Annals of the Academy of Romanian Scientists
Series on Agriculture, Silviculture and Veterinary Medicine Sciences
ISSN 2069 – 1149 Volume 6, Number 1/2017

EFFECT OF FERTILIZER-CONTAINING-MICROORGANISMS ON MAIZE GROWTH AND ROOT LENGTH

Brigitta TÓTH[1]

Abstract. *The general objective of this research was deliver and implement viable alternatives to mineral fertilizers by developing strategic combinations of BEs with alternatives to the current practice of mineral fertilizations, such as organic farming, low input agriculture, or the use of fertilizers based on recycling products. In this study, 13 bacterial and fungal isolations were tested as BEs. During this investigation the main plant physiological parameters (relative chlorophyll content, height of plants, root growth, dry and fresh weight) of maize (cv Maxxis) were examined. Maize is well known to be particularly sensitive to low phosphorous (P) availability, as well as to show responsiveness to applications of microbial BEs. The experiment was conducted in pots (2 kg soil) under controlled growth chamber conditions with P as the major limiting nutrient.*

Keywords: crop production, microorganism, phosphorous, plant growth, root length

1. Introduction

Phosphorus (P) is one of the major essential macro-elements required for maximizing crop growth and production. With increasing demand of agricultural production and as the peak in global production will occur in the next decades, phosphorus (P) is receiving more attention as a nonrenewable resource [1]. One unique characteristic of P is its low availability due to slow diffusion and high fixation in soils. All of this means that P can be a major limiting factor for plant growth.

Root-induced chemical and biological changes in the rhizosphere play a vital role in enhancing the bioavailability of soil P [2]. These root-induced changes mainly involve proton release to acidify the rhizosphere, carboxylate exudation to mobilize sparingly available P by chelation and ligand exchange, and secretion of phosphatases or phytases to mobilize P by enzyme-catalyzed hydrolysis [3].

Some soil and rhizosphere microorganisms can also enhance plant P acquisition by directly increasing solubilization of P to plants, or by indirect hormone-induced stimulation of plant growth [4]. P-solubilizing microorganisms account for approximately 1% to 50% in P solubilization potential [5].

[1]Title: PhD, Institute of Nutrition, University of Debrecen, Debrecen, Hungary (e-mail: btoth@agr.unideb.hu).

They may mobilize soil P by the acidification of soil, the release of enzymes (such as phosphatases and phytases), or the production of carboxylates such as gluconate, citrate, and oxalate [6].

In this study, 13 different bacterial and fungal inoculants (so called bio-effectors: BEs) were tested on the basic plant physiological parameters which are the followings: plant height, shoot,- root fresh and dry weight total root length and fractional root length based on root diameter.

2. Material and Methods

The experimental plant was *Zea mays* L. cv. Maxxis (R. A. G. T. Saaten Deutchland GmbH). The experiment was conducted in pots (2 kg soil) under controlled environmental conditions. The seedlings were grown for 30 days in a greenhouse. A low P organic farming soil free of postharvest residues was obtained from the plough depth (top 20 cm) in Kleinhohenheim (research station of Hohenheim University, Stuttgart, Germany) (clay loam pH 6.8, 20 mg CAL-P/kg soil). The soil was moisture until 60 % WHC, water losses were replaced after gravimetric determination (every other day, later on every day). The following basal fertilization was applied: N was applied at the rate 100 mg N kg^{-1} ($Ca(NO_3)_2$) in all treatments. There were two non-inoculated controls; one was without added P (Contr.) and the other with added 150 mg P kg^{-1} ($Ca(H_2PO_4)_2$) (Contr.+P).

13 bacterial and fungal isolations were tested as BEs. Table 1shows the applied bacterial and fungal inoculants.

Height of plants was measured on the 9^{th} and 30^{th} days after sowing with folding rule. The dry weight was measured by thermogravimetric method. The samples were dried for three days at 65 °C. Measurement of the root samples was performed with WinRhizo Pro (Regent Instrument, Quebec, Canada), an interactive scanner-based image analysis system. The experiment was arranged in completely randomized design, and the data were analyzed in Sigma Plot version 12.0 version.

Table 1) List of the name of applied products, the treatments short name and the contents of the applied BEs

Name of product	Tr. Short name	BEs	Beneficial effects
Trichoderma-WG	T-WG	*Trichoderma harzianum*	P-solubilization
OmG-08	T-OmG	*Trichoderma harzianum*	root growth, P-solubilization
Vitalin T50	T. Vit.T50	*Trichoderma harzianum*	root growth, P-solubilization
Trianum-P	T-Tria-P	*Trichoderma harzianum* T22	resistance to diseases and drought stress
Biological Fertilizer DC	P.B.F.-DC	*Penicillium sp*. PK 112	root and shoot growth stimulation, P-solubilization
Proradix WG	Ps. Pro.WG	*Pseudomonas* sp. DSMZ 13134	root growth stimulation, resistance to biotic and abiotic stress, mycorrhization, P-solubilization, pathogen suppression
Rhizovital 42	B.am-Rvt	*Bacillus amyloliquefaciens* FZB42	Root growth stimulation, P solubilization, yield, tolerance biotic (soil borne disease) and abiotic stress
Bacillus simplex R41	Bs.R41	*Bacillus amyloliquefaciens*	Root growth stimulation at temperatures below 12 °C, yield
Bacillus antrophaeus	B.atr	*Bacillus antrophaeus*	
Bacillus spec.,	B. spec	*Bacillus* spec	
Phylazonit	Phylazo.	*Azotobacter chrococcum Bacillus megaterium Azospirillum brasilens*	P-solubilization
Bactofil	Bacto.	*Azotobacter vinelandii Bacillus megaterium Bacillus polymyxa Pseudomonas fluorescens Sterptomyces albus*	P-solubilization
Meganit	Mega.	*Azotobacter chroccoccum Azospirillum ssp Bacillus megaterium Bacillus subtilis*	P-solubilization

3. Results and Discussions

The height of plants was measured on the 9th and 30th days after sowing (DAS). Among all treatments at 9 DAS, there was no difference in plant height between treatments (results are not shown). Among all treatments at 30 DAS, there was no

significant increase in plant height with the inoculation of any bio-effector in comparison to the corresponding control treatment without added P. Among all treatments at 30 DAS, only the non-inoculated control with added P (Contr.+P) was taller than the non-inoculated control without added P (Contr.). Inoculation with *Bacillus spec*. resulted in smaller plant height compared to the control without added P (results are not shown). At the end of the experiment, the dry weight of roots and shoots were measured. The results can be seen in Table 1. The non-inoculated control with added P (Contr.+P) showed significantly higher shoot fresh weight compared to all other treatments. The shoot fresh weight of control with P-fertilization was approx. 22 % higher than the fresh weight of P-deficient value (Contr.). There was no significant difference among other treatment. There was no difference in shoot dry weight among the other treatments.

Wu et al. [7] observed moderate increment in plant biomass due to the increase of nutrients either in chemical or organic form.

The fertilizer effect of plant growth was more pronounced after inoculation of AMF and its combination with beneficial bacteria. The results suggested that the dual inoculation of beneficial bacteria and AM fungi could compensate the nutrient deficiency in soils of maize.

Table 2) Shoot and root fresh and dry weight of maize (g plant^{-1}) inoculated with different bio-effectors (BEs) on 30 day after sowing (DAS). Difference letters show significant difference between treatments (One Way ANOVA, Tukey-test, $^{a}p<0.05$, $^{b}p<0.01$)

Treatments	*Shoot FW*	*Shoot DW*	*Root FW*	*Root DW*
Contr.	15.864± 0.924^{b}	1.557± 0.137	18.940± 1.429	0.783± 0.063
T-WG	14.356± 0.652^{b}	1.417± 0.056	19.288± 1.201	0.798± 0.047
T-OmG-08	14.276± 0.876^{b}	1.416± 0.095	21.146± 3.438	0.654± 0.115
T-Vit. T50	14.630± 1.225^{b}	1.474± 0.151	20.210± 4.463	0.712± 0.079
T-Tria-P	14.212± 1.569^{b}	1.365± 0.127	17.482± 2.102	0.699± 0.084
P.B.F.-DC	14.958± 0.356^{b}	1.487± 0.048	17.918± 1.444	0.754± 0.041
Ps.-ProWG	15.334± 1.615^{b}	1.558± 0.142	17.748± 1.764	0.853± 0.076
B.am-Rvt	14.868± 1.484^{b}	1.526± 0.161	18.380± 0.763	0.853± 0.067
Bs-R41	15.034± 0.946^{b}	1.508± 0.123	18.168± 2.663	0.799± 0.098
B.atr	16.014± 0.890^{b}	1.622± 0.122	17.458± 2.299	0.819± 0.089
B.spec	14.878± 0.828^{b}	1.527± 0.069	16.280± 1.370	0.792± 0.065
Phylazo	15.254± 0.786^{b}	1.541± 0.115	16.200± 2.278	0.842± 0.052
Bacto.	14.366± 0.786^{b}	1.445± 0.082	16.220± 2.278	0.763± 0.042
Mega.	14.830± 1.112^{b}	1.508± 0.137	16.540± 1.674	0.810± 0.096
Contr.+P	20.342± 1.173^{a}	2.144± 0.117	15.740± 1.673	0.780± 0.081

Root growth and development are sensitive for early P uptake by plants since P is relatively unavailable and immobile in many soils [8]. Root growth depends on the P status of the plant. P deficiency leads to higher root:shoot ratio [9]. Anghinoni and Barber [11] observed increased root length and dry weight on 12-d-old maize when P-deficiency increased between 1 and 6 days. On the other hand, Khamis et al. [12] did not observe an effect of P deficiency on maize root biomass. There are many reports of P fertilization increasing root length and biomass on a wide range of plants (e.g. maize, wheat, bean, cucumber) under different experimental conditions (hydroponic, - soil experiments) [13]. Root architecture refers to the complexity of root system spatial configurations that arise in response to soil conditions [14]. Soil P limitations is a primary effector of root architecture and is known to impact all aspects of root growth and development [15]. Williamson et al. [15] found that growth under P-deficient conditions resulted in a redistribution of root growth from the primary root to lateral roots. Reduced primary root elongation under low P conditions was accompanied by increased lateral root density and elongation. The abundant development of lateral roots associated with P-deficiency induced alterations in root architecture in almost invariable accompanied by increased root hair density and length. Phosphorus helps in development of meristematic tissue, in stimulation of early root growth.

Using the WinRHIZO digital image analysis system allowed for a categorization of the root system length, according to diameter class. Roselem et al. [13] observed that maize with higher efficiency in the acquisition and use of nutrients showed a larger proportion of thin roots. Schenck and Barber [16] suggested that the formation of thinner and longer roots is a mechanism that plants use to increase the nutrient absorption.

Figure 1. shows the total root length of 30-d-old maize treated with different BEs. The total root length was 133.6 - 241.5 m plant^{-1}. The longest root length was measured at T.-Vit.T50 treatment.
This value is higher with approx. 11 % compared to non-inoculated control without additional P-fertilization. There was no significant difference between two non-inoculated treatments. The total root length was significantly shorter at T.-WG compared to both of non-inoculated controls.

Plants are able to respond to P starvation by changing their root architecture, including root morphology, topology, and distribution patterns. Increases in root/shoot ratio, root branching, root elongation, root topsoil foraging, and root hairs are commonly observed in P-deficient plants, while the formation of specialized roots such as cluster roots occurs in a limited number of species [17].

P deficiency has been shown to reduce growth of primary roots and enhance length and density of root hairs and lateral roots in many plant species [17]. The P-efficient genotypes of common bean (*Phaseolus vulgaris*) have more shallow roots in the topsoil where there are relatively high contents of P resources [18]. Some plant species, including white lupin (*Lupinus albus*), can develop cluster roots with dense and determinative lateral roots, which are covered by large numbers of root hairs [19].

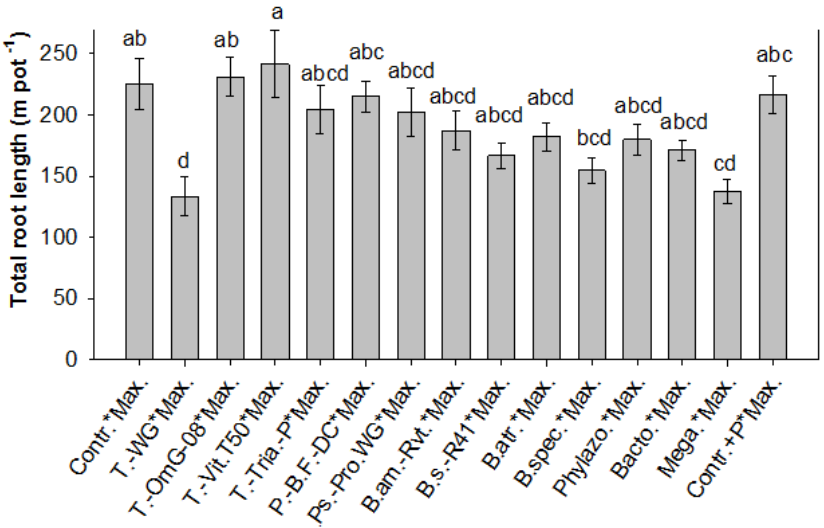

Fig. 1. Total root length of maize (m plant^{-1}) inoculated with different BEs on 30 DAS. Different letters show significant difference between treatments (One Way ANOVA, Tukey-test, $p<0.05$)

Therefore, root architecture plays an important role in maximizing P acquisition because root systems with higher surface area are able to explore a given volume of soil more effectively [19]. The distribution of root length based on diameter is very similar in all treatments (Figure 2). The longest roots diameter class belongs to <0.2 mm. Approx. 82-88 % of total root length belong to the <0.4 mm diameter group.

According to Clarkson and Hanson [19] a root system with a large number of smaller diameter roots is more effective in the absorption of nutrients. Costa et al. [20] observed that the biggest contribution for the total root length came from roots with diameter classes between 0.2 and 0.4 mm.

Pallant et al. [21] reported that 70 % of the roots belongs to the very thin roots classes (<0.5 mm). The biggest distribution to the length of roots with a diameter smaller than <0.5 mm has been believed to be a strategy that the plant uses, in low P availability conditions.

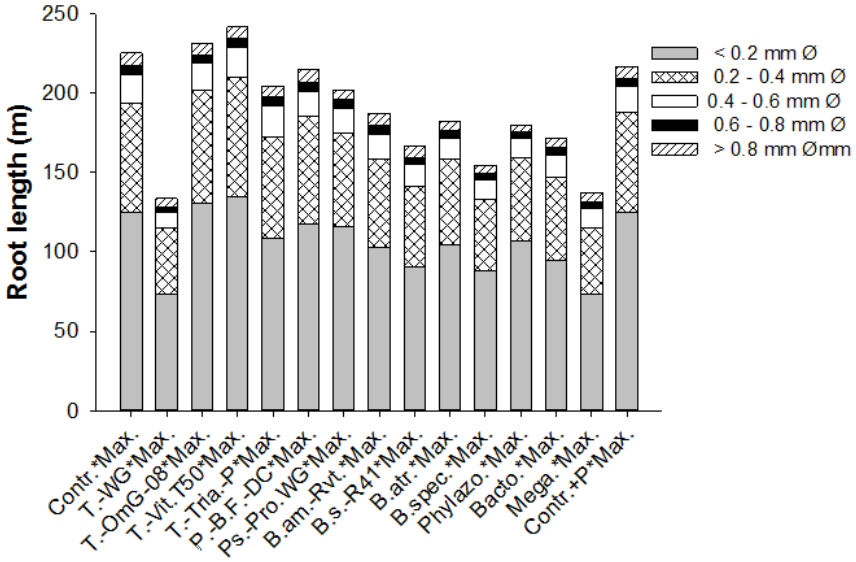

Fig. 2. Distribution of roots according to diameter class (<0.2 mm; 0.2-0.4 mm; 0.4-0.6 mm; 0.6-0.8 mm; >0.8 mm) in maize inoculated with different bio-effectors.

Conclusions

There was no observed significant difference between the two non-inoculated controls in some measured parameters. The shoot fresh and dry weight was higher in the non-inoculated control with additional P-fertilization. But, the root dry and fresh weight was not different under P-deficient and P-sufficient supply. Even though, the P-deficiency should theoretically affect root length, there was no significant difference between the P-deficient (Contr.) and P-sufficient (Cont.+P) control. An explanation could be that the roots tried to darned additional soil to uptake sufficient phosphorus which resulted in a longer root length. The advantages of bacterial and fungal inoculations are not questionable in agriculture, even thought our experiment showed no significant differences. A possible explanation for this is the soil used contained adequate nutrients for the plants, resulting in little differences between two controls. The good availability of nutrients, including phosphorus, could shadow the effect of microorganisms.

Acknowledgment

The author is grateful for the support of the Hungarian Scholarship Board (Hungarian Eötvös Fellowship).

Abbreviations

Bacto: Bactofil, B.am-Rvt: Rhizovital WG, B.atr:Bacillus antrophaeus, B. spec: Bacillus spec., Bs.R41: Bacillus simplex R41, BEs: bioeffectors, Contr: negative control (-P), Contr.+P: positive control (+P), DAS: day after sowing, DW: dry weight, FW: fresh weight, Mega: Meganit, P.B.F.-DC: Biological Fertilizer DC, Phylazo: Phylazonit, Ps. Pro. WG: Proradix WG, T-OmG: OmG-08, T.Vit. T50: Vitalon T50, T-Tria-P: Trianum-P, T-WG: Trichoderma-WG

R E F E R E N C E S

[1] D. Cordell , J. O. Drangert, S. White, *Glob Environ Change* 19, 29-305 (2009)

[2] P. Hinsinger, *Bioavailability of soil inorganic P in the rhizosphere aaffected by root-induced chemical changes: a review*. Plant Soil **237**, 173-195 (2001)

[3] G. Neumann and V. Römheld V. (2002). *Plant Roots, The Hidden Half*, (Marcel Dekker, Inc. New York) p. 617-649 (2002)

[4] A. E. Richardson, J. M. Barea A. M. McNeil, C. Prigent-Combaret, *Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms*. (2009). Plant Soil **321**, 305-339 (2009)

[5] Y. P. Chen, P. D. Rekha, A. B. Arunshen, W. A. Lai, C. C. Young, *Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities* (2006). Appl Soil Ecol **34**, 33-41 (2006)

[6] D. L. Jones, E. Oburger (2011). *Phosphorus in Action.* (Springer, New York, pp. 169-198 (2011)

[7] S. C. Wu, Z. H. Cao, Z. G. Li, K. C. Cheung, M. H. Wong, *Effects of biofertilizer containing N-fixer, P and K solubilizers and AM fungi on maize growth: a greenhouse trial*. Geoderma 125, 155-166 (2005)

[8] S. A. Barber, *Soil nutrient bioavailability: a mechanistic approach*. New York, Wiley-Inter Science (1984)

[9] W. J. Horst, M. Abdon, F. Wiesler, (1996). *Zeitschrift für Pflanzernaehrung und Bodenkunde* **159**, 155-161 (1996)

[10] I. Anghinoni and S. A. Barber S. A. *Phosphorus Influx and Growth Characteristics of Corn Roots as Influenced by Phosphorus Supply*. Agronomy Journal **72**, 685-688 (1980)

[11] S. Khamis, S. Chaillou, T. Lamaze, *CO_2 Assimilation and Partitioning of Carbon in Maize Plants Deprived of Orthophosphate*. Journal of Experimental Botany **41**, 1619-1625 (1990)

[12] C. A. Roselem, J. S. Assis, A. D. Santiago, *Root growth and mineral nutrition of corn hybrids as affected by phosphorus and lime*. Communications in Soil Science and Plant Analysis 25, 2491-2499 (1994)

[13] J. P. Lynch and K. M. Brown, *Topsoil foraging-an architectural adaptation of plants to low phosphorus availability*. Plant and Soil **237**, 225-237 (2001)

[14] L. C. Willianson, S. P. Ribrioux, A. H. Fitter, H. M. *Phosphate availability regulates root system architecture in Arabidopsis*. Leyser, Plant Physiology **126**, 875-882 (2001)

[15] N. K. Schenk and S. A. Barber, *Potassium and phosphorus uptake by corn genotypes grown in the field as influenced by root characteristics*. Plant and Soil **54**, 65-76 (1980)

[16] J. López-Bucio, A. Cruz-Ramirez, L. Herrera – Estralla, *The role of nutrient availability in regulating root architecture*. Curr Opin Plant Biol **6**, 280-287 (2003)

[17] H. Lambers, M. W. Shane, M. D. Cramer, S. J. Pearse, E. J. Veneklaas, *Root Structure and Functioning for Efficient Acquisition of Phosphorus: Matching Morphological and Physiological Traits*. Ann Bot **98**, 693-713 (2006)

[18] J. P. Lynch and K. M. Brown K. M. *The Ecophysiology of Plant-Phosphorus Interactions*. (Springer, Dordrecht, The Netherlands, pp. 83-116) (2008)

[19] D. T. Clarkson and J. B. Hanson, *The Mineral Nutrition of Higher Plants*. Annual Review of Plant Physiology **3**, 239-298 (1980)

[20] C. Costa, L. M. Dwyer, X. Zhou, P. Dutilleul, C. Hamel, L. M. Reid, D. L. Smith, *Root Morphology of Contrasting Maize Genotypes*. Agronomy Journal **94** 96-101 (2002)

[21] E. Pallant, R. A. Holmgren, G. E. Schuler, K. L. McCraken, B. Drbal, *Using a fine root extraction device to quantify small diameter corn roots (≥0.025 mm) in field soils*. Plant and Soil **153**, 273-279 (1993)