Cytokinin and Growth of Excised Roots of *Bryophyllum calycinum*

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**ABSTRACT** Excised roots of *Bryophyllum calycinum* require for growth both auxin and cytokinin. This is demonstrated by the poor growth of 2-mm root tips in a basal medium of mineral salts, sucrose, and vitamins supplemented with either an auxin or a cytokinin, and much better growth when the basal medium is supplemented with both auxin and cytokinin. However, both substances are synthesized by the root, as is demonstrated by the growth of large inocula (dry wt 6–7 mg) through many successive passages in a medium limited to mineral salts, sugar, and vitamins.

The roots of *Bryophyllum calycinum* require for growth both auxin and cytokinin. This conclusion follows from the response of 2-mm root tips cultivated in a basal solution of mineral salts, sucrose, and vitamins. The addition of a cytokinin—kinetin, 6-benzylaminopurine, or zeatin—to the basal medium induced 3- to 5-fold lengthening of the root, but no branch roots were produced. In medium supplemented with the auxin naphthalene-2-acetic acid (NAA), limited growth, characterized by short branch roots and a heavy coat of root hairs, occurred. Growth was substantial if both auxin and cytokinin were added to the medium.

Root tips of *B. calycinum* about 2-mm long were excised from roots growing from foliar embryos of leaf pieces cultivated under sterile conditions in the light in a solution of mineral salts, sucrose, and vitamins (1). Two root tips were placed in each flask. They were grown in the dark at about 28°C in 25 ml of a basal solution of mineral salts, sucrose, and vitamins in 50-ml Erlenmeyer flasks. The basal medium was supplemented with NAA at 1.25 μg/25 ml, 6-benzylaminopurine at 1.25 μg/25 ml, or with both. After 70 days (Fig. 1) little growth had occurred in the solution supplemented with the cytokinin (6-benzylaminopurine); the auxin (NAA) induced some growth, especially branching; but when both auxin and cytokinin were present, growth was substantial. After 120 days little change had occurred in the roots in the cytokinin medium; the branches had lengthened considerably in the auxin medium; growth in the medium with both auxin and cytokinin was several times that in the medium containing cytokinin only. Dry weights per root after 129 days were 11.2 mg for the roots in the medium with both growth substances, 1.2 mg for those in the auxin medium, and 0.6 mg for those in the cytokinin medium.

The poor growth of root tips in the cytokinin medium suggests that the root tip contained too little auxin, either because of failure to synthesize an adequate amount or because auxin in the excised root was leached out into the surrounding medium. Growth in the auxin medium, evidenced especially by the production of branch roots, indicates that the root tip contained some cytokinin when excised and synthesized some during the incubation period, but not enough for maximum growth.

Although 2-mm excised root tips required an external supply of both auxin and cytokinin for good growth in these (still) cultures, larger inocula grew well without either growth substance in the medium. We have carried cultures of excised roots of *B. calycinum* through more than 30 successive passages, extending over nearly 5 years, in a medium limited to mineral salts, sucrose, and vitamins. The inoculum for these cultures consisted of a mass of roots, dry weight 6–7 mg, taken from an established culture.

The response of cultures with inocula ranging between 2 mm in length and 6–7 mg dry weight varied, especially in response to NAA. In some instances, growth was excellent; in others poor. We interpret this to mean that sometimes the excised roots used for inocula contained (or made during

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**Abbreviation:** NAA, naphthalene-2-acetic acid.
incubation) sufficient cytokinin to permit good growth in the presence of the auxin NAA, in other instances they did not.

Since our earlier report (1) we have found that riboflavin or its products is injurious to Bryophyllum roots, especially if the roots are grown in the light. We now omit riboflavin from the vitamin mixture.

We conclude that the roots of B. calycinum require for growth both auxin and cytokinin, but synthesize both compounds. The effect of these two hormones in the medium in which excised roots are grown is influenced by the size and nature of the inoculum and the conditions of culture.

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