

**Short Communication**

**The Use of Dimethylsulfoxide as a Solvent in the Tobacco Bioassay for Cytokinins**

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The use of dimethylsulfoxide as a solvent for synthetic purine and urea derivatives in the tobacco callus bioassay for cytokinin activity (10) has simplified the test procedure at two stages. First, the high solubility of these compounds in DMSO<sup>1</sup> has made it possible to test the less active materials, or those which are only slightly water-soluble, through a wide range of concentrations; secondly, full strength DMSO stock solutions act as sterilizing agents. Test compounds in DMSO solutions can be added directly to the cooling agar medium after autoclaving, thus avoiding destructive heating, and reducing the work, time, and equipment needed for the bioassay.

In this laboratory DMSO was first employed as a solvent in the bioassay of diphenylurea. Bruce and Zwar (1) have reported the

the medium without inhibiting growth was between 0.2 and 0.4%, as shown in Figure 1. Concentrations of 0.8% or higher reduced the yield by half or more, but the tissues remained a healthy white color. Comparative tests with ethanol, also added after

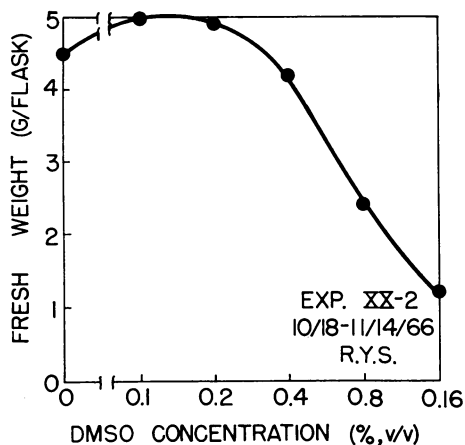


FIG. 1. Effect of DMSO concentration on yield of tobacco callus. Tissue cultured on Linsmaier and Skoog revised medium with  $2 \times 10^{-2}$   $\mu$ M zeatin.

assay of an extensive series of urea derivatives which were added to the culture media as ethanolic solutions before autoclaving. Their preliminary tests indicated no alcohol toxicity (about 1% final strength) and no loss of activity as a result of the autoclaving process. However, in our experience there was a marked loss in activity when DPU was added before autoclaving, while its low solubility in water and also in alcohol precluded its assay in a full range of concentrations. DMSO effectively dissolved DPU, which could then be added to the medium in a small volume of solvent, to obtain concentrations in the medium as high as 20  $\mu$ M for maximum growth, and 200  $\mu$ M for bud production.

The highest concentration of DMSO which could be added to

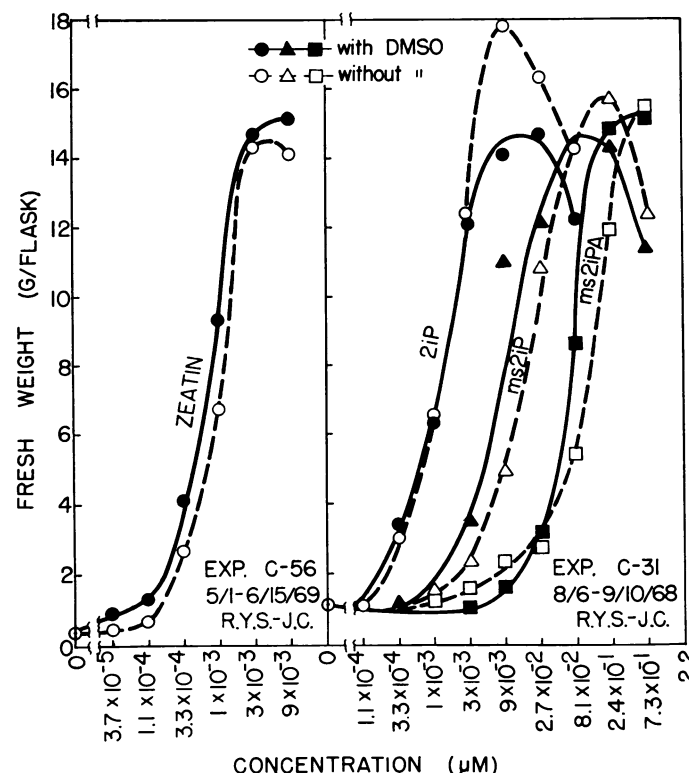


FIG. 2. Effect of 0.05% (v/v) DMSO on yields of tobacco callus on media with serial concentrations of cytokinins. Solid lines: 0.05% DMSO; dashed lines: no DMSO (cytokinins filter-sterilized). A: Zeatin [6-(4-hydroxy-3-methyl-*trans*-2-butenylamino)purine]; B: circles: 2iP [6-(3-methyl-2-butenylamino)purine]; triangles: ms2iP [6-(3-methyl-2-butenylamino)-2-methylthiopurine]; squares: ms2iPA [6-(3-methyl-2-butenylamino)-2-methylthio-9- $\beta$ -D-ribofuranosylpurine].

autoclaving, showed it to be somewhat more toxic than DMSO, and in growth-inhibiting concentrations it caused browning of the tissue. The concentration of DMSO employed in the cytokinin assays is 0.05%. The possibility of growth stimulation in the presence of this level of DMSO in the medium was tested. Several cytokinins including zeatin [6-(4-hydroxy-3-methyl-*trans*-2-butenylamino)purine], 2iP [6-(3-methyl-2-butenylamino)purine], and 6UP [6-phenylureidopurine] were added to the

<sup>1</sup> Abbreviations: DMSO: dimethylsulfoxide; DPU: diphenylurea.

media in filter-sterilized solutions and in DMSO solutions. Figure 2 shows the results of two such tests. Occasionally the presence of DMSO resulted in a slight increase in growth.

On the basis of the above results the tobacco bioassay procedure has been modified as follows. Compounds to be tested are dissolved in DMSO (J. T. Baker Chemical Corporation), and a series of 3-fold dilutions are prepared to cover the full range of concentrations expected to promote callus growth. The initial cytokinin concentration is adjusted so that the aliquot size is 0.025 ml of DMSO solution per 50 ml of nutrient medium (7) for each test substance at each concentration. In a few instances where either solubility or the total amount of a compound has been limiting, the aliquot size at the highest concentration has been tripled, without apparent effect on growth. For ease and uniformity of application a Schwarz Biopette with a 0.025-ml adapter was used. Since DMSO acts as a sterilizing agent, the solutions may be pipetted directly into the agar medium just before it has reached the gelation point. Thus, with the exception noted above, all media contain 0.05% DMSO and any slight effect of DMSO on growth will be equalized.

The use of DMSO as a solvent requires caution since it has been found to have several biological effects, including increased cell permeability (5). Thus it has been shown to increase phosphorus (3) and iron (6) uptake by plants. Recently Delmar and Mills (2) have reported the use of a 10% DMSO solution in the study of tryptophan synthetase activity in whole cells of *Nicotiana tabacum* var. Wisconsin 38 (the strain used in the tobacco bioassay) to be without inhibitory effects on enzyme activity. Although preincubation in a 1% DMSO solution had no effect on subsequent enzyme activity, preincubation in 5% or higher solutions reduced activity because of leaching of the serine pool. In general, low concentrations of DMSO do not appear to be inhibitory to plants; a 2% DMSO solution did not alter the effect of added kinetin on *Datura* spp. (9). However, instances of

DMSO inhibition have been reported in work with animal tissues, where DMSO has been shown to compete with aldehyde in an alcohol dehydrogenase reaction (8) and to increase benzene metabolism and the toxicity of other aromatic hydrocarbons (4).

In view of the possible effects of DMSO on tissue cultures, its inclusion in nutrient media is not recommended as a routine practice in physiological studies. In the case of assaying synthetic cytokinins, however, dilute solutions of DMSO have simplified the tobacco bioassay and made possible the testing of purine and urea derivatives which are difficult to dissolve in water.

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