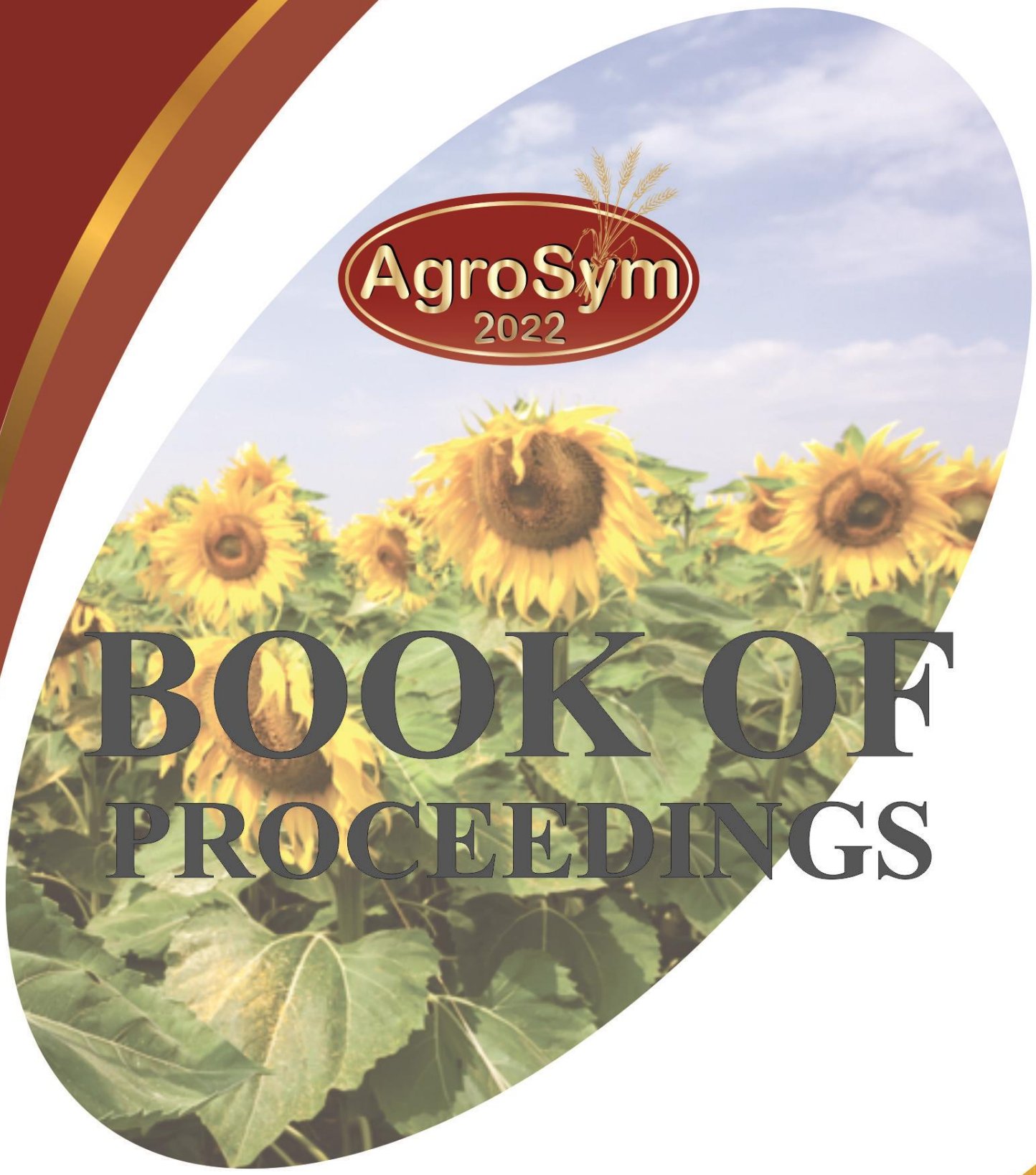

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CONTENTS

STATIC MAGNETIC FIELD IMPROVES EFFECTS OF BIOPRIMING BY *AZOTOBACTER CHROOCOCCUM* F8/2

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Abstract

Seed inoculation (biopriming) represents an agronomic practice directed towards improving germination, as well as fostering beneficial plant-microbe interaction from the very beginning of plants' life. Besides biopriming, static magnetic field (SMF) is studied as an abiotic factor affecting germination and plant growth. This paper is aimed to examine the combined effect of *Azotobacter chroococcum* F8/2 and SMF of 90 mT on germination. *A. chroococcum* F8/2 has been proven as a successful biopriming agent, with beneficial effect on cucumber, tomato, wheat, and soybean germination. This research starts from the hypothesis that the combined effect of *Azotobacter* inoculation and SMF could lead to synergistic improvement of germination parameters, compared to already shown effects of biopriming itself. The research was conducted with following cultivable plants: basil, cucumber, tomato, wheat, and soybean. Seed treatment was performed by 1h-immersion of surface-sterilized seeds into bacterial suspension (10^7CFU/ml), followed by exposure to SMF of 90 mT for 5 min and 15 min. The germination test was conducted with 100 seeds per treatment and lasted 7 days. The highest improvement of germination percentages was observed in cucumber and basil (an increase for 35-41% and 41-45%, respectively), compared to biopriming without SMF treatment. Tomato and wheat germination were not improved by addition of SMF treatment to biopriming. The obtained results indicate that the application of SMF can affect the germination parameters that are changed by biopriming. There is a need for further research in order to explain the differences between plant species' response.

Keywords: *Azotobacter chroococcum, biopriming, germination, static magnetic field.*

Introduction

Biopriming is a hydration of seeds with a saline/suspension of biological component that can be bioactive molecule (saliclic acid, giberellines) or Plant Growth Promoting Rhizobacteria, PGPR (Ashraf and Foolad, 2005; Hamayun et al., 2010). Microbial inoculation by PGPR represents a backbone of biopriming. Selected strains used as biocomponents characterize diverse Plant Growth Promoting (PGP) potential. Numerous studies highlight PGP properties of *Azotobacter* and affirm its representatives as biofertilisers, biostimulators, and biocontrol agents (Sumbul et al., 2020; Pirttila et al., 2021). In previous studies, the selected strain *A. chroococcum* F8/2 demonstrated a significant enhancing effect on the germination of cucumber, tomatoes, wheat, and soybeans (Kerecki et al., 2021). Since the germination is the most delicate stage of the plant's life cycle, the important question is how much abiotic factors can influence it. Rising temperatures, salinity, and changes in soil pH are all known to have a negative impact on seed fate and germination. On the other side, some abiotic environmental factors have been studied as promoters of germination and plant growth, and possibilities for their use are being studied. The static magnetic field (SMF) is known to be a ubiquitous and unavoidable abiotic factor that affects the living world. In the case of SMF, its

strength and duration of exposure, plant species, and environmental factors all influence whether it has a positive or negative effect on plant growth (Zhang et al., 2017). These are the critical moments and data on the SMF impact is quite often contradictory. Nonetheless, the potential application of SMF and other forms of magnetic activity in sustainable plant production is supported by numerous scientific studies that indicate positive effects on germination (Bhardwaj et al., 2012), early-stage plant development (Souza et al., 2015), and final yield (Vashisth et al., 2013). Previously published data show that SMF can have a positive or negative effect on the growth and activity of microorganisms depending on the strength and timing of exposure, but it can also cause structural changes in nucleic acids and cell membrane characteristics (Belyavskaya 2004; Goodman et al., 1995). Unfortunately, there is a limitation of data on the influence of SMF on PGPR activity, as well as plant-microbe interaction. Studies have mainly been focused on either plants or microbes, without insight into SMF effect on overall plant-microbe community. The main aim of the present study was to determine the impact of 90mT SMF on the germination parameters (germination percentage, germination index, mean germination time, and vigor I) of different plant inoculated seeds and thus to characterize the relationship between the three priming actors: plants, inoculant, and magnetic fields.

Material and methods

Bacterial strain

Azotobacter chroococcum F8/2 belongs to the collection of The Department of Environmental Microbiology of the Faculty of Agriculture, University of Belgrade. The strain has been identified for the purposes of previous research, some of the PGP activities have been confirmed, as well as the strain potential to be used as a bioprimig agent (Kerečki et al., 2021).

Plant species and seed treatment

Seeds of plant cultures of basil (*Ocimum basilicum* L.), cucumber (*Cucumis sativus* L.), tomato (*Solanum lycopersicum* L.), wheat (*Triticum aestivum* L.), and soybeans (*Glycine max* L.) were homogenized and sterilized for 2 minutes with 70% alcohol (v/v) and 0.02 % of NaOCl (v/v), rinsed with sterile deionized water, and left to dry in sterile conditions. To verify sterilization success, ten seeds from each plant species were chosen, positioned on MPA medium, and incubated for 24 hours at 30 °C. The absence of bacterial growth indicated successful seed sterilization.

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 (KS 260, IKA, Germany) for 1 hour at the temperature of 28±2 °C/130 rpm.

Following inoculation and drying, two portions of the seeds were subjected to 90 mT SMF for 5 and 15 minutes before being placed in Petri boxes for germination. The SMF exposure system was set up according to Jovičić-Petrović et al. (2021).

Germination assay

Three different treatments per plant species were tested: inoculated seeds, not subjected to SMF; inoculated seeds exposed to 90 mT for 5 min; and inoculated seeds exposed to 90 mT for 15 min. Using the modified filter paper method outlined by Kerečki et al. (2021) seeds germinated in Petri boxes at natural light and an average room temperature of 25°C. The periodical addition of sterile water supply ensured optimal humidity. For the next seven days, the number of germinated seeds was recorded daily. Germination parameters (final germination percentage - FGP, germination index - GI, mean germination time - MGT, and vigor I) were calculated using a germination measurement tool (Argon Info-Tech).

Statistical analysis

Tukey's test was used to compare the differences in means of the obtained results from inoculated seeds not treated in SMF versus inoculated seeds exposed to SMF, at a 5% level of probability.

Results and discussion

The results revealed that SMF of the given exposure-system had a beneficial impact on the germination of inoculated basil and cucumber seeds. Differences were not observed in soybean inoculated seeds, while some germination parameters of bioprimed wheat and tomato decreased, except in case of wheat exposed to SMF for 5 min (Table 1.).

Table 1. The effect of SMF on germination parameters achieved by *Azotobacter chroococcum* F8/2 biopriming

Plant	Treatment	FGP	GI	MGT	Vigor I
basil	T	58±8	3.00±0.31	5.01±0.27	294±48
	T5	82±5*	6.11±0.45	4.03±0.15*	363±32*
	T15	84±18*	5.56±0.77*	4.20±0.42*	336±72
wheat	T	89±7	14.38±1.53	1.97±0.13	1883±242
	T5	86±5	14.14±1.67	1.88±0.20	1445±212
	T15	80±3*	11.78±0.17*	2.16±0.16	1320±181
soybean	T	55±4	3.73±0.69	4.63±0.48	501±161
	T5	60±3	3.62±0.54	4.75±0.11	434±133
	T15	56±7	3.12±0.70	4.84±0.55	616±215
tomato	T	99±2	6.64±0.42	3.97±0.18	992±324
	T5	90±2*	6.66±0.33	3.60±0.14*	818±87
	T15	86±5*	6.40±0.87	3.87±0.43	729±104*
cucumber	T	71±2	7.39±0.72	2.81±0.15	1293±168
	T5	100±0*	14.87±1.12	2.09±0.38*	1688±96
	T15	96±5*	14.49±1.36	2.18±0.26*	1673±184

T-seeds bioprimed with *A. chroococcum* F 8/2; T5 – seeds bioprimed with *A. chroococcum* F 8/2 and subjected to 90mT SMF for 5 min; T15 – seeds bioprimed with *A. chroococcum* F 8/2 and subjected to 90mT SMF for 15 min; ± std. dev. * indicate statistically significant differences between the parameter in inoculated seeds (T), and combined biopriming-SMF treatments (T5 and T15) at $p \leq 0.05$

SMF application caused an increase of the basil and cucumber germination percentage by 35-41% and 41-45%, respectively. Basil also showed a 44% increase in GI and a 23% increase in vigor. This is in accordance to Jovičić-Petrović et al. (2021) who reported an increase in

germination percentage of white mustard by 53.2% as a result of the combined effects of biopriming and SMF of 90 mT / 15 min.

The five-minute treatment in SMF reduced MGT of basil by 24.3%, while SMF reduced MGT in both exposure durations in cucumbers by 34.5% and 28.9%. Although SMF decreased germination percentage of tomato, MGT was reduced by 10.3% when SMF was applied for 5 min. This is in accordance with Feizi et al. (2020) who reported a decrease of MGT of mustard seeds exposed to SMF. In basil, the positive SMF impact was reflected in the promotion of all observed germination parameters, whereas while in the case of wheat, SMF caused significant reduction of FGP and GI. The observed differences between tested plant species supported an earlier statement that the effect of SMF is determined by the plant`s genotype (Hernandez - Aquilar et al., 2009).

Furthermore, the result indicated that exposure time impacts the SMF effect, since differences in certain parameters were observed depending on the time of exposure, which is in the line with observation of Zhang et al. (2017). Similarly, Jovičić-Petrović et al. (2021) claimed that SMF effects on seed germination metrics are modulated by duration of seed exposure.

Although the metabolic events affected by SMF are not completely understood, we could take into account increased water assimilation and increased enzyme activity (peroxide, catalysis, super-oxide dysmutasis, glutathione-transferase, particularly amylase), to be critical moments in promoting germination parameters (Shine et al., 2011; Shine and Gurupasad, 2012; Maffei 2014). The fostered permeability of the cell wall of the seed, enabling greater absorption of water and energy molecules (Reina et al., 2001; Aladjadjiyan 2002), could result in richer metabolic activity (Iqbal et al., 2012). According to Kastenios et al. (2016), when SMF is applied to seeds, there is a significant increase α-amylase activity (Katsenios et al., 2016), that influences carbohydrate mobilization and degradation to monosaccharides necessary for seedlings development (Kataria et al., 2017).

It is important to note that the magnetic field can reduce or eliminate the negative effects of other abiotic factors (drought, increased salinity, and high heat) that cause germination, plant growth, and yield suppression (Kataria et al., 2017). In such cases, SMF can trigger the production of prolin, a biochemical indicator of stressful environmental conditions that stabilizes macromolecules, allowing plant cells to recover faster from oxidative stress (Matysik et al., 2002; Singh et al., 1973).

Previous research had highlighted the relevance of SMF on biopriming, probably caused by metabolic changes in both plant and biopriming agent (Jovičić-Petrović et al., 2021). This research showed that SMF can lead to the increase of bacterial indole-acetic acid production, as well as changes in abscisic acid production in plants. The overall effect on plant germination makes a good base to propose the use of new technique for seed revitalization and germination promotion, named biomnagnertic priming. In basil and cucumber, we can assume that similar metabolic changes contributed to the positive effect on germination. However, observed differences in response of different plant species indicate the need for further evaluation of specific interactions, and do not provide enough support general recommendations of the biomagnetic priming use in agriculture.

Conclusion

According to shown data carefully chosen plant, inoculant, and optimal exposure system of SMF (type of magnet, intensity, polarity, orientation, and exposure time) can improve germination and thus enhance later stages of growth and final yield. However, the effect of SMF combined with microbial inoculation is highly dependent on plant species and SMF exposure time. Further research is needed to provide sufficient data to define recommendations in terms of biomagnetic priming application in crop production.

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