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Effect and after-effect of barley seed coating with phosphorus on germination, photosynthetic pigments and grain yield

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Abstract

Phosphorus is an important macronutrient for barley, but commonly it has low availability in soils. Therefore, seed coating with readily available phosphorus is used in agricultural practice. The aim of this work was to investigate the influence of barley seed coating with phosphorus on germination, the content of pigments of green plastids in shoots, the seed yield and the presence of an after-effect on the second generation. The coating of barley seeds with phosphorus depressed germination in the beginning of the development of plants, but there was a positive influence of the coating on the amount of pigments of green plastids in the shoots, and the yield of barley seeds increased by 3 to 91 %. The treatment of barley seeds with the phosphorus influenced positively the physiological activity of next generation seeds. The usage of phosphorus-treated barley seeds is effective: physiological activity of the seeds increases as well as the yield of the seeds. Also, phosphorus uptake is improved, which contributes to better environmental protection.

Key words: mineral nutrition, phosphorus seed coating, photosynthetic pigments, seed germination, spring barley, yield.

Introduction

Phosphorus is the second limiting element after nitrogen for plant growth – plants can not grow without a sufficient supply of this nutrient. Phosphorus plays an essential role in all physiological and biochemical processes in plants (Hartmann 1997; Schachtman et al. 1998; Marschner 1999; Lott et al. 2000). The amount of phosphorus in plants ranges from 0.05 % to 0.50 % of total dry weight (Bieleski 1973; Theodorou, Plaxton 1993; Marschner 1999). It is a component of key molecules such as nucleic acids, phospholipids, and ATP. Phosphorus is also involved in controlling key enzyme reactions and in the regulation of metabolic pathways (Theodorou, Plaxton 1993; Marschner 1999; Abel et al. 2002). Although bound phosphorus is quite abundant in many natural soils, it is largely unavailable for uptake. Phosphorus availability in soils declines with rapid formation of insoluble complexes with cations and by use of soil microorganisms. Therefore, to improve yields, the phosphorus content in soils needs to be supplemented by means of mineral nutrition. However, even under adequate phosphorus fertilisation, only 20 % or less of that applied is removed by the first year's growth (Bieleski 1973; Schachtman et al. 1998;

Vance 2001). This results in phosphorus loading of prime agricultural land. Runoff from phosphorus loaded soils is a primary factor in eutrophication and hypoxia of lakes and marine estuaries in the developed world. As noted by Abelson (1999), a potential phosphate crisis looms for agriculture in the 21st century.

Low availability in bulk soil limits phosphorus uptake by plants. More soluble minerals such as potassium move through the soil via bulk flow and diffusion, but phosphorus is moved mainly by diffusion. Since the rate of diffusion of phosphorus is slow (10^{-12} to 10^{-15} $m^2 s^{-1}$), high plant uptake rates create a zone around the root that is depleted of phosphorus (Schachtman et al.1998).

Therefore, to achieve more rational use of phosphorus fertilisation, specifically phosphorus treated seeds are used (Stramkale et al. 2004). Usage of phosphorus-treated seeds improves utilisation of mineral nutrient, avoiding contamination of the environment (Gravalos et al. 2000).

A seed coating creates a nutritious environment around germinating seed providing nutritional support in early phase of crop development (Taylor, Herman 1990). The main nutrient supplied in a form of seed coating is phosphorus (Taylor et al. 1998).

Seed coating containing phosphorus has been shown to be an effective way of promoting early seedling growth in phosphorus-deficient soils (Valdes et al. 1987; Refalka et al. 1993; Ros et al. 2000). Even in soils with sufficiently high phosphorus content, a seed coating with phosphorus can be beneficial due to imporved availability of the nutrient (Scott et al. 1991).

Seed coating with phosphorus has been succesfully used in agricultural practice in different regions (Scott 1989). However, there is only limited information available on the exact mechanisms of how phosphorus coating promotes early development of seedlings (Stramkale et al. 2004) and on the possible after-effects of using phosphorus coated seeds on the next generation of crops. The aim of the present experiments was to investigate the effect of phosphorus coating on germination characteristics, photosynthetic pigment content, and the grain yield of barley. A special attention was focused on the effect of phosphorus coating on early seedling development in the next generation using un-coated barley seeds grown from phosphorus coated seed material.

Materials and methods

Spring barley (*Hordeum vulgare* L.) 'Anabell' bred in Germany was used in the laboratory and field tests (in 2002 and 2003). The cultivar provides high yield with excellent quality, it demonstrates good resistance to different plant diseases and has high logging resistance. This cultivar is well suited for brewery and also for food and forage.

In the experiments the seeds treated with phosphorus-powdered nutrition were provided by Finnish company "Kemira Grow How". The nutrition is fixed to the seeds with the help of a binding agent. The patent for this treatment method (iSeed^{TM}) belongs to the Finnish company "Kemira Grow How" and the patent for the binding agent belongs to "Kemira Grow How" and "Fortum Oil and Gas".

The addition, barley seeds of the second generation from field grown barley plants established from phosphorus treated seeds were used. This helped to determine whether the phosphorus treatment has an effect also on the next generation.

In the laboratory tests the seeds were germinated in the dark at 20 °C in a Petri dish lined with two layers of filter paper. The seeds were damped with 5 ml of distilled water. To avoid drying of the seeds, a filter paper bridge connected the Petri dish with a distilled water dish. To prevent evaporation the Petri dish was covered with a lid, and a narrow gap was left to allow air circulation. The percentage of germinated seeds was determined every 5 h. A seed with at least 1 mm of protruded radicle was detected as germinated. The number of repetitions was five. The number of seeds in every repetition was 50. As soon as seed lobes appeared, the Petri dish was placed under light conditions to promote formation of pigments of green plastids.

In the laboratory experiments the following were determined:

(i) Germination, germinating power and germination energy of barley seeds. Germination power is the final percentage of germinated seeds. Germination energy is germination percentage at the time of the most intensive germination (Hartmann et al. 1997; Kutschera 1998).

(ii) Content of pigments of green plastids in 7-day old shoots was measured spectrophotometrically in acetone extract, by determining the optical density (D) at wave-lengths corresponding to absorption maxima of chlorophyll *a*, chlorophyll *b* and carotenoids. Concentration of pigments (C; mg l^{-1}) was calculated according to the following equations (Gavrilenko, Zigalova 2003):

$$C_{Chla} = 9.784\ D_{662} - 0.990\ D_{644};$$
$$C_{Chlb} = 21.426\ D_{644} - 4.650\ D_{662};$$
$$C_{car} = 4.695\ D_{440.5} - 0.268\ C_{Chla} + C_{Chlb}.$$

Field tests were performed in 2002 and 2003 at the Latgale Scientific Agricultural Centre in Eastern Latvia. In 2002, barley was sown on April 24 and harvested on August 20, and in 2003, it was sown on May 8 and harvested on September 6. The field test design was random blocks with four repetitions. The total area for a block was 2 m × 10 m = 20 m^2. The total space of the test was 1259 m^2. The soil type was humus podzolic gley. The content of organic substances in the soil was 38 - 52 g kg^{-1}, pH_{KCl} – 7.3, P – 63 - 99 mg kg^{-1}, K – 98-147 mg kg^{-1}. The pre-plant was wheat. As a basic fertiliser "Kemira Grow How" complex mineral nutrition 18:9:9 was used, the mineral supplement was ammonium nitrate (the reccomendations of the supplier). In field tests the yield of barley was determined. The barley was cropped in the seed ripening phase using a seed combine harvester Sampo-130.

During the field test, May 2002 was warm but dry (there was no rainfall in three succesive decades). The mean temperature in June was 16.2 °C, rain from 3.4 mm (in the 1st decade) up to 16.6 mm (in the 3rd decade). July was warm and sunny. The mean temperature in July was 20.1 °C, which exceeded the mean monthly temperature by 3.6 °C. Precipitation was insufficient – only 38.7 % of the monthly mean. Hot and dry weather favoured grain ripening at the end of a summer of 2002. May 2003 was warm and wet. The sufficient moisture level positively influenced barley seed germination. June was hot and dry. In July, precipitation increased over the mean by 1.4 times. During the harvest (in the 1st decade of August) precipitation exceeded the mean by about 4.2 fold. Unfortunately, this factor affected the yield negatively.

The mathematical data processing (standard error, standart error of difference) and figures were produced using *MS Excel*.

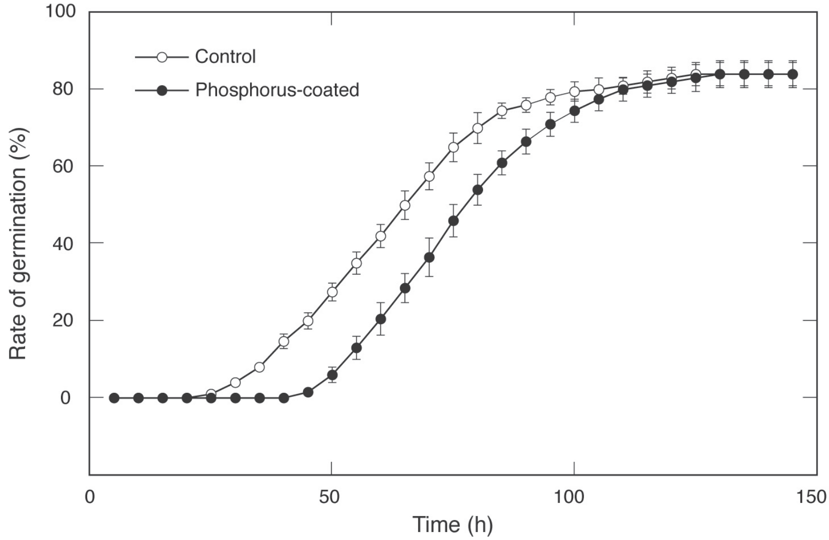

Fig. 1. Effect of phosphorus coating on germination rate of barley (*Hordeum vulgare* L.) seeds.

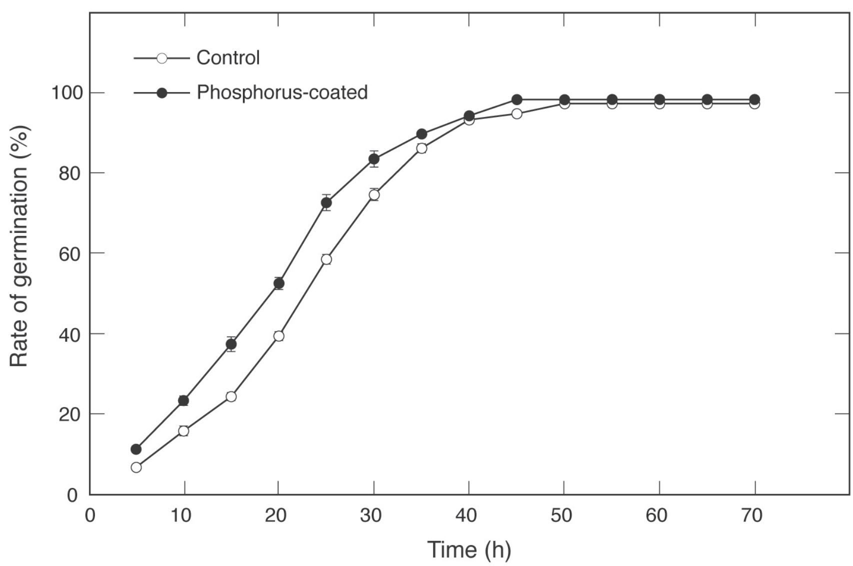

Fig. 2. After-effect of phosphorus coating on germination rate of barley (*Hordeum vulgare* L.) seeds of the second generation.

Results

Effect of phosphorus coating on germination characteristics and pigment content

The germination of non-treated barley seeds started within 22 h after the start of the experiment. The first signs of germination of phosphorus-treated seeds were visible only after 44 h. However, at the end of the experiment, the germination percentage of phosphorus treated barley seeds reached that of control seeds (Fig. 1).

Germination of the second generation obtained from barley seeds treated with phosphorus was considerably more intense than the control (Fig. 2). In 2003 the second generation of seeds both for the phosphorus variant and control began intensive germination (Fig. 2), in contrast with the germination in 2002 (Fig. 1). This may be due to the different origin of seeds: in 2002 seeds were from Finnish company "Kemira Grow How", but in 2003 from Scientific Agricultural Centre of Latgale.

The germination energy of control barley seeds was 2.3 times higher than the germination energy of phosphorus-treated seeds. However, the germination energy of the second generation obtained from phosphorus treated seeds was 3.3 times higher than germination energy of control seeds (Fig. 3). Significant differences were not observed for indicators of germination power (data not shown).

In order to determine how phosphorus treatment of seeds affected the content of photosynthetic pigments in newly established barley seedlings, concentrations of chlorophyll *a*, chlorophyll *b* and carotenoids were determined in 7-day-old shoots. Both the chlorophyll *a* and chlorophyll *b* levels were significantly higher in the shoots of barley grown from phosphorus-treated seeds (Fig. 4). The concentrations of chlorophyll *a* (32 %) and chlorophyll *b* (41%) were higher in the leaves of shoots from phosphorus-treated seeds. Significant differences in carotenoid content were not observed (Fig. 4).

The concentrations of chlorophyll *a* (46 %), chlorophyll *b* (41 %) and carotenoids (43 %) were higher in the shoots of the second generation arising from seeds coated with phosphorus than in the control shoots (Fig. 4).

Effect of phosphorus coating on barley yield

The field tests demonstrated that when the barley 'Anabell' was fertilised with different nitrogen, phosphorus and potassium fertilisers, the plants germinated and grew better if

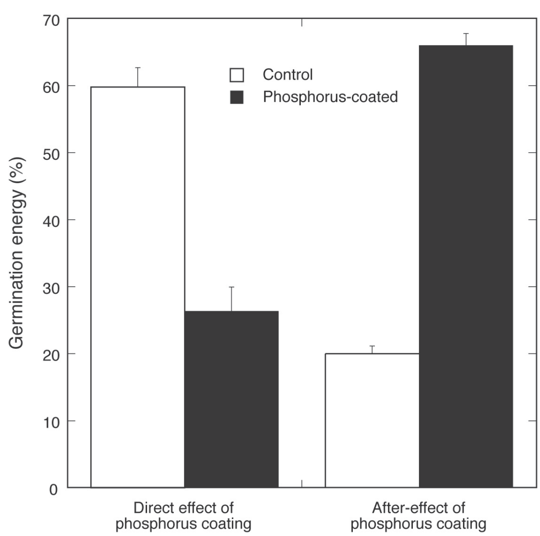

Fig. 3. Effect and after-effect of phosphorus coating on germination energy of barley (*Hordeum vulgare* L.) seeds.

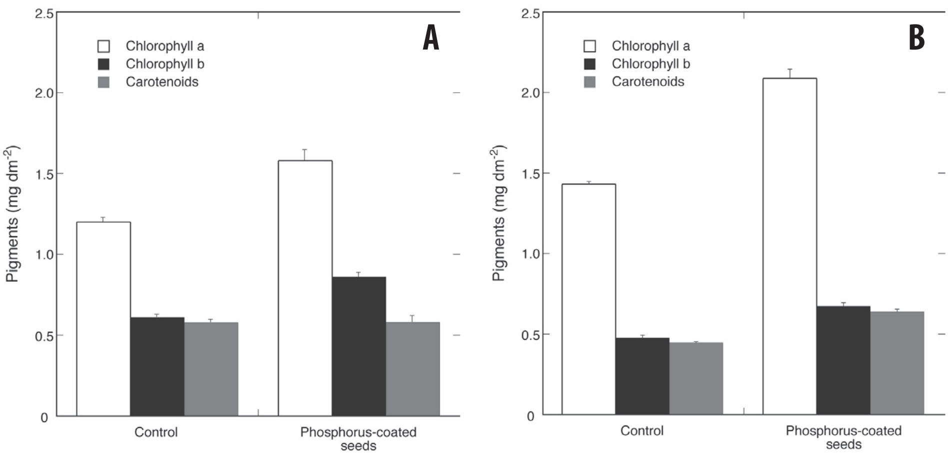

Fig. 4. Effect (A) and after-effect (B) of phosphorus coating on the total amount of pigments of green plastids of barley (*Hordeum vulgare* L.) seeds.

the seeds were coated with phosphorus. The barley yield increased by 3 to 91 %, when the seeds were treated with phosphorus coupled with nitrogen, phosphorus and potassium fertiliser application, compared to the control test (Table 1). The highest yield of the barley seeds 'Anabell' (7.21 t ha^{-1}) was obtained from plants grown from the phosphorus-treated seeds and using mineral nutrition with the general amount of nitrogen 120 kg ha^{-1} consisting from the basic fertiliser 90 kg ha^{-1} and supplement fertiliser 30 kg ha^{-1}: N_{120} (N_{90} 18:9:9 – 500 kg ha^{-1} + N_{30} – 220 kg ha^{-1} ammonium nitrate).

The yield of barley for control variants was greater in 2002 in comparison to 2003 which can be explained by meteorological factors. Nitrogen, phosphorus and potassium fertilisation had a much more greater effect on grain yield in 2003 in comparison to 2002, when there was insufficient amount of rain. Estimations of the standard error of difference showed that the increase in barley grain yield by phosphorus coating was significant, except in the case of phosphorus-treated seeds without fertiliser in 2002.

Discussion

Barley germination of control seeds was 1 to 22 % more intensive than for seeds coated with phosphorus. It is possible that the phosphorus concentration used in the present experiments for the barley seed coating was too high for optimal germination. A species-specific inhibitory effect of phosphorus seed coating has been shown earlier (Taylor 1997). Increased concentrations of phosphorus were shown to delay seedling emergence of lucerne (Scott, Blair 1989). Bieleski (1973) and Ragothama (1999) estimated that the concentration gradient from the soil solution to the plant root cell exceeds 2000-fold for phosphorus. In the soil solution the free phosphorus concentration is on average 1 μM. This concentration is well below the K_M for plant uptake. The possible cause of the poorer germination of phosphorus-treated seeds may be explained by the increased inhibiting impact of osmotic potential. An increased non-organic phosphorus (P_n) concentration in the direct vicinity of seeds may delay water uptake by change in osmotic potential, which affects germination process.

Table 1. Barley (*Hordeum vulgare* L.) cv. 'Anabell' grain yield as influenced by seed phosphorus coating and different levels of mineral nutrition in 2002 and 2003

Variants of fertilizer	Grain yield			
	2002		2003	
	t ha^{-1}	%	t ha^{-1}	%
Control	4.61	100.0	3.78	100.0
Control + P seeds (iSeeds™)	4.75	103.0	4.33	114.6
N_{120} (N_{90} 18:9:9 – 500 kg ha^{-1} + N_{30} – 88 kg ha^{-1} ammonium nitrate)	5.20	112.8	6.80	179.9
N_{120} (N_{90} 18:9:9 – 500 kg ha^{-1} + N_{30} – 88 kg ha^{-1} ammonium nitrate) + P seeds (iSeeds™)	5.48	118.9	7.21	190.7
N_{120} (N_{45} 18:9:9 – 250 kg ha^{-1} + N_{75} – 220 kg ha^{-1} ammonium nitrate)	5.75	124.7	5.42	143.4
N_{120} (N_{45} 18:9:9 – 250 kg ha^{-1} + N_{75} – 220 kg ha^{-1} ammonium nitrate) + P seeds (iSeeds™)	5.95	129.3	5.66	149.7
	$\gamma_{0.05} = 0.18$		$\gamma_{0.05} = 0.20$	

The sufficient store of phosphorus in seeds is vital for seed germination and successful seedling growth (Lott et al. 2000). It is possible that seed coating with phosphorus promotes the synthesis of storage compounds in seeds during the process of the formation of the yield.

The early vigour of barley seedlings was ensured by promotion of the formation of photosynthetic pigments and accordingly, higher rates of photosynthesis. This apparently supported for future development, as shown by an increase of grain yield (Table 1). Other studies have described an increase in dry matter and the amount of tillers which may be cosequence of increased photosynthesis and photosynthate production (Scott et al. 1985).

To summarize, the coating of seeds with well available phosphorus influenced germination, the content of the chlorophyll in shoots, and the yield of seeds of spring barley 'Anabell'. The yield of barley seeds increased by 3 to 91 % when the seeds were treated with phosphorus and different nitrogen, phosphorus and potassium fertilisers were used. Treatment of seeds with phosphorus effected positively the physiological activity of the next generation seeds: increased seed germination and germination power, anf higher concentrations of chlorophyll and carotenoids in the shoots.

In conclusion, the usage of phosphorus-treated barley seeds is effective: the physiological activity of the seeds increases as well as the yield of the seeds. Also, phosphorus usage is improved, which contributed to better environmental protection.

References

Abel S., Ticconi C.A., Delatorre C.A. 2002. Phosphate sensing in higher plants. *Physiol. Plant.* 115: 1–8.

Abelson P.H. 1999. A potential phosphate crisis. *Science*. 283: 2015.

Bieleski R.L. 1973. Phosphate pools, phosphate transport, and phosphate availability. *Annu. Rev.*

Plant Physiol. 24: 225–252.
Gavrilenko V.F., Zigalova T.V. 2003.*The Laboratory Manual for the Photosynthesis.* Academia, Moscow. 254 p. (in Russian).
Gravalos E., Garcia A., Cuerda G.C. 2000. *Seminis Vegetable – Seeds Monograph.* - Institute for Regional Development, Spain. 26 p.
Hartmann T.H., Kester E.D., Davies T.F., Geneve L.R. 2002. P*lant Propagation: Principles and Practices.* Prentice Hall, New Jersey. 880 p.
Kutschera U. 1998. *Grundpraktikum zur Pflanzenphysiologie*. Quelle und Meyer, Wiesbaden. 160 p.
Lott J.N.A., Ockenden I., Raboy V., Batten G.D. 2000. Phytic acid and phosphorus in crop seeds and fruits: a global estimate. *Seed Sci. Res.* 10: 11–33.
Marschner H. 1999. *Mineral Nutrition of Higher Plants.* Academic Press, London. 889 p.
Ragothama K.G. 1999. Phosphate acquisition. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 50: 665–693.
Rebafka F.P., Bationo A., Marschner H. 1993. Phosphorus seed coating increases uptake, early growth and yield of pearl millet (*Pennisettum glaucum* R.Br.) grown on an acid sandy soil in Niger, West Africa. *Fert. Res.* 35: 151–160
Ros C., Bell R.W., White P.F. 2000. Phosphorus seed coating and soaking for improving seedling growth of *Oryza sativa* (rice) cv. IR66. *Seed Sci. Technol.* 28: 391–401.
Schachtman D.P., Reid R.J., Ayling S.M. 1998. Phosphorus uptake by plants: from soil to cell. *Plant Physiol.* 116: 447–453.
Scott J.M. 1989. Seed coatings and treatments and their effects on plant establishment. *Adv. Agron.* 42: 43–83.
Scott J.M., Blair G.J. 1989. Phosphorus seed coatings for pasture species. I. Effect of source and rate of phosphorus on emergency and early growth of phalaris (*Phalaris aquatica* L.) and lucerne (*Medicago sativa* L.). *Austr. J. Agric. Res.* 39: 437–445.
Scott J.M., Hill C.B., Jessop R.S. 1991. Comparison of phosphorus applied as banded granules or as seed coatings to wheat sown in soils differing in P-sorption capacity. *Fertil. Res.* 29: 281–287.
Scott J.M., Mitchell C.J., Blair G.J. 1985. Effect of nutrient seed coating on the emergence and early growth of perennial reygrass. *Austr. J. Agric. Res.* 36: 221–231.
Stramkale V., Jukāma K., Vikmane M., Kondratovičs U. 2004. The study of physiological action of the phosphorus treated rape seeds. *Agron. Vēstis* 6: 68–74.
Taylor A.G. 1997. Seed storage, germination and quality. In: Wien H.C. (ed) *The Physiology of Vegetable Crops*. Wallingford, U.K.: CAB International. pp. 1–36.
Taylor A.P., Allen P.S., Bennett M.A., Bradford K.J., Burris J.S., Misra M.K. 1998. Seed enhancements. *Seed Sci. Res.* 8: 245–256.
Taylor A.G., Herman G.E. 1990. Concepts and technologies of selected seed treatments. *Annu. Rev. Phytopathol.* 28: 321–339.
Theodorou M.E., Plaxton W.C. 1993. Metabolic adaptations of plant respiration to nutritional phosphate deprivation. *Plant Physiol.* 101: 339–344.
Valdes V.M., Bradford K.J. 1987. Effect of seed coating and osmotic priming on the germination of lettuce seeds. *J. Amer. Soc. Hort. Sci.* 112: 153–156.
Vance C.P. 2001. Update on the state of nitrogen and phosphorus nutrition. Symbiotic nitrogen fixation and phosphorus acquisition. Plant nutrition in a world of declining renewable resource. *Plant Physiol.* 127: 390–397.

Miežu sēklu fosfora apstrādes ietekme un pēcietekme uz dīgšanu, fotosintēzes pigmentiem un sēklu ražu

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Kopsavilkums

Fosfors ir nozīmīgs augu makroelements. Augsnē fosfors lielākoties ir augiem grūti pieejamā formā. Tāpēc kultūraugu sējumos lieto īpašas ar fosforu apstrādātas sēklas. Darba mērķis bija noskaidrot, kā miežu sēklu apstrāde ar fosforu ietekmē to dīgšanu, fotosintēzes pigmentu saturu lapās un graudu ražu; kā arī to, kāda ir sēklu apstrādes pēcietekme uz nākamās paaudzes sēklu dīgšanu. Nosakot sēklu dīgšanu procentos un pigmentu saturu dīgstos spektrofotometriski, konstatēja, ka sēklu apstrāde ar fosforu kavēja sēklu dīgšanu miežu augšanas sākumā, bet pigmentu daudzums dīgstos fosfora ietekmē palielinājās. Par 3 līdz 91% pieauga miežu graudu raža. Sēklu apstrāde ar fosforu pozitīvi ietekmēja nākamās paaudzes sēklu fizioloģisko darbību. Var secināt, ka ar fosforu apstrādāto miežu sēklu izmantošana ir efektīva: palielinās sēklu fizioloģiskā aktivitāte un sēklu raža, tiek uzlabota fosfora izmantošana un notiek vides saudzēšana.