ENDOSPERM OF *TAXILLUS VESTITUS* WALL. : A SYSTEM TO STUDY THE EFFECT OF CYTOKININS *IN VITRO* IN SHOOT BUD FORMATION

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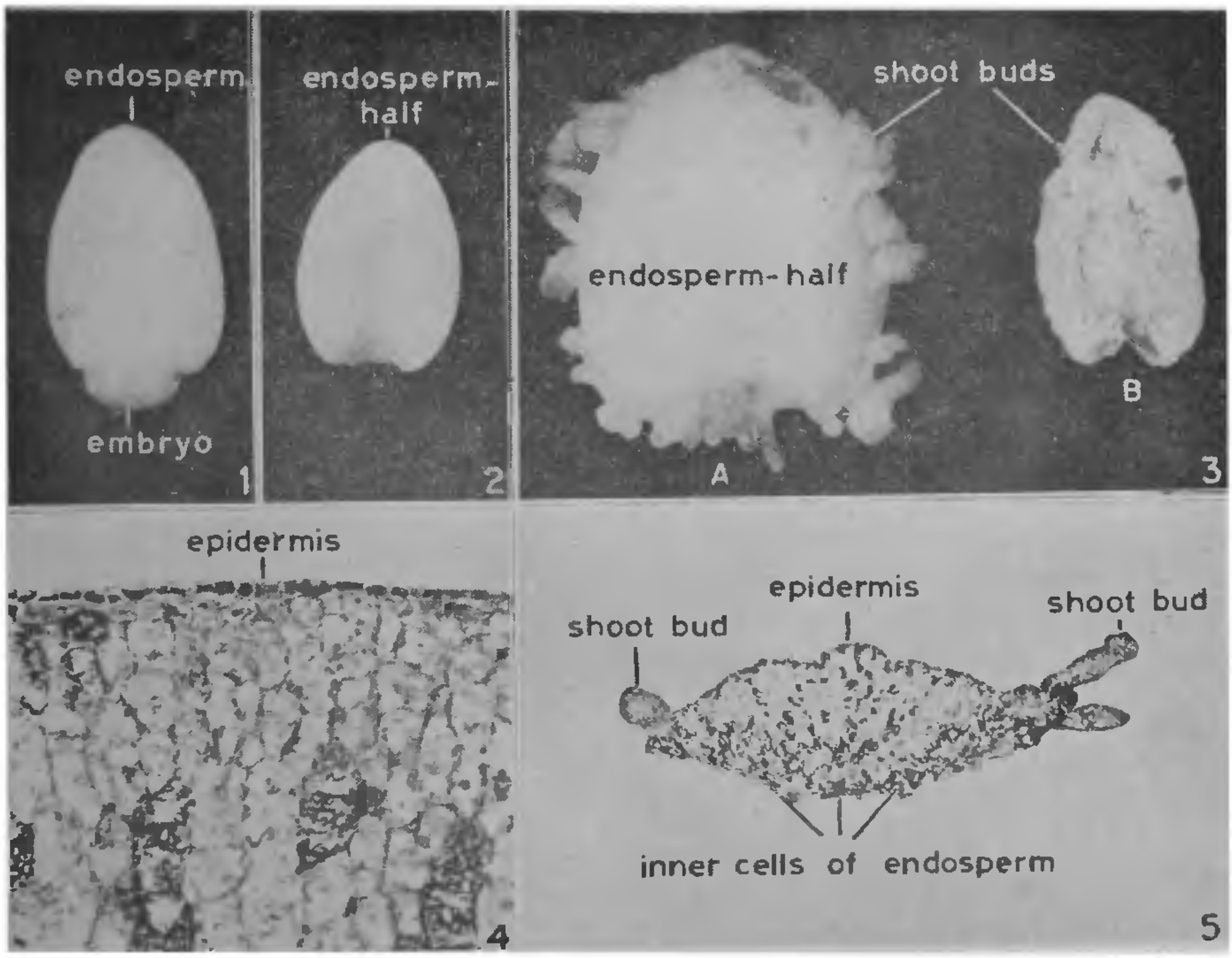
CYTOKININS promote the formation of shoot buds in many plant tissues cultured *in vitro*.[1] However, a few buds also develop in such cultures even in the absence of cytokinins, *i.e.*, in the controls.[2] In the present communication we report the regulation of the differentiation of shoot buds by cytokinins from the epidermal cells of mature endosperm of *Taxillus vestitus*. This tissue does not form shoot buds either under natural conditions, or in cultures in the absence of a cytokinin.

Ripe fruits of *T. vestitus* (Loranthaceæ) were washed with a detergent, surface sterilized with 90% ethyl alcohol, and the 'seeds' (endosperm + embryo ; there is no testa) (Fig. 1) excised aseptically. The mature endosperm (which encloses the embryo except at the radicular end) comprises about 20–22 layers of parenchymatous cells, containing starch or starch and tannin, surrounded by the small, densely cytoplasmic epidermal cells (Fig. 4). Like the endosperm of any other angiosperm, it lacks vascular elements.

The seed was cut longitudinally and after removing the embryo the endosperm-halves (Fig. 2) were implanted in two positions : cut



FIGS. 1–5. Organogenesis in endosperm cultures of *Taxillus vestitus*. Fig. 1. Mature endosperm (entire) with embryo intact, × 6·1. Fig. 2. Same cut longitudinally and embryo removed, showing only one endosperm-half with the cut surface exposed : stage of implantation, × 6·1. Fig. 3. 10-week-old culture of endosperm-half with cut surface in contact with the medium – WM + kinetin (10 ppm). there were as many as 18 shoot buds around the periphery, (A); only 2 small buds developed in the endosperm-half with cut surface away from the medium (on the epidermal side, B), × 11·4. Fig. 4. A portion of endosperm in section showing epidermis and inner cells ; stage of implantation, × 100·9. Fig. 5. Transection of an endosperm-half as in Fig. 3A, showing shoot buds developed on the periphery, × 14·6,

surface in direct contact with the nutrient medium, and cut surface away from the medium. The seed was also cut transversely into 3 or 4 pieces and only the pieces of endosperm were implanted with one of the cut surfaces in contact with the medium. Modified [without indoleacetic acid (IAA)] White's agar (0·8%) medium[3] containing 2·0% sucrose (WM) served as the control. Supplements used either individually or in different combinations included IAA, indolebutyric acid (IBA), naphthaleneacetic acid (NAA), 2, 4-dichlorophenoxyacetic acid (2, 4-D), 2, 4, 5-trichlorophenoxyacetic acid (2, 4, 5-T), adenine, and some substituted aminopurines : 6-benzylaminopurine, 6-benzylamino-9-(2-tetrahydropyranyl)-purine, 3-(γ, γ-dimethylallylamino)-purine, 6-(γ, γ-dimethylallylamino)-purine, 6-furfurylaminopurine (kinetin), 6-furfurylamino-8-azapurine, 6-isoamylaminopurine, and 6-methylaminopurine. For each treatment 24 cultures were maintained at $25 \pm 3°$ C and $55 \pm 5\%$ relative humidity under diffuse daylight (10–15 ft-c). Each experiment was repeated thrice.

On WM alone, the endosperm proliferated only slightly at the cut end and the callus was highly ephemeral. Callusing was somewhat better on WM containing an auxin (2×10^{-5} M): IAA, IBA, NAA, 2, 4-D or 2,4,5-T. However, none of these media induced the formation of shoot buds.

When longitudinally or transversely cut pieces of endosperm were grown on WM supplemented with a cytokinin, after 3 to 4 weeks shoot buds developed along the cut surfaces in contact with the medium (Fig. 3 A). Anatomical studies showed that repeated division of epidermal cells, near the injured portion, was followed by the differentiation of buds and vascular tissues (Fig. 5, later stage). Cytological preparations of the developing shoot buds showed a normal triploid condition ($3n = 27$). The position of the explant on the medium had a remarkable influence on the differentiation of buds. Endosperm pieces with the cut surface in contact with the medium enlarged appreciably and produced as many as 13 shoot buds, while only 2 buds developed when the cut surface was away from the medium and the growth of explant remained arrested (Fig. 3 A, B).

The period required for the initiation of shoot buds, number of buds produced on each explant, and shape and size of buds varied from cytokinin to cytokinin and its concentration. At 10 p.p.m. of kinetin cent per cent cultures showed shoot buds after 6 weeks, while at 5 p.p.m. 9 weeks were required. There was practically no response with adenine, 3-(γ, γ-dimethylallylamino)-purine and 6-methylaminopurine ; with 6-benzylamino-9-(2-tetrahydropyranyl)-purine irregular swellings (not well-organized shoot buds) were noticed along the cut surface ; but progressive response was observed in inducing shoot buds with 6-furfurylamino-8-azapurine, 6-isoamylaminopurine, 6-furfurylaminopurine, 6-benzylaminopurine and 6-(γ, γ-dimethylallylamino)-purine (Fig. 7).

Injury to the explant resulted in the promotion of shoot bud differentiation on media containing cytokinins. Majority of shoot buds were confined to the injured portions which were in direct contact with the medium ; a few shoot buds were also formed around the injured surface even if they were not in direct contact with the medium. However, injury alone did not replace the effect of a cytokinin.

When the entire endosperm with the enclosed embryo was implanted, bud initiation was delayed by about 5 weeks, and the number of cultures showing shoot buds also diminished (by about 60% at 10 p.p.m. of kinetin ; Fig. 6).

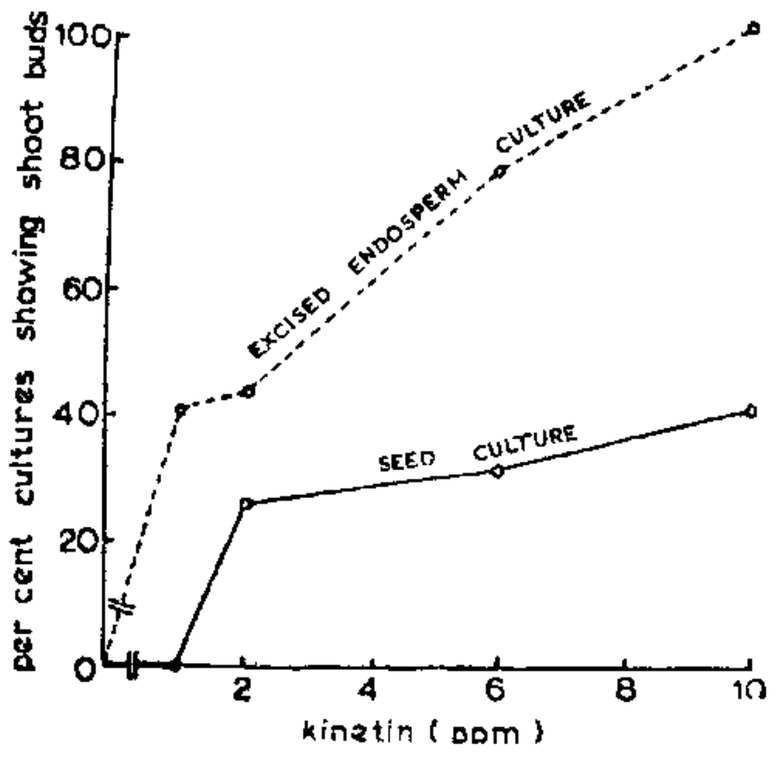


FIG. 6. Effect of kinetin on shoot bud formation from mature endosperm. Curve with dotted line represents the percentage of cultures with shoot buds in 6-week-old cultures, endosperm-half was implanted with the cut surface in contact with the medium ; solid line represents the shoot buds differentiated 9 weeks after culturing the entire endosperm with embryo intact.

Furthermore, there were only about 3 buds per culture in contrast to 13 when only endosperm-half was cultured.

Addition of an auxin to WM containing a cytokinin had an inhibitory effect on bud formation : NAA, 2, 4-D and 2, 4, 5-T (2×10^{-5} M)

inhibited bud formation completely ; IBA and IAA reduced the number of cultures showing shoot buds as well as the number of buds developing on each explant. In this species we have succeeded in chemically controlling the formation of shoot and haustorium from endosperm (to be published later).

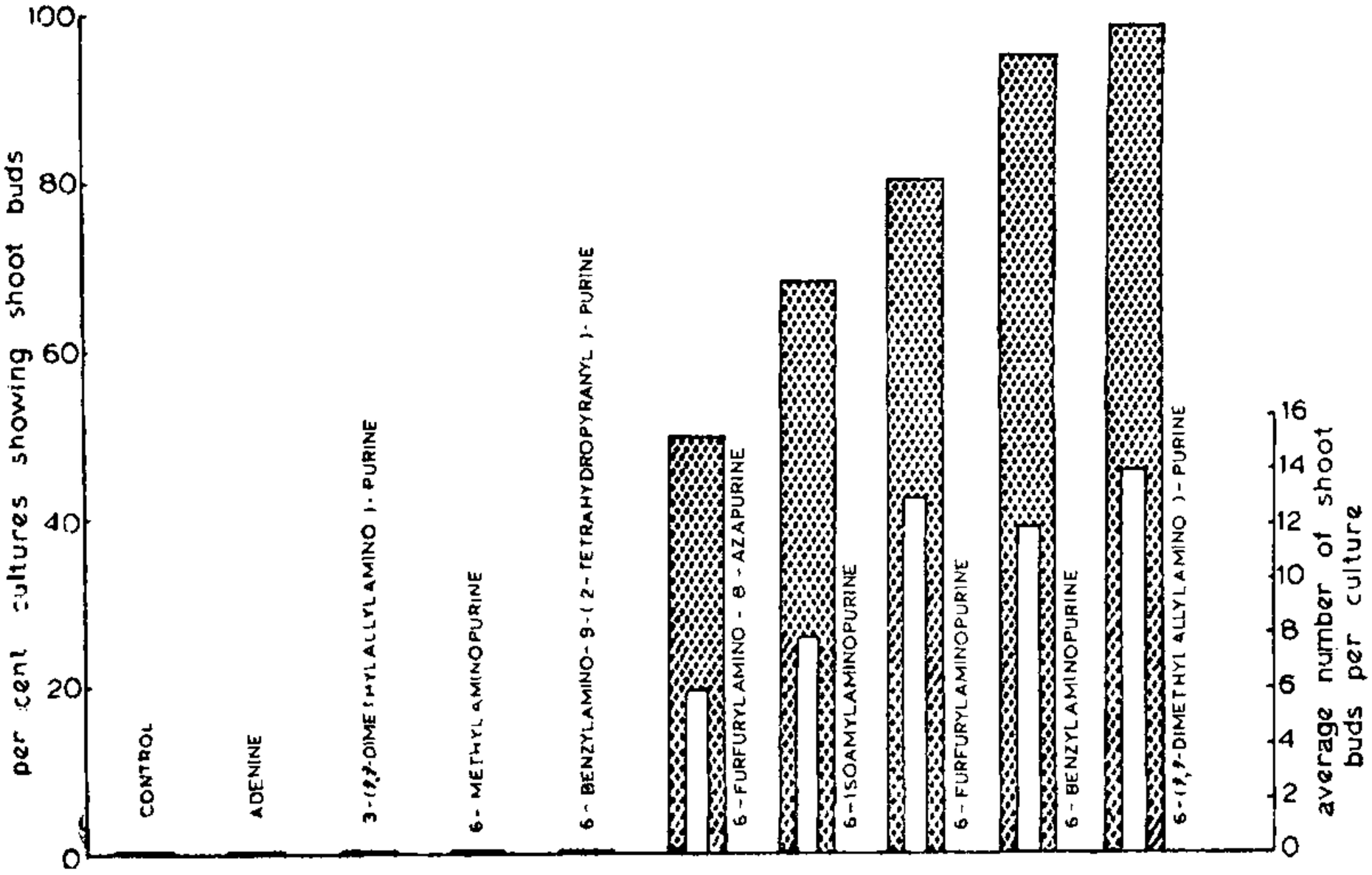


FIG. 7. Effect of some substituted aminopurines (and adenine*) at 2×10^{-5}M on shoot bud formation from endosperm-half implanted with cut surface in contact with the medium, in 8-week-old cultures.

Epidermal cells of some angiosperms, both *in vivo* and *in vitro*, have been reported to form buds,[4] and embryoids.[5,6] In these instances attention has not been drawn either to the specific sites of bud formation, or to a specific chemical leading to this phenomenon. Cytokinins enhance the differentiation of shoot buds in a number of plant tissues cultured aseptically. Recently, Paulet (1965)[7] showed a spectacular enhancement of the buds in tobacco tissue culture (approximately 300 buds with 1 mg./l of kinetin against 32 without kinetin in *Nicotiana suaveolens*). However, as mentioned by Miller (1961),[2] in all those tissues in which the cytokinins promoted the formation of shoot buds, a few buds also developed in the controls, *i.e.*, without the presence of exogenous cytokinins. As far as we know, the present findings in *T. vestitus* is the first report in which shoot buds do not differentiate in the controls, *i.e.*, in the absence of cytokinins.

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