SOIL INVERTASE AND LEVANSUCRASE ACTIVITIES UNDER SOIL WATER STRESS, ELEVATED COPPER LEVEL, AND SOYBEAN SEED INOCULATION WITH THE LEVAN-PRODUCING BACTERIA *PSEUDOMONAS AUREOFACIENS*

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Scopul acestui studiu a fost de a examina efectul inoculării seminţelor de soia cu bacteria producentă de levan *Pseudomonas aureofaciens* asupra acumulării biomasei de soia şi a activităţii invertazice şi levansucrazice în rizosferă în condiţii de stres hidric şi conţinut înalt de cupru. A fost observată o majorare a activităţii levansucrazice în rizosfera plantelor inoculate şi supuse stresului hidric, fapt ce corespunde ipotezei noastre generale. Inocularea bacteriană a dus la majorarea masei verzi de soia în cazul nivelului hidric redus (35% CRA). Soiul de soia Zodiac nu a prezentat sensibilitate faţă de conţinutul înalt de cupru în sol (300 ppm), însă activitatea enzimelor studiate a fost semnificativ redusă ($P<0.05$) atât în solul rizosferic inoculat, cât şi în cel neinoculat. Sunt necesare cercetări ulterioare pentru a elucida funcţia biochimică a levansucrazei bacteriene, manifestarea acestei enzime în condiţiile stresului ecologic şi utilitatea ei în ameliorarea proprietăţilor fizice ale solului.

Soil invertase and levansucrase activities are of great agricultural importance because of their role in the carbon cycle and soil aggregation [12]. Invertase (β-D-fructofuranosid – fructohydrolase, EC 3.2.1.26) is an extracellular enzyme that catalyzes the hydrolysis of sucrose liberating glucose and fructose. Levansucrase (sucrose-6-fructosyltransferase, EC 2.4.1.10) catalyzes levan (polyfructan) synthesis from sucrose by transfructosylation [8,12]. The relationship between levansucrase and invertase activities in rhizosphere soil has not been determined, even though both enzymes require sucrose as substrate, which is commonly secreted into the rhizosphere by roots. According to Kiss and Dragan-Bularda [10-11] the synthesis of levan in toluene- and sucrose-treated soil was due to extracellular levansucrase in soil, although proliferating microorganisms could have participated as well. Since levan synthesis does not require ATP and only requires a single enzyme – levansucrase, it is expected that this exopolysaccharide could be important in bacterial protection under different stress conditions (e.g., by complexing metals thereby reducing their toxicity or by imbibing water under water limiting conditions) as well as in soil structure improvement and water retention.

A *Pseudomonas* sp. strain has been demonstrated to increase its exopolysaccarides (EPS) production under low water conditions [14]. We isolated the levan-producing strain *Pseudomonas aureofaciens* from soybean (cultivar Zenit) roots growing in a Moldovan chernozem soil and demonstrated its beneficial effect on soybean plant wet and dry biomass accumulation (cultivars Zenit, Bucuria, Aura, Alina) under water and metal stress condition [5-6].

The objective of this research was to study the effect of seed inoculation with a levan-producing bacteria *Pseudomonas aureofaciens* on the biomass production of a new soybean cultivar Zodiac and on soil properties, including assessment of invertase and levansucrase activities in soybean rhizosphere soil.

Materials and Methods

A carbonate-rich chernozem soil from the Moldovan Academy of Sciences Experimental Station was used in a greenhouse experiment. Soybean seeds (*Glycine* max L.) of the new cultivar "Zodiac" [2] were used without surface sterilization. A total of 48 pots were incubated under several moisture and copper amendment conditions to mimic commonly encountered field cultivation stresses. Three specific soil treatments were used (16 pots each): **NS1** – nutrition status 1 - soil fertilized with 50 mg N kg^{-1} of dry soil, and 90 mg P kg^{-1} of dry soil; **NS2** – nutrition status 2 - non-fertilized soil, resulting in N and P deficiencies; **NS2 + Cu300 ppm** - non-fertilized soil + a toxic level of copper amendment, 300 ppm. Two sets of pots - 24 pots for optimal soil water content (70% **WHC -** water holding capacity) and 24 pots for reduced water (35% WHC) – were established in a greenhouse. For each group of 24 pots, 12 of them were planted with inoculated seeds, and 12 with non-inoculated seeds.

The *Pseudomonas aureofaciens* strain was cultivated in liquid AS media at 28°C for 48-72 h. A bacterial suspension (10^9 CFU ml^{-1}) was used for seed treatment. At the blossom phase, plants were subjected to 14 days of water stress. In one set of 24 pots, soil water content was adjusted to 70% of WHC, while in the second 24 pots soil water content was 35% of WHC. Then plants were harvested and root-adhering soil samples were collected and analyzed. Prior to analysis soil samples were passed through a 1 mm sieve and kept air-dried at 10-12°C. Soil invertase (**INV**) activity was determined by a method adapted from Chiunderova [4,13] and Galstean [11]. Soil levansucrase (**LS**) activity was measured by a method adapted from Kiss [8]. Soil dehydrogenase (**DH**) activity was determined by the modified method of Galstean [7]. Microsoft Excel for Windows XP (Microsoft Office) was used for statistical analysis.

Results and Discussion

Comparison of all the studied soil enzyme activities under soybean plants with that ones measured in initial soil before soybean planting has shown that all resulting activities were higher in fertilized soil, at the initial level in non-fertilized, and significantly lower in Cu-polluted soil.

Generally, soil **DH** was higher in NP-fertilized soil (NS1). Additionally, DH activity in the fertilized soil was not affected by water deficiency, whereas DH in the non-fertilized soil (NS2) was significantly lower at soil water content 35%WHC. Introduction of the levan-producing bacterial population into the soil with inoculated soybean seeds did not enhance soil DH activity, actual activity levels could be significantly ($P<0,05$) 11-18 % less than with the non-inoculated soil. Interestingly in the non-fertilized (NS2) but inoculated soil (+Inoc) under water deficiency (35%WHC), the DH activity was not reduced but was equal to that observed in fertilized soil. DH activity was strongly reduced in soil amended with 300 ppm Cu. Interpretation of these data is limited by the fact that copper can prevent formazan formation in the DH assay [3].

The soil **INV** activity was always statistically greater in fertilized soil. Additionally, this increased activity was not dependent on soil water content being maintained in soybean root zone with or without N and P fertilizer addition (NS1 and NS2), with only a single exception. INV activity was reduced significantly by 26% at 35% WHC in fertilized and inoculated soil. Inoculation of the rhizosphere soil in most cases did not change the level of soil INV activity, although in 1 case out of 6, a significant reduction of soil INV activity at 70% WHC (by 25%) after inoculation of non-fertilized soil was observed. Cu-pollution reduced INV activity in both non-inoculated and inoculated soils, although the differences were not always statistically significant.

Soil **LS** activity was stable even in the presence of water stress and a toxic level of 300 ppm of Cu; that is, the differences were not statistically significant. NP fertilizers were the strongest influencing factor at 70% WHC, resulting in an enhancement of LS activity by 8-35%. Contrary to expectations, inoculation of soil with levan-producing bacteria did not significantly alter the level of soil LS activity. The only exception occurred with inoculation of non-fertilized soil and subsequently subjected to reduced soil water. In this case, the soil LS activity reached the level observed in fertilized soil. Some contribution of levan-producing bacteria at more severe constraints could be assumed for water stress but not for chemical stress.

The coefficients of variation (**CV**) were in the range of 3-15% for DH activity, 4-27% for INV activity, and 6-33% for LS. For the copper amended soil, the data were highly variable. Thus, the LS activity revealed a higher range of deviation, compared with DH and INV activities. This fact accentuates the adaptive function of LS activity in the changeable environment.

The Pearson's coefficient r-matrix (Table) shows that the investigated enzymes activities exhibited very high and significantly positive relationships with each other independently of nutrition status, water level and presence or absence of the inoculated levan-producing bacteria. A main observation is that soil INV and LS activities were always positively correlated despite the fact that the same substrate was necessary for expression of both invertase and levansucrase. Thus, in the majority of the cases the competition for common substrate was not observed. A meaningful observation is the apparent reduction of the relationship between the two indicated enzymes in inoculated soil incubated under reduced water, as noted by the lower correlation coefficient for this situation (**$r = 0.586$**). The tendency for the increase of soil LS activity accompanied with a reduction of soil INV activity as a result of levan-producing bacteria activity in soil at water stress condition is convincing. Especially when it is considered together with the fact that soil DH activity in all circumstances had very high correlation to both enzymes involved in carbohydrate metabolism. It is worthy of mentioning that the enhanced soil LS activity in inoculated non-fertilized soil was concomitant with enhanced DH activity at the same treatment.

Table

Correlation matrix r (r^2)[a] between soil enzyme activities[b]

	Invertase	**Levansucrase**	**Dehydrogenase**
<u>Non-inoculated</u>			
70% WHC			
Invertase	1	0.985 (0.97)	0.901 (0.81)
Levansucrase		1	0.814 (0.66)
Dehydrogenase			1
35% WHC			
Invertase	1	0.972 (0.95)	0.810 (0.66)
Levansucrase		1	0.925 (0.86)
Dehydrogenase			1

	Invertase	Levansucrase	Dehydrogenase
Inoculated (+Inoc)			
70% WHC			
Invertase	1	0.998 (1.00)	0.803 (0.65)
Levansucrase		1	0.837 (0.70)
Dehydrogenase			1
35% WHC			
Invertase	1	0.586 (0.34)	0.702 (0.49)
Levansucrase		1	0.988 (0.98)
Dehydrogenase			1

Values in parenthesis are the determination coefficient, r^2, which measures the proportion of variance for a variable, that is explained by the variation of another estimated variable. The lower limit of significance is $r \geq 0.45$, $r^2 \geq 0.20$, and $P \leq 0.05$ [1]; conditions see Material and Method.

The new soybean cultivar, Zodiac, was significantly sensitive to water and N, P nutrient deficiencies. The plant green mass (**PGM**) was reduced by water stress by 25-29% in the fertilized soil, and by 33-41% - in the non-fertilized treatment. It is interesting to note that the cultivar was not sensitive to high copper levels (300 ppm) at either soil water contents. Inoculation of the soybean seeds with the levan-producing *P. aureofaciens* strain before planting was not accompanied by a significantly positive effect on biomass production, although there was a tendency for an increase in PGM production under the water stress condition. Probably, more replicates in the experiment with cultivar Zodiac could confirm a positive effect of seed inoculation for plant development under reduced water supply. Additionally, there was a high number of indigenous EPS-producing bacteria in the calcareous chernozem soil used in this study, thereby negating any potential impact of additional seed inoculation.

For all treatments, **PGM** had a significant positive correlation with all three soil enzymatic activities. Although the invertase activity correlated more with plant characteristics and levansucrase activity correlated more with soil properties. Apparently, the inoculation of soil with levan-producing bacteria resulted in a dissociation of the positive correlation between INV and LS in favor of an increase in LS activity (Table), thus changing their relationship.

Conclusions

1. Inoculation of soybean Zodiac's seeds before planting by levan-producing *P. aureofaciens* PsB-03 strain revealed a steady tendency of increased plant green mass (PGM) under water stress. PGM had a significant positive correlation with all three soil enzymatic activities examined.
2. At reduced soil water content (35% WHC), the inoculation of soil with levan-producing bacteria resulted in a lower value of the Pearson correlation coefficient (r = 0.586) between soil INV and LS activities than for the non-inoculated soil (r = 0.972).
3. The soybean cultivar Zodiac was not sensitive to copper concentration (300 ppm) at either level of soil water content.
4. All of studied soil enzyme activities were higher in fertilized soil than in non-fertilized soil, and significantly lower in Cu-polluted soil.

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