Short Communication

Effects of Different Cytokinins on the Senescence of Detached Oat Leaves

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Summary. Senescence is delayed (chlorophyll retained) in oat leaf sections by kinetin and benzyladenine, but not by the natural cytokinins, zeatin and isopentenyladenine.

Because tissue culture bioassays for cytokinins in plant extracts are laborious and time-consuming, other, more rapid cytokinin responses have been used instead. Some assays have been based upon the retarding effect of kinetin on the senescence of detached leaf sections in the dark (Letham, 1967; Martin and Thimann, 1972). Leaves of Xanthium (Osborne and McCalla, 1961) and of Rumex (Goldthwaite, 1972) respond also to other substances than cytokinins and are therefore unsuitable. Leaves of barley (Kende, 1964, 1965) and of oats (Thimann and Sachs, 1966) react more specifically to kinetin; barley sections respond also to zeatin and zeatin riboside (Engelbrecht, 1971), oat leaves to benzyladenine (Thimann and Sachs, 1966). To evaluate the suitability of oat leaves for bioassay, we investigated their sensitivity to natural and synthetic cytokinins.

Oat seedlings (Avena sativa L. cv. Victory) were grown for 7 days on Hoagland’s nutrient solution in the greenhouse. The apical 4 cm of the first leaves, 10 leaves per Petri dish, 9 cm diameter, were layed on a slide placed on filter paper which was wetted with 2 ml of distilled water. A 10-μl drop of a cytokinin solution, containing 10 mg/l Triton X-100, was applied to the centre of each leaf piece and the closed Petri dishes were incubated for 4 days in the dark at 27°C. Triplicate determinations of chlorophylls a and b were made after Bruinsma (1963).

An isolate of soybean callus, obtained from Dr. C. O. Miller, Indiana University, Bloomington, Ind., USA., was maintained at 27°C in the dark. The assay was performed according to Miller (1963), using tissues of about 100 mg fresh weight.

Adenine, kinetin and isopentenyladenine (6-γ,γ-dimethylallylamino)-purine) were obtained from Sigma Chem. Comp., St. Louis, Mo., USA., benzyladenine from Schuchardt GmbH, Munich, GFR, and zeatin from Calbiochem, Los Angeles, Calif., USA. Solutions of 10⁻⁴ M in bidistilled water were made by ultrasonic vibration and checked spectrophotometrically. The surfactant was added afterwards.
Kinetin and benzyladenine were active in the chlorophyll retention bioassay, but the natural cytokinins, zeatin and isopentenyladenine, were much less so, hardly more than adenine (Fig. 1). By contrast, all four cytokinins stimulated callus growth in vitro in the soybean test; using $10^{-6}$ M concentrations, fresh weight increase in 5 weeks ranged between ca. 25- and ca. 48-fold (adenine: 5.7×, controls: 1.3×).

The poor activity of the natural cytokinins in the chlorophyll retention assay cannot be ascribed to insufficient penetration of these substances. Zeatin and its riboside are known to penetrate from their activity in the oat leaf mobilization assay (Dekhuijzen and Staples, 1968; Bezemer-Sybrandy and Veldstra, 1971). Changes of the surfactant in the cytokinin solutions did not change the activity pattern. Taking into account the large concentration range, metabolic inactivation of the natural cytokinins is no probable explanation for their lack of activity.

The difference in response to natural and to synthetic cytokinins renders oat leaves a doubtful material for bioassay. It also raises the general question as to the significance of conclusions from experiments.