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BRIEF COMMUNICATION

A Modified *Amaranthus* Betacyanin Test for Cytokinin Bioassay

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Abstract. A modified procedure for the extraction of betacyanin from *Amaranthus* seedlings is described. Application of this modification increased the absorbance of cytokinin-treated *Amaranthus* explants in most cases. The modified *Amaranthus* test is compared with the soybean callus test in the bioassay of kinetin, 6-benzylaminopurine, and 6(δ,δ-dimethylallyl) aminopurine.

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The detection and identification of cytokinin in relatively small samples of plant material and quantities below 0.1 μg, were carried out using gas chromatography combined with mass spectrometry (SKOOG and ARMSTRONG 1970). However, a simple, rapid and sensitive test for assaying the several fractions obtained after paper-, column-, or thin-layer-chromatographic separation of plant extracts will still be needed. Most cytokinin bioassays, their sensitivities and specificities were reviewed and compared by LETHAM (1967a, b). The *Amaranthus* betacyanin assay was originally worked out by KÖHLER and CONRAD (1966), developed and applied to various cytokinins by BIGOT (1968) and simplified by BIDDINGTON and THOMAS (1973). This assay has received relatively little attention from plant physiologists. The present investigation is an attempt to develop a simple and rapid procedure for the extraction of *Amaranthus* betacyanin. Moreover, it was also intended to compare the modified version with the most widely recommended bioassay for cytokinins, *i.e.* with the soybean callus test (MILLER 1965).

Cytokinins. Kinetin (K), 6-benzylaminopurine (BA) and 6(δ,δ-dimethylallyl) aminopurine (DA) were purchased from Fluka AG, Buchs SG, Switzerland.

Bioassay Methods.

(A) **Amaranthus betacyanin assay.** This test was performed essentially as described by BIDDINGTON and THOMAS (1973) and may be briefly summarized as follows: the cytokinin, or the

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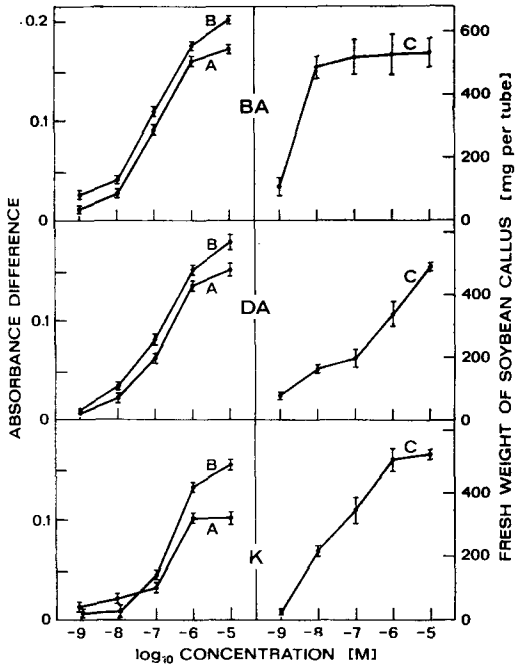

Fig. 1. Activities of 3 cytokinins in 3 tests. The respective control values have been subtracted. *A* and *B*: *Amaranthus* test. Each point is the mean of 9 replicates. Extraction of betacyanin by: *A*: freezing and thawing, *B*: mortar and centrifuge. *C*: soybean callus test (3 replicates). Vertical bars represent the standard errors at the 5% level. *K*: Kinetin, *DA*: 6-(δ, δ-dimethylallylamino) purine and *BA*: 6-benzylaminopurine.

sample, was dissolved in 2 ml of phosphate buffer solution (1/75 M; pH = 6.3) containing *l*-tyrosine at 1 mg ml^{-1}. Solutions to be tested, were added to 2 layers of filter paper in Petri dishes, each of which received 10 explants consisting of the upper portion of the hypocotyl plus the cotyledons of 3-day-old *Amaranthus caudatus* L. The Petri dishes were incubated at 25 °C for 24 h in the dark, after which the betacyanin of the explants was extracted either by subjecting the explants for two cycles of freezing (—20 °C) and thawing at room temperature in 2 ml of distilled water (BIDDINGTON and THOMAS 1973), or by grinding the explants in a small mortar (diameter of 45 mm) with 1 ml distilled water. The material was transferred to a plastic centrifuge tube, and after completing the volume to 2 ml the slurry centrifuged at 5000 rev. min^{-1} for 25 min. In either case, the quantity of betacyanin was determined as the difference between the absorbances at 542 nm and 620 nm (BIGOT 1968).

(B) **Soybean Callus Test.** The procedure was carried out as described by MILLER (1965), except that only one tissue piece was planted on 15 ml of medium in a test tube (15 × 230 mm). Three tubes were used for each concentration of cytokinin. The tubes were maintained at 27 °C, 70 % relative humidity and continuous illuminance of about 440 lx. The fresh callus yield was weighed after two weeks. Long growth period did not experimentally change the trend of the activities obtained from the tested pure cytokinin, as the growth of the present callus tissue was rapid enough. However, for the detection of the natural occurrence of cytokinin in the plant tissue, it is preferable to extend the growth period to three weeks.

Fig. 1 gives the relationship between cytokinin concentrations and either absorbance differences or callus fresh weight after subtraction of the respective control values. Fig. 1 shows that the curves representing the two extraction procedures (*A* and *B*) run more or less parallel, curve *B* (mortar and centrifuge) following the higher course except in the case of kinetin at 10^{-9} and 10^{-8} M. The control values (mean $\bar{X} \pm$ standard error $S\bar{x}$ and degrees of freedom ν) were as follows: *A* (freezing and thawing): 0.011 ± 0.002 ($\nu = 8$); *B* (mortar and centrifuge): 0.030 ± 0.002 ($\nu = 8$); *C* (callus test): 12 ± 2.1 mg ($\nu = 2$). The 3 cytokinins can be arranged in the following descending order of activity in the *Amaranthus* test: *BA*, *DA* and *K*. Except

at the lowest concentrations of kinetin, the modified procedure consistently gives higher readings than the original. Although these differences are statistically significant only at the higher concentrations, the modified procedure has the advantage of being faster than repeated freezing and thawing. On the whole, the modified procedure can be regarded as an improvement on the original method (BIGOT 1968) and that of BIDDINGTON and THOMAS (1973).

The sensitivity of the modified *Amaranthus* test was compared with that of the soybean callus test for cytokinin bioassay (Fig. 1). In the case of *BA*, the range of the ascending linear relationship between response and concentration is much smaller in the callus test than in the modified *Amaranthus* test. So far as the quantitative determination of this compound within the range of 10^{-8} to 10^{-5} is concerned, the modified *Amaranthus* test is preferable. The two tests seem to be equally sensitive to variation in the concentrations of *DA* in the whole range ($10^{-9}-10^{-5}$ M), while the callus test is advantageous at low concentrations of kinetin.

Since the time needed for the callus test is 2 or 3 weeks, the modified *Amaranthus* test can be recommended as a rapid and simple cytokinin test for preliminary bioassay of large numbers of unknown separated chromatographic fractions. Meanwhile, the soybean callus test can be used in the second step to ascertain the activity of a limited number of fractions after the examination by the modified *Amaranthus* test.

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