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Effect of Phosphate Deficit on Root Growth, Production of Reactive Oxygen Species and Hormone Content in Barley Plants

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Abstract. The article presents the results of research into the effect of removing phosphate from a nutrient medium on the content of auxins and cytokinins in roots, root elongation and content of reactive oxygen species in root tips of the barley 'Steptoe'. In our experiments, the growth response was detected as root elongation after a 4-day exposure to a phosphate-free medium. Activation of linear root growth was preceded by changes in hormonal balance and in the level of reactive oxygen species. Auxin content in the roots increased after 6 h of phosphate starvation and a two-fold increase in the concentration of auxins in roots was detected by the end of the first day of the exposure to the phosphate deficit conditions. Staining with diaminobenzidine revealed an increased level of reactive oxygen species in the root tips of phosphate-starved plants after 6 h of exposure. However, after one day (24 h), a reverse pattern was observed: the level of staining was higher in the plants supplied with phosphates. Immunolocalisation of cytokinins in the root tips, where the zones of cell division and extension determining root elongation are located, showed a decreased content of zeatin in the cells under the effect of phosphorus deficit. The obtained data suggest that the detected rise in the amount of reactive oxygen species was due to the increased concentration of auxins accumulated as a result of the phosphate deficit effect on the barley plants. The increase in ROS and auxins contents could in turn influence the level of cytokinins and, in the end, affect root elongation. Further experiments are needed to test this hypothesis.

Keywords: *Hordeum vulgare*, phosphate deficit, root growth, auxins, cytokinins, reactive oxygen species.

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Влияние дефицита фосфора на рост корней, продукцию активных форм кислорода и содержание гормонов в растениях ячменя

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Аннотация. В работе представлены результаты изучения влияния удаления фосфатов из питательного раствора на содержание ауксинов и цитокининов в корнях, удлинение корней и содержание активных форм кислорода в кончиках корней растений ячменя. Ростовая реакция, проявившаяся в наших экспериментах в удлинении корней растений ячменя сорта Steptoe, была отмечена после 4 суток воздействия бесфосфатной среды. Активации линейной скорости роста корней предшествовали изменения гормонального баланса и уровня реактивных форм кислорода. С помощью метода иммуноферментного анализа через 6 часов воздействия дефицита фосфора было отмечено достоверное возрастание, а к концу первых суток – двукратное увеличение концентрации ауксинов в корне. На фоне фосфатного голодания окрашивание корней диаминобензидином позволило выявить повышенный уровень реактивных форм кислорода в кончиках корней через 6 часов; по истечении первых суток (24 ч) наблюдали обратную картину – уровень окрашивания корней снабженных фосфатами растений был выше. Результаты иммунолокализации цитокининов в кончиках корней, где и находятся определяющие рост корней в длину зоны деления и растяжения, показали снижение содержания зеатина в клетках, находящихся под влиянием дефицита фосфора. Полученные данные позволяют предположить, что выявленное накопление в корнях активных форм кислорода может быть обусловлено повышенной концентрацией ауксинов, накопившихся в результате воздействия дефицита фосфора на растения ячменя. Повышение уровня ауксинов и реактивных форм кислорода в свою очередь могло повлиять на уровень цитокининов и, в конечном счете, на удлинение корней. Требуются дальнейшие исследования для проверки этого предположения.

Ключевые слова: *Hordeum vulgare*, дефицит фосфора, рост корней, ауксины, цитокинины, активные формы кислорода.

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Introduction

Regulation of the rate of root growth and development is an important mechanism of plant adaptation to phosphate starvation. Allocation of new biomass to root growth (Hermans et al., 2006; Wang et al., 2015), changes in the rates of primary roots elongation and the pattern of root branching influence root ability to explore different layers of soil and the nutrient uptake capacity of plants (Lynch, 2011). Nevertheless, the mechanisms of root growth response to phosphate starvation are still not completely clear (Aibara and Miwa, 2014). A deficit in phosphates influences hormone concentration in plants (Ribot et al., 2008; Rubio et al., 2009) and reactive oxygen species (ROS) production, while both hormones and ROS are capable of affecting root growth and development (Tyburski et al., 2010). However, little attention has been given to possible interaction of these factors under phosphate starvation conditions. In the present paper we study the effect of removing phosphate from the nutrient medium on auxins and cytokinins contents in roots, root elongation and ROS content in the root tips of barley plants (up to the zone of root hairs).

Materials and methods

Barley plants (*Hordeum vulgare* L. 'Steptoe') were grown on 0.1 strength Hoagland-Arnon (H-A) nutrient medium (0.5 mM KNO_3, 0.5 mM $Ca(N0_3)_2$, 0.1 mM KH_2PO_4, 0.1 mM $MgSO_4$, 0.5 mM $CaSO_4$) in which potassium phosphate was substituted with sodium phosphate (modified H-A). After stratification and germination, half of the seedlings were transferred to a medium without phosphates (P-). Plants were grown at the 14-h photoperiod with the irradiance of 400 μmol m^{-2} s^{-1} PAR, temperature of 25/18°C (day/night) and relative air humidity (RH) of 60–70%. Preliminary experiments had showed that substitution of potassium for sodium phosphate did not inhibit the growth of plants.

Auxin content was determined by means of enzyme immunoassay (Vysotskaya et al., 2003) and ROS level in roots was detected using diaminobenzidine (DAB) staining (the protocol was adapted from Daudi et al., 2012) after 6 and 24 hours of phosphate starvation. For immunolocalisation of cytokinins, root tips were fixed in a mixture of aldehydes and carbodiimide (Kudoyarova et al., 2014) on the second day after removing phosphates from the nutrient solution. The intensity of staining on the photographs was estimated in arbitrary units using the ImageJ program (the minimum and maximum values were taken for 0% and 100%, respectively). Root length was measured on the fourth day of the experiment.

Results and discussion

Auxin content in the roots increased after 6 h of phosphate (P) starvation (97 ± 11 and 160 ± 17 ng/g of root fresh weight on the medium with and without P, respectively; mean±SE, n=9) and after 24 h it was 2 times higher than in the control (72±6 and 120±9 ng/g of root fresh weight on the medium with and without P, respectively; mean±SE, n=9). These results agree with the literature data (Nacry et al., 2005). DAB staining revealed an increased ROS level in the root tips of the plants grown without phosphates during 6 h: the intensity of staining increased approximately 3-fold (Fig. 1). After 24 h of exposure, a reverse pattern was observed and the level of staining was 3.5 times higher in plants supplied with phosphates (Fig. 2).

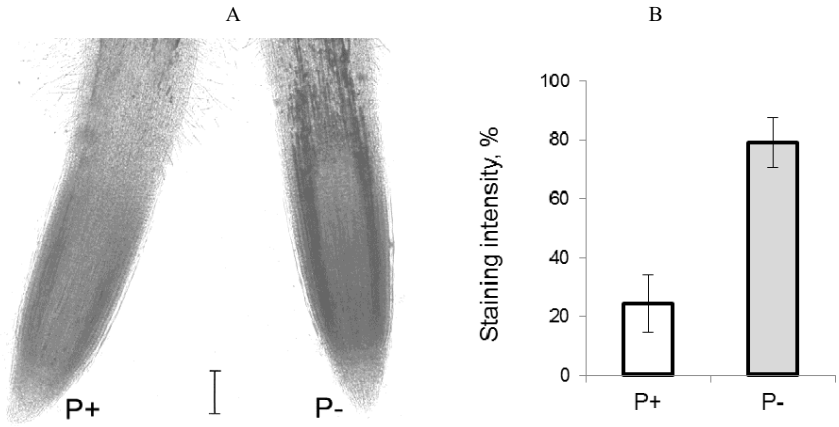

Fig. 1. (A) Level of ROS in root tips of barley plants (intensity of DAB staining) grown on modified Hoagland-Arnon medium (P+) and 6 h after removing phosphates (P-) from the nutrient medium. Scale bar: 200 µm. (B) Diagram presents the results of the semiquantitative assay of intensity of staining of root tips of 'Steptoe' obtained using the ImageJ program (as described by Sharipova et al., 2016). The images of nine independent sections per treatment were taken. The intensity of staining was expressed in arbitrary units, with maximum and minimum staining intensity taken for 100% and 0%, respectively. Error bars are standard error, n=9

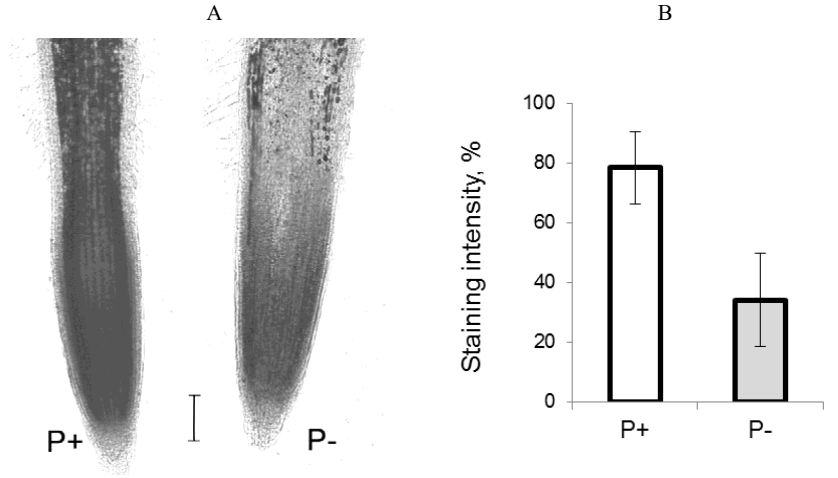

Fig. 2. (A) Level of ROS in root tips of barley plants (intensity of DAB staining) grown on modified Hoagland-Arnon medium (P+) and 1 d after removing phosphates (P-) from the nutrient medium. Scale bar: 200 µm. (B) Diagram presents the results of the semiquantitative assay of intensity of staining of root tips of 'Steptoe' obtained using the ImageJ program (as described by Sharipova et al., 2016). The images of nine independent sections per treatment were taken. The intensity of staining was expressed in arbitrary units, with maximum and minimum staining intensity taken for 100% and 0%, respectively. Error bars are standard error, n=9

Auxins are known, on the one hand, to induce ROS production and, on the other hand, to contribute to their inactivation brought about by up-regulation of the genes coding for antioxidant enzymes (Krishnamurthy and Rathinasabapathi, 2013). Auxin-induced ROS production may link the elevated auxin concentration with the initial increase in the ROS level in the roots of P-starved plants, while the hormone-induced inactivation of ROS may explain the subsequent decline in ROS detected under P deficit.

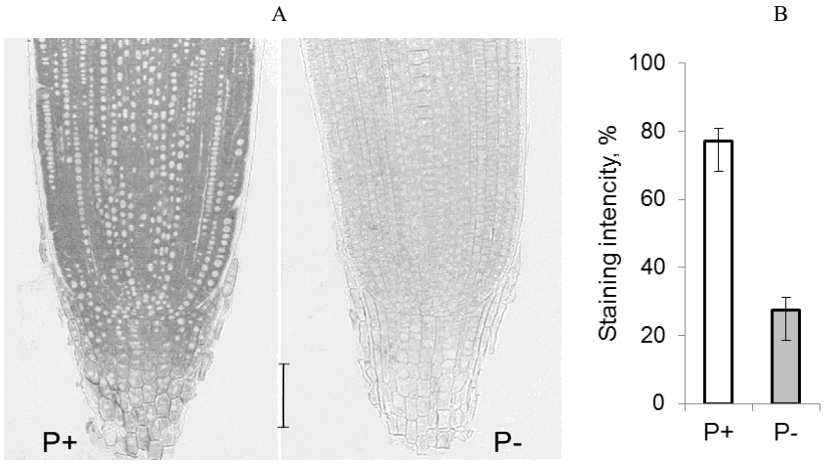

Fig. 3. (A) Immunohistochemical localisation of cytokinin zeatin in root tips of barley seedlings grown on modified Hoagland-Arnon medium (P+) and placed for 2 days on the medium without phosphates (P-). Scale bar: 50 μm. (B) Diagram presents the results of the semiqunatative assay of intensity of staining of root tips obtained using the ImageJ program (as described by Sharipova et al., 2016). The images of nine independent sections per treatment were taken. The intensity of staining was expressed in arbitrary units, with maximum and minimum staining intensity taken for 100% and 0%, respectively. Error bars are standard error, n=9

In the present experiments, phosphate deficit stimulated a 16% elongation in roots on the medium without P after 4 days of exposure (29.5±1.1 and 34.3±1.3 cm on the medium with and without P, respectively; mean±SE, n=40).

ROS are known to influence cell extension (Tyburski et al., 2010) suggesting their possible involvement in the changes in root elongation induced by P deficit. However, the data are contradictory as both stimulatory and inhibitory effects of ROS have been reported (Tsukagoshi et al., 2010).

Immunolocalisation of cytokinins in root tips showed a decline in zeatin content in the cells influenced by P deficit (Fig. 3). Since cytokinins are known to inhibit root growth at the expense of cell division (Ivanov and Filin, 2018), acceleration of root growth under P deficit is likely to be due to a decrease in the level of cytokinins in cells. The decline in the level of cytokinins could result from either a transitory increase in the ROS level exerting cytokinins decay through their oxidation or accumulation of auxins able to activate enzymatic destruction of cytokinins (Hare and van Staden, 1994).

Conclusion

The obtained results suggest the following succession of events: a phosphate deficit causes changes in the ROS level brought about by accumulation of auxins which finally results in the changes in cytokinins level and elongation of roots. Further research is needed to test this hypothesis.

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