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Dimethyl Sulfoxide: A Solvent for Cytokinins in the *Amaranthus* Bioassay

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Abstract. Dimethyl sulfoxide present in the agar medium at concentration 0.2 % (v/v) and lower does not inhibit cytokinin-induced betacyanin synthesis in the *Amaranthus caudatus* seedlings. The activity of kinetin, N^6-(Δ^2-isopentenyl)adenine and *trans*-zeatin is the same when these cytokinins are dissolved in either water or dimethyl sulfoxide and incorporated into the medium after autoclaving. A simple method is described which allows the cytokinin activity of slightly water-soluble and thermolabile compounds, *e.g.* aromatic urea and thiourea derivatives, to be determined in the *Amaranthus* bioassay.

Dimethyl sulfoxide (DMSO) has been successfully used as a solvent for cytokinins in the tobacco callus bioassay (Schmitz and Skoog 1970). The advantages of this procedure are the high solubility of adenine and urea derivatives in the DMSO which acts also as a sterilizing agent allowing direct addition of test compounds to the cooling agar medium after autoclaving. The cytokinin activity of slightly water soluble and/or thermolabile compounds therefore can be determined. Reports of effects of DMSO on the synthesis of plant pigments indicate that the use of DMSO in bioassays based on stimulation of plant pigment synthesis may be complicated by effects of the solvent (Raymundo *et al.* 1967, Pecket and Hathout Bassim 1974, Dumortier and Vendring 1978). We report bellow the use of DMSO as a solvent for studying of the effects of adenine and urea derivatives on betacyanin synthesis in the *Amaranthus* bioassay.

MATERIAL AND METHODS

Slightly modified *Amaranthus* bioassay of Conrad (1974) was used. *Amaranthus caudatus* seeds (2 g) were sterilized in 10% aqueous chloramine B solution (w/v) for 10 min, washed 10 times in sterile distilled water and germinated aseptically on filter paper disc in Petri dishes (11 cm) containing 2 g of charcoal saturated with sterile distilled water. After 48 h germination in the dark at 26 °C the seedlings were planted aseptically on agar medium in Petri dishes (9 cm in diameter, 3 cm deep). Each dish contained 60 seedlings and 10 ml of medium consisting of 40 mg L-tyrosine, 400 mg KNO_3, 55 mg

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freshly distilled phenol. 190 mg $Na_2HPO_4.2H_2O$, 580 mg KH_2PO_4, 40 mg streptomycinsulphate and 4 g agar (Difco) and made up to 400 ml with twice distilled water. The pH of the medium was adjusted to 6.3 using H_3PO_4. Substances to be tested for cytokinin activity were added to the medium either before autoclaving dissolved in water or after autoclaving in DMSO

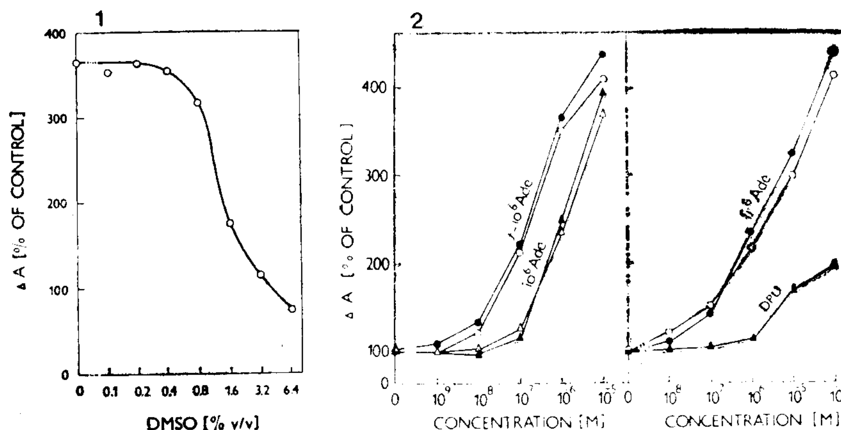

Fig. 1. The effect of DMSO concentration on betacyanin content in *Amaranthus* seedlings grown in the dark on medium containing 3×10^{-5} M of kinetin.

Fig. 2. The effect of *trans*-zeatin (*t*-io⁶Ade), N^6-(Δ^2-isopentenyl)adenine (i⁶Ade), kinetin (fr⁶Ade) and diphenylurea (DPU) on betacyanin content in dark-grown *Amaranthus* seedlings. Compounds were dissolved either in water (open symbols) or in DMSO (solid symbols) and incorporated into the culture medium. The final DMSO concentration was 0.25% (v/v). There is no statistically significant difference between the activities of compounds dissolved in water and DMSO (5% confidence limit).

solution. The seedlings were grown in the darkness at 26 °C for further 68 h. Fifty seedlings from each dish were then transferred to test tubes containing 5 ml of distilled water. The betacyanin pigment was extracted by a twice repeated freezing and thawing procedure. The concentration of the pigment was calculated as the difference between absorbance at 542 nm and 620 nm (ΔA). The activity of diphenylurea (DPU) dissolved in DMSO was also tested in the tobacco callus bioassay using the original procedure of SCHMITZ and SKOOG (1970).

RESULTS

As shown in Fig. 1 a concentration of DMSO in the test medium of 0.2% (v/v) and lower does not reduce the kinetin-induced betacyanin synthesis in *Amaranthus* seedlings. There is no statistically significant difference in activities of *trans*-zeatin, N^6-(Δ^2-isopentenyl)adenine and kinetin when applied either in water or in DMSO solution (0.25% v/v final concentration) to the test media over a wide range of concentrations (Fig. 2). With respect to low solubility of DPU in water this compound was applied to the test medium dissolved only in DMSO. At high concentrations DPU does not give, however, the maximum cytokinin response in the *Amaranthus* bioassay (Fig. 2) it does in the tobacco callus test (Fig. 3).

These results show that DMSO can be used in the *Amaranthus* bioassay. For routine assays compounds being tested are dissolved in DMSO and aliquots of 25 μl are distributed to the Petri dishes containing 10 ml of sterilized test medium just before gelation giving a final DMSO concentration of 0.25% (v/v). The other steps of the bioassay, *i.e.* preparation of media, planting and cultivation of seedlings and betacyanin estimation, are as described in the methods.

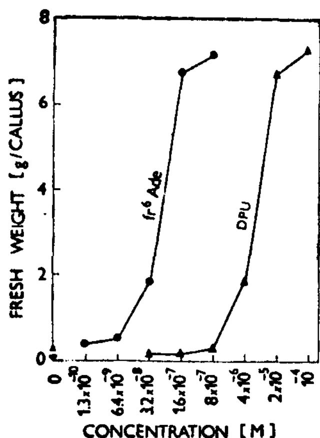

Fig. 3. The effect of kinetin (fr^6Ade) and diphenylurea (DPU) on yields of tobacco callus tissue. Compounds were dissolved in DMSO and incorporated into the culture medium after autoclaving. The final DMSO concentration was 0.05% (v/v).

DISCUSSION

The inhibition of betacyanin synthesis in the *Amaranthus* seedlings by DMSO requires higher concentration than the inhibition of growth in the tobacco callus bioassay (SCHMITZ and SKOOG 1970). This allows larger volumes of DMSO, and therefore more test compound, to be added to the medium.

Using DMSO as a solvent the activity of slightly water-soluble urea derivatives can be tested easily in the *Amaranthus* bioassay. Our preliminary results show that the thiourea derivatives are also active. The stimulation of betacyanine synthesis by these urea derivatives is much smaller than that by the adenine-type cytokinins. Similar difference in activities of kinetin and DPU was also found by CONRAD and KÖHLER (1967). The use of DMSO as a solvent allows the high final concentrations of these substances necessary for stimulation of betacyanin synthesis to be achieved.

DMSO is known to affect the permeability of biological membranes (LYMAN *et al.* 1976, REEVES and HALL 1977) and to increase the penetration of some growth regulators (SCIUCHETTI 1967) and other substances (KEIL 1967, LEONARD 1967, DELMER and MILLS 1969) into plant cells. It also changes the activity of several enzymes when applied at concentration 10% and higher (RAMBLER 1967). At low concentrations (0.5%) it inhibits nitrate reductase in potato slices (PALMER 1979). These effects, however, are unlikely to interfere with the effect of cytokinins on betacyanin synthesis once the DMSO concentration does not exceed 0.2% (v/v) in the medium.

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