Direct Proof of Phosphorus Oxytriamide in Exudates of Decapitated Phaseolus vulgaris Plants

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Abstract. Phosphorus oxytriamide PO(NH₂)₃ was proved chromatographically in the exudate of decapitated Phaseolus vulgaris plants. This fact verified the presumption that covalent compounds of phosphorus and nitrogen enter roots without previous hydrolysis.

In great numbers of vegetation pot experiments, we found that some phosphorus-nitrogen compounds (oligomeric cyclic phosphorus nitroamides, amidophosphates) caused a statistically significant increase in grain yield, in comparison with other commonly used nitrogen and phosphorus salts (Ondráček et al. 1970 b, c). The explanation of this effect has not yet been published.

The mechanism of the uptake of covalent phosphorus-nitrogen compounds by plant roots is one of the possible factors positively influencing the grain yield. We stated in the above-mentioned paper that the movement of ³²P from trimeric phosphorus nitridoamide [PN(NH₂)₂]₃ solution into barley plants is more intensive than that of ³²P from diammonium orthophosphate (NH₄)₂HPO₄ solution. We further found (Ondráček et al. 1970a) that phosphorus, taken up by plants from trimetric phosphorus nitridoamide, could be readily leached into distilled water below the level of (NH₄)₂HPO₄ check plants. We found similar results with tetrameric phosphorus nitridoamide [PN(NH₂)₂]₄ as well. The above mentioned experiments indicate that N—P compounds enter plants without previous hydrolysis following the laws of the osmosis and diffusion.

In this paper, we support this statement with a direct chromatographic proof of one of the investigated N—P compounds, phosphorus oxytriamide PO(NH₂)₃, in the exudate of decapitated Phaseolus vulgaris plants. We selected phosphorus oxytriamide PO(NH₂)₃ because it can be easily proved by means of paper chromatography.

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Material and Methods

The Phaseolus vulgaris L. seeds were germinated in moist sawdust. After six days of germination, 12 plants were transferred into 12 cm adapted Petri dishes and sealed. Each Petri dish contained 200 ml of 0.1 M PO(NH$_2$)$_3$ solution prepared according to KLEMEMT and KOCH (1954). Illuminance and temperature were maintained in the same way as in earlier experiments (ONDRAČEK et al. 1970b). After 24 h the experimental plants were decapitated 1 or 2 cm above the hypocotylus and glass tubes 2 or 3 cm long stoppered with cotton wool plugs were put on the decapitated plants. The glass tubes were sealed by smearing them with lanolin. After another 24 h the accumulated exudate was removed and analysed by means of descendent paper chromatography, using Whatman No. 1 paper and the method described by BIEBERACHER (1956). Chromatograms were developed in 1% ammonium molybdate (NH$_4$)$_2$MoO$_4$ solutions and by UV irradiation. The nutrient solution was analysed in the same manner.

The position of the spots on the tracks of the analysed samples was compared with the position of the spots on the check track. A mixture of PO(NH$_2$)$_3$ and NH$_4$PO$_2$(NH$_2$)$_2$ solutions was applied at the start of the check track. The same position of spots indicates the presence of the same compounds for the given system, as KOUKIL (1968) had demonstrated by means of preparative and X-ray methods. The pH values of the exudate samples were determined by the pH indicator paper Phan. A more exact determination was not possible due to the small amount of exudate.

Results and Discussion

Both PO(NH$_2$)$_3$ and NH$_4$PO$_2$(NH$_2$)$_2$ are present in the exudate, which is evident in Fig. 1; the lowest row of spots represents probably diammonium amidophosphate (NH$_4$)$_2$PO$_3$NH$_2$ or ammonium hydrogen amidophosphate NH$_4$HPO$_3$NH$_2$. Only PO(NH$_2$)$_3$ and ammonium diamidophosphate NH$_4$PO$_2$(NH$_2$)$_2$ can be observed in the chromatogram of the nutrient solution (Fig. 2). PO(NH$_2$)$_3$ is hydrolyzed a little faster when passing through plant tissues than in nutrient solution, probably due to the influence pH 6.5 of plant sap. The assimilation of nonhydrolyzed molecules of an N—P compound taken up passively without any loss of energy remains unknown.

We suppose that either N—P compounds are hydrolyzed spontaneously or that enzymatic systems able to hydrolyze N—P bonds can be synthetized by plants following the induction by substrate, as in the case of the induction of nitrate reductase by nitrate (see e.g. INGLE and HAGEMAN 1966). The hydrolysis of amidophosphates can be catalyzed by the enzyme phosphoamidase demonstrated in amoeba (MATTENHEIMER and MILLER 1957). The hydrolysis of the molecules of N—P compounds could also be catalyzed by some oligoelements. For example VAŠÍČKOVÁ (1964) reported an appreciable influence of bismuth salts on PO(NH$_2$)$_3$ hydrolysis.

As soon the splitting of N—P bonds is achieved and such compounds as for example (NH$_4$)$_2$PO$_3$NH$_2$ appear in plants, different biological reactions can be assumed to take place, e.g. the reaction with adenosine monophosphate (NIELSEN 1966) according to the equation

$$\text{O} - \text{OPNH}_2 + \text{O} - \text{PO}^- \overset{\text{H}_2\text{O}}{\longrightarrow} \text{O} - \text{OP} - \text{O} - \text{PO}^- + \text{NH}_4^+$$

The splitting of N—P bonds undoubtedly needs some time, which is in agreement with the observation of CHYTILOVÁ (1967) who found that the dry matter of alfalfa plants 20 days after the application was lower in the case of N—P compounds than with common fertilizers. M. DVOŘÁK (personal