

Influence of arbuscular mycorrhiza, P limitation and Cd-stress on polyamine contents of plants

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Arbuscular mycorrhiza is the most ancient and widespread type of mycorrhiza symbiosis. Phosphorus uptake and growth of host plants are stimulated by arbuscular mycorrhizal (AM) fungi. In return, host plants provide carbohydrates for their fungal partners (Smith and Read 1997). Growth and photosynthesis of the host plants are often improved due to the better mineral nutrition. The positive growth response to AM formation, however, usually declines under high P availability. Physiology of AM plants are prone to significant changes due to other, direct responses of plant metabolism, like hormonal balances, which are often masked by the nutritional benefits (Smith and Read 1997).

There are profound changes in plant polyamine (PA) metabolism under several environmental challenges including mineral nutrient deficiencies or atmospheric pollutants, like cadmium (Bouchereau et al. 1999). Put seems to be a stress signal molecule and its concentration is usually increased under these conditions. Information is very scarce about the role of PAs in plant-microbe symbioses (Walters 2000). They may have a role in the molecular signalling events between the symbiotic partners (El Ghachtouli et al. 1995).

Materials and Methods

P limitation experiment

Plantago lanceolata L. plants were grown in pots on a gamma-irradiated mixture of loam and sand in a greenhouse for three months. Temperature was around 24/17°C day/night and photosynthetic photon flux was maximum 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in the beginning, but increased up to 600-700 $\mu\text{mol m}^{-2} \text{s}^{-1}$ by the second half of the experiment.

Nutrients were provided in the beginning. KH_2PO_4 was added in 2 concentrations (P_1 and P_2) while it was not added in one case (P_0). Plant-available soil phosphorus contents determined by an ICP spectrometer were 1.7 (P_0), 32.7 (P_1) and 135.3 (P_2) $\mu\text{g g}^{-1}$.

Soil-based inoculum of *Glomus fasciculatum* originated from the European Bank of Glomales (BEG 53) was used. Mycorrhizal colonization was quantified according to Phillips and Hayman (1970) and Trouvelot et al. (1986). Total percentage plant phosphorus contents (% of d. wt) were measured by an ICP spectrometer. $^{14}\text{CO}_2$ fixation capacities were determined according to Láng et al. (1985) with a liquid scintillation apparatus.

Cd-stress experiment

Ri T-DNA transformed carrot (*Daucus carota* L.) roots were provided by G. Bécard (Lab. de Mycol. Pole de Biotech. Veget., Auzeville, Castanet Tolosan, France). Mycorrhizal root cultures were colonized by *Glomus intraradices*. Roots were cultivated on a minimal medium described by Bécard and Fortin (1988). After subculturing, roots were grown for six weeks in dark at room temperature.

During treatments, roots were transferred to 1 L bakers containing 100 ml $1/4$ -strength Hoagland solution. For Cd treatment, $\text{Cd}(\text{NO}_3)_2$ in $3 \cdot 10^{-6}$ M and $5 \cdot 10^{-5}$ M concentrations were used. One of the controls was measured immediately after opening the Petri dish, while the other control was soaked for 6 hours on a shaker with the Cd-treated ones. After treatments, root fragments were randomly homogenized and thoroughly cleaned of agar remnants.

Polyamine quantification

Quantities of free PAs were determined by thin-layer chromatography (Rác et al. 1996) and a Jobin Yvon-Spex Fluoromax-2 spectrofluorometer. Putrescine (Put) content compared to the sum of spermidine (Spd) and spermine (Spm) contents (Put/(Spd+Spm) ratio) was calculated.

Statistics

Two-way ANOVA were used by the software SPSS 7.5. Means were compared between treatments by the least significant difference (LSD) test or the Student's t-test.

Results and Discussion

P limitation experiment – growth effects

Inoculated plants were heavily colonised by the AM fungus at every P levels. Colonization intensity decreased with increasing P availability, as documented before (Smith and Read 1997). Percentage P contents and CO_2 fixation capacities of mycorrhizal (M) plants were higher than those of non-mycorrhizal (NM) ones. Effect of AM colonization on growth had a strong interaction with P availability. NM plants showed a typical yield response curve for P supply, therefore P was truly limiting. Growth parameters of M plants remained at the same levels, which were significantly higher than those of NM ones at P_0 level, in accordance with other results (Smith and Read 1997). This difference not only disappeared at P_1 , but a negative growth response to AM colonization could be detected at the highest P level, as

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indicated before (Smith and Read 1997).

Polyamine content – P limitation and Cd stress

Role of PAs in the AM symbiosis was firstly shown by El Ghachtouli et al. (1995), who detected that exogenously applied PAs increased colonization frequency in pea. According to El Ghachtouli et al. (1996), PA biosynthesis inhibitors reduced root growth and AM colonization, which were reversed by added Put, but there was no difference in PA concentrations due to AM colonization. In contrast, Goicoechea et al. (1998) observed, that AM alfalfa plants maintained higher Spd and Spm concentrations under water stress.

In contrast to the earlier results, we found, that M plants had mostly lower or equal free PA concentrations than NM ones, but differences seem to be related to P supply. PA concentrations were equivalent in NM and M plants when growth parameters were similar at P_1 level, irrespectively of their different P contents. There were significantly higher PA concentrations in NM leaves and also roots at P_0 level, so these differences seem to be of mostly plant origin also in the roots. Most dramatic differences were recorded in the case of Put, as observed under several stress conditions including mineral nutrient (K) deficiency (Bouchereau et al. 1999). It has not been indicated yet in case of P limitation.

The ratio of PAs was documented to be a good indicator of stress (Bouchereau et al. 1999). Despite the great differences in concentrations, there were not any significant alterations in PA ratios between NM and M leaves. On the contrary, significantly higher ratios were found in NM roots at P_0 and P_1 levels, that is until growth parameters of NM plants were below or the same than that of M ones.

It is concluded that P deficiency stress, as well as the demand for AM symbiosis may be reflected by PA concentrations and ratio. A given state of P deficiency stress appears to exist, below which PA concentrations and ratio are increased in NM plants. According to previous results, PAs might mediate phytohormone action and influence the colonization process (El Ghachtouli et al. 1996). Present study seems to be the first indication of the possible role of PAs in the P nutrition-dependent regulation of AM symbiosis.

As regards Cd-stress experiment, differences in PA concentrations were less characteristic. In contrast to the earlier results on barley (Soós 2000), Put concentrations of NM roots were lower after Cd treatment, and their Spd concentrations also decreased. There were no changes, however, in the PA concentrations of M roots, except their increase of Spm contents when treated with Cd. M roots had, therefore, significantly higher Spm concentrations under Cd treatment compared to the NM ones.

Since the minimal medium used to grow roots contained nutrients – including phosphate – in small amounts, results were compared to the low P level of the P limitation experiment. Similarities were found in that Put contents of NM roots were higher, than those of M ones and NM roots had significantly higher Put/(Spd+Spm) ratios in every case.

In summary, it seems that P limitation had the same effect in both experiment, but more studies are needed for the safe confirmation of the connections between PAs, P nutrition and arbuscular mycorrhiza and to characterize the effects of Cd-stress.

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References

- Bécard G, Fortin JA (1988) Early events of vesicular-arbuscular mycorrhiza formation on Ri T-DNA transformed roots. *New Phytol* 108:211-218.
- Bouchereau A, Aziz A, Larher F, Martin-Tanguy J (1999) Polyamines and environmental challenges: recent development. *Plant Sci* 140:103-125.
- El Ghachtouli N, Paynot M, Morandi D, Martin-Tanguy J, Gianinazzi S (1995) The effect of polyamines on endomycorrhizal infection of wild-type *Pisum sativum*, cv. Frisson (nod+myc+) and two mutants (nod-myc+ and nod-myc-). *Mycorrhiza* 5:189-192.
- El Ghachtouli N, Martin-Tanguy J, Paynot M, Gianinazzi S (1996) First report of the inhibition of arbuscular mycorrhizal infection of *Pisum sativum* by specific and irreversible inhibition of polyamine biosynthesis or by gibberellic acid treatment. *FEBS Lett* 385:189-192.
- Goicoechea N, Szalai G, Antolín MC, Sánchez-Díaz M, Páldi E (1998) Influence of arbuscular mycorrhizae and Rhizobium on free polyamines and proline levels in water-stressed alfalfa. *J Plant Physiol* 153:706-711.
- Láng F, Sárvári É, Szigeti Z (1985) Apparatus and method for rapid determination of photosynthetic CO₂ fixation of leaves. *Biochem Physiol Pflanzen* 180:333-336.
- Phillips JM, Hayman DS (1970) Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans Brit Mycol Soc* 55:158-160.
- Rác I, Kovács M, Lásztity D, Veisz O, Szalai G, Páldi E (1996) Effect of short-term and long-term low temperature stress on polyamine biosynthesis in wheat genotypes with varying degrees of frost tolerance. *J Plant Physiol* 148:368-373.
- Smith SE, Read DJ, ed., (1997) *Mycorrhizal symbiosis*. Academic Press, London, etc.
- Soós V (2000) Effect of abiotic and biotic stress on polyamine contents of barley and tobacco. M.Sc. Theses.
- Trouvelot A, Kough JL, Gianinazzi-Pearson V. Mesure du taux de mycorrhization VA d'un système racinaire. Recherche de méthodes d'estimation ayant une signification fonctionnelle. In: *Mycorrhizae: physiology and genetics-Les mycorrhizes: physiologie et génétique*. - Proceedings of the 1st ESM/1er SEM, Dijon, 1-5 July 1985-INRA, Paris: 217-221, 1986.
- Walters DR (2000) Polyamines in plant-microbe interactions. *Physiol Mol Plant Pathol* 57:137-146.