Journal of Applied Botany and Food Quality 82, 35 - 40 (2008)

Institute of Agricultural and Nutritional Sciences, Professorship Plant Nutrition, Martin-Luther University Halle-Wittenberg, Halle (Saale), Germany

Phytohormonal effects on rhizosphere processes of maize (*Zea mays* L.) under phosphorus deficiency

L. Wittenmayer, A. Deubel, W. Merbach

(Received November 13, 2007)

Summary

Effects of the hormones indole-3-acetic acid (IAA), gibberellic acid (GA_3), and *trans*-zeatin (*t*-Z) on growth, P status and rhizosphere processes of maize (*Zea mays* L., cv. 'Bezemara') were investigated in a pot experiment at two levels of phosphorus availability (+P: water soluble phosphate and -P: sparingly soluble tricalcium phosphate). Six weeks after seed germination, plants were harvested and analysed for dry weight, shoot length, root surface, P concentration, acid phosphatases activity (acid Pase) in shoot and rhizosphere and the content of carboxylic acids and sugars in the rhizosphere. ANOVA was used to estimate the effects of treatments on measured parameters. Hormone application via rhizosphere had a highly significant effect on the growth of whole plants, their P status and rhizosphere processes. GA_3 and *t*-Z promoted quantitatively shoot and root growth and morphological changes, whereas IAA affected the chemical composition of the rhizosphere. In several parameters, the effects of hormone treatment depended on the P status of plants indicating different sensitivity of +P and -P plants to plant growth regulator (PGR) application (significant interaction of hormone application × P availability). The findings help to improve our knowledge, why PGR treatments and plant growth promoting rhizo-microorganisms have varying effects on plants depending on growth conditions.

Abbreviations

acid Pase: acid phosphatases activity, IAA: indole-3-acetic acid, GA_3: gibberellic acid, PGR: plant growth regulator, *t*-Z: *trans*-zeatin

Introduction

The plant growth promoting effects of rhizo-microorganisms include the production of a vast range of metabolites (biologically active substances) that may affect plant growth directly after being taken up by the plant, or indirectly by modifying the soil environment (WALKER et al., 2003). Phytohormones like auxins (indole-3-acetic acid, IAA), gibberellins (gibberellic acid, GA_3) or cytokinins (*trans*-zeatin, *t*-Z) belong to this group of substances (FRANKENBERGER and ARSHAD, 1995). It is well documented that cultures of several plant growth promoting rhizo-microorganisms (PGPR) possess a great potential to produce these phytohormones in considerable amounts (MARTINEZ-TOLEDO et al., 1988; SCHOLZ-SEIDEL and RUPPEL, 1992; GYANESHWAR et al., 2002) and, thus, it is assumed that they might support plants in responding to environmental stress (ITAI, 1999). Unfortunately, a direct link between microbial capacity for hormone formation and the plant growth promoting effects of hormones in the rhizosphere is still missing. It is well known that hormonal effects depend on time of application (developmental stage of plant) and its duration (ARTECA, 1996). Sensitivity of plant tissue to PGR also plays an important role (TREVAWAS, 1981).

There are very few experiments with applications of plant growth regulators to the rhizosphere of intact plants. LEINHOS and BERGMANN (1995) and LIPPMANN et al. (1995) conducted experiments with a single application of IAA to maize plants. Due to its fast metabolisation in the presence of maize roots within a few hours (CROZIER et al., 1988), the hormonal effect of a single application in the rhizosphere is temporarily limited and hardly comparable with microbial, continuous hormone formation at the root surface. In order to understand the microbial effect of hormone formation in soil on plant growth, observations on a long-term scale are necessary, i.e. the PGR effect should exceed its half-life time substantially and correspond with the timescale of plant development. Thus, the hormonal effects have to be investigated within days or weeks rather than within a few hours. Stress conditions are a good background for elucidating plant growth promoting effects and their mechanisms. Nutrient deficiency is a common and very important abiotic stress factor. Particularly, mechanisms of plant response to P starvation are being intensively investigated (SCHILLING et al., 1998; NEUMANN and RÖMHELD, 2001; RANDALL et al., 2001; YUN and KAEPPLER, 2001; ABEL et al., 2002; LYNCH and HO, 2005). They include morphological, physiological and biochemical processes (JONES, 1998; AHMAD et al., 2001; PINTON et al., 2001). There is data available about the effects of hormones on root architecture (LEINHOS and BERGMANN, 1995; LIPPMANN et al., 1995; LÓPEZ-BUCIO et al., 2002). Their effect on rhizosphere physiology and chemistry is still poorly understood.

The aim of this experiment was to investigate the long-term effects of the three plant growth promoting phytohormones indole-3-acetic acid (IAA), gibberellic acid (GA_3), and *trans*-zeatin on plant growth and rhizosphere processes depending on phosphorus availability. A continuous microbial biosynthesis of phytohormones in the rhizosphere was simulated by controlled delivery of plant growth regulators by hormone-containing nutrient solutions. Thus, this experiment is an approach to elucidate long-term effects of phytohormone-producing plant growth promoting rhizobacteria on plants coping with nutrient deficiency.

Material and methods

Plant cultivation

The maize cultivar 'Bezemara' was used in a pot experiment. Seeds were germinated in quartz sand (1 to 2 mm particle size) wetted with saturated $CaSO_4$ solution. After seedlings developed a root length of about 1 cm, six germinated maize seeds were transferred to cultivation pots containing 1.5 kg of quartz sand. The pots were covered inside by a proliferated plastic bag. For harvest, this bag was pulled out of the pot carrying the intact root system. After four days, the seedling number per pot was adjusted to four.

The Ruakura nutrient solution (SMITH et al., 1983) was used in this experiment with the following modifications: iron citrate was replaced by an equimolar amount of Fe(III) chloride. In treatment with low phosphorus availability (-P), the sole P source (65 mg P per pot) was sparingly soluble $Ca_3(PO_4)_2$ mixed with quartz sand before planting. The quantities of other nutrient elements involved in this replacement (potassium, sulphur, calcium) were adjusted to the concentration of the full nutrient solution (+P). Their values are given in Tab. 1.

Tab. 1: Element concentration (mg l^{-1}) in applied +P and -P nutrient solutions.

Nutrient element	+P	-P
N	261.6	261.6
P	40.5	0
K	241	241
S	60.9	88.6
Ca	128.2	128.2
Mg	21.1	21.1
Cu	0.0406	0.0406
Zn	0.253	0.253
Mn	0.507	0.507
Fe	10.1	10.1
B	0.507	0.507
Mo	0.0102	0.0102
Cl	29.3	29.3
Na	14.6	14.6

Plants were cultivated in a vegetation station under ambient conditions from 15 May to 28 June and harvested six weeks after germination.

Hormone application

For hormone treatment, stock solutions of indole-3-acetic acid (IAA), gibberellic acid (GA_3) and *trans*-zeatin (*t*-Z) were prepared using pure ethanol as a solvent. 400 µl of the appropriate stock solution were added to one litre of +P or -P nutrient solution resulting in 0.1 m*M* (IAA or GA_3) or 0.01 m*M* (*t*-Z) hormone-containing application solution. In the control treatment ('0'), 400 µl of pure ethanol were used. Hormone-containing nutrient solutions were prepared every day immediately before application to plants to ensure precise original doses of growth regulators avoiding their chemical or microbial destruction. During the first two weeks, the daily hormone dose per plant was 0.6 µmol (IAA or GA_3) and 0.06 µmol (*t*-Z). Considering plant biomass, these amounts have been increased every two weeks by 100% of the initial values.
During the experiment, the total volume of nutrient solution used for one pot was 1650 ml. This amount is equivalent to 65 mg of P – the quantity used in -P treatment. Thus, total P amounts in +P and -P treatments were the same.

Analysis of rhizosphere solution

In order to collect the rhizosphere solution, plants were transferred into two-litre beakers by lifting the root system with the attending substrate by the plastic bags. Then the plastic bags were removed carefully with scissors and one litre of water was added. Gently dipping of the maize roots in the solution removed the sand from the root surface. After two minutes, the roots were pulled out of the solution. Then the dipping solution was filtered through glass wool, frozen in liquid nitrogen and lyophilized (Saarnio et al., 2004).
Water-soluble neutral (carbohydrates) and acidic (carboxylic acids) compounds in the maize rhizosphere were collected and determined by HPLC as described elsewhere (Gransee and Wittenmayer, 2000). In order to separate acidic and neutral compounds, the solid phase extraction technique was applied (Johnson et al., 1996) before chromatographic quantification.
Water soluble phosphorus in the rhizosphere solution was measured according to Murphy and Riley (1962).
The Sigma Diagnostics procedure number 104 (Sigma Diagnostics Inc, St. Louis, MO, USA) was used to measure acid phosphatases activities (acid Pase) in the rhizosphere using p-nitrophenyl phosphate as a substrate (Tabatabai and Bremner, 1969). For the determination of rhizosphere acid Pase, aliquots of root dipping solutions were frozen in liquid nitrogen and lyophilized at -30°C. The dry residue was dissolved in an appropriate volume of a tris buffer. Protein quantification was conducted according to Bradford (1972).

Determination of growth characteristics

The root surface was estimated using the methylene blue method according to Sattelmacher et al. (1983). In order to characterize the P status of plants, P concentration in shoots and roots was determined using ammonium vanadate molybdate (Gericke and Kurmies, 1952) and the acid phosphatase activity was measured in the third and fourth fully developed leaves under the same conditions as for rhizosphere solution.

Statistical analysis

A two-factorial experimental design was used in ANOVA: factor A – hormone application (0, IAA, GA_3 and *t*-Z), factor B – phosphorus availability (+P water soluble phosphate; -P sparingly soluble tricalcium phosphate). Each treatment included four replicates. The total number of pots was $a \times b \times r = 4 \times 2 \times 4 = 32$.
For statistical analysis, SAS software 9.1.2 (SAS Institute Cary, NC USA) was used. The t test ($P < 0.05$) was used for comparison of ±P means for the same hormone treatment. Significant differences are indicated by boldface font in Tab. 2-4. For comparisons of mean values between different hormone treatments of similar P availability, Tukey test ($P < 0.05$) was used (Dörfel and Bätz, 1980; Warnstorff, 2000).

Results

Plant growth

Phosphorus nutrition of maize plants exclusively from sparingly soluble tricalcium phosphate resulted in a substantial increase in root surface (+17% in control treatment, Tab. 2).

The application of hormones via nutrient solution had a similar effect. In +P treatment, the IAA, GA_3 and *t*-Z applications induced an 8%, 30% and 36% increase in root surface, respectively. In -P plants, root surface was increased by 11%, 23%, and 26% by the respective hormone treatments. The hormones affected not only the root system, but also the shoot. GA_3 and *t*-Z significantly increased the shoot length but decreased its dry weight indicating remarkable morphological changes. Phosphorus nutrition from sparingly soluble tricalcium phosphate resulted in a decrease of plant dry weight in all treatments. The differences between +P and -P plants were relatively small compared to experiments using P free substrates for P-deficiency induction (e. g. Gaume et al., 2001). In control treatment, the -P induced decrease in plant dry weight was only 11%. Nutrition with $Ca_3(PO_4)_2$ affected mainly the shoot. In all treatments, the -P plants had a significant lower shoot dry weight than the +P plants (0: -19%, IAA: -18%, GA_3: -14%, and *t*-Z: -10%), whereas the roots tend to increase their dry weight in P limited conditions. Different responses of shoot and root to P availability resulted in different root : shoot ratios of +P and -P plants. In control treatment, this value was increased by 37% due to P deficiency. IAA application of +P

Tab. 2: Effect of hormone application, phosphorus availability (+P = high, -P = low) on growth of maize cultivar 'Bezemara'.

Parameter		Hormone application and P availability*								LSD** (Tukey, P < 0.05)
		0		IAA		GA3		*t*-Z		
		+P	-P	+P	-P	+P	-P	+P	-P	
Dry weight in g	Shoot	**10.7**	**8.7**	**9.4**	**7.7**	**8.5**	**7.3**	**8.6**	**7.7**	0.8
	Root	3.2	3.6	3.1	3.3	2.3	2.6	2.6	2.9	0.6
	Plant	**13.8**	**12.3**	**12.5**	**11.0**	10.8	9.9	11.2	10.6	1.3
Root : shoot ratio		**0.30**	**0.41**	**0.33**	**0.43**	**0.28**	**0.36**	**0.30**	**0.38**	0.06
Shoot length in cm		**100**	**92**	**98**	**91**	**130**	**121**	**120**	**109**	8
Root surface in dm²		**84**	**98**	**91**	**110**	**109**	**121**	**114**	**125**	2

*) significant differences between +P and -P treatment at the same hormone application are indicated by boldface (t test, P<0.05)
**) for comparison of +P or -P means of the different hormone applications.

plants also increased this parameter slightly by 10% resembling this P deficiency response of maize plants. The highest root : shoot ratio was observed in IAA treatment of P deficient plants (0.43).

Rhizosphere solution

Within eight hours, the original P concentration of 1.31 mM in the +P nutrient solution was lowered in the rhizosphere of maize plants to 0.27 mM (Tab. 3).

In the rhizosphere of -P plants, the concentration of water-soluble P was half as high. IAA and *t*-Z applications had no effect on P concentration in the rhizosphere neither in +P nor in -P maize plants. In contrast, GA_3 tended to increase the concentration of water-soluble P in the -P rhizosphere, indicating an inhibited P uptake by roots rather than an improved solubilization of the sparingly soluble tricalcium phosphate. In this treatment, P concentration and P uptake by organs and by total plant were the lowest as well. Because of the lower shoot dry matter production combined with lower P concentrations, also IAA and *t*-Z reduced total P uptake of plants in +P treatments, whereas P uptake in -P treatment was not affected. Generally, plants supplied with water-soluble phosphate had a significant higher P concentration in shoot and in root.

Acid phosphatases activity (acid Pase) in the shoot is considered to be an indicator for P stress. The maize cultivar 'Bezemara' did not show significant differences in enzyme activity between +P and -P plants in control and IAA treatments, but in GA_3 and *t*-Z (Tab. 3). Higher activity of acid Pase in the rhizosphere is assumed to be an adaptation process to low available P in soil. Highest rhizosphere-enzyme activity was observed in IAA plants independent of P availability, the lowest in GA_3 treatment (Tab. 4). Significant differences depending on available P were measured in control and *t*-Z treatments, surprisingly with higher activities in the +P rhizosphere.

The pH of the diluted rhizosphere solution varied depending on P availability and hormone treatment. Though the original pH value of both nutrient solutions was identical (6.0), the pH of the rhizosphere solution was different. Nutrition with water-soluble phosphate increased the pH in comparison to nutrition with tricalcium phosphate. Furthermore, the rhizospheres of control plants at both P levels were more acidic than those of corresponding hormone treatments. No substantial differences between hormone treatments could be observed.
Among water soluble carbohydrates, sucrose, glucose and fructose were the quantitatively most important compounds. P deficiency

Tab. 3: Effect of hormone application and phosphorus availability on P status of maize cultivar 'Bezemara'.

Parameter		Hormone application and P availability*								LSD** (Tukey, P < 0.05)
		0		IAA		GA3		*t*-Z		
		+P	-P	+P	-P	+P	-P	+P	-P	
water-soluble P in rhizosphere in m*M*		**0.27**	**0.11**	**0.26**	**0.13**	**0.28**	**0.15**	**0.27**	**0.13**	0.05
P concentration in mg g^{-1} dw	Shoot	**2.66**	**1.46**	**2.34**	**1.52**	**1.98**	**1.30**	**2.27**	**1.51**	0.24
	Root	**1.49**	**0.79**	**1.32**	**0.99**	**1.09**	**0.76**	**1.13**	**0.88**	0.25
P uptake inmg per pot	Shoot	**28.5**	**12.7**	**22.0**	**11.7**	**16.7**	**9.5**	**19.5**	**11.6**	1.9
	Root	**4.8**	**2.8**	**4.1**	**3.3**	2.6	2.0	2.9	2.6	0.9
	Plant	**33.3**	**15.6**	**26.2**	**15.0**	**19.3**	**11.5**	**22.4**	**14.1**	2.2
Acid Pase in shoot in nmol s^{-1} mg^{-1} protein		5.7	6.9	6.6	6.6	**6.1**	**11.3**	**4.6**	**8.4**	3.2

For explanation of * and ** see Tab. 2.

Tab. 4: Effect of hormone application, phosphorus availability on rhizosphere characteristics of maize cultivar 'Bezemara'.

Parameter		Hormone application and P availability*								LSD** (Tukey, $P < 0.05$)
		0		IAA		GA3		*t*-Z		
		+P	-P	+P	-P	+P	-P	+P	-P	
Acid Pase in rhizosphere in nmol s^{-1} mg^{-1} protein		**14.0**	**3.2**	21.2	23.6	4.5	4.2	**10.6**	**3.3**	8.4
pH of rhizosphere solution. (1 : 10 v/v) diluted		**6.95**	**5.90**	**7.26**	**6.32**	**7.22**	**6.27**	**7.19**	**6.26**	0.31
Neutralized Ca derived from $Ca_3(PO_4)_2$ solubilisation in mmol		0	0.754	0	0.726	0	0.556	0	0.686	0.046
Concentration in rhizosphere in m*M*	sucrose	0.04	0.06	**0.09**	**0.04**	**0.06**	**0.02**	0.04	0.05	0.05
	glucose	1.13	0.62	1.35	0.92	0.48	0.26	0.50	0.33	0.73
	fructose	**0.51**	**0.20**	**0.61**	**0.32**	0.12	0.08	0.14	0.07	0.25
	citrate	**0.74**	**0.10**	**0.66**	**0.41**	0.19	0.08	**0.40**	**0.06**	0.16
	trans-aconitate	**0.41**	**0.05**	**0.32**	**0.08**	0.13	0.03	**0.26**	**0.03**	0.15
	oxalate	0.12	0.06	0.08	0.09	0.03	0.05	0.10	0.03	0.07
	succinate	**1.47**	**0.67**	1.04	0.73	0.22	0.33	0.56	0.25	0.62
	malate	**0.80**	**0.08**	0.45	0.32	0.15	0.06	**0.47**	**0.05**	0.24

For explanation of * and ** see Tab. 2.

reduced monosaccharide exudation partially significant in all hormone treatments. Lowest glucose and fructose contents were observed in GA_3 and *t*-Z treatments at both P levels.

The most common carboxylic anions were trans-aconitate, citrate, succinate, malate and oxalate. Due to their marginal role in P-solubilization, the identified monocarboxylic acids lactate and acetate are not included in this table. Generally, +P plants contained in their rhizosphere more acid anions than -P plants. Furthermore, GA_3 induced a general reduction in acid anions in the rhizosphere.

Discussion

By using the method of continuous application of hormone containing nutrient solution it is possible to investigate hormonal long-term effects on plant growth, P status and rhizosphere processes of the maize cultivar 'Bezemara'. The factorial analysis of variance in the experiment showed that hormone treatment had a significant effect on the investigated parameters.

Both phosphorus deficiency and hormone application resulted in an increase of root surface. Thus, phytohormones resemble P deficiency response in roots. Considering the data of other maize cultivars (Deubel et al., 2007), the effect of hormone treatment on root surface was significantly modified by genotype. Thus, there was a genotype-induced variation in response-sensitivity to hormone treatment. Root morphology and architecture play an important role in nutrient acquisition in marginal soils (Schubert and Mengel, 1989; Liao et al., 2001). Particularly, the formation of root hairs is significant in phosphorus uptake by the plant (Jungk, 2001). Föhse et al. (1991) observed that up to 90% of total P uptake is contributed to root hairs. In our experiment, an increase in the root surface area did not result in an improved P acquisition. In soil, root hairs can penetrate small soil pores, as if 'mining' nutrients therein (Michael, 2001). In coarse quartz sand used in this experiment, this adaptation response would not result in an increased availability of phosphorus because P was not caught in small soil compartments accessible only to root hairs. In this context, it should also be mentioned that another phytohormone not considered in this experiment has a strong influence on root morphology: ethylene (Schmidt, 2001; Arshad and Frankenberger, 2002).

The most frequent interaction occurred between hormone application and P status of plants indicating that the same phytohormone treatment of +P and -P plants may result in a different response to the plant growth regulator applied. López-Bucio et al. (2002) observed a different response of *Arabidopsis* to plant growth regulator treatment depending on P status of plants. The authors assume that P status of plants influences sensitivity to exogenously applied hormones. Varying growth response due to changing sensitivity of plant roots to auxin production by microorganisms is also discussed by Björkman (2004). Inactivation of hormones by the formation of conjugates (Ljung et al., 2001, 2002; LeClere et al., 2002), increased catabolism (Kerk et al., 2000) or reduced transport to the sensitive tissue (Ljung et al., 2001) may affect sensitivity of plant tissue to PGR. Crozier et al. (1988) observed that maize roots were making the predominant contribution to catabolism of rhizosphere IAA in colonized roots.

The activity of acid phosphatases is considered to be a good indicator for P deficiency in plants (Barrett-Lennard and Greenway, 1982; McLachlan and De Marco, 1982) particularly in the rhizosphere (Tarafdar and Jungk, 1987; Ascencio, 1997). Comparing the acid Pase of 23 barley cultivars, Römer et al. (1995) found that not all cultivars respond to P deficiency by increasing acid Pase activity in the shoots. In several cases the authors observed higher enzyme activities in +P plant than in -P plants in comparison with different cultivars. Thus, the acid Pase was more strongly affected by genotype than by P availability. In our experiment with the cultivar 'Bezemara', the enzyme activity in shoots was influenced by P only in GA_3 and *t*-Z treatments. Thus, a general influence of a singly investigated growth parameter, e.g. P availability, on the enzyme activity, cannot be concluded.

It is well documented that in root exudates carboxylic acids may dissolve $Ca_3(PO_4)_2$ replacing phosphate by carboxylic anions (Jones et al., 2003, Jones, 1998). In order to solubilise P amounts covering the P content of maize plants in -P treatments (Tab. 3), equivalent quantities of calcium ions had to be neutralised by carboxylic anions

(Tab. 4). Thus, from the measured concentration of carboxylic anions in the rhizosphere solution conclusions about their exudation rates cannot be drawn because a manifold portion of carboxylic acids have been bound in insoluble calcium compounds. Due to the low solubility of calcium salts (calculation according to SEIDELL and LINKE, 1952) with citrate and oxalate of 1.5 and 0.5 mmol l^{-1}, respectively, a precipitation of the exuded organic acid anions in the vicinity of root surface might have been occurred as observed for lupine plants in a calcareous soil by DINKELAKER et al. (1989). This would explain the lower concentration of organic acids in -P treatments. Fore example, citrate concentration in the control -P treatment was 0.1 m*M*. Considering the volume of the rhizosphere solution (0.11 l), 0.011 mmol of dissolved citrate were found in this treatment. To neutralise the released Ca amount of 0.754 mmol, a 46 times higher citrate amount is necessary. Even the sum of all identified dissolved organic acids of the rhizosphere solution is only a small fraction of the quantity necessary for neutralisation of the calcium. The assumption of a higher carboxylic acid exudation rate of -P plants is supported by the substantial lower pH values in their rhizosphere indicating additional proton release by co-exudation with carboxylic anions.

The content of sugars and carboxylic anions in the maize rhizosphere was promoted by the applied IAA doses. From our data, it cannot be concluded that other hormones do not affect rhizosphere chemistry. The observation of negligible effects of GA_3 and *t*-Z is valid only for the applied doses of these phytohormones. For IAA is known that depending on its concentration, this hormone can stimulate or inhibit root growth (PILET, 1996, 1998a,b; BJÖRKMAN, 2004). WERNER et al. (2001) found that in cytokinin-deficient plants, root growth was promoted. In our pot experiment, the applied *t*-Z amount stimulated root growth. Obviously, not only the strength but also the direction of hormone effects (stimulation/inhibition), depends on their applied doses (LUDWIG-MÜLLER, 2004). Since hormonal effects depend on PGR doses, for elucidation of the optimum effect of a specific PGR on plant growth and particular rhizosphere parameters, various concentrations should be tested. Furthermore, only single hormonal factors were tested here. In a plant-rhizosphere continuum, multiple hormonal interactions occur. There are indications that only the combination of various hormones (e.g. IAA, gibberellic acid, and kinetin) can induce rhizosphere effects similar to those produced by plant growth promoting microorganisms (TIEN et al., 1979). Thus, in future experiments, applications of various doses of PGR and their combinations should be tested for their long-term rhizosphere processes.

Acknowledgments

The authors want to thank Mrs. Chr. Birke and Mrs. E. Pfeil for their technical assistance. The English Text was revised by G.C. Calderone, Trenton NJ, USA.

References

ABEL, S., TICCONI, C.A., DELATORRE, C.A., 2002: Phosphate sensing in higher plants. Physiol. Plant. 115, 1-8.

AHMAD, Z., GILL, M.A., QURESHI, R.H., 2001: Genotypic variation of phosphorus utilization efficiency of crops. J. Plant Nutr. 24, 1149-1171.

ARSHAD, M., FRANKENBERGER, W.T., Jr., 2002: Ethylene. Agricultural Sources and Applications. Kluwer Academic/Plenum Publishers, New York, Boston, Dordrecht, London and Moscow.

ARTECA, R.N., 1996: Plant Growth Substances: Principles and Applications. Chapman and Hall, New York, Albany, Bonn, Boston, Cincinnati, Detroit, London, Madrid, Melbourne, Mexico City, Pacific Grove, Paris, San Francisco, Singapore, Tokyo, Toronto, Washington.

ASCENCIO, J., 1997: Root secreted acid phosphatase kinetics as a physiological marker for phosphorus deficiency. J. Plant Nutr. 20, 9-26.

BARRETT-LENNARD, E.G., GREENWAY, H., 1982: Partial separation and characterization of soluble phosphatases from leaves of wheat grown under phosphorus deficiency and water deficit. J. Exp. Bot. 33, 694-704.

BJÖRKMAN, T., 2004: Effect of *Trichoderma* colonization on auxin-mediated regulation of root elongation. Plant Growth Regul. 43, 89-92.

BRADFORD, M.M., 1972: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248-254.

CROZIER, A., ARRUDA, P., JASMIM, J.M., MONTEIRO, A.M., SANDBERG, G., 1988: Analysis of indole-3-acetic acid and related indoles in culture medium from *Azospirillum lipoferum* and *Azospirillum brasilense*. Appl. Environ. Microbiol. 54, 2833-2837.

DEUBEL, A., WITTENMAYER, L., MERBACH, W., 2008: Einfluss von Phytohormonen und P-Versorgung auf Wachstum und Wurzelabscheidungen von Mais. In: Merbach, W., Deubel, A., Augustin, J. (eds.), Ökophysiologische Interaktionen in der durchwurzelten Bodenschicht. Grauer, Beuren, Stuttgart, 69-76.

DINKELAKER, B., RÖMHELD, V., MARSCHNER, H., 1989: Citric acid and precipitation of calcium citrate in the rhizosphere of white lupin (*Lupinus albus* L.). Plant Cell Environ. 12, 285-292.

DÖRFEL, H., BÄTZ, G., 1980: Mittelwertvergleiche in mehrfaktoriellen Versuchen bei signifikanter Wechselwirkung [Comparison of mean values in multiple-factorial experiments]. Arch. Acker- Pflanzenb. Bodenk. 24, 323-328.

FÖHSE, D., CLAASSEN, N., JUNGK, A., 1991: Phosphorus efficiency of plants. II. Significance of rood radius, root hairs and cation-anion balance for phosphorus influx in seven plant species. Plant Soil 132, 261-272.

FRANKENBERGER, W.T., JR., ARSHAD, M., 1995: Phytohormones in Soils: Microbial Production and Function. Marcel Dekker, New York, NY.

GAUME, A., MÄCHLER, F., LEÓN, C.D,, NARRO, L., FROSSARD, E., 2001: Low-P tolerance by maize (*Zea mays* L.) genotypes: Significance of root growth, and organic acids and acid phosphatase root exudation. Plant Soil 228, 253-264.

GERICKE, S., KURMIES, B., 1952: Die kolorimetrische Phosphorbestimmung mit Ammonium-Vanadat-Molybdat und ihre Anwendung in der Pflanzenanalyse [Colorimetric quantitation of phosphorus by ammonium vanadate molybdate and its application in plant analysis]. Z. Pflanzenern. Bodenk. 59, 235-247.

GRANSEE, A., WITTENMAYER, L., 2000: Qualitative and quantitative analysis of water-soluble root exudates in relation to plant species and development. J. Plant Nutr. Soil Sci. 163, 381-385.

GYANESHWAR, P., NARESH KUMAR, G., PAREKH, L.J., POOLE, P.S., 2002: Role of soil microorganisms in improving P nutrition in plants. Plant Soil 245, 83-93.

ITAI, C., 1999: Role of phytohormones in plant responses to stress. In: Lerner, H.R. (ed.), Plant Responses to Environmental Stresses. From Phytohormones to Genome Reorganization, 287-301. Marcel Dekker, Inc., New York and Basel.

JOHNSON, J.F., ALLAN, D.L., VANCE, C.P, WEIBLEN, G., 1996: Root carbon dioxide fixation by phosphorus-deficient *Lupinus albus*. Contribution to organic acid exudation by proteoid roots. Plant Physiol. 112, 19-30.

JONES, D. L., 1998: Organic acids in the rhizosphere – a critical review. Plant Soil 205, 25-44.

JONES, D.L., DENNIS, P.G., OWEN, A.G., VAN HEES, P.A.W., 2003: Organic acid behavior in soils – misconceptions and knowledge gaps. Plant Soil 248, 31-41.

JUNGK, A., 2001: Root hairs and the acquisition of plant nutrients from soil. J. Plant Nutr. Soil Sci. 164, 121-129.

KERK, N.M., JIANG, K., FELDMAN, L.J., 2000: Auxin metabolism in the root apical meristem. Plant Physiol. 122, 925-932.

LECLERE, S., TELLEZ, R., RAMPEY, R.A., MATSUDA, S.P.T., BARTEL, B., 2002: Characterization of a family of IAA-amino acid conjugate hydrolases from *Arabidopsis*. J. Biol. Chem. 277, 20446-20452.

Leinhos, V., Bergmann, H., 1995: Influence of auxin producing rhizobacteria on root morphology and nutrient accumulation of crops. II. Root growth promotion and nutrient accumulation of maize (*Zea mays* L.) producing *Pseudomonas* strains and by exogenously applied IAA under different water supply conditions. Angew. Bot. 69, 37-41.

Liao, H., Rubio, G., Yan, X., Cao, A., Brown, K.M., Lynch, J.P., 2001: Effect of phosphorus availability on basal shallowness in common bean. Plant Soil 232, 69-79.

Lippmann, B., Leinhos, V., Bergmann, H., 1995: Influence of auxin producing rhizobacteria on root morphology and nutrient accumulation of crops. I. Changes in root morphology and nutrient accumulation in maize (*Zea mays* L.) caused by inoculation with indole-3-acetic acid (IAA) producing *Pseudomonas* and *Acinetobacter* strains or IAA applied exogenously. Angew. Bot. 69, 31-36.

Ljung, K., Bhalerao, R., Sandberg, G., 2001: Sites and homeostatic control of auxin biosynthesis in *Arabidopsis* during vegetative growth. Plant J. 28, 465.

Ljung, K., Hull, A.K., Kowaczyk, M., Celenza, J., Cohen, J.D., Sandberg, G., 2002: Biosynthesis, conjugation, catabolism and homeostasis of indole-3-acetic acid in *Arabidopsis thaliana*. Plant Mol. Biol. 49, 249-272.

López-Bucio, J., Hernández-Abreu, E., Sánchez-Calderón, L., Nieto-Jacobo, M.F., Simpson, J., Herrera-Estrella, L., 2002: Phosphate availability alters architecture and causes changes in hormone sensitivity in the *Arabidopsis* root system. Plant Physiol. 129, 244-256.

Ludwig-Müller, J., 2004: From auxin homeostasis to understanding plant pathogen and plant symbiont interaction: editor's research interests. J. Plant Growth Regul. 23, 1-8.

Lynch, J.P., Ho, M.D., 2005: Rhizoeconomics: Carbon costs of phosphorus acquisition. Plant Soil 274, 45-56.

Martinez-Toledo, M.V., de la Rubia, T., Moreno, J., Gonzalez-Lopez, J., 1988: Root exudates of *Zea mays* and production of auxins, gibberellins and cytokinins by *Azotobacter chroococcum*. Plant Soil 110, 149-152.

McLachlan, K.D., De Marco, D.G., 1982: Acid phosphatase activity of intact roots and phosphorus nutrition in plants. III. Its relation to phosphorus garnering by wheat and a comparison with leaf activity as a measure of phosphorus status. Aust. J. Agric. Res. 33, 1-11.

Michael, G., 2001: The control of root hair formation: suggested mechanisms. J. Plant Nutr. Soil Sci. 164, 111-119.

Murphy, J., Riley, J.P., 1962: A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta 27, 31-36.

Neumann, G., Römheld, V., 2001: The release of root exudates as affected by the plant's physiological status. In: Pinton, R., Varanini, Z., Nannipieri, P. (eds.), The Rhizosphere: Biochemistry and Organic Substances at the Soil-Plant Interface, 41-93. Springer, Tokyo, Berlin, Heidelberg, New York, Barcelona, Hong Kong, London, Milan, Paris, Singapore.

Pilet, P.-E., 1996: Root growth and gravireaction: a reexamination of hormone and regulator implications. In: Waisel, Y., Eshel, A., Kafkafi, U. (eds.), Plant Roots: The Hidden Half, 285-305. Marcel Dekker, New York, Basel, Hong Kong.

Pilet, P.-E., 1998a: Applied indol-3yl-acetic acid on the cap and auxin movements in gravireacting maize roots. J. Plant Physiol. 152, 135-138.

Pilet, P.-E., 1998b: Some cellular and molecular properties of abscisic acid: its particular involvement in growing plant roots. Cell. Mol. Life Sci. 54, 851-865.

Pinton, R., Varanini, Z., Nannipieri, P., 2001: The rhizosphere as a site of biochemical interactions among soil components, plants and microorganisms. In: Pinton, R., Varanini, Z., Nannipieri, P. (eds.), The Rhizosphere: Biochemistry and Organic Substances at the Soil-Plant Interface, 1-17. Springer, Tokyo, Berlin, Heidelberg, New York, Barcelona, Hong Kong, London, Milan, Paris, Singapore.

Randall, P.J., Hayes, J.E., Hocking, P.J., Richardson, A.E., 2001: Root exudates in phosphorus acquisition by plants. In: Ae, N., Arihara, J., Okada, K., Srinivasan, A. (eds.), Plant Nutrient Acquisition: New Perspectives, 71-100. Springer, Tokyo, Berlin, Heidelberg, New York, Barcelona, Hong Kong, London, Milan, Paris, Singapore.

Römer, W., Beissner, L., Schenk, H., Jungk, A., 1995: Einfluß von Sorte und Phosphordüngung auf den Phosphorgehalt und die Aktivität der sauren Phosphatasen von Weizen und Gerste. – Ein Beitrag zur Diagnose der P-Versorgung von Pflanzen – [Influence of cultivar and phosphorus application on P concentration and acid phosphatase activity in wheat and barley. – A contribution to the diagnosis of P supply of plants –] Z. Pflanzenern. Bodenk. 158, 3-8.

Saarnio, S., Wittenmayer, L., Merbach, W., 2004: Rhizospheric exudation of *Eriophorum vaginatum* L. – Potential link to methanogenesis. Plant Soil 267, 343-355.

Sattelmacher, B., Klotz, F., Marschner, H., 1983: Vergleich von zwei nicht-destruktiven Methoden zur Bestimmung von Wurzeloberflächen [Comparison of two non-destructive methods for determination of root surface areas]. Z. Pflanzenern. Bodenk. 146, 449-459.

Schilling, G., Gransee, A., Deubel, A., Lezovic, G., Ruppel, S., 1998: Phosphorus availability, root exudates, and microbial activity in the rhizosphere. J. Plant Nutr. Soil Sci. 161, 465-478.

Schmidt, W., 2001: From faith to fate: Ethylene signalling in morphogenic responses to P and Fe deficiency. J. Plant Nutr. Soil Sci. 164, 147-154.

Scholz-Seidel, C., Ruppel, S., 1992: Nitrogenase- and phytohormone activities of *Pantoea agglomerans* in culture and their reflection in combination with wheat plants. Zentralbl. Mikrobiol. 147, 182-191.

Schubert, S., Mengel, K., 1989: Important factors in nutrient availability: root morphology and physiology. Z. Pflanzenern. Bodenk. 152, 169-174.

Seidell, A., Linke, W.F., 1952: Solubilities of Inorganic and Organic Compounds: A Compilation of Solubility Data from the Periodical Literature. Van Nostrand, New York, 1254.

Smith, G.S., Johnston, C.M., Cornforth, I.S., 1983: Comparison of nutrient solutions for growth of plants in sand culture. New Phytol. 94, 537-548.

Tabatabai, M.A., Bremner, J.M., 1969: Use of *p*-nitrophenyl phosphate for assay of soil phosphate activity. Soil Biol. Biochem. 1, 301-307.

Tarafdar, J.C., Jungk, A., 1987: Phosphatase activity in the rhizosphere and its relation to the depletion of soil organic phosphorus. Biol. Fertil. Soils 3, 199-204.

Tien, T.M., Gaskins, M.H., Hubbell, D.H., 1979: Plant growth substances produced by *Azospirillum brasilense* and their effect on the growth of pearl millet *Pennisetum americanum.* Appl. Environ. Microbiol. 37, 1016-1024.

Trevawas, A., 1981: How do plant growth substances act? Plant Cell Environ. 4, 203-228.

Walker, T.S., Bais, H.P., Grotewold, E., Vivanco, J.M., 2003: Root exudation and rhizosphere biology. Plant Physiol. 132, 44-51.

Warnstorff, K., 2000: Comment on "Forage Production, Grain Yield, and Protein Content in Dual-Purpose Triticale Grown for Both Grain and Forage" by L.F. García del Moral, A. Boujenna, J.A. Yañez, and J.M. Ramos. Agron. J. 87, 902-908. Agron. J. 92, 1042-1045.

Werner, T., Motyka, V., Strnad, M., Schmülling, T., 2001: Regulation of plant growth by cytokinin. Proc. Nat. Acad. Sci. USA 98, 10487-10492.

Yun, S.J., Kaeppler, S.M., 2001: Induction of maize acid phosphatase activities under phosphorus starvation. Plant Soil 237, 109-115.

Address of authors:
Dr. Lutz Wittenmayer, Dr. Annette Deubel, Prof. Dr. Wolfgang Merbach (corresponding author), Institute of Agricultural and Nutritional Sciences, Pofessorship Plant Nutrition, Martin-Luther University Halle-Wittenberg, Adam-Kuckhoff-Straße 17b, D-06108 Halle, Germany.

E-mail: wolfgang.merbach@landw.uni-halle.de
lutz.wittenmayer@landw.uni-halle.de
annette.deubel@landw.uni-halle.de