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Anticytokinin effects on *in vitro* response of embryogenic and nonembryogenic genotypes of *Dactylis glomerata* L.

M. M. Somleva[1], V. Kapchina[1], V. Alexieva[2]* & E. Golovinsky[3]

[1] *Faculty of Biology, University of Sofia, 8 D. Tzankov Bul., 1421 Sofia, Bulgaria*

[2] *Acad. M. Popov Instute of Plant Physiology, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl. 21, 1113 Sofia, Bulgaria*

[3] *Institute of Molecular Biology, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl. 21, 1113 Sofia, Bulgaria*

* *Author for correspondence*

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Abstract

The effect of triazine and carbamate type of anticytokinins on *in vitro* response of embryogenic and nonembryogenic genotypes of orchardgrass (*Dactylis glomerata* L.) was studied. Both compounds stimulated callus production. Anticytokinins influenced both the efficiency of somatic embryogenesis and frequency of embryoid formation.

1. Introduction

Somatic embryogenesis can occur in tissue or cell culture of a number of plant species, usually in response to the presence of exogenous plant growth regulators. Graminaceous tissues do not appear to display the culture response typical for many dicotyledons where *in vitro* development is controlled by the relative auxin:cytokinin levels. Graminaceous cultures require only an exogenous auxin for induction of both callus and morphogenesis. No general aspects can be attributed to the cytokinins. Their presence in the culture medium often has little or no influence, or may even be inhibitory. It is known that only if a responsive tissue is taken as starting material is the phytohormone content of the culture medium a major factor regulating growth and differentiation of cereal tissue *in vitro* [for review see 6].

Leaf explants from a highly embryogenic genotype of *D. glomerata* L. (orchardgrass) provides a reliable system for direct (without callus) or indirect somatic embryogenesis. The embryogenic response in this species is highly genotype dependent [3]. Differences in endogenous cytokinin levels between the embryogenic genotype and nonembryogenic ones have been reported by Wenck *et al.* [10]. In view of this, application of anticytokinins to the culture medium could give additional information for the role of endogenous cytokinins in *D. glomerata*.

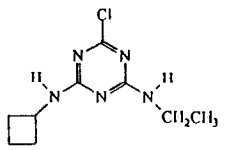
There are 6 classes of cytokinin antagonists [4, 5]. Anticytokinin effects are studied mostly in relation to cytokinin receptors. A few reports are available about possibilities for application of these compounds [5, 11].

The aim of our investigation was to compare the effect of two anticytokinins, structural analogues of purine and phenylurea cytokinins, on *in vitro* response of leaf explants from the embryogenic and nonembryogenic genotypes of *Dactylis glomerata*.

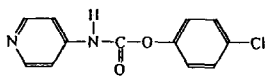
2. Materials and methods

2.1 *Compounds tested*

The following compounds were studied:



2-chloro-4-cyclobuthylamino-6-ethylamino-1,3,5-triazine (I)



N-(4-pyridyl)-O-(4-chlorophenyl)carbamate (II)

The compounds were kindly provided by Prof. H. Iwamura from Kyoto University, Japan.

2.2 *Plant material*

Leaf explants from embryogenic and nonembroygenic genotypes of *D. glomerata* L. were chosen and cultured according to Conger *et al.* [2]. Six segments (2–3 mm long) from the basal portions of the innermost two leaves were surface sterilised and plated onto 0.8% agar SH medium [8]. The medium contained 6.6 $g.l^{-1}$ 3,6-dichloro-o-anisic acid (dicamba) for induction of callus and somatic embryo formation.

2.3 *Evaluation of anticytokinin effects*

Filter sterilised anticytokinins were added to the culture medium at final concentrations of 10^{-7}, 10^{-6}, 10^{-5} and $10^{-4}M$ of *I* and 10^{-7}, 10^{-6}, and $10^{-5}M$ of *II* for estimation of their influence on *in vitro* response of the explants from both genotypes. After four weeks growth at 25 °C in the dark callus dry weight was determined.

In order to describe somatic embryogenesis, two definitions characterising this process were used: Firstly, efficiency of embryogenesis denotes the number of explants forming somatic embryos in relation to all cultured explants. The number of calli which exhibited at least one embryoid was totalled and used to calculate the number that failed to exhibit somatic embryogenesis. Secondly, intensity of somatic embryogenesis denotes the number of embryoids formed directly or indirectly and is expressed as a percentage to the control.

The data presented are from two experiments (8 replicates per variant).

The statistic calculations were performed by the procedure of Fisher.

3. Results and discussion

Orchardgrass leaf explants cultured on a medium lacking any cytokinin provides a suitable system for elucidation of an anticytokinin effect directly on endogenous cytokinins. Callus production allows the comparison of the *in vitro* response of two genotypes of *D. glomerata*. Their response to a range of anticytokinin concentrations was different (Fig. 1). Both compounds tested showed a concentration-dependent effect on the growth of the embryogenic callus. Triazine anticytokinin (*I*) at 10^{-7} and $10^{-6}M$ did not significantly stimulate this process. However, at higher concentration its effect was inhibitory. Carbamate stimulated callus growth of embryogenic genotype at the whole concentration range used (Fig. 1B). Its effect was stronger at $10^{-7}M$ (158.8% to the control value) and decreased with an increment of concentrations. It is evident that both anticytokinins have a similar effect on the growth of nonembryogenic callus. The compounds caused an enhancement of callus production, but a dose-response relationship was not so clear. The stimulating action of the phenylurea derivative (compound *II*) was stronger than *I*. Phenylcarbamate ($10^{-7}M$ and $10^{-6}M$) increased callus dry weight from the nonembryogenic genotype by 44.00% and 86.63% respectively. The triazine derivative caused the same growth stimulation at concentrations 10-fold higher than those of *II*.

The differences between the growth responses of both the genotypes of *D. glomerata* are not surprising. Wenck *et al.* [10] reported that basal leaf portions of the nonembryogenic genotypes of orchardgrass contained 3.5-fold more endogenous cytokinins than those of the embryogenic genotype. Inhibition of the cytokinin action by cytokinin antagonists (biosynthesis, transport, physiological effects etc.) in the nonembryogenic genotype could be one of the reasons for the effects observed.

On the basis of these results it is difficult to explain whether anticytokinins act on the initiation of callus or influence its proliferation.

The presence of anticytokinins (triazine- or carbamate type) in the medium influenced both the efficiency of somatic embryogenesis (Fig. 2) and frequency of embryoids produced indirectly and directly (Figs. 3 and 4). Apparently, the triazine anticytokinin promotes

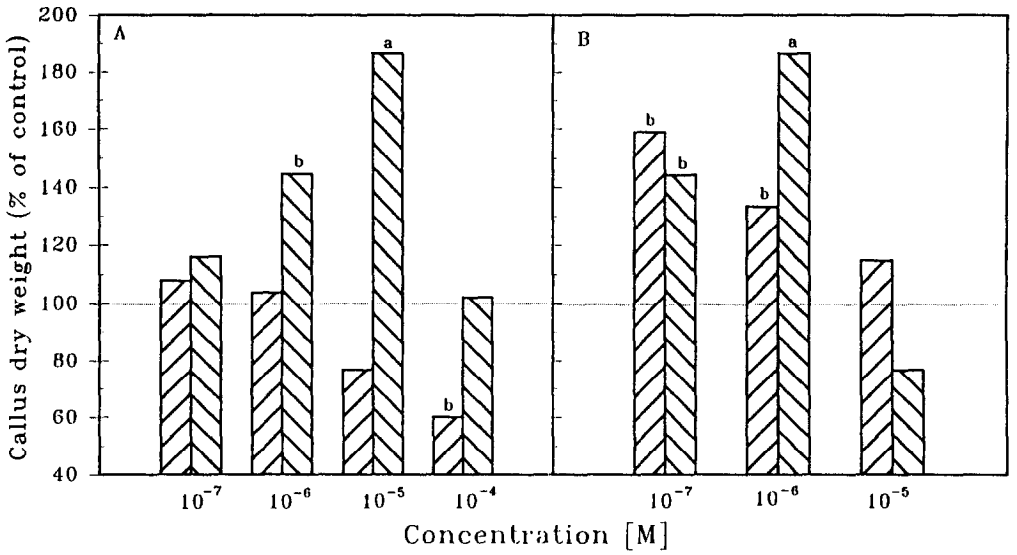


Fig. 1. Effect of 2-chloro-4-cyclobuthylamino-6-ethylamino-1,3,5-triazine (A) and N-(4-pyridyl)-O-(4-chlorophenyl) carbamate (B) on callus growth from orchardgrass leaf explants. ▨ – embryogenic genotype; ▩ – nonembryogenic genotype. a – significant at 0.01; b – significant at 0.005.

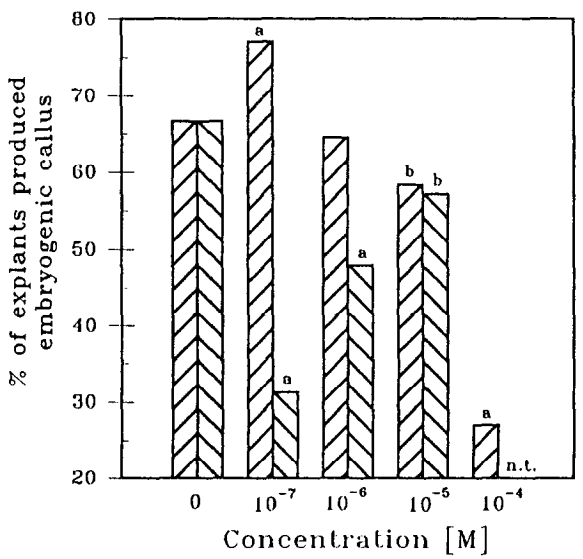


Fig. 2. Efficiency of somatic embryogenesis from orchardgrass leaf explants in the presence of 2-chloro-4-cyclobuthylamino-6-ethylamino-1,3,5-triazine (*I*) and N-(4-pyridyl)-O-(4-chlorophenyl) carbamate (*II*). ▨ – *I*; ▩ – *II*; n.t. – not tested. a – significant at 0.01; b – significant at 0.05.

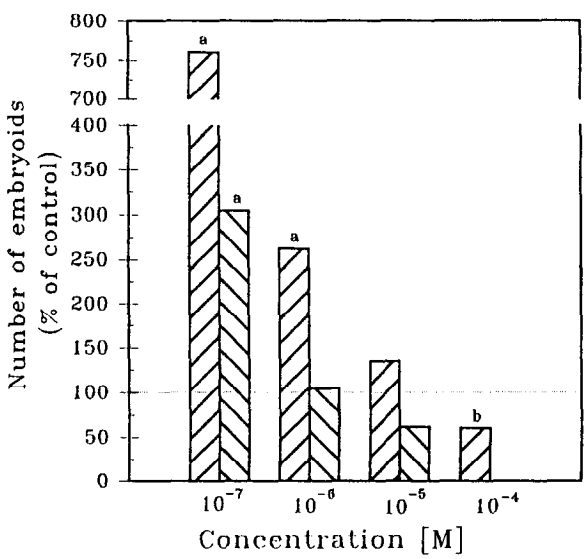


Fig. 3. Influence of 2-chloro-4-cyclobuthylamino-6-ethylamino-1,3,5-triazine on the intensity of somatic embryogenesis from leaf explants of *D. glomerata*. ▨ – indirect embryoids; ▩ – direct embryoids. a – significant at 0.01; b – significant at 0.05.

a significantly higher intensity of embryogenesis than carbamate. The optimal concentration of *I* was $10^{-7}M$ (760.4% and 304.5% for indirect and direct embryogenesis, respectively) beyond which the number of embryoids decreased. The total stimulation of somatic embryogenesis observed at $10^{-7}M$ suggests that compound *I* increased the number of somatic cells competent to form embryoids. Indirect embryogenesis was

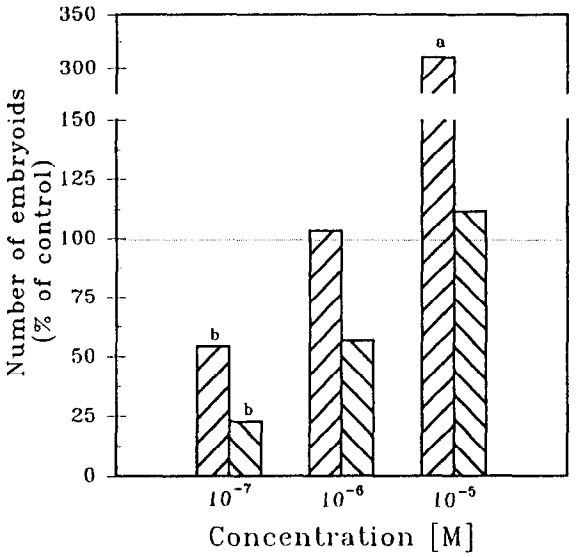


Fig. 4. Influence of N-(4-pyridyl)-O-(4-chlorophenyl) carbamate on the intensity of somatic embryogenesis from leaf explants of *D. glomerata*. ▨ – indirect embryoids; ▩ – direct embryoids. a – significant at 0.01; b – significant at 0.05.

strongly suppressed by triazine at $10^{-4}M$ (61.5% to the control) and total inhibition of direct embryo formation was observed. These results suggest a herbicidal action of triazine at the highest concentration applied.

The expression of embryogenic capacity in *Gramineae* is very sensitive to exogenous cytokinins [1, 7, 9]. The addition of zeatin even at 0.001 μM to the culture medium significantly suppressed embryo formation from orchardgrass leaf explants [10]. The strong inhibition of embryogenesis caused by carbamate at $10^{-7}M$ indicated some cytokinin activity of this compound at the lowest concentration used. Carbamate-type anticytokinin did not stimulate direct embryogenesis. The embryogenic callus grown at the highest concentration had greater capability for somatic embryogenesis than that grown at lower concentrations. It remains unclear whether these effects of *II* reflect its direct action on the embryogenic cells. Alternatively this may be due to such metabolic and physiological changes in the callus which improve indirect embryogenesis.

Both anticytokinins used in our experiments stimulated more effectively indirect somatic embryogenesis than direct embryo formation.

In general, *I* and *II* influence *in vitro* response of orchardgrass leaf explants presumably through changes in the endogenous cytokinin effects. Most probably some of these changes favour the expression of embryogenic capacity. More detailed studies are needed for elucidation of this physiological effect.

Application of anticytokinins provides a new approach for investigation of the mechanisms by which cytokinins regulate somatic embryogenesis in cereals. They also reveal new possibilities for practical application of this class of plant growth regulators. Available evidence indicates that the present results are the first in relation to anticytokinin effects on plant embryogenesis.

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