ORIGINAL SCIENTIFIC PAPER

Biopriming: multiple effects on soybean germination metrics

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Abstract
Biopriming is a pre-sowing seed inoculation technique based on beneficial microorganisms. The aim of the study was to estimate biopriming potential of microbial consortium on soybean seeds. Obtained results showed a stimulative effect of biopriming resulting in 8% higher germination percentage. Inoculated seeds were characterised as more vigorous according to vigor I, vigor II and electrical conductivity results. Accelerated aging test decreased germination percentage of both treatments to approximately 66±2%. Inoculated seeds were marked as more vigorous according to vigor I index. Biopriming of soybean seeds resulted in an increase of germination parameters that suggest stimulative effects on seed quality.

Key words: biopriming, germination, soybean, microbial inoculant

Introduction

Seed quality is of crucial importance from the standpoint of seed production, directly affecting plant growth and yield (Miladinov et al., 2018). Numerous methods of seed pretreatment are being applied to improve all aspects of seed quality, prolonge storage and slow seed aging (Miladinov et al., 2018). Among numerous, biopriming, a pre-sowing technique that implies treatment with plant growth-promoting microorganisms (PGPM), gained special attention. This group of soil organisms poses multifarious traits with beneficial effects on seeds, seedlings and plant development. Bioprimed seeds have enhanced germination while bioprimed plants pose a higher level of resistance to environmental stress (Mitra et al., 2021; Sarkar et al., 2021). Seed biopriming can improve the efficiency of nutrient management and increase resistance to phytopathogens by activating the immune system (Devika et al., 2021; Sarkar et al., 2021). The most usual plant growth-promoting rhizobacteria (PGPR) representatives used as biopriming agents are among *Bacillus* sp., *Pseudomonas* sp., *Azotobacter* sp., *Azospirillum* sp., and *Burkholderia* sp. (Mitra et al., 2021). Soybean (*Glycine max* L. Merr.) is one of the world's most valued crops with broad utilization in the food, feed, pharmaceutical and chemical industries (Staniak et al., 2021). It has low mineral fertilization requirements and is involved in nitrogen fixation by the *Bradyrhizobium japonicum*.
The aim of the study was an estimation of biopriming effects of mixed Plant Growth-Promoting Bacteria (PGPB) inoculum on germination parameters of soybean seeds.

Material and methods

The experiment was performed in the Laboratory of Environmental Microbiology at the Faculty of Agriculture (Belgrade, Serbia). Soybean seeds cultivar Wendy (maturity group I, PZO Seed GmbH), and a commercial inoculant BioGnezdo (Biofor system, Serbia) were used in the study.

PGPB inoculant is a consortium consisted of: *Azotobacter chroococcum, Bacillus licheniformis, Bacillus circulans, Bacillus megaterium,* and *Bacillus amiloliquefaciens.*

Soybean seeds were surface sterilized by immersion in 70% ethanol (v/v) for 2 min, followed by 2 min exposure to 2% NaOCl (v/v) and thorough rinsing with sterile deionized water. Seeds were inoculated by immersion into bacterial suspension for 15 min/28 ± 2 °C/120 rpm (KS 260, IKA, Germany). The control seeds were immersed in sterile water.

Germination test was performed on filter paper in glass Petri dishes (Ø 20 cm). Total 100 seeds per treatment were maintained at 25°C for a week. Seeds with radicles of 2 mm or longer were counted, and the number of germinated seeds was recorded daily. The following parameters were calculated:

- The germination percentage (G) was measured on the eighth day using the formula:

G (%) = (total number of germinated seeds/ total seeds) x 100 (ISTA 2009);

- The germination speed (GS) was calculated by formula:

GS = (No of germinated seeds/days of first count) + (No of germinated seeds/days of final count) (Ruan et al., 2002);

- The germination index was calculated by formula: GI = n/d

'n' number of emerged seeds in day d, d is day after planting (Abdul-Baki and Anderson, 1973);

- Vigour index I and vigour index II were calculated by formula:

Vigor I (VI) = (Germination percentage × Seedling length)/100;

Vigor II (VII) = Germination percentage × Seedling dry weight (Abdul-Baki, Anderson 1973);

Electrical conductivity test was performed by immersion of 2x50 seeds in 250 ml of deionized water. Previously, the seeds were weighed with a precision scale of 2 decimal places. After 24 h at 25°C the solutions containing the seeds were slightly stirred and immediately measured by the conductivity meter Eutech cond 6^+ (Thermo Scientific, MA, USA). Obtained results were calculated according to the formula: $\mu S\ cm^{-1} g^{-1}$ = Conductivity ($\mu S\ cm^{-1}$)/Sample weight (g) (Hampton and TeKrony, 1995)

Accelerated aging test was conducted in plastic boxes containing 50 seeds placed 2 cm above water level and exposed to temperature of 41°C and humidity of 95% for 72 h. After that, the germination test was set up. The germination percentage, germination speed, germination index, vigor I and vigor II, seedling length and dry biomass were recorded 8 days after. The test was performed in three replicates. The ability of PGPB inoculant to antagonize *Fusarium graminearum* was assessed using a confrontation assay on potato dextrose agar (Himedia, India) plates in three replicates. Petri dishes were incubated at 25 ± 2°C, for seven days. Statistical analyses of variance were conducted by T-test at the 5% level of probability. All statistical analyses were performed using Statistica 12.0 (StatSoft, Tulsa, OK, USA).

Results and discussion

The presented study was conducted with the aim to estimate biopriming potential of a mixed consortium of selected PGPB. The effects were estimated through standard germination parameters (Table 1). The comparison of germination percentage confirmed the stimulative effect of biopriming on soybean seeds increasing by 8%. This result is in accordance to Miladinov et al. (2018) who reported 1-9% increase in several soybean lines as a result of seed priming. Kerečki et al. (2021) reported 15% increase of germination percentage as a result of soybean seeds biopriming with *Azotobacter chroococcum* F8/2. In our study, inoculated seeds were also characterized with higher germination speed and germination index.

Table 1. Germination parameters of soybean seeds and seedlings length and dry biomass (LSD 5%)

Treatment/Parameters	Control seeds	Inoculated seeds
G (%)	76±2.00[b]	82±1.50[a]
GS	95±2.50[b]	102±2.20[a]
GI	6.67±0.58[b]	10.67±2.08[a]
Vigor I	2.71±1.20[b]	8.28±3.20[a]
Vigor II	654±8.10[b]	713±7.30[a]
Conductivity	5.21±0.48[a]	1.17±0.05[b]
Lenght (cm)	3.57±0.15[b]	10.10±0.13[a]
Dry biomass (g)	8.61±0.15[a]	8.70±0.25[a]

The results showed that inoculated seeds are characterized by higher vigor index. Statistically, significant difference was observed in vigor I which was 3-fold higher while vigor II was increased by 12%. Kerečki et al. (2021) reported an increase of 23% of vigor II as a result of biopriming. The vigor is a good measure of seed quality and viability providing the producer with a more accurate picture of crop performance in real conditions (Marcos-Filho, 2015). There are several ways of vigor index determination and the electrical conductivity test is one of them (Catão and Caixeta, 2019). Reported data showed that seed inoculation led to a fall of this parameter, indicating more vigorous seeds compared to control. Namely, damaged seeds, seeds that are possibly less vigorous, release more exudates to the outside of the cell, resulting in higher electrical conductivity values and *vice versa* (Matera et al., 2019). Nevertheless, all seeds whose electrical conductivity is < 25 $\mu S\ cm^{-1} g^{-1}$ are considered highly vigorous (Milošević and Kobiljski, 2011). The results on seedling´s length and biomass (Table 1) confirmed that inoculated seedlings are significantly longer (almost 3-fold longer) while no differences were recorded in dry biomass. This is in accordance with Kerečki et al. (2021) who reported an increase of 100% of bioprimed seedlings´ length while no differences were recorded in dry biomass. Accelerating aging test is a suitable way for evaluation of vigor index and is commonly used in case of soybean seeds providing promt information regarding storage capacity and field performance (Matera et al., 2019; Santos Suñé et al., 2021). The germination percentage of control and bioprimed seeds decreased from 76 and 82% to 68 and 64%, respectively. Similarly, Rastegar et al. (2011) reported a germination percentage of 65% of soybean seeds after accelerated aging. Also, germination speed and vigor II was similar in both treatments (Table 2). The vigor I results make difference and qualify inoculated seeds as more vigorous compared to control. Matera et al. (2019) claim that seeds characterised as high vigor in this test keep their viability when exposed to severe abiotic conditions.

The seedlings that emerged after the accelerated aging test significantly differ among treataments (Table 2). Inoculated seeds gave 70% longer seedlings with 8% higher dry biomass suggesting a role of biopriming in the alleviation of temperature and humidity stress.

A comparison of results obtained in the standard germination test (Table 1) to those obtained after the accelerated aging test (Table 2) revealed negative effects of accelerated aging on seed vigor.

Table 2. Germination parameters of soybean seeds and seedlings length and dry biomass after accelerated aging test (LSD 5%)

Treatment/Parameters	Control seeds	Inoculated seeds
G (%)	68±1.50^{a}	64±2.50^{a}
GS	61±1.50^{a}	58±1.20^{a}
GI	2.67±0.58^{a}	7±1.00^{b}
Vigor I	2.37±0.20^{b}	3.08±0.15^{a}
Vigor II	334±6.10^{a}	340±4.30^{a}
Lenght (cm)	3.48±0.05^{b}	5.93±0.06^{a}
Dry biomass (g)	4.91±0.10^{b}	5.32±0.19^{a}

Namely, the germination percentage of control and bioprimed seeds was decreased by 11% and 22%, respectively. Seedlings length and dry biomass also decreased. According to the obtained results, aging treatment resulted in a reduction in germination metrics. This in accordance with Rastegar et al. (2011) who claimed that accelerated aging affects germination percentage, rate, vigor due to biochemical changes in a seed. The additional value of applied consortia was revield by a confrontation test which show high antagonistic activity towards *Fusarium graminearum* whose growth was decreased by 77%. In some areas, *Fusarium graminearum* is regarded as a key pathogen of soybean, producing seed and root rot, as well as pre- and post-emergence damping off (Chiotta et al., 2016). Germination metrics monitored in the study confirm the potential of consortia designed in this study for soybean´s seed quality improvement. The antagonistic potential of applied consortia provides additional services to seeds and seedlings by raising the level of protection against phytopathogens. Nowadays, biopriming is perceived as a revolutionary method based on principles of sustainability which leads to the better establishment, adaptation and health of plants resulting, ultimately, in increased yield (Deshmukh et al., 2020; Chitra and Jijeesh, 2021).

Conclusion

Germination test and accelerated aging test provide insight into seed physiological quality and biopriming effects on the same. According to obtained results it can be concluded that biopriming rise germination percentage, speed and index and bioprimed seeds are more vigorous compared to control. Further, biopriming lower down seeds electrical conductivity suggesting more vigorous seeds compared to control and accelerated aging test showed that inoculated seeds are more vigorous and gave longer seedlings with higher dry biomass. It can be concluded that PGPB can be used as a cost-effective tool for biopriming of soybean seeds.

Acknowledgement

The results presented in the paper was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No. 451-03-68/2022-14/200116).

References

Abdul-Baki A., Anderson J.D. (1973). Vigor determination in soybean seed by multiple criteria. Crop Science. 13: 630-633.

Catão H.C.R.M., Caixeta F. (2019). Electrical conductivity test in soybean seeds with reduced imbibition period. Revista de Ciências Agrárias. 42: 387-393.

Chiotta M.L., Alaniz Zanon M.S., Palazzini J.M., Scandiani M.M., Formento A.N., Barros G.G., Chulze S. N. (2016). Pathogenicity of *Fusarium graminearum* and *F. meridionale* on soybean pod blight and trichothecene accumulation. Plant Pathology 65: 1492-1497.

Chitra P., Jijeesh C.M. (2021). Biopriming of seeds with plant growth promoting bacteria Pseudomonas fluorescens for better germination and seedling vigour of the East Indian sandalwood. New Forests. 52: 1-13.

Deshmukh A.J., Jaiman R.S., Bambharolia R.P., Patil V.A. (2020). Seed biopriming- a review. International Journal of Economic Plants. 7: 038-043.

Devika O.S., Singh S., Sarkar D., Barnwal P., Suman J., Rakshit A. (2021). Seed priming: a potential supplement in integrated resource management under fragile intensive ecosystems. Frontiers in Sustainable Food Systems. 5: 1-11.

ISTA. (2009). Seed health testing. International rules for seed testing. [ISTA: 2009]. Bassersdorf, Switzerland: International Seed Testing Association.

Hampton J.G., Te Krony D.M. (1995). Handbook of Vigour Test Methods, 3rd Edition, 1-117. Zurich, Switzerland: International Seed Testing Association.

Kerečki S., Jovičić-Petrović J., Kljujev I., Lalević B., Karličić V., Petrović I., Raičević V. (2021). Biopriming: a sustainable support for crop establishment. In *The Proceedings of the XII International Scientific Agricultural Symposium "Agrosym 2021"*, Kovačević D. (ed.), 188-194. Jahorina, Republic of Bosnia and Herzegovina: East Sarajevo: Faculty of Agriculture.

Matera T.C., Pereira L.C., Braccini A.L., Krzyzanowski F.C., Scapim C.A., Piana S.C., Marteli D.C.V., Pereira R.C., Ferri G.C., Suzukawa A.K. (2019). Accelerated aging test and its relationship to physiological potential of soybean seeds. Journal of Seed Science. 41: 301-308.

Marcos-Fliho J. (2015). Seed vigor testing: an overview of the past, present and future perspective. Scientia Agricola. 72: 363-374.

Miladinov Z., Balešević-Tubić S., Đukić V., Ilić A., Čobanović L., Dozet G., Merkulov-Popadić L. (2018). Effect of priming on soybean seed germination parameters. Acta Agriculturae Serbica. 45: 15-26.

Milošević M., Kobiljski B. (2011). Semenarstvo, Milošević M., Kobiljski B. (eds), 1-400. Novi Sad, Republic of Serbia: Institute of field and vegetative crops.

Mitra D., Mondal R., Khoshru B., Shadangi S., Mohapatra P.K.D., Panneerselvam P. (2021). Rhizobacteria mediated seed bio-priming triggers the resistance and plant growth for sustainable crop production. Current Research in Microbial Sciences. 2: 1-5.

Santos Suñé A., da Silva Almeida A., Figueiredo J.C., dos Reis B.B., de Avila Nunes C., Saraiva C.R.C., Kroessin L., Correa W.L., de Tunes L.M.W. (2021). Adequacy of the accelerated aging test for soybean seeds. Colloquium Agrariae. 17: 1-13.

Sarkar D., Singh S., Perihar M., Rakshit A. (2021). Seed bio-priming with microbial inoculants: A tailored approach towards improved crop performance, nutritional security, and agricultural sustainability for smallholder farmers. Current Research in Environmental Sustainability. 3: 1-10.

Staniak M., Stepień-Warda A., Czopek K., Kocira A., Baca E. (2021). Seeds quality and quantity of soybean (*Glycine max* (L.) Merr.) cultivars in response to cold stress. Agronomy. 11: 1-13.

Rastegar Z., Sedghi M., Khomari S. (2011). Effects of Accelerated Aging on Soybean Seed Germination Indexes at Laboratory Conditions. Notulae Scientia Biologicae. 3:126-129.

Ruan S., Xue Q., Thlkowska K. (2002). Effect of seed priming on germination and health of rice (*Oryza sativa* L.) seeds. Seed Science Technology. 30: 451-458.