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INDUCED APOGAMY AND APOSPORY IN *ADIANTUM CAPILLUS - VENERIS* L.

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Cytokinins especially Kinetin (Kn), was found to shorten the biological life-cycle by 40 days in *Adiantum capillus-veneris*. Concommitantly its effect was also studied to induce apogamy and apospory for multiplication. Whereas, 10^{-5} M Kn with 1 or 2% sucrose induced apogamy, that with 7% sucrose induced apospory. The supra optimal concentration of sucrose seems to creat osmotic stress and hence simulate starvation condition. The callus was formed when 2,4-D, IAA or a mixture of 2,4-D and IAA was used. The results ascertain that this species is quite good for experiential studies.

Key words : Cytokinins, sucrose, Apogamy, Apospory.

In an earlier communication (Gupta and Bhambie, 1991), it was asserted that *Adiantum capillus-veneris* being a cosmopoliton species is a better material for experimental studies than *Ceratopteris richardii* which has restricted distribution (Hickok *et al.*, 1987). White (1979) has reviewed this aspect but as reports are still coming in, it has been thought proper to report important findings with Kinetin on the induction of apogamy and apospory in *Adiantum capillus-veneris*. In all eight growth regulators have been tried (Pdhyay and Mehta, 1981; Sheffield and Bell, 1981; Bir and Goyal, 1982; Hickok and Kiriluck, 1984; Sharma and Vangani, 1989; Kuriyama *et al.*, 1990).

MATERIAL AND METHODS

Fertile fronds of *Adiantum capillus-veneris* were collected locally and spores grown aseptically on Knop's medium to get gametophytes and sporophytes in 60-65 days (Bir and Anand, 1982). The aspectically developed gametophytes were inoculated in Knudson medium supplemented with Kinetin (Kn, 6-furfurylamino purine) 10^{-8}M to 10^{-4}M and 2% (w/v) sucrose. For apospory, injured broken, or surgically cut pieces of juvenile leaves, young rhizomes or petioles were inoculated in Knop's medium supplemented with Kinetin (10^{-4} to 10^{-8}M) and sucrose (Su) varying 1 to 8% (w/v). Redifferentiation of cultures was done on Knop's and Murashige and Skoog media (Gupta and Bhambie, 1991, 1992). All the cultures were incubated at 25 ± 2°C with continuous light of four ft. four fluorescent tubes.

OBSERVATIONS

Full-fledged gametophytes were inoculated in Knudson's medium Kn (10^{-4} to 10^{-8}M) and Su 1 to 2% (w/v). On the medium with 10^{-6}M Kn and 2% sucrose the gametophytes after 2-3 days developed thick pads of callus around their margins. Subsequently the callus pads were converted into an irregular mass consisting of numerous buds intermingled with unicellular hairs (Figs. 1, 4). Later on, these buds developed leafy appendages around them (Fig. 7). The buds were separated by surgical manipulations and when grown on basal medium, each gave rise to a new sporophytic plant (Fig. 7). A single gametophyte thus could produce a number of haploid apogamous sporophytes. A bud when observed in thick longitudinal section depicted a layer of columnar cells in between two leafy appendages. One of the central cell by oblique divisions gave rise to a pyramidal, triangular cell. A root bud has developed after 20 to 25 days after the onset of appendiges.

Injured, broken or cut pieces of juvenile leaves rhizomes and petioles were inoculated in Knops medium supplimented with Kn (10^{-5}M) and sucrose (7%). After 45 days in 30% cultures injured broken or cut pieces of juvenile leaves at the region of injury or cut started producing callus. These callus outgrowths after 20 days gave rise to numerous thick penetrating or prostrate gametophytes. These gametophytes varied in shape from cordate. strapshaped or dichotomously branched (Fig.6). Numerous gametophytes are thus produced from one cut piece of juvenile leaf.

Callus was obtained with 10^{-5}M, 10^{-5} MKn, 10^{-5}M, 10^{-6}M IAA (indole-3-acetic acid) 10^{-5}, 10^{-6}M, 2,4.D (2-4 dichlorophenoxy acetic acid) and 10^{-5} M Kn plus 10^{-5} M IAA along with 1 or 2% sucrose in Knudson medium (Figs. 8, 9). In some cases the callus got converted into gametophytes or was further rediffe-

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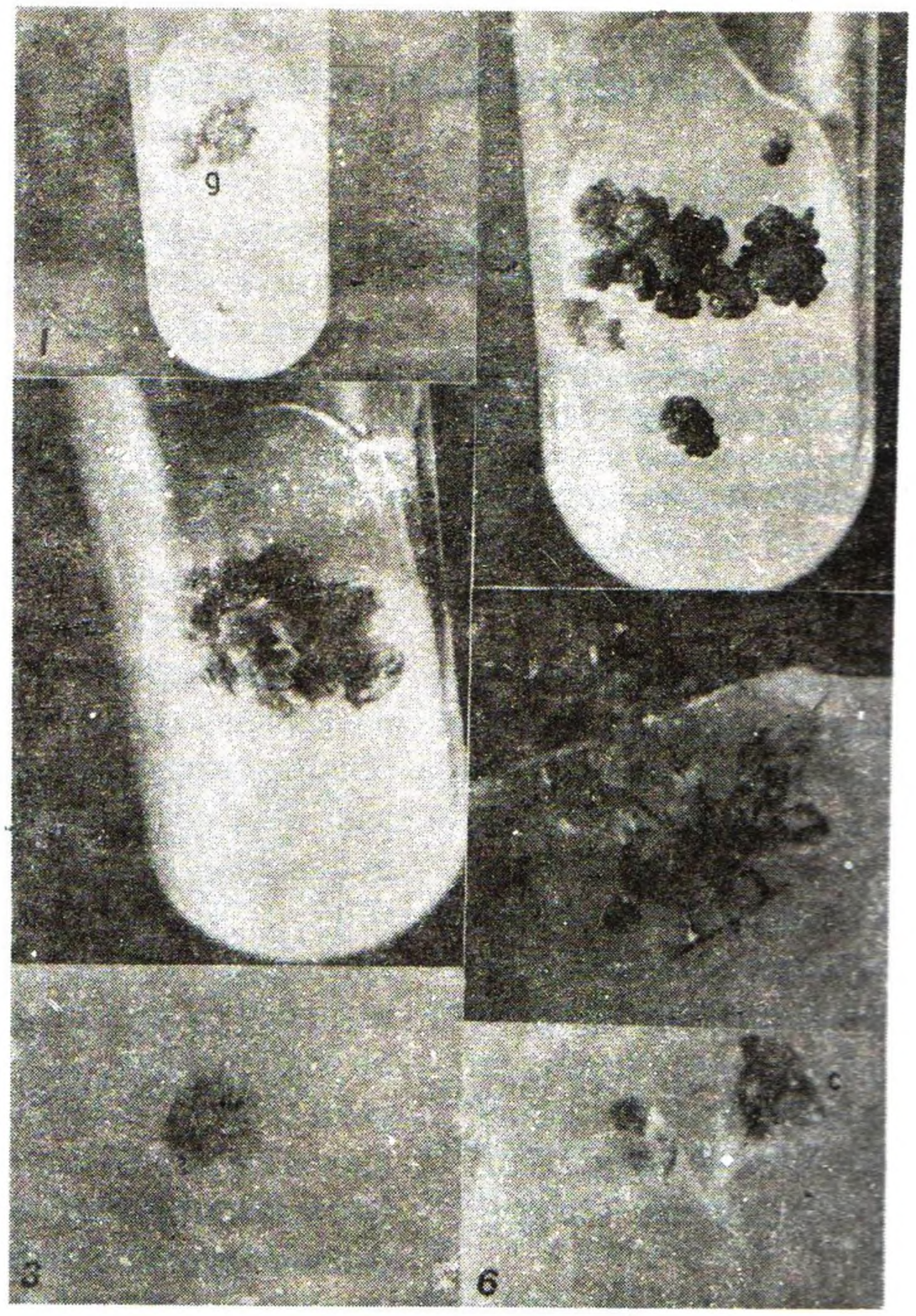

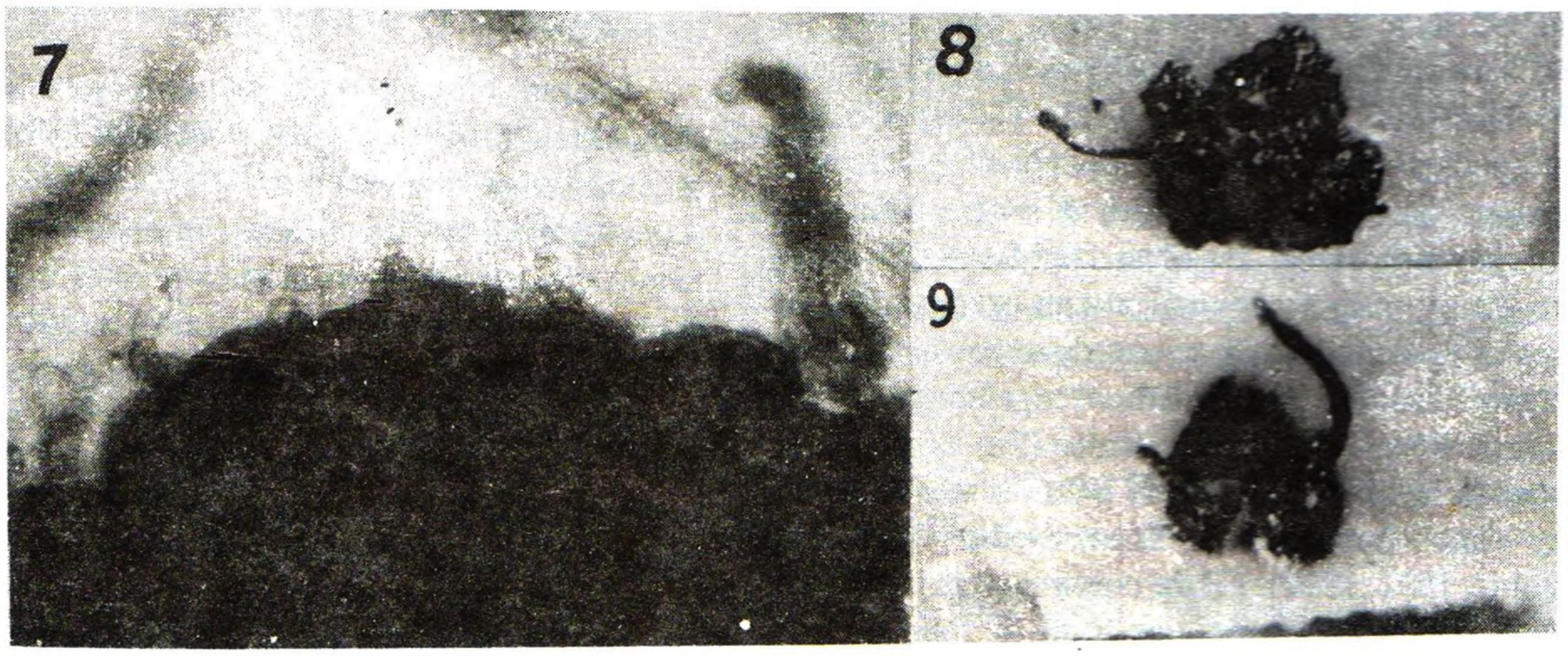

Figure 1&2 Differentiation of gametophytes from callus on kinetin supplemented Knudson medium. Fig. 3 Callus subcultured with 2,4-D showing profuse rhizoidal growth and differentiation of a few gametophytes. Fig. 4 Differentiation of green cells in the buds for further aporophytic germination in Kinetin on Knudson medium. Fig. 5 Regeneration of sporophyte from excised coiled leaf giving rise to numerous young leaves and a few gametophytes. Fig. 6 Regeneration of sporophyte from an exoised young leaf. Fig. 7 Showing various types of hairs and leafy appendages from an apogamous sporophyte in a thick 1. S. Fig. 8 &9 Subcultured callus with kinetin showing differentiation from petiole and rhizome pieces; note the occurrence of cylindrical leafy appendages in fig.9.

Table 1: Various media supplemented with sugars and growth regulators for studying callus initiation, apogamy and apospory

Medium	Concentration of sucrose (w/v)	Concentration of growth regulators (Molar)	Observations
Knudson	1%; 2%	10^{-5}M Kn 10^{-6}M Kn 10^{-5}M 2,4-D 10^{-5}M IAA	Gametophyte Vegetative/Reproduction Gametophyte
-do-	1%	No	Gametophyte Slow growth Gametophyte
-do-	2%	No	Gametophyte Vigorous growth Gametophyte
-do-	2%	10^{-6}M Kn	Gametophyte Apogamously sporophyte
-do-	15	10^{-5}M 2,4-D	Gametophyte--Callus
-do-	1%	10^{-5}M 2,4-D	Callus--Initially profuse rhizoids—Later on few gametophytes
-do-	1%	10^{-5}m Kn	Callus——Callus—Gametophytes
-do-	2%	10^{-5}M Kn+10^{-5}m IAA	Rhizome pieces —Callus (having budlike structures)
-do-	1%	10^{-5}M 2,4-D	Petiole pieces—Callus
Knops	1 to 10%	10^{-5}, 10^{-6}M Kn;2, 4-D (10^{-5})	Best results in 7% sucrose
-do-	7%	10^{-5}M Kn	Injured broken or cut pieces of lamina-gametophytes aposporously- normal or strapshped or dichotomously branched-sex organs-sporophytes.
Kundson	7%	-do-	-do-
M S	7%	-do-	-do-

rentiated (Fig. 6). However, in 2,4-D it initially produced gametophytes having profuse simple, basal bulbous shaped or branched rhizoids around it and later on formed a few gameto phytes (*see* Table 1, Fig. 5). In some cases a sporophyte was developed from young juvenile leaf after 40 days or so (*see* Table 1; Fig.6).

DISCUSSION

Kinetin has been reported to enhance apogamous development of sporophytes in *Equisetum arvense* and *Pteridium aquilinum* (Pdhyay and Mehta 1981; Kuriyama *et al.*, 1990; Sheffield and Bell, 1981). It has also been reported to induce apospory from cut pieces of juvenile leaves in *Pteridium aquilinum*. Present work thus corroborates the earlier work of Sheffield and Bell, (1981) and Sheffield (1985).

Apogamy and apospory have been explained by 'Gane Block Hypothesis' i.e., four gene blocks are said to be responsible for the differentiation of four parts viz. stem leaf, root and gametophyte. Each gene has subunits. Functioning of each subunit depends on the switching on and off of the major gene block. (Mehta, 1979). However, the occurrence of subunits of a gene has been questioned by some workers, (Sattler, 1988; Gupta and Bhambie, 1992). These gene block counted the activation of sporophytic gene and the concurrent supression of the gametophytic genes and vice versa. Usually starved condition are considered to be responsible for apospory. However, in present studies 7% sucrose and 10^{-6} M Kn have given the best results which may be partially due to osmotic stress and hence simulate the starvation conditions i.e., a type of stress. The concentration of sucrose is supra-opitmal and therefore, acting negatively for apospory. In certain cases metabolic and morphogenetic effect of sucrose has not been found to be of fundamental importance in inducing apospory (Sheffield and Bell, 1981).It is well known that concentration of metabolites is also very important for experimental designing of tissue culture work (White, 1979).

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