BIOPRIMING: A SUSTAINABLE SUPPORT FOR CROP ESTABLISHMENT

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Abstract

Crop yields are highly depended on germination and early stages of plant growth. Numerous priming techniques are being developed aimed to improve germination. Biopriming represents a sustainable approach based on seed treatment in bacterial suspension of selected plant growth promoting strains. One of the most promising plant growth promoting bacteria is *Azotobacter chroococcum*. The aim of the research was to evaluate the effects of *A. chroococcum* F8/2 as a biopriming agent on germination of various cultivable plants: basil, white mustard, cucumber, tomato, wheat, canola, and soybean. After surface sterilization, seeds were bioprimed in the bacterial suspension (10^7CFU/ml). Uninoculated seeds represented control treatment. Germination test was conducted with 100 seeds per treatment and the germination was monitored for 7 days. Following germination parameters were determined: germination percentage, germination index, mean germination time, vigor I, vigor II, length and dry biomass of the seedlings. The bacterial inoculation caused higher germination percentages of cucumber, tomato, wheat and soybean. The highest increase in germination index was observed in wheat (an increase of 19.8%). Tomato and basil were the only plants where vigor I was not increased by inoculation. Generally, the most favorable effects of *A. chroococcum* biopriming were observed in wheat where vigor I was increased more than twice, and vigor II was higher by 75.4% in inoculated seeds. The results indicate a significant potential for *A. chroococcum* use in biopriming. The observed effects of seed priming on germination parameters were crop-specific, with the most prominent potential in wheat biopriming.

Keywords: *Azotobacter chroococcum, Biopriming, Germination, Seedlings' growth.*

Introduction

The current agricultural, plant production concerning the needs of the world human population is out of step. Limited soil resource, its increased compaction, salinity, absence of organic matter, presence of pollutants are just some of the abiotic factors that compromise this production. However, the disturbed balance in the agroecosystem reflects its destructive impact on all other ecosystems and their living components. The introduction of new technologies that respect natural laws are and will be the key to sustainable crop production. Among them, bioprimig technology contributes to increasing the yield and quality of crops by using natural potentials while respecting the principles of sustainability. Biopriming involves the use of bioagents in seed treatment by various methods. Microbiological inoculation is the most desirable and most frequently used biopriming method based on the application of rhizosphere microorganisms as bioagents in the form of bacterial suspension (Ashraf and Foolad, 2005). This significant population of microorganisms from the soil of the narrow root zone (rhizosphere) has properties that in the interaction with the plant promote physiological events resulting in the good yield.

Therefore, they are defined as plant growth bacteria (Plant Growth Promoting Bacteria / Rhizobacteria, PGPR) (Kloepper and Schroth, 1978). They are active participants in the plant's nutritional cycle, phytohormonal modulation, biocontrol and elimination of toxic substances (Sumbul *et al.*, 2020).

Since the seeds are the starting material in plant production and carry a huge potential responsible for plant growth and development, biopriming is focused on the seeds and the initiation of their potential, which occurs in germination. Germination is defined as a crucial and critical phase in the life of a plant and its good start is a prerequisite for the promotion of yield and quality (Sanchez *et al*., 2014; Houle et al., 2001**).** The yield can be increased in the range of 25-65% (Ahmad *et al.*, 2016) with an improvement in crop quality at the same time (Revillas *et al.*, 2000). By microbiological inoculation, selected PGPR strains will successfully colonize the seed structure **(**Mahmood and Kataoka, 2018) and after stimulating germination and seedlings' growth, will continue to actively participate by various mechanisms in the plant life in the rhizosphere (Yadav *et al*., 2015). Among the most common inoculants is *Azotobacter* due to its prestigious PGP traits such as the ability to provide nutrients to plants, especially nitrogen through the process of nitrogen fixation, synthesis of phytohormones, siderophores, antibiotics, participation in biocontrol and degradation of toxic compounds (Nosrati *et al.*, 2014; Sumbul *et al*., 2020; Kumari *et al*., 2017). However, the positive effect is far greater and reflected in the improvement of the quantitative and qualitative properties of the soil while preserving its diversity (Jeffries *et al.*, 2003).

Bearing in mind the importance of biopriming, the aim of this work was focused on the bioagent, *Azotobacter chroococcum* F8/2 and its influence on germination and seedlings´ condition. The success of germination was determined by germination parameters such as final germination percentage (FGP), germination index (GI), average germination time (MGT), vigor I and II, seedling length, and their dry weight. In addition to the choice of inoculant, the results of germination are influenced by numerous factors, among which the plant species is of exceptional importance. Thus, different plant species are used to test the effect of the proposed biopriming.

Material and methods

Bacterial strain

The isolated strain belongs to the collection of the Department for Ecological Microbiology, the Faculty of Agriculture. Growing on the selective Fiodorov medium, based on cell morphology, colony appearance, and biochemical profile the isolate was identified as *Azotobacter chroococcum.*

Plant species and seed pre-treatment

Seven plant species are selected, important for national and worldwide agricultural production: basil (*Ocimum basilicum*), mustard (*Synapsis alba*), cucumber (*Cucumis sativus*), tomato (*Solanum lycopersicum* L.), wheat (*Triticum aestivum*), canola (*Brassica napus* L.), and soybean (*Glycine max.* L.). All seeds were uniform, compact in structure, and subjected to sterilization with 70% alcohol (v/v) / 2 min and 0.02% NaOCl (v/v)/ 2 min. After thorough rinsing with sterile deionized water, they were set to dry in sterile conditions. Ten seeds were selected for each plant species, placed on MPA medium, and incubated for 24 hours at a temperature of 30 °C to check the success of sterilization.

Inoculum preparation and seed inoculation

48h-old bacterial culture of *A. chroococcum* F8/2 was "scratched" from solid media, and resuspended in the sterile saline (0,9% NaCl) until the inoculum suspension of 10^7 CFU /ml was reached. Previously prepared seeds were immersed in the inoculum suspension and incubated in a rotary shaker (130 rpm) for 1 hour at the temperature of 28±2 °C.

Germination assay

Germination test was performed by applying the slightly changed filter paper method (ISTA; 1999). Sterile Petri dishes were used in which sterile filter papers were placed on the bottom. Before placing the seeds, the filter paper was moistened with sterile water. 100 seeds for each plant species were arranged in 4 replicates of 25 seeds, except for soybeans, where due to the size of the seeds, the number of replicates was 10 with 10 seeds each. Non-inoculated seeds were set up in a completely identical way as control. Previously dried and inoculated seeds were placed on Petri dishes and left at natural light and an average room temperature of 25°C for 7 days. Sterile water was added when needed to maintain optimal humidity.

The number of germinated seeds was recorded daily based on a radical emergence and its length of 2 mm. After 7 days, representative seedlings from each replicate were chosen, randomly for measuring their length. Thereafter, they were dried overnight at 90 °C and their dry weight was recorded. Advanced germination measurement tool (Argon Info-Tech) was used to calculate germination parameters such as the final germination percentage, germination index (GI), mean germination time (MGT), vigor I, and vigor II.

Statistical analysis

The analysis was conducted by Tukey's test comparing the differences between means of obtained results from the inoculated seeds vs control at the 5% level of probability.

Results and discussion

Data obtained from the conducted experiment show differences in plant species response to the applied biopriming with A. chroococcum F8/2. Significant increases in FGP were achieved in cucumber (12.70%), tomato (8.79%), soybean (14.58%), and wheat which showed the largest increase of 19.58% compared to the control (Tab. 3, 4, 5, and 7). Although a desirable reduction in MGT was observed in some plants, it did not indicate a significant difference between biopriming and control treatments. A slight increase in germination index (GI) was observed in canola, tomato, and wheat, while GI of inoculated treatments was significantly higher compared to control in cucumber for 28.30%, and soybeans for 56.72% (Tab. 3 and 7).

Table 1. Germination parameters of basil (Ocimum basilicum) bioprimed by Azotobacter chroococcum F8/2

Treatments	FGP (%)	MGT (day)	GI	Vigor I	Vigor II	Seedlings lenght (cm)	Seedlings dry mass (g)
Inoculation	58 ± 4	5.01 ± 0.13	3.16 ±0.16	293.90 ± 13.79	0.0392 ±0,0040	5.1 ±0.23	0.0007 ±0.0001
Control	69 ± 4	5.16 ± 0.09	3.74 ±0.25	321.45 ± 24.73	0.0355 ±0.0027	4.63 ±0.29	0.0005 ±0.0001

*Data show means ±Std. Err.; * indicates that the parameter in control and inoculation treatment are significantly different at p≤0.05*

Table 2. Germination parameters of white mustard (*Sinapsis alba* L.) bioprimed by *Azotobacter chroococcum* F8/2

Treatments	FGP (%)	MGT (day)	GI	Vigor I	Vigor II	Seedlings lenght (cm)	Seedlings dry mass (g)
Inoculation	100 ± 0	1.06 ± 0.02	24.52 ±0.34	1266.4 ±77.8	0.5192 ±0.0298*	12.65 ±0.67	0.0052 ±0.0010*
Control	100 ± 0	1.08 ± 0.05	24.57 ±0.37	1186.8 ±56.2	0.3967 ±0.0252*	11.87 ±0.67	0.0040 ±0.0008*

*Data show means ±Std.Err.; * indicates that the parameter in control and inoculation treatment are significantly different at p≤0.05*

Table 3. Germination parameters of cucumber (*Cucumis sativus*) bioprimed by *Azotobacter chroococcum* F8/2

Treatments	FGP (%)	MGT (day)	GI	Vigor I	Vigor II	Seedlings lenght (cm)	Seedlings dry Mass (g)
Inoculation	71 ± 1*	2.82 ± 0.07	7.40 ±0.36*	1293.09 ±48.43*	1.3242 0.0789	18.19 ±0.62*	0.0186 ±0.0011
Control	63 ± 2*	3.14 ± 0.17	5.76 ±0.08*	549.40 ±30.05*	1.0991 0.0842	8.71 ±0.42*	0.0174 ±0.0012

*Data show means ±Std. Err; * indicates that the parameter in control and inoculation treatment are significantly different at p≤0.05*

Table 4. Germination parameters of tomato (*Solanum lycopersicum* L.) bioprimed by *Azotobacter chroococcum* F8/2

Treatments	FGP (%)	MGT (day)	GI	Vigor I	Vigor II	Seedlings lenght (cm)	Seedlings dry Mass (g)
Inoculation	99 ± 1*	3.97 ± 0.09	6.64 ± 0.21	991.75 ± 93.54	0.1931 ± 0.0125*	9.25 ± 0.29*	0.0020 ± 0.0001*
Control	91 ± 2*	3.78 ± 0.07	6.48 ± 0.08	1076.17 ± 33.741	0.143 ± 0.0065*	11.82 ± 0.33*	0.0016 ± 0.0001*

*Data show means ±Std. Err.; * indicates that the parameter in control and inoculation treatment are significantly different at p≤0.05*

Table 5. Germination parameters of wheat (*Triticum aestivum*) bioprimed by *Azotobacter chroococcum* F8/2

Treatments	FGP (%)	MGT (day)	GI	Vigor I	Vigor II	Seedlings lenght (cm)	Seedlings dry Mass (g)
Inoculation	89 ± 3*	1.97 ± 0.10	14.38 ± 0.76	1883.44 ± 69.81*	1.8277 ± 0.0573*	21.18 ± 0.69*	0.0204 ± 0.0005*
Control	77 ± 1*	1.84 ± 0.08	12.00 ± 0.62	894.52 ± 48.66*	1.0421 ± 0.0396*	11.64 ± 0.66*	0.0135 ± 0.0005*

*Data show means ±Std.Err.; * indicates that the parameter in control and inoculation treatment are significantly different at p≤0.05*

Table 6. Germination parameters of canola (*Brassica napus* L.) bioprimed by *Azotobacter chroococcum* F8/2

Treatments	FGP (%)	MGT (day)	GI	Vigor I	Vigor II	Seedlings lenght (cm)	Seedlings dry Mass (g)
Inoculation	43 ± 6	1.66 ± 0.20	8.48 ± 0.87	591.84 ± 36.32*	0.1303 ± 0.0119*	13.77 ± 0.46*	0.003 ± 0.0002
Control	44 ± 0	1.75 ± 0.25	8.36 ± 0.91	397.99 ± 36.22*	0.1397 ± 0.0119*	9.04 ± 0.46*	0.0031 ± 0.0002

*Data show means ±Std. Err.; * indicates that the parameter in control and inoculation treatment are significantly different at p≤0.0*

Table 7. Germination parameters of soybean (*Glycine max* L.) bioprimed by *Azotobacter chroococcum* F8/2

Treatments	% FGP	MGT (day)	GI (seed/day)	Vigor I	Vigor II	Seedlings lenght (cm)	Seedlings dry Mass (g)
Inoculation	55 ± 2*	4.64 ± 0.24	3.73 ± 0.25*	500.87 ± 46.37*	10.4019 ± 0.5542*	9.26 ± 0.98*	0.1811 ± 0.0106
Control	48 ± 2*	5.20 ± 0.05	2.38 ± 0.25*	222.64 ± 29.50*	8.4321 ± 0.5201*	4.63 ± 0.60*	0.1759 ± 0.0108

*Data show means ±Std. Err.; * indicates that the parameter in control and inoculation treatment are significantly different at p≤0.05*

Vigor I was determined as the common value of the germination percentage and the length of seedlings. Statistically significant increases were observed in wheat of 110%, soybean (124%), and canola (48.71%). However, a significant decrease of 46.61% was presented in cucumber. Analyzing the data of Vigor II, increases were recorded in soybeans (23.36%), followed by mustard (30%), tomatoes (37.39%), and wheat of 75.39%, which was the most significant increase achieved by Azotobacter biopriming. Considering the seedlings' length, the promotion was noted in cucumber (108.84%), soybean (100%), and wheat (81.96%). The inhibitory effect of inoculation treatment was evident in tomatoes where a reduction of the seedlings length was 27.78%. Although the inoculation had an inhibitory effect on the length of seedlings in tomatoes, it contributed to an increase in their dry matter by 25%. Also, an increase of 30% in dry mass was recorded in mustard. However, according to the results, the most prominent dry mass increase was in the wheat of even 51.11%.

Since germination is a complex physiological event that determines the entire future plant development and its final production, all of the studied parameters were in purpose for a better assessment of germination success influenced by Azotobacter biopriming. Biopriming stimulates pre-germinative processes in the seed contributing to better germination (Bradford, 1986). The selected bioagent, A. chroococcum F8/2, initiated significant promotion of the germination percentage in most of the plants, as the best in wheat. Although the germination percentage is an important determinant of germination success it is not crucial. Previous studies have given priority to vigor as a powerful seed potential in the realization of final yield under given conditions (TeKrony, 2003). The phytohormonal activity of A. chroococcum is obvious by analyzing seedling length. The ability to modulate hormonal balance among ethylene, GAs, and auxin is most likely the reason for the increase in seedling length and the direct reason for the improvement of vigor I. The stimulating effect of Azotobacter inoculation on seedlings'

development is reflected through dry weight increase that affects vigor II as an indicator of seedlings' ability to continue to develop successfully in the later life stages. Also, germination and vigor are genetic traits (Wu et al., 2017). It is known that germination depends largely on the vitality of the embryo and the depth of dormation (Martinez, 2013). The result of germination may be influenced by the different strain affinity for a certain plant species and its exudates (Lugtenberg et al.,2001; Kumar et al., 2007; Compant, 2010) that are released in the imbibition phase, significantly affecting the colonization process and metabolic activity of biopriming agents (Brelwey and Black, 1993).

Conclusion

The effect of biopriming using *Azotobacter chroococcum* F8/2 is specifically dependent on plant species, with best results in germination promotion in wheat. *A. chroococcum* biopriming of soybean, wheat, tomato and cucumber seeds may trigger the promotion of germination, but also could help the creation of a compatible connection between inoculant and plants providing useful activities in the rhizosphere in later crop development stages. The final results of the application of *Azotobacter* biopriming could be more far-reaching, including agronomic, ecological, and economic aspects.

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