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CYTOKININS AND UREA DERIVATIVES STIMULATE SEED GERMINATION IN *LOTUS CORNICULATUS* L.

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Abstract – We studied the effects of various cytokinins and urea derivatives on germination of aged seeds of in *Lotus corniculatus* L. The following substances were applied: N^6-isoprenoid cytokinins (isopentenyl adenine and zeatin), adenine sulfate, N^6-aromatic cytokinins (kinetin, benzyladenine) and their N^9-ribosides, N-benzyl-9-(2-tetrahydropyranyl)adenine, and urea derivatives (diphenylurea, thidiazuron, and chloro-pyridyl phenylurea). With the exception of adenine sulfate, all cytokinins increased the percentage of seed germination up to twofold, depending on their kind and concentration. It is concluded that cytokinins may be among the missing factors in aged seeds of *L. corniculatus* contributing to the implementation of their full germination potential. They could be used to improve germination of both freshly harvested and aged seed samples, if necessary.

Key words: Cytokinins, seed germination, urea derivatives.

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INTRODUCTION

It is well known that the viability of seeds in different plant species is highly variable, ranging from a few days to many years. Germination capacity depends on the species and external conditions, while the physiological state of seeds also plays a very significant role in this process. It is assumed that some important growth factors deteriorate during storage, thus diminishing the percentage of viable seeds. Application of various growth regulators may in many instances substitute for missing factors, but no general opinions and rules exist as to their natural role. As for cytokinins, it seems that they exert their action mostly in stressed seeds, such as those exhibiting salinity-induced dormancy (K a h n et al., 2004) those affected by heavy metals Cu and Zn (G a d a l l a h and E l - A n a n y, 1999), or by oxidative stress (C h a i t a n y a and N a i t h a n i, 1998). On the other hand, cytokinins do not affect germination in large-seeded grain legumes (M a l i k and S a x e n a, 1992) or even inhibit light-induced germination in photoblastic seeds of *Paulownia tomentosa* (G r u b i š i ć, unpublished). Stored in standard conditions, seeds of the perennial forage legume *L. corniculatus* gradually lose their germination capacity within several years after harvest. In a previous work treating the effect of cytokinins on *in vitro* seedling morphogenesis (N i k o l i ć et al., 2006), we noted that cytokinins increased the percentage and rate of seed germination as a side effect. In freshly harvested seeds the germination percentage was increased from two- to threefold, reaching the values of 80-90% at optimum cytokinin concentrations. In seeds four or more years old with much lower viability, the relative cytokinin effect was similar, but the maxima did not exceed 50%. It seemed to us that these effects warrant a more detailed study. We therefore used eleven cytokinins and cytokinin-like urea derivatives in the present experiments, and a broader range of their concentrations was applied.

MATERIAL AND METHODS

Seed harvest and germination

Seeds of bird's foot trefoil, *L. corniculatus* L. cv. Bokor (M i j a t o v i ć et al., 1986), produced in the experimental field of the Center for Agricultural and Tech-

nological Research at Zaječar were used in this work. The seeds were mechanically collected during the summer of 1999, and samples were air dried and stored in paper bags at room temperature in the laboratory.

For germination, 30 seeds were placed in a 6-cm Petri dish containing 2 mL of an aqueous solution of the tested chemical or 2 mL of distilled water in the controls. In all solutions, the fungicide Nistatin was supplied at a concentration of 500 mg L^{-1} in order to suppress fungal infections. The urea derivatives were first dissolved in DMSO, then diluted with water. Germination was determined as protrusion of the radicle. The number of germinated seeds was scored at 3-day intervals throughout an 18-day incubation period. The seeds were incubated at a constant temperature of 25 ± 2°C in darkness. Three replicates were prepared for each treatment and the mean germination percentage was calculated. Germination data were arcsin transformed for statistical analysis and analyzed using Statgraphics Plus, V2.1. They were subjected to the analysis of variance (ANOVA), and the LSD test was used to determine significant differences among mean values of the treatments ($P<0.05$).

Chemicals

The following substances were used in germination experiments: two isoprenoid cytokinins, 2iP [6-(γ,γ-dimethylallylamino)purine, ICN Biomedicals Inc.] and ZEA (zeatin, mixed isomers, Sigma Co.); two aromatic cytokinins, KIN (kinetin, Sigma Co.) and BA (6-benzyladenine, Sigma Co.) and their ribosides KINR (ICN Biomedicals Inc.) and BAR (Sigma Co.); the synthetic aromatic cytokinin NBA [N-benzyl-9-(2-tetrahydro-pyranyl)adenine, Sigma Co.]; the presumably inactive AS (adenine sulfate, Sigma Co.); the urea derivatives with cytokinin-like actions DPU (1,3-diphenylurea, ICN Biomedicals Inc.), TDZ [thidiazuron, 1-phenyl-3-(1,2,3-thidiazol-5-yl)urea, Sigma Co.], and 4-CPPU [N-(2-chloro-4-pyridyl)-N'-phenylurea, Sigma Co.]. The following concentrations were applied: 10^{-7}, $3x10^{-7}$, 10^{-6}, $3x10^{-6}$, 10^{-5}, $3x10^{-5}$, and 10^{-4} M. In some experiments gibberellin (GA_3, Serva Co.) in various concentrations was added to 10^{-6} M BA. Fusicoccin (FC, Sigma Co.) at 10^{-5} M and ethephon at 3.3 mg L^{-1} were also tested.

RESULTS AND DISCUSSION

The mean germination percentage in control seeds ranged from 22 ± 3% to 32 ± 2%, with an average value

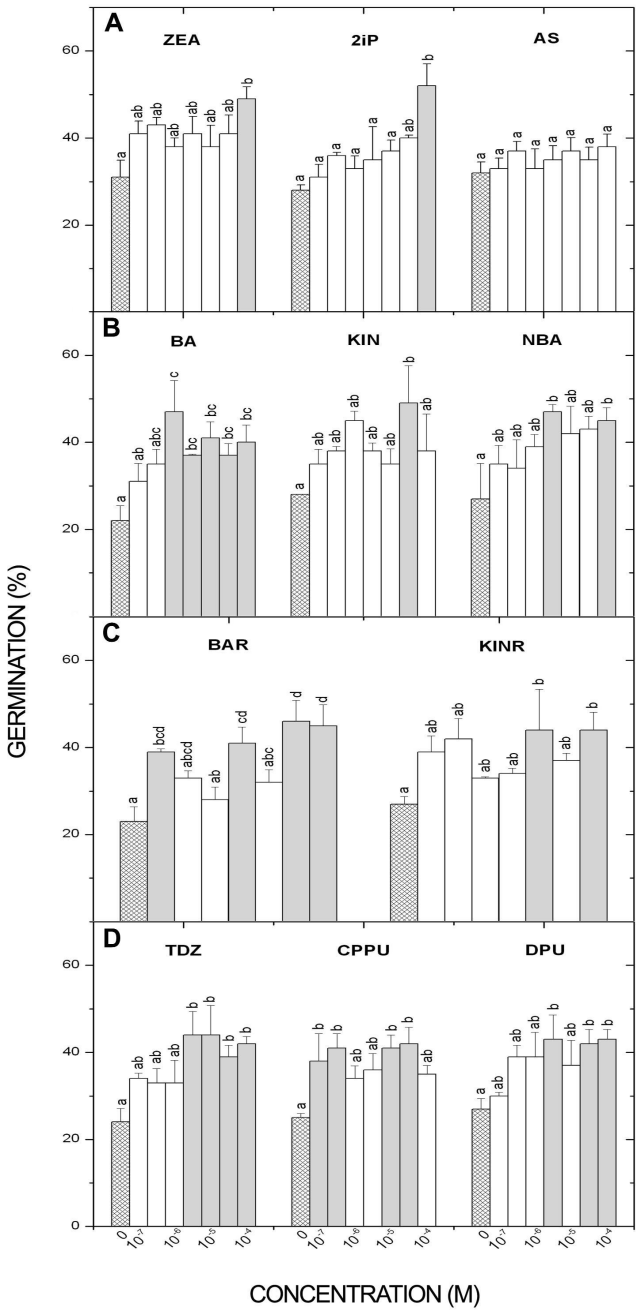

Fig. 1. Effects of cytokinins and urea derivatives on the percentage of *L. corniculatus* seed germination at 0, 10^{-7}, $3x10^{-7}$, 10^{-6}, $3x10^{-6}$, 10^{-5}, $3x10^{-5}$, and 10^{-4} M. **A**: N^6-isoprenoid cytokinins (2iP and ZEA) and AS; **B**: aromatic cytokinins (BA, KIN, and the synthetic NBA); **C**: ribosides of aromatic cytokinins BAR and KINR; **D**: urea derivatives (TDZ, CPPU, and DPU). The germination percentage was determined after 18 days. Cytokinin effects were compared with controls (0); vertical bars indicate ± SE of three replicates containing 30 seeds each. Shaded columns – values significantly different from controls ($P<0.05$, LSD test).

of 27%. For *L. corniculatus* these values can be judged as very unsatisfactory, since a good, commercially acceptable sample of seeds should have a germination percentage of no less than 70%. Although the variation was rather high from treatment to treatment, all cytokinins and urea derivatives used stimulated seed germination over their respective control values. The only exception was adenine sulfate, which was included in the experiments as a presumably inactive control (Fig. 1). However, the hormonal effect of cytokinins displayed certain unusual traits, such as the lack of a concentration-dependent response in all experiments. The lowest concentrations used (10^{-7} M) did not produce significantly different results from the controls, except in the case of BAR; use of concentrations lower than 10^{-7} M would therefore have been pointless. Although the range of concentrations used extended over four log units (10^{-4} – 10^{-7} M), a clear-cut dose-response curve typical of hormonal effects was not obtained. Optimum germination percentages tended to be at higher concentrations, but occasionally occured at lower ones. Seed germination is a complex sequence of events. It would appear that if the requirement for cytokinin was saturated by a constant low concentration, while other interacting factors may have been irreversibly lost during storage. In a few pilot experiments, we added GA_3, ethephon, or fusicoccin with or without cytokinins, but the results were disappointing and are not presented here. Cell membrane lesions were reported to occur in dehydrated *L. corniculatus* seeds (McKersie and Stinton, 1980), and if this happened in let us say 40% of our seeds, it can hardly be expected that their germination percentage could be improved over 60% by adding any growth regulator generally known to stimulate that process. An interesting question is whether cytokinins could protect membranes in *L. corniculatus* seeds from deterioration if added some time after harvest. Such an experiment might point to a specific role for cytokinins and indicate molecular differences between isoprenoid and aromatic cytokinins, as well as between these and urea derivatives. Moreover, treatment of seeds with a urea derivative could be a plausible way to prolongate viability of seeds used in selection processes.

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ЦИТОКИНИНИ И ДЕРИВАТИ УРЕЕ СТИМУЛИШУ КЛИЈАЊЕ СЕМЕНА *LOTUS CORNICULATUS* L.

РАДОМИРКА НИКОЛИЋ, НЕВЕНА МИТИЋ, СУЗАНА ЖИВКОВИЋ, Д. ГРУБИШИЋ и МИРЈАНА НЕШКОВИЋ

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Испитиван је ефекат различитих цитокинина и деривата урее на клијање старих семена *Lotus corniculatus* L. Употребљене су следеће супстанце: N^6-изопреноидни цитокинини (изопентенил аденин и зеатин), аденин сулфат, N^6-ароматични цитокинини (кинетин, бензиладенин), и њихови N^9-рибозиди, N-бензил-9-(2-тетрахидропиранил)аденин и деривати урее (дифенилуреа, тидиазурон и хлоро-пиридил фенилуреа). Сви цитокинини су, изузев аденин сулфата, повећавали проценат клијања семена до два пута у зависности од врсте цитокинина и концентрације. Закључено је да цитокинини могу бити један од недостајућих фактора у старим семенима *L. corniculatus*, који доприносе остварењу њиховог пуног потенцијала за клијање. Ако је потребно, цитокинини могу бити искоришћени за побољшање клијања свеже убраних и старих семена.