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EVALUATION OF PRODUCTIVITY IN A MAIZE CROP TREATED WITH *AZOTOBACTER CHROCCOCCUM*, *AZOTOBACTER VINELANDII* AND *BACILLUS MEGATERIUM*

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Abstract. *While in the atmosphere nitrogen is abundant, this element plays a limited role as nutrient for plants. Cereal crops, as an example, are uncapable to directly use freely available nitrogen gas. As a consequence, their optimal growing and, in general, the productivity of such crops relies mainly on the use of chemical fertilizers, which, under the European Green Deal and the Farm to Fork Strategy are under scrutiny with plans of reduction of their use. As increasing crop production is a vital target for agriculture and food systems, the role of nitrogen fixers could play a fundamental role to match the targets of increasing the food production by decreasing the use of chemical fertilizers. Also, such approach is very appropriate from the perspective of sustainable production and conservative agriculture. The present work has the objective to examine the role of nitrogen fixers on the maize yield, respectively the productivity in harvested maize crop treated with Azotobacter chrococcum, Azotobacter vinelandii and Bacillus megaterium, three different nitrogen-fixing bacteria. The investigations were based on field tests. Thus, there at the beginning the seeds were inoculated before seeding; and during the vegetation the crops were treated with the three bacteria species. The fields experiment was organized in four variants (one non-treated variant and three with the bacteria Azotobacter chrococcum, Azotobacter vinelandii and Bacillus megaterium) and three replicates. The examination of collected samples revealed a general increase in productivity in the variants treated with Azotobacter vinelandii.*

Keywords*: maize crop, yield, inoculation, Azotobacter chrococcum, Azotobacter vinelandii and Bacillus megaterium.*

INTRODUCTION

Concerns regarding the positive effect of bacterial inoculation of maize are for long time ago, there being reported positive results regarding plant performance due to increase of nitrogen and phosphorus content in maize plants (PANDEY et al., 1998). Thuse, the use of *Azotobacter choroccocum* for inoculation of the seed of a barley was reported since the 70s by HARPER et al (1979) cited by PANDEY et al., (1998). Use of microorganisms for the inoculation of non-leguminous crops can diminish the dose of nitrogen fertilizers, the researches on endophyte diazotrophic bacteria having promising results (RIGGS et al., 2001), Thus, such results were demonstrated also by OLIVEIRA et al. (2017) for the bacteria *Azospirillum brasilense* that increased the maize productivity at low nitrogen fertilization doses due to the ability to produce growth promoting phytohormones. Other research referring to the maize inoculation with phosphate solubilizing bacteria have demonstrated the potential by influencing plant growth and yield (VIRUEL et al., 2014). Other literature sources have demonstrated that nitrogen-fixing bacteria inoculated on maize have the capacity at least partially to decreased the negative impact of salinity stress (ROJAS-TAPIAS et al., 2012; MARULANDA et al., 2010).

Thus, in literature can be found numerous researches revealing that growth-promoting rhizobacteria and mycorrhizae are able to influence the growth and productivity in different environmental stress conditions by different direct and indirect metabolic mechanisms (OLIVEIRA et al., 2018; BELTRAN-MEDINA et al., 2023; KOUL et al., 2022; FERRAREZI ET AL., 2022).

Other research suggests that pre-crop effect and different weather conditions could determine very opposite results of the inoculation of maize with microorganism consortia (bacteria with mycorrhizae) from a year to other (BRADÁČOVÁ et al., 2019; HETT et al., 2023).

The purpose of the work was to test three nitrogen-fixing bacteria inoculated on maize in a field test and asses their impact on the production in condition of high temperature and drought stress.

MATERIAL AND METHODS

The materials used for the experiment were:

- nitrogen-fixing bacteria *Azotobacter chrococcum*, *Azotobacter vinelandii* and *Bacillus megaterium*;
- an early maize hybrid;
- fertilizer NPK 20-20-20 (80 kg active substance / ha).

The experiment was set in the field of the Didactic Station of the University of Life Sciences ”King Michael I of Romania” from Timișoara. The soil type in the experimental field is black chernozem (IANOȘ et al.,1997). The climate is temperate continental, with a multiannual temperature of the air of 10.6 ºC and multiannual precipitations amount of 592 mm (METEOROLOGICAL STATION TIMIȘOARA, 2023).

Maize crop was seeded on 29 June 2023 at a density of 85 000 seeds/ha. The experimental plot was divided in four variants and three replicates. Each plot had 255 square metres (5 m x 51 m); each plot was seeded with 5 rows with a distance between rows of 70 cm. Experimental variants were: V1 – non-treated control; V2 – treated with *Azotobacter chrococcum*; V3 - treated with *Bacillus megaterium*; and V4 - treated with *Azotobacter vinelandii.*

The seed from the treated variants was inoculated with each of the bacteria mentioned above, respectively there was applied the equivalent of 250 ml of preparate in 1 litre of water for a quantity of 100 kg of seed for each of the three bacteria species.

After sowing there were applied two foliar treatments with a suspension preparate containing the same nitrogen-fixing bacteria used in the inoculum at a dose of 1 l/ha. The treatments applied in vegetation were applied as it follows:

- on 29 July 2023, respectively one month after sowing;

- on 22 August 2023.

On 21 October 2023, the maize crop was harvested, and the yield data were collected for statistical analysis, respectively the maize grain yield (t/ha) and cobs (t/ha).

Other data analysed in this work were referring to the impact of nitrogen-fixing bacteria on the germination of the maize crop seeded at a late moment, respectively 29 June 2023.

Statistical analysis method used was one-way ANOVA, and the software used for the processing of the data was JASP 0.18.1.0 (JASP Team, 2023).

RESULTS AND DISCUSSIONS

The results referring to the situation of germination under the influence of the treatments (Figure 1), expressed as plants per hectare shows that all treatments have higher values in comparison with the control. Thus, control had 48026.14 plants/hectare, while V4 had 63111.11 plants/hectare.

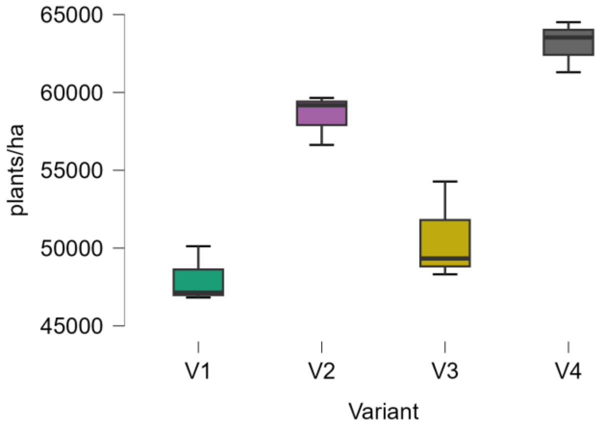

Figure 1. Boxplots analysis of the maize germination (plants/ha) (2023)

One-way ANOVA statistical analysis (Table 1) shows the existence of significant differences among the analysed experimental variants ($p < 0.001$) in the case of maize crop germination, expressed as plants/hectare.

Table 1

One-way ANOVA analysis of maize plants/ha

Cases	Sum of Squares	df	Mean Square	F	p
Variant	$4.366\times10^{+8}$	3	$1.455\times10^{+8}$	30.926	<0.001
Residuals	$3.765\times10^{+7}$	8	$4.706\times10^{+6}$		

Note. Type III Sum of Squares

For the identification of the statistically significant results regarding the germination of maize was performed the Tukey post-hoc test. Thus, comparing the non-treated variant with the treated ones there was noticed that V4 treated with *Azotobacter vinelandii* had a highly significant positive significance (p < 0.001***), but also, the results obtained in V2 (with *Azotobacter chroccocum*) were significant (p = 0.002**). Significant results were obtained also comparing V4 with V3 (p < 0.001***) and V3 with V2 (p = 0.009**).

Table 2

Post-hoc comparisons among the compared experimental variants of maize plants/ha

			95% CI for Mean Difference				
		Mean Difference	Lower	Upper	SE	t	p_{tukey}
V1	V2	-10457.516	-16129.926	-4785.106	1771.326	-5.904	0.002 **
	V3	-2614.379	-8286.789	3058.031	1771.326	-1.476	0.493
	V4	-15084.967	-20757.377	-9412.557	1771.326	-8.516	<0.001 ***
V2	V3	7843.137	2170.727	13515.547	1771.326	4.428	0.009 **
	V4	-4627.451	-10299.861	1044.959	1771.326	-2.612	0.115
V3	V4	-12470.588	-18142.998	-6798.178	1771.326	-7.040	<0.001 ***

* p < 0.05, ** p < 0.01, *** p < 0.001

Note. P-value and confidence intervals adjusted for comparing a family of 4 estimates (confidence intervals corrected using the Tukey method).

The plant densities lower in comparison with the seeding density (85000 seeds/ha) in all the experimental variants is due mainly to the very late seeding of the maize crop, respectively 29 June 2023.

In the following is analysed the maize grain production under the influence of the treatments with nitrogen-fixing bacteria (Figure 2), the obtained results showing that all treatments were determined higher values in comparison with the control. Thus, in control was obtained an average grain yield of 5.65 t/ha, while in V4 the yield 8.63 t/ha.

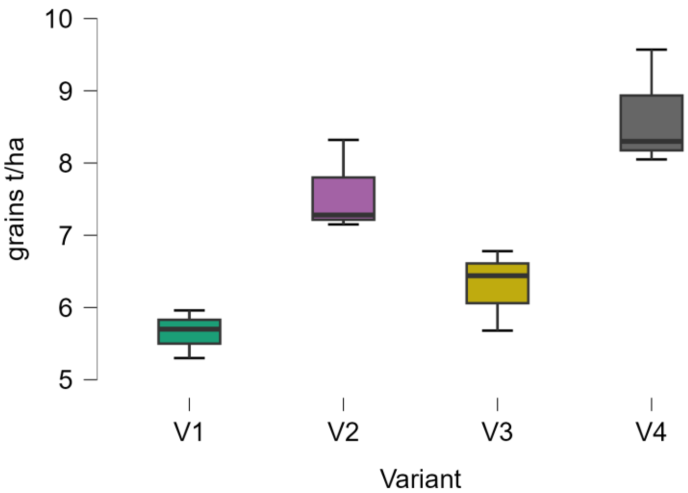

Figure 2. Boxplots analysis of the maize grains yield (t/ha) (2023)

After the analysis of the maize grains yield (t/ha) with one-way ANOVA (Table 2) we noticed that the differences among the treatment variants are significant (p = 0.001).

Table 3

One-way ANOVA analysis of maize grains yield (t/ha)

Cases	Sum of Squares	df	Mean Square	F	p
Variant	15.977	3	5.326	14.171	0.001
Residuals	3.007	8	0.376		

Note. Type III Sum of Squares

In Table 4 is presented the post-hoc comparisons among the compared experimental variants of maize grains yield. Comparing the grain yield of the non-treated variant with the treated ones highlighted the existence of statistical significance in V4 treated with *Azotobacter vinelandii* (p = 0.002**) and in V2 (with *Azotobacter chroccocum*) (p = 0.02*). Other significant differences were identified between V4 and V3 (p = 0.007**).

Table 4

Post-hoc comparisons among the compared experimental variants of maize grains yield (t/ha)

		Mean Difference	95% CI for Mean Difference Lower	95% CI for Mean Difference Upper	SE	t	p_{tukey}
V1	V2	-1.930	-3.533	-0.327	0.501	-3.856	0.020 *
	V3	-0.647	-2.250	0.956	0.501	-1.292	0.592
	V4	-2.987	-4.590	-1.384	0.501	-5.967	0.002 **
V2	V3	1.283	-0.320	2.886	0.501	2.564	0.123
	V4	-1.057	-2.660	0.546	0.501	-2.111	0.228
V3	V4	-2.340	-3.943	-0.737	0.501	-4.675	0.007 **

* p < .05, ** p < .01

Note. P-value and confidence intervals adjusted for comparing a family of 4 estimates (confidence intervals corrected using the Tukey method).

Secondary production of the maize kernels, respectively the cobs yield, was also considered in this research (Figure 3). The greatest maize cobs yield (t/ha) was determined in V4 (1.73 t/ha) and the lowest in V1 (1.21 t/ha).

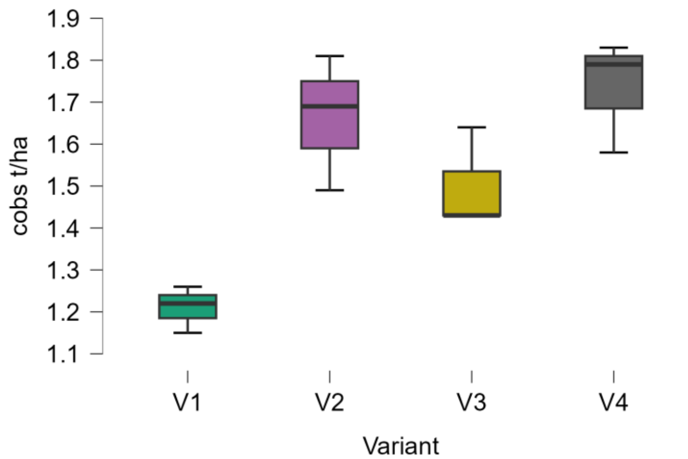

Figure 3. Boxplots analysis of the maize cobs yield (t/ha) (2023)

According with the results from one-way ANOVA regarding the maize cobs yield (t/ha) (Table 5) there was identified the existence of significant differences among the analysed experimental results.

Table 5

One-way ANOVA analysis of maize cobs yield (t/ha)

Cases	Sum of Squares	df	Mean Square	F	p
Variant	0.487	3	0.162	10.482	0.004
Residuals	0.124	8	0.015		

Note. Type III Sum of Squares

In Table 6 is presented the post-hoc comparisons among the compared experimental variants of maize cobs yield (t/ha). The significant results in comparison with the control were identified in V4 (p = 0.004*) and in V2 (p = 0.009).

Table 6

Post-hoc comparisons among the compared experimental variants of maize cobs yield (t/ha)

			95% CI for Mean Difference				
		Mean Difference	**Lower**	**Upper**	**SE**	**t**	**p_{tukey}**
V1	V2	-0.453	-0.779	-0.128	0.102	-4.461	0.009 **
	V3	-0.290	-0.615	0.035	0.102	-2.854	0.082
	V4	-0.523	-0.849	-0.198	0.102	-5.150	0.004 **
V2	V3	0.163	-0.162	0.489	0.102	1.607	0.426
	V4	-0.070	-0.395	0.255	0.102	-0.689	0.899
V3	V4	-0.233	-0.559	0.092	0.102	-2.296	0.178

* p < .05, ** p < .01

Note. P-value and confidence intervals adjusted for comparing a family of 4 estimates (confidence intervals corrected using the Tukey method).

The best experimental variant for all the analysed parameters of the maize crop was from far the one treated with *Azotobacter vinelandii* (V2), followed relatively closely by the variant treated with *Azotobacter chroccocum*.

CONCLUSIONS

There was noticed that both variants treated with *Azotobacter* species had statistically significant differences in comparison with non-treated control in the case of germination, but the highest significance was obtained in case of *Azotobacter vinelandii.* Thus, the production results have the same trend as in the case of germination. This fact shall be investigated in future because could have implications in the increase of the maize plant resistance to high temperature stress and even to drought. Such potential use of the nitrogen-fixing bacteria, could have great importance for maize production in the context of climate change.

The same situation regarding the statistical significance in comparison with the control variant was noticed for grain yield and cobs yield.

There will be developed future investigations to identify other implication of the application of nitrogen-fixing bacteria on maize crop production.

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