RHIZOSPHERE SOIL PROPERTIES AFTER SOYBEAN SEED INOCULATION BY LEVAN-PRODUCING STRAIN *PSEUDOMONAS AUREOFACIENS*

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Flow and retention of soil water is a function of soil structure. This research was designed to evaluate the hypothesis that a sustained improvement of soil aggregate structure in the rhizosphere is attained through inoculation of soybean seeds with exopolysaccharide producing Pseudomonas spp. The effect of inoculation of soybean seeds with levan-producing Pseudomonas aureofaciens on rhizosphere soil structure, water infiltration rate, soil bulk density, and water stable aggregate production was evaluated in greenhouse and field studies. Soils in the field were water stressed by a severe drought experienced in Moldova in the summer of 2007. Water infiltration, measured with a Mini-Disc Infiltrometer at a suction 2 cm, was faster ($P<0.05$) in soils planted with inoculated seeds than soils that received non-inoculated seeds. Also, at the end of field experiment, the inoculated soils had significantly lower bulk densities at 0-10 cm and 10-20 cm depths than did the non-inoculated soils. A water-stable aggregate index of soil sampled from 10-20 cm depth was 13% greater at the grain forming phase, but returned to the control level to the end of vegetative growth, when root exudates ceased. Inoculated soils contained more of agronomic valuable meso-aggregates (size 0.25-7 mm) than the control throughout the top 30 cm of soil, with the largest difference recorded within the 20-30 cm depth interval. Thus, inoculation of soybean rhizosphere soil by the levan-producing pseudomonad improved soil physical parameters under drought conditions.

***Key words**: rhizosphere soil, water infiltration, bulk density*

Drought and erosion by water are two of the main causes of land degradation. Water stress in soil is a major factor limiting crop production.

Bacterial production of exopolysaccharides (EPSs) in rhizosphere soil enhances water retention in the microbial and root environment, and regulates the diffusion of carbon sources [7, 8]. When inoculated to wheat roots, the EPS Levan produced by *Paenibacillus polymixa* improved aggregation of root adhering soil [4]. Similar effects were noted with inoculation of wheat with *Pantoea agglomerans* [2]. Inoculation of sunflower seeds by *Rhizobium* sp. YAS34 resulted in modified soil porosity and soil aggregate cohesion around root, and as a consequence increased nutrient uptake and prevention from water stress [1]. In the rhizosphere, plants produce exudates and stimulate bacterial activities that amplify the effect of bacterial EPS on soil property modifications. Mechanisms involved in this dynamic process are not well understood [3]. Levan and alginate are the simplest EPSs produced by rhizobacteria [12]. Effects of copper ion concentration on alginate biosynthesis by pseudomonad have been reported [11]. A *Pseudomonas* sp. strain increased EPS production during desiccation [13]. Thus, strains of *Pseudomonas* spp. that produce high levels of EPS should enhance soil aggregation, improving growth conditions for both soil microbes and higher plants. An EPS-producing strain *Pseudomonas aureofaciens* isolated from soybean roots growing in a Moldavian chernozem had beneficial effect on plant wet and dry biomass accumulation after soybean rhizosphere soil or seeds inoculation [9].

The aim of this research was to evaluate the effect of soybean seed inoculation with levan-producing *Pseudomonas aureofaciens* on rhizosphere soil structure, water infiltration rate, soil bulk density, and water stable aggregate production.

MATERIAL AND METHOD

Experimental design in greenhouse condition. The experiment mimicked two types of unfavorable condition for plant cultivation: deficiency of water supply and soil pollution by toxic copper at 300 ppm. A carbonate-rich chernozem soil from the Experimental Station of Moldovan Academy of Sciences (ESMAS, Chisinau, Moldova) was used. Selected soil properties were as follow: pH of 7.4; humus content of 2.47 %; particle density of 2.56 g cm^{-3}; total cation exchange capacity (CEC) of 14.1 meq 100^{-1} g of dry soil; and water holding capacity (WHC) of 47.6 % of dry soil. For the greenhouse experiment the soil was sieved to pass 10 mm openings. A total of 64 plastic pots with a volume of 7.5 kg were each filled with 5.2 kg of dry soil. In 32 of the pots, soil was contaminated by thoroughly mixing it with $CuSO_4 \cdot 5H_2O$ to achieve a copper concentration of 300 ppm. In each of the two sets of 32 pots (representing contaminated and non-contaminated soil), the following four treatments, each replicated eight times, were set up: 1) inoculated seeds, 2) non-inoculated seeds, 3) water content at 70% WHC (WC1 is equal to 33.4% of soil dry weight) and 4) water content at 35% WHC (WC2 - 16.7% of soil dry weight).

Seed inoculation and plant growth condition. Soybean seeds (*Glycine max L.*) of the new cultivar “Zodiac” [6], grown on ESMAS were selected for the experiments. *Pseudomonas aureofaciens* strain PsB-03 was isolated from soybean root-adhering soil (RAS) in 2003 [9]. Bacterial suspension (10^9 colonies forming units ml^{-1}) was used for seed treatment at a rate of $5 \cdot 10^7$ cells g^{-1} of dry seed. Four seeds per pots were planted at a depth of 2 cm. Initial soil water content was 50% of WHC (23.8% of dry weight). At blossom, plants were subjected to 14 days of water stress. Soil water content treatments (70% WHC and 35% WHC) were established in subsets of pots with contaminated and non-contaminated soil. After 14 days of water stress, the plants were harvested and root-adhering soil samples were collected and analyzed.

Experimental design in the field conditions. The research site was located at the ESMAS (Chisinau, Moldova). Experiment surface was 230.9 m^2 (2.7m x 85.5 m), and consisted of 2 plots, each with 3 lines of plants Zodiac from non-inoculated (control), and inoculated seeds, respectively. The distance between lines was 45 cm. Each of two plots inside was subdivided in 3 replicates. Bacterial suspension ($4.5 \cdot 10^8$ CFU ml^{-1}) was used for seed treatment at a rate of $1 \cdot 10^7$ cells g^{-1} of dry seed. Seeds were planted on 4 May, 2007 with a mini-tractor supplied with seed-drill.

Soil sampling. Field plots were sampled twice. The first sampling took place in July, 2007 and was for characterization of bulk density and texture (day/night air temperature were 31-33°C/23-24°C; 2.5 month without rain and irrigation). The second sampling was conducted in August 2007 (at the end of vegetative growth) and included bulk density and infiltration rate (day/night air temperature were 25-27°C/18-20 °C; 2 weeks after raining). Nine replicate soil samples (n=9) were extracted from depths: 0-10 cm, 10-20 cm, 20-30 cm, and 30-40 cm.

Bulk density, particle density, and water content of soil. The soil bulk density (D_b) - the ratio of the mass of dry solids to the bulk volume of the soil [5] - was determined with the core method, and included the effect of both the intra- and inter-aggregate pore spaces. Cores of 5.8 cm diameter x 4 cm height were used for extraction of undisturbed soil samples according to Kacinski [10]. Soil dry mass as well as soil water content were determined after drying at 105°C for 6 h.

Particle-size distribution, soil structure, and aggregate water-stability determination. Soil structure of studied soils was determined by dry sieving analysis according to Savinov [14]. Five hundred grams of soil were passed consequently through a set of sieves. Fractions collected on each sieve were weighted, and percents of nine fractions were calculated. Three size groups of structural separables were considered: micro-aggregates (< 0.25 mm); meso-aggregates (between 0.25 mm and 7-10 mm - agronomic valuable), and macro-aggregates (> 7-10 mm). A coefficient of soil structure was calculated as a ratio: K = percent of meso-aggregates / (percent of macro- + micro-aggregates). Particle analyses of soil samples were made using a sedimentation procedure.

Infiltration rate determination. A mini Disk Infiltrometer (Decagon Devices Inc., USA) was used for measuring of infiltration rate at a suction of 2 cm at time intervals of 30 seconds. In the greenhouse experiment, the mini Disk infiltrometer was placed on the soil surface at the center of each of 8 pot-replicates for each of 4 treatments and the cumulative infiltration (cm) was measured. The first measurement took place in September, 2007 and the second in April, 2008. Infiltration rate was calculated from a graph of cumulative infiltration vs. square root of time, where the slope represents the infiltration rate (i, cm s^{-1}).

RESULTS AND DISCUSSIONS

Infiltration rates were evaluated: i) under soil water stress condition; ii) under copper-contamination condition; iii) under double stress that is water and metal stress.

At an optimal value of 70% WHC, the average infiltration rate (n=8) of soil planted with inoculated seeds were greater than soil planted with non-inoculated seed (*figure 1*), but the difference was not statistically significant because of high variability among replicates. On the other hand, infiltration rates of soil maintained at a 35% WHC were significantly ($P < 0.05$) greater for soil planted with inoculated seeds when measurements were done at the end of the growing period (September

2007, *figure 1*). The difference remained significant (P<0.01) when measurements were repeated in the following spring (April 2008, *figure 1*).

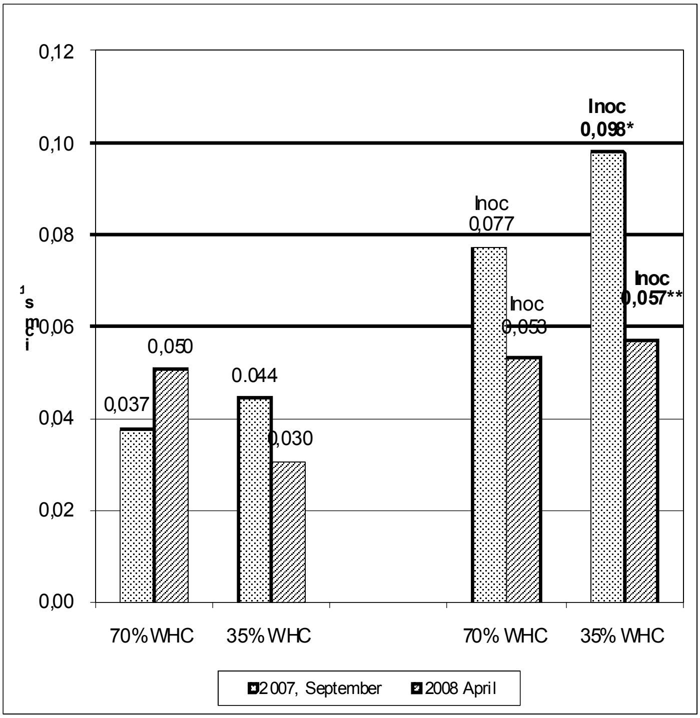

Figure 1 **Soil water infiltration rates, i, from the greenhouse experiment measured at two dates. Soil was not contaminated with copper. Plants were from either inoculated (Inoc) or non-inoculated seeds and in both cases soil-plant systems were subjected to a two-week period of water stress before equilibration to two contrasting soil water contents (70% and 35% WHC); *P<0.05, **P<0.01, n=8**

The same effect was registered for the case of elevated (300 ppm) copper concentration in soil (*figure 2*). Seed inoculation contributed significantly (P < 0.05) to higher water infiltration rates of metal stressed soil (at optimal water supply of 70% WHC) after winter season. Inoculated treatments under double (metal and water) stress and single water stress revealed comparable infiltration rates, and it is interesting that soil of the non-inoculated treatment under double stress exhibited a similar infiltration rate as soil of the inoculated treatment (*fig.2*). Possibly, soil indigenous EPS-producing bacteria are more adapted to conditions of double (water and metal) stress. Thus, unfavorable factors capable to inducing intensive bacterial synthesis of exopolymers in soil may result in greater infiltration rates.

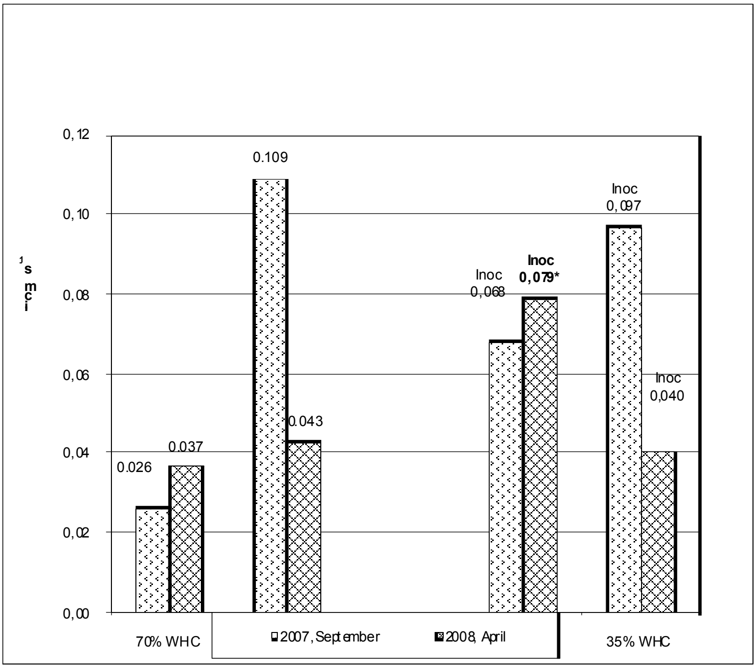

Figure 2 **Soil water infiltration rates, i, from the greenhouse experiment measured at two dates. Soil was contaminated with copper (300 ppm). Plants were from either inoculated (Inoc) or non-inoculated seeds and in both cases soil-plant systems were subjected to a two-week period of water stress before equilibration to two contrasting soil water contents; *P<0.05, **P<0,01, n=8**

Under a severe drought condition (summer 2007), soils in the field exhibited the same tendency than soil in the greenhouse experiment, i.e., greater infiltration rates in soil planted with inoculated soybean seeds. However, the effect was weaker; possibly because the density of bacteria cells applied to seeds planted in the field was five times smaller than in the greenhouse experiment. The number of observations was not enough to prove a statistically significant difference between control and experimental field plots. On the hand, bulk densities of field soils planted with inoculated seeds were significantly lower ($P < 0.05$, n=9) than in the soils planted with non-inoculated seeds in three out of the four sampled layers. In addition, a water-stable aggregate index (% of fractions > 0.25 mm) of soil sampled from 10-20 and 20-30 cm depth were 13% and 25% greater at the soybean grain forming phase in soil planted with inoculated seeds, but returned to the control level to the end of vegetative growth when root exudates ceased. At the grain forming phase soils planted with inoculated seeds contained more homogenous distribution of agronomic valuable meso-aggregates (size 0.25-7 mm) than the control throughout the top 30 cm of soil, with the largest difference recorded within 20-30 cm depth interval.

CONCLUSIONS

Inoculation of soybean seeds by the levan-producing pseudomonad improved soil physical properties of the rhizosphere soil under drought conditions. Water infiltration rate was faster ($P<0.05$) in soils planted with inoculated seeds than in soil planted with non-inoculated seeds. Also, at the end of field experiment, soils from inoculated seeds had significant lower bulk densities.

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