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Bud Induction with Cytokinin[1]

A LOCAL RESPONSE TO LOCAL APPLICATION

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ABSTRACT

A portion of the surface of detached *Graptopetalum paraquayense* E. Walther leaves can be used to assay small amounts of reagents in lanolin for their ability to induce shoots only at the site of application. The cytokinins benzyladenine, kinetin, and 6-(γ,γ-dimethylallylamino)purine (DMAAP) were tested, and DMAAP was most effective in bud induction at concentrations below 1%. The higher the hormone concentration, the sooner the appearance of leaf primordia and the higher the ultimate yield of buds. Leaves treated with DMAAP for 2 days developed buds as rapidly as those with longer treatments.

The incorporation of cytokinin into plant tissue culture growth medium at sufficient levels commonly enhances budding (4, 6). However, these observations do not prove that cytokinin is required to be present, or present at especially high concentrations, at the actual site of bud initiation.

We present evidence in favor of the direct involvement of cytokinin at the bud forming site: single local applications of cytokinin usually cause single bud formation under the applied hormone. In this system, larger applications yield larger single buds, as against many small buds. Further, application for just a 2-d period is sufficient for induction, the new bud appearing at the site of previous application.

Reports of a local response to a locally applied phytohormone are limited. In early work by Camus (1), changes in parenchymal, cambial, and vascular tissue were brought about by buds grafted to callus. Clutter (2) was able to induce vascular tissue in pith sections of tobacco with auxin via inserted glass pipets. More recently, work by G. Steucek (unpublished data) has led to localized bud induction on the lower residual meristem of *Graptopetalum*. He used 1% BA in lanolin. Our work extends his observations.

MATERIALS AND METHODS

Plants of the succulent *Graptopetalum paraquayense* E. Walther were obtained from a greenhouse at the Carnegie Institution of Washington, Stanford, CA. Mature leaves were detached from stems and stored in airtight plastic containers. A ring of aligned cells normally surrounds the point of previous attachment to the stem (Fig. 1). The whole ring can be regarded as a residual meristem, though normally only the upper part produces buds (7).

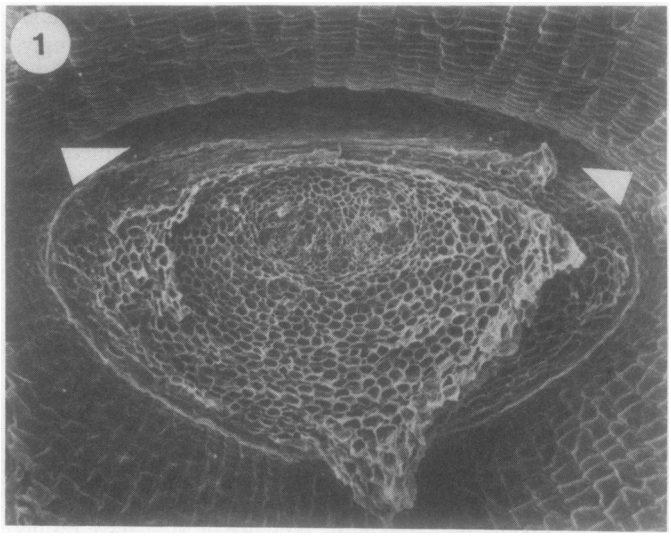

FIG. 1. Scanning electron micrograph of leaf base taken 2 d after leaf removal. Low power (× 31). The recessed area (between arrows) above the abscission scar is the upper residual meristem from which the shoot is normally produced. Micrograph courtesy of Kenton E. Brooks.

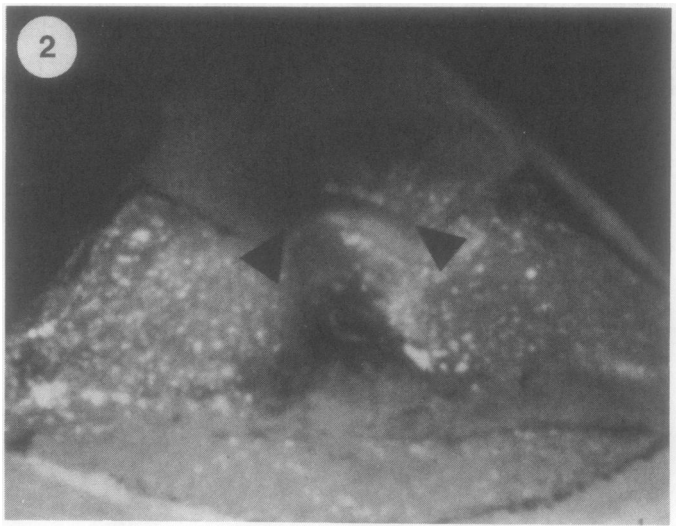

FIG. 2. Typical surgical preparation for hormone application. The abscission scar and upper residual meristem have been excised, and the lower residual meristem (recessed area between arrows) is the only region now capable of giving rise to buds in the time course of these experiments (× 13)

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Table I. *Percentage of Buds Induced by Three Cytokinins on the Lower Residual Meristem of G. paraquayense*

Controls consisted of meristems treated with two drops of plain lanolin.

Cytokinin Tested	Concn (w/w)	No. of Leaves Treated	Percentage of Lower Residual Meristems with Buds
	%		
Control		52	16
Kinetin	0.5–1	27	63
BA	0.5–1	25	60
DMAAP	0.5–1	47	75
Kinetin	2–32	39	39
BA	2–32	45	89
DMAAP	2–32	46	87

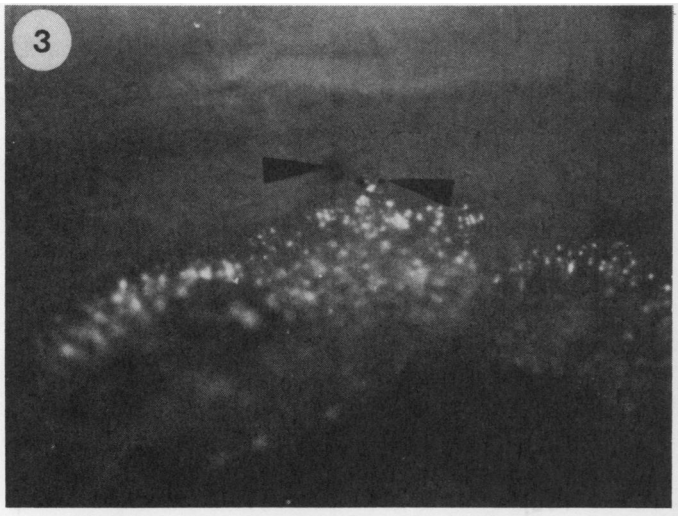

FIG. 3. Three d of bud growth at 8 d after leaf removal and 7 d after application of hormone paste. Tungsten beads (between arrows) mark the site of application (× 69)

Within 2 h of picking the leaf, the upper residual meristem was removed with a single-edge razor blade (Gem Blue Star, Staunton, VA). Vascular tissue was then removed with triangles of "strap" razor blades (Gillette Techmatic, Boston, MA) prepared according to Green and Brooks (3). This operation leaves the lower residual meristem intact (Fig. 2). One d after surgery, the cut edges of the leaves were coated with 3% methyl cellulose to minimize dehydration. Leaves were kept in plastic containers in the dark between observations.

Hormones. The cytokinins used were BA (U.S. Biochemical Corp., Cleveland), kinetin (Sigma), or DMAAP[4] (Sigma). All hormones were applied as small droplets (0.15–0.3 mm in diameter) of a paste of anhydrous lanolin (Baker and Adamson Products, Morriston, NJ) to the lower residual meristem (Fig. 2). The paste was prepared by stirring a known mass of hormone into a predetermined mass of lanolin with a wooden applicator. To make the paste highly visible, tungsten spheres 9 to 18 μm in diameter (Particle Information Service, Grants Pass, OR) were mixed in. Application of droplets was done with a glass ball-tipped applicator, made on a De Fonbrune microforge. To improve adhesion of the paste to the applicator, the ball tip was made rough by spraying it with an acrylic coating (#1303A; Borden, Inc., Columbus, OH). Application was done under the dissecting microscope,

[4] Abbreviation: DMAAP, 6-(γ,γ-dimethylallylamino)purine.

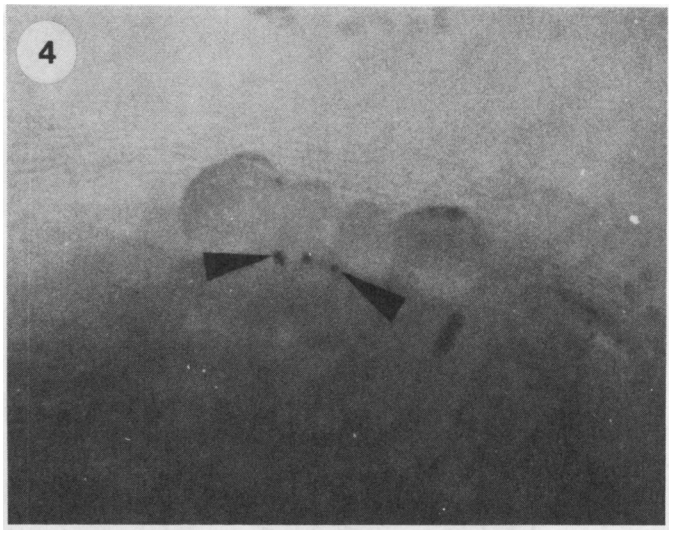

FIG. 4. Three d of bud growth at 11 d after hormone application. The lanolin paste to the left (between arrows) contains 0.1% DMAAP; the paste to the right serves as an internal control. Four leaf primordia have formed on a short stem. Tungsten beads have been displaced by bud tissue. (× 78)

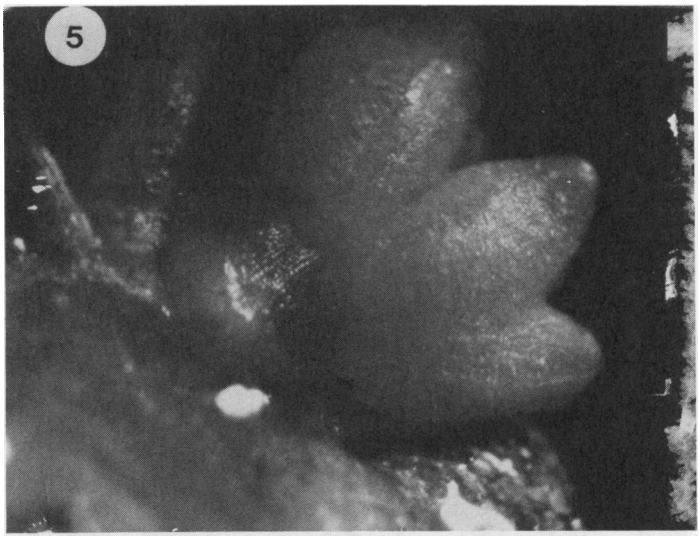

FIG. 5. Typical response to applied 0.0125% DMAAP. Specimen at 14 d after hormone treatment. (× 44)

using a De Fonbrune micromanipulator to allow precise placement of spots. On each lower residual meristem, two equivalent bead-containing droplets were placed, one to the left and the other to the right of the middle of the crescent-shaped surface. One droplet contained the hormone; the other was a control.

In experiments requiring removal of hormone paste, the tissue was coated with Elmer's acrylic latex contact cement or 'glue-all' acrylic (Borden, Inc.). Excess glue was removed by suction with a Pasteur pipet attached to rubber tubing. After the glue had dried to a film, it was removed with forceps. This technique was repeated three times to remove all lanolin. Silicone rubber cement, previously applied to surrounding tissue, ensures easy removal of glue.

RESULTS

A comparison of the amount of bud induction by the cytokinins BA, kinetin, and DMAAP is presented in Table I. At the lowest concentrations tested, DMAAP was the most effective at inducing

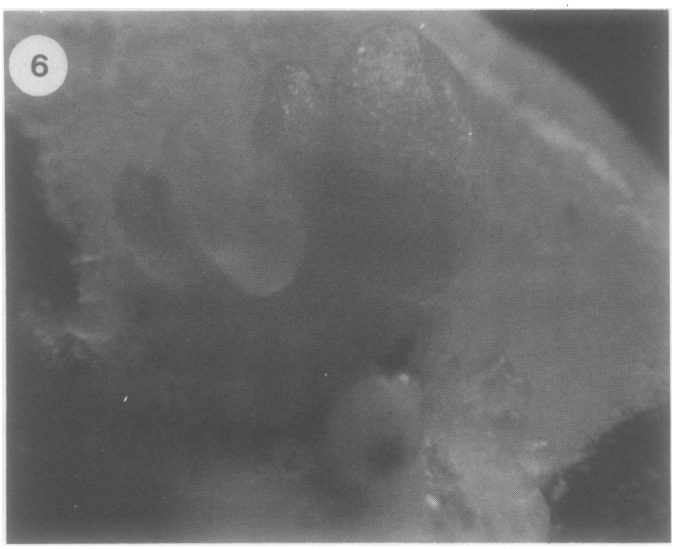

FIG. 6. Response to applied DMAAP. Specimen at 13 d after cytokinin treatment. In addition to the larger plantlet growing from the site of 1.0% DMAAP application, a smaller plant has begun to emerge from the untreated region of the lower residual meristem. (× 40)

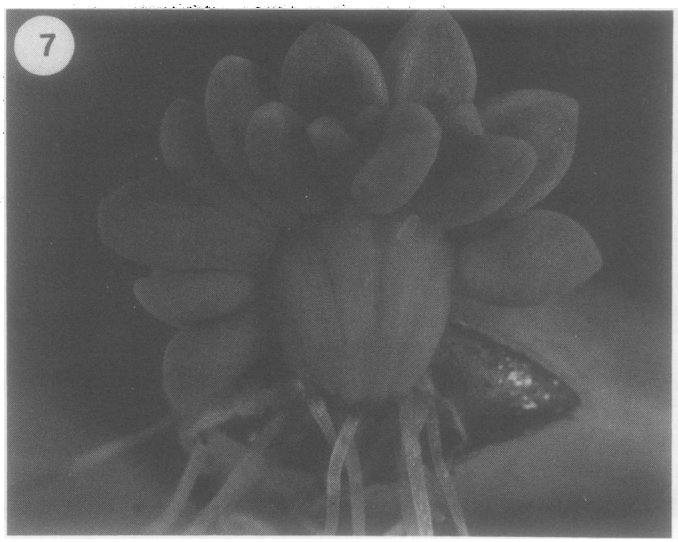

FIG. 7. Response to applied DMAAP. Specimen at 57 d after application of 0.5% DMAAP. One wide stem has formed with a large number of leaves at the apex. (× 7)

budding.

The gross course of stem formation on the lower residual meristem, induced by 0.1% DMAAP, is illustrated in Figures 3 and 4. Tungsten beads mark the site of application. The lanolin paste to the left contains DMAAP, while the paste to the right serves as control. Two weeks after application of the drops, a cylindrical shoot is typically seen beneath the cytokinin paste. Roots do not form beneath this paste. If the diameter of the droplet is increased, a larger stem forms. In control plants which have not undergone surgery to remove the upper residual meristem, only roots emerge from the lower residual meristem. Even if the lower residual meristem is excised, roots still form from the region it previously occupied. Thus, cytokinin application suppresses root initiation while promoting bud formation.

The response to applied cytokinins is somewhat variable. In addition to the local effect (Figs. 3–5), some general effects are

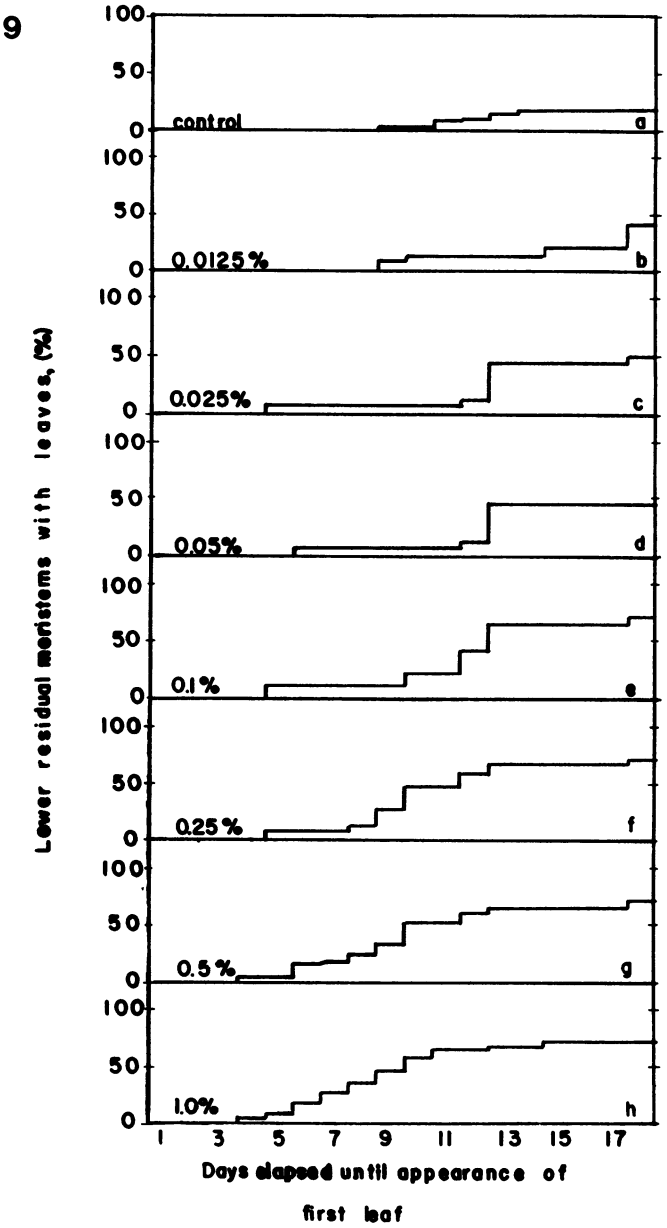

FIG. 9. Time course for appearance of leaf primordia at different concentrations of DMAAP. a, Control; b, 0.0125%; c, 0.025%; d, 0.05%, e, 0.1%; f, 0.25%; g, 0.5%, h, 1.0%.

seen. At higher concentrations of DMAAP (0.5–1.0%), formation of a plantlet at the application site is sometimes followed by bud initiation over the remaining surface of the lower residual meristem (Fig. 6). Branched stems occasionally form. Another variation involves the formation of a gigantic stem, covering the width of the lower residual meristem, with numerous leaves at its apex (Fig. 7).

The relation between concentration of DMAAP and the time before the first appearance of leaf primordia on the bud is given in Figure 9. The final yield of new buds with emergent leaf primordia ranged from 16% for control leaves to 71 to 73% for DMAAP concentrations of 0.1 to 1.0%.

A delay between surgical preparation of the leaf and treatment with DMAAP of from 2 to 6 d had no effect on the percentage of plants induced. However, certain developmental differences were noted. A delay in hormone application resulted in an increase in

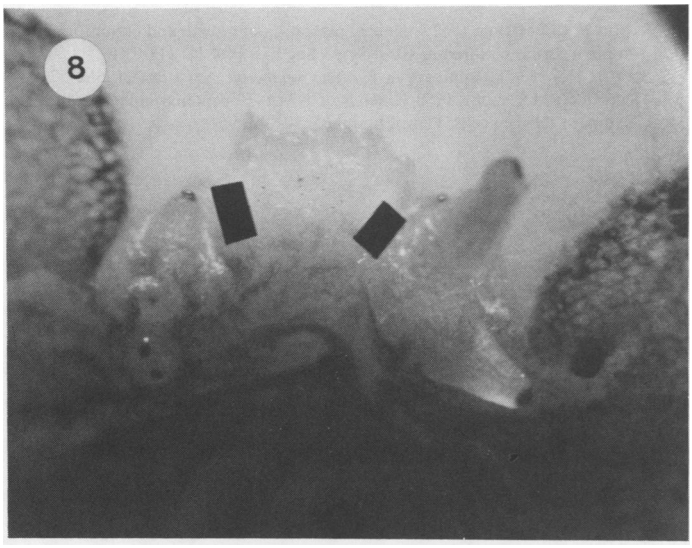

FIG. 8. Callus formation 10 d after 0.5% DMAAP application. Treatment was 4 d after surgery. Callus is between bars. (× 43)

the amount of callus formed from the lower residual meristem before the appearance of buds (Fig. 8). In addition, appearance of roots was greatly accelerated relative to stem formation, the two organs often appearing simultaneously.

Results of experiments in which the hormone paste was left on the assay surface for fixed times are presented in Figure 10. The formation of buds occurred as rapidly after 2 d of hormone treatment as it did with longer treatment times.

DISCUSSION

The production of shoots from the lower residual meristem is clearly enhanced by application of cytokinin. The new buds arise directly under the localized source of hormone. Once the site of bud initiation has been established, the course of stem formation is very similar to that seen in the upper residual meristem as described by Green and Brooks (3): leaves form and grow rapidly and the stem forms beneath the leaves. The stem then enlarges to form a short cylinder with prominent vertical files of epidermal cells.

Cytokinins applied to the upper residual meristem do not affect normal stem formation. The lower residual meristem never gives rise to stems in normal plants. After surgery, it does so infrequently. Because of this, as well as the successes of Steucek (unpublished data) with bud induction there, this surface was chosen for the assay. An important feature of the assay was that it was necessary for the assay surface to be uninterrupted. If it was wounded, stems were sometimes induced on one or both sides of the cut, depending on local tissue conditions, even without hormone application. Preventing desiccation of the assay surface was found to be a prerequisite for bud initiation there.

Using excised *Nicotiana rustica* leaves, Mothes and Engelbrecht (5) demonstrated a kinetin-induced directed transport, the kinetin-treated area functioning as a translocation sink and locus of predominant synthesis. This is a possible explanation for the evidence presented in Figure 10 for a minimum effective application time for bud formation. Once organogenesis has been initiated, any further requirement for cytokinin (if one indeed exists) is apparently met by the bud itself. The failure of buds to appear sooner with longer applications of DMAAP is consistent with findings of Terrine and Laloue (8). Using tobacco cells grown as cell suspensions, they found that cytokinin degradative activity

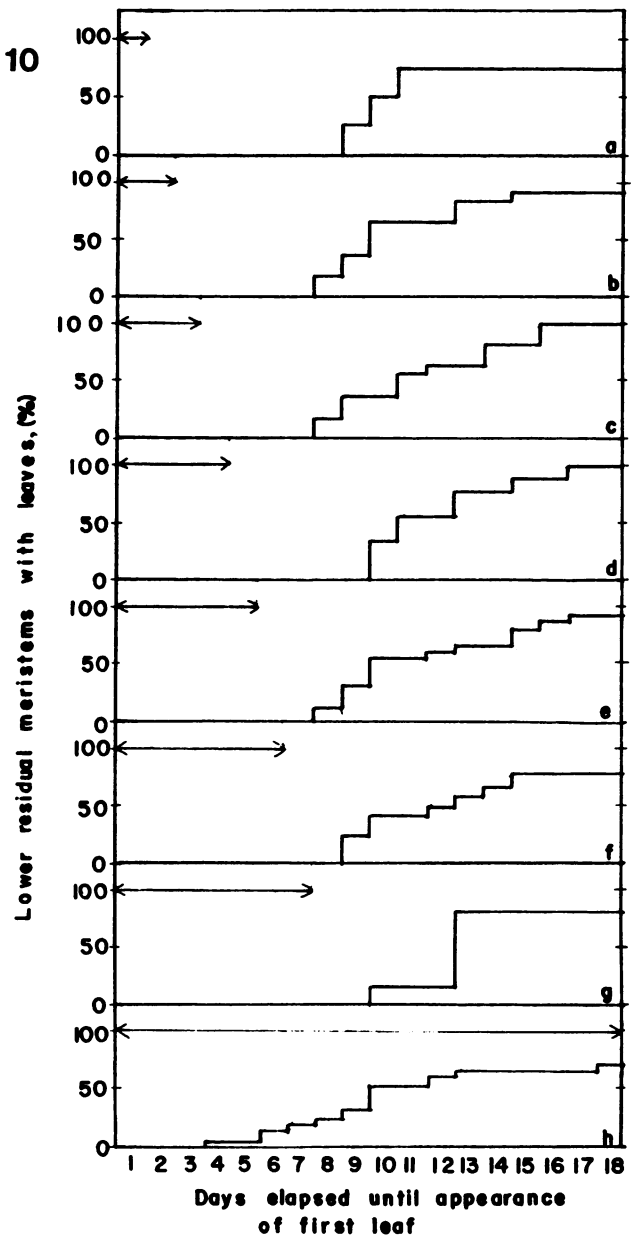

FIG. 10. Time course for appearance of leaf primordia with 0.5% DMAAP in contact with assay surface for various times (indicated by arrows). a, 1 d; b, 2 d; c, 3 d; d, 4 d; e, 5 d; f, 6 d; g, 7 d; h, 13 d.

was apparently regulated by the level of cytokinin itself. A possible explanation is that the lanolin paste serves as a physical constraint to expansion of the young plantlet. This seems unlikely, since coating the entire assay surface with lanolin failed to inhibit bud formation and growth. Results of this work strongly support the hypothesis that organogenesis proceeds from cellular changes, involving cytokinin, occurring in a limited area. In addition to its possible uses as an assay, the *Graptopetalum* system presents possibilities for studying the histological basis of hormone action. Unlike the situation with conventional tissue culture, the site of bud initiation can be controlled.

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