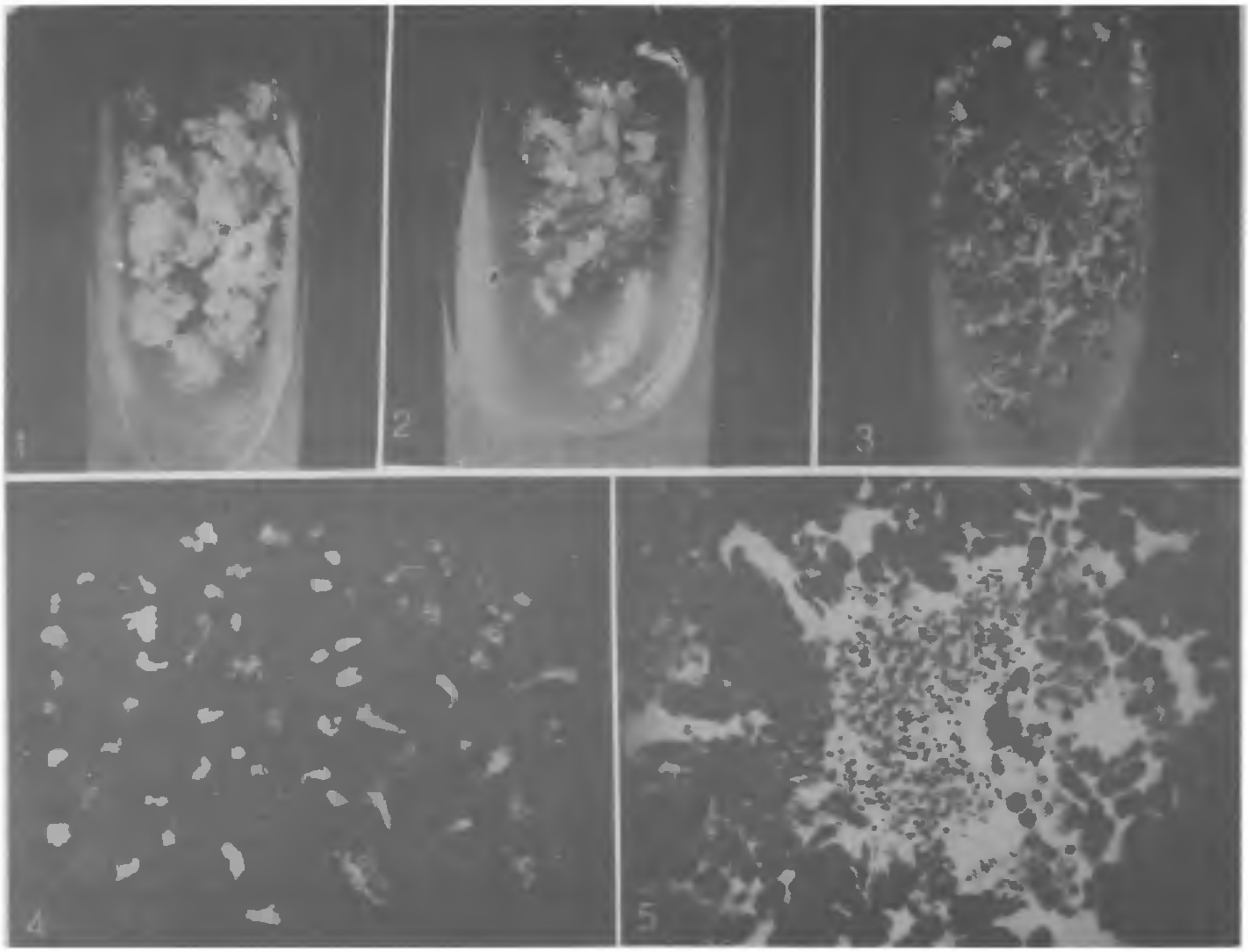
IN VITRO study in Liliaceous species has earlier been carried out[1–4]. However in the genus *Asparagus*, despite its horticultural and medicinal importance attempts towards *in vitro* propagation are meagre. In *Asparagus*, culture in artificial medium has been reported to be successful[1–3]. One of the important advantages of *in vitro* growth, is the scope of securing somatic embryos in well-defined medium. Such somatic embryos provide ideal materials for rapid propagation of stable regenerants. Moreover, they offer the possibility of securing genetic variants. In *Asparagus*, somatic embryogenesis so far has been reported in *A. officinalis*[1,5]. The present investigation deals with induction of somatic embryoids in *A. cooperi*.

Asparagus cooperi Baker was collected from Chandra Nursery, Sikkim and grown in the experimental garden of this university.

For explants, young shoots of 6–15 cm in length were cut into 2–5 mm segments which were washed with 5% Teepol for 6–8 min, followed by three washes with distilled water. They were then surface-sterilized with 0.1% mercuric chloride for 9–10 min and rinsed with sterile distilled water.

The basal medium used was Murashige and Skoog's[6] (MS) supplemented with 100 mg/l myoino-



Figures 1–5. **1.** Friable callus 120-days-old; **2.** Globular embroids (→) developing from friable calli; **3, 4.** Embryoids at different stages of development, and **5.** Squash preparation of embryogenic calli, showing small embryogenic cells surrounded by large non-embryogenic cells.

sitol, 0.5 mg/l nicotinic acid, 0.5 mg/l pyridoxin HCl, 1 mg/l thiamine HCl, 30,000 mg/l sucrose. Growth regulators 2,4-dichlorophenoxyacetic acid, α-naphthalene acetic acid (NAA) and kinetin (Kn) were added to the media in various concentrations according to the experimental objectives. After 120 days of culture, 1000 mg/l KNO_3 was added to the media. For solidification 7000 mg/l agar was added and the pH adjusted to 5.7.

The explants were placed in culture tubes each containing 20 ml medium. The cultures were kept under 16/8 h light/dark period at 25±2°C and 50–60% relative humidity. Subculturing was done after every 40 days.

The MS basal media containing 1 mg/l NAA+ 1 mg/l Kn. Within 15 days, the explants became swollen, expanded in size and turned yellowish brown. After 30 days of inoculation calli were initiated from cut ends and from adaxial surface of the explants. The initial callus was compact and smooth. Subculturing was done after 40 days when the full explant with the callus was transferred on the fresh medium. After 120 days, when the third subculturing was done, 1000 mg/l KNO_3 was added to the media.

After 15 days of the third subculture, callus became smooth, glossy, nodular and yellowish green. Gradually, globular nodule-like structures appeared on the calli (figures 1, 2). The nodules consisted of small spherical cells with thick cytoplasm and large nucleus from which embryos derived. Further, the development of these nodular structures gave rise to a cluster of embryoids of different stages such as globular, banana-like and torpedo-like (figures 3–5).

The present study demonstrate that somatic embryo can be induced from callus in *A. cooperi* following KNO_3 treatment as reported earlier[7,8]. Further study is in progress to secure large scale production of regenerants and to explore the possibility of securing variant, if any, often noted in regenerants from callus.

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ORGANOGENESIS IN ANTHER-DERIVED CALLUS CULTURE OF COWPEA [*VIGNA UNGUICULATA* (L.) WALP]

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THE culture of the whole anther and subsequent development of plants was successfully demonstrated with *Datura innoxia*[1]. Raising of anther callus as reported in cultured anthers of *Cajanus cajan*[2,3], *Phaseolus aureus*[4] and in *Winged bean*[5], and subsequent attempts for plant regeneration from the callus is a significant step towards legume improvement programme[6,7]. A similar attempt was made in *Vigna unguiculata*, an economically important drought-resistant food legume.

Flower buds of cowpea of different sizes were taken and anthers at several stages of development were excised and cultured. Callusing was observed only in young anthers containing thin-walled microspores at late uninucleate stage. Cultured anthers kept under weak light (250 lux) or continuous dark conditions for initial 20 days only callused while no callusing occurred in continuous light condition (250 lux). Further, callusing was obtained in the culture chamber at a relatively lower temperature (23 ± 1°C).

Kinetin or BAP in combination with IAA or IBA gave best results towards anther callusing. 1 mg/l Kn/BAP+0.5 mg/l IAA/IBA gave 85% response to anther callusing. A good response (50–60%) was also obtained on MS medium supplemented with 2 mg/l Kn/BAP+1 mg/l IAA/IBA. NAA and 2,4-D did not elicit any anther response when used in place of IAA/IBA. Cytokinins and auxins (0.5–5 mg/l) when used singly in the agar MS medium failed to induce anther callusing. Similarly