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THE INFLUENCE OF PHYTOPATHOGENIC FUNGI ON THE QUALITY PARAMETERS OF GREEN BEAN SEEDS IN PERIOD 2018-2022

UTICAJ FITOPATOGENIH GLJIVA NA PARAMETRE KVALITETA SEMENA BORANIJE U PERIODU 2018 - 2022

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ABSTRACT

Green beans are among the most essential legumes because of their nutritional value. However, the production of green beans in Serbia suffers major decline due to significantly reduced yields, as well as the sown areas. Diseases caused by phytopathogenic fungi not only reduce yield and have the negative impact on total germination and seed quality of green beans, but also some phytopathogenic fungi can produce mycotoxins, adversely affecting human health. In this paper, the quality parameters of two green bean varieties (GB1 and GB2) were monitored for quality traits over a period of five years on the territory of Smederevska Palanka. The energy germination and total germination were the highest in the first observed year and amounted to 63% and 88% for GB1, respectively. For GB2 the energy germination and total germination (2018) were 71% and 75%, respectively. A significant decline in total germination was determined in 2020. and amounted to 75% for GB1 and 67% for GB2($p<0.05$). The lowest energy germination and total germination were obtained in the last observed year (2022) in both genotypes and statistically increased infection with Alternaria sp. and Fusarium sp. The presence of Alternaria sp. was detected for GB1 and GB2 in the range of 2-3% (2018); Fusarium sp. was 3%. In the last observed year, the presence of other fungi was determined, apropos a higher percentage of diseased seeds with Alternaria sp. and Fusarium sp. (5-6%). Other phytopathogenic fungi were detected in both genotypes: Aspergillus sp. (1-3%), Penicillium sp. (1-4%), Rhizopus sp. (1-3%) and Rhizoctonia solani (2-3%). The moisture was 8.5-10.9 for both genotypes and did not differ statistically between varieties ($p>0.05$). The impact of phytopathogenic fungi on green beans was reflected in the reduction of total germination, and therefore in seed quality and yield. The priority of future research is the application of biotreatments that will contribute to seed protection and improve total germination, and thus the yield in the field.

***Key words**: effects, germination, phytopathogenic fungi.*

REZIME

Boranija zbog svoje nutritivne vrednosti spada u najvažnije mahunarke u Srbiji. Međutim, proizvodnja boranije u Srbiji je u značajnom opadanju usled značajno smanjenih zasejanih površina i prinosa. Fitopatogene gljive koje se prenose putem semena utiču na smanjene prinose i kvalitet boranije. Takođe, pored negativnog uticaja na ukupnu klijavost i kvalitet semena neke fitopatogene gljive mogu produkovati miktoksine koje imaju štetan uticaj na zdravlje ljudi. U ovom radu su praćeni parametri kvaliteta dve sorte boranije (GB1 i GB2) na teritoriji Smederevske Palanke u periodu od pet godina. Energija klijanja i ukupna klijavost bile su najbolje u prvoj posmatranoj godini i iznosile su 63% i 88% za GB1, respektivno. Za GB2 energija klijanja i ukupna klijavost (2018) bile su 71% i 75%, respektivno. U 2020. godini utvrđen je značajan pad ukupne klijavosti i iznosio je 75% za GB1 i 67% za GB2 ($p<0,05$). Najmanja energija klijanja i ukupna klijavost dobijena je u poslednjoj posmatranoj godini (2022) kod oba genotipa i statistički su značajno povećane infekcije sa Alternaria sp. i Fusarium sp. Prisustvo Alternaria sp. detektovana je za oba genotipa GB1 i GB2 u rasponu od 2-3%; Fusarium sp. iznosio je 3% (2018). U poslednjoj posmatranoj godini utvrđen je veći procenat obolelog semena (5-6%) sa Alternaria sp. i Fusarium sp. Druge fitopatogene gljive su detektovane su kod oba genotipa: Aspergilus sp. (1-3%), Penicillium sp. (1-4%), Rhizopus sp. (1-3%) i Rhizoctonia solani (2-3%). Vlaga semena je iznosila u rasponu 8,5-10,9 za oba genotipa, koja se nisu statistički značajno razlikovala između sorti ($p>0,05$). Uticaj fitopatogenih gljiva na boraniju ogledao se u smanjenju ukupne klijavosti, a samim tim i kvaliteta semena i prinosa. Prioritet budućih istraživanja je primena biotretmana koji će doprineti zaštiti semena i poboljšati ukupnu klijavost, a samim tim i prinos na polju.

***Ključne reči:** uticaj, klijavost, fitopatogene gljive.*

INTRODUCTION

Green beans (*Phaseolus vulgaris*) are one of the most important legumes crops worldwide (Amani Machiani et al., 2019). Beans have an exceptional agrotechnical importance for agricultural production, because they contain some of the unique characteristics. Primarily, they enrich the soil with nitrogen because of symbiosis with bacteria that bind nitrogen from the air. These nutrients serve as food for growth and development of green beans, but they also remain in the soil and improve its physical properties (Ketta & Hewedy, 2021). According to the data from 2019 (Statistical yearbook RS, 2020), beans are grown on about 16,500 ha in Republic of Serbia, with an average yield of nearly 1 t/ha. Healthy, pathogen-free seed material is the most important input for the crop production Some areas under beans are still grown as a joint crop with maize, while green beans are grown in the field. (Todorović et al., 2008). However, decline in green bean production is significant in recent years and one of the most important factors affecting it are various diseases caused by phytopathogenic fungi, bacteria and viruses as well as harmful insects. Seed-borne pathogens can drastically reduce the germination of seeds stored for genetic preservation or plant reproduction. Significant losses in bean yield have been caused by more than 200 reported diseases (Assefa et al., 2019; Taboada et al., 2022). Conversely, the beans used for the food that are previously contaminated with fungi can contain mycotoxins that make the beans unsafe for consumption (Waller et al., 2001). Analysis of seed infection is a useful research tool for predicting the development of seed-borne diseases (Yoder, 2005). One of the most aggressive pathogens is considered *Fusarium solani* limiting bean productivity and being one of the most damaging Fusarium species (Toghueo et al. 2016). The aim of this work was to determine the presence of seed-borne fungi and their influence on certain parameters (germination energy, total germination, moisture) of seed quality of green bean collected in the territory of Smederevska Palanka in Serbia.

MATERIAL AND METHOD

Two varieties of green bean seeds were collected from the field in Smederevska Palanka in the period of 2018-2021 (coded as GB1 and GB2). The analysis includes non-commercial genotypes of green bean seeds. All samples were stored in paper bags in the laboratory at a temperature of 20-22 ºC. Seed testing was performed using standard methods for assessing the quality and seeds health (ISTA, 2020). Green bean seed quality was assessed through germination parameters (germination energy and total germination), moisture and seed health.

Germination energy and total seed germination were determined using the standard filter paper method. Samples of two different green bean genotypes consisting of a total of 400 seeds (100 per replicate) were placed in Petri dishes with filter paper moistened with distilled water. These quality parameters, especially germination, detect abnormal seeds that cannot develop by the end of the tested time and will not eventually develop into a normal plant. The seeds were incubated for 7 and 14 days at 20 ºC. The final count of seedlings was made after 14 days.

Determination of the moisture content of green bean seeds was measured by the thermogravimetric method. Seeds samples (5 g) was kept at a temperature of 105 ºC ± 2 ºC for 17 h ± 1 h. Seed water is defined as seed moisture (SW) and is calculated according to the following formula (1):

$$SW(\%) = \frac{(m_2 - m_3)}{m_2 - m_1} \tag{1}$$

where: m_1 (g) - the mass of a container and lid; m_2 (g) - the mass of a container, lid, and contents before drying; m3 (g) - the mass of a container, lid, and contents after drying.

The seed health of green bean genotypes was tested for presence of *Fusarium* spp. and *Alternaria* spp. Seed testing was performed with the standard method on filter paper (ISTA, 2020). According to the Rules on the Quality of Seeds of Agricultural Plants (47/87), the limited percentage of infected seeds for both plant pathogenic fungi is under 5% (2).

$$Seed\ health(\%) = \frac{(\text{number of infected seeds})}{(\text{total number of seeds})} \cdot 100 \tag{2}$$

Also, the mycopopulation on the outside of the green bean seed was tested using of potato dextrose agar (PDA). Twenty grams of green bean seeds was soaked for 1 min in 1 % sodium hypochlorite and washed with distilled water. After that, samples were soaked in sterile 10 mM phosphate buffered saline - PBS (containing: Na_2HPO4 x $12H_2O$ 2.7 g; NaH_2PO4 x $2H_2O$ 0.4 g; NaCl 8.0 g; Distilled water 1000 mL) over night at 4 °C. Samples were shaken for 2 h at room temperature (24 °C) and 115 rpm, then filtered and centrifuged at 11 000 g for 20 min at 10 °C. The supernatant was discarded and the pellet was resuspended in 1 mL of sterile distilled water. The suspension was added to PDA using the dilution method. After 7 days the results were read from the Petri dish and the morphological characteristics were determined of fungi by using the microscope.

Statistical analysis was performed using SPSS software (version 23, IBM, USA). The effects of factors were evaluated by ANOVA (F test) and the Tukey's Multiple Range Test ($p \leq 0.05$) to determine effects of their means. Differences of $p < 0.05$ were considered as significant.

RESULTS AND DISCUSSION

This study the presence of the pathogenic fungi population in seeds of two different green bean genotypes and their influence on seed quality parameters (germination energy, total germination, moisture) obtained in the seasons of 2018-2021. All tested parameters were compared between two green bean genotypes and showed significant differences in the period of five years ($p<0.05$) (Table 1). The highest energy germination and total germination were detected in GB1 and amounted to were 83% and 88% in 2018, respectively. A slight decrease in these two parameters was registered in 2019 and amounted to 79% (germination energy) and 85% (total germination). Significant decline was obtained from samples in 2020 and germination energy was 69 %, while total germination was 75%. Germination energy and total germination were 70% and 74% in 2021. The lowest energy germination and total germination were evaluated in 2022 and dropped to 58% and 65%, respectively.

Table 1. Analysis of parameters of quality (energy, total germination and moisture) in a five-year period (2018-2022).

Green bean genotype	Quality parameters (%)	Year				
		2018	2019	2020	2021	2022
GB1	Energy	$83^{\pm1a}$	$79^{\pm1a}$	$69^{\pm1c}$	$70^{\pm2b}$	$58^{\pm2cb}$
	Total germination	$88^{\pm2a}$	$85^{\pm1a}$	$75^{\pm2a}$	$74^{\pm1b}$	$68^{\pm1b}$
	Moisture	$8.5^{\pm0.5b}$	$8.9^{\pm0.1a}$	$10^{\pm0.9a}$	$8.7^{\pm0.3b}$	$10^{\pm0.9a}$
GB2	Energy	$71^{\pm2a}$	$73^{\pm1a}$	$63^{\pm2a}$	$63^{\pm3}$	$59^{\pm1b}$
	Total germination	$75^{\pm1a}$	$75^{\pm3a}$	$67^{\pm1a}$	$73^{\pm1b}$	$66^{\pm1b}$
	Moisture	$9^{\pm0.2b}$	$10^{\pm0.1a}$	$11^{\pm0.5a}$	$9^{\pm1b}$	$9^{\pm0.5a}$

Different small letters mean significant effect: a-statistically significance between the genotypes; b- no statistically significance between the genotypes; c-difference between years ($p \leq 0.05$); Tukey's Multiple Range test). Values are mean ±standard error of the mean.

The genotype GB2 had significantly lower quality parameters than GB1 in all observed years: energy germination 71 % and 75% total germination in 2018. Energy germination was 73% and total germination 75% (2019), while decline was obtained 2020 and amounted to 63% (energy germination) and 67% (total germination). In 2021, energy germination was the same as in 2020, whereas total gemination significantly increased to 73% in comparison to 2020. In the last observed year (2022), energy germination was 59% and total germination was 66% (Table 1).

The moisture for GB1 was 11.5% and for GB2 was 12.5% in 2018 and there were no statistical differences between genotypes. In the period from 2019 to 2022, seed moisture was in the range of 8.5-10.9 % for both genotypes (Table 1). The quality parameters indicate a significant decrease in overall germination in the last year and an increase in fungal infections. One of the important factors that affects seed quality is infection by fungi. Many seed-borne fungi may drastically reduce the germination of seeds stored for plant reproduction (Amza, 2018).

In general, the obtained results revealed that the seedborne fungi were present in all green bean seed samples and they significantly affected total germination. The seed health of GB1, examined with filter paper method detected different range of fungi between 2018 and 2022: *Alternaria* spp. 2-5% and *Fusarium* spp. in range from 4 to 6%. Similarly, the presence of seed-borne *Fusarium* spp. and *Alternaria* spp. ranged from 3-5% for genotypes GB2. A higher percentage of the presence of fungi was detected in GB2, which can be associated with reduced germination given that the highest percentage was registered in the last observed year (Table 2). Further, the percentage of other fungi present on seed coat in both genotypes was: *Aspergillus* sp. (1-3%), *Penicillium* sp. (1-4%), *Rhizopus* sp. (1-3%) and *Rhizoctonia solani* (2-3%) (Table 2).

Table 2. Presence of phytopathogenic fungi in the seeds of green bean genotypes.

Green bean genotype	Seed-borne fungi (%)	2018	2019	2020	2021	2022
GB1	*Alternaria* sp.	$2^{\pm1}$	$4^{\pm1}$	$3^{\pm1}$	$4^{\pm1}$	$5^{\pm1}$
	Fusarium sp.	$3^{\pm2}$	$5^{\pm2}$	$3^{\pm0}$	$4^{\pm1}$	$6^{\pm0}$
	Aspergillus sp.	$1^{\pm0}$	$2^{\pm1}$	$1^{\pm0}$	$1^{\pm0}$	$3^{\pm1}$
	Penicillium sp.	$1^{\pm1}$	$3^{\pm0}$	$2^{\pm1}$	$4^{\pm0}$	$3^{\pm1}$
	Rhizopus sp.	$1^{\pm0}$	$1^{\pm0}$	$3^{\pm0}$	$2^{\pm1}$	$3^{\pm0}$
	Rhizoctonia solani	$2^{\pm1}$	$3^{\pm1}$	$2^{\pm0}$	$4^{\pm1}$	$4^{\pm0}$
GB2	*Alternaria* sp.	$3^{\pm1}$	$4^{\pm1}$	$4^{\pm1}$	$3^{\pm1}$	$5^{\pm1}$
	Fusarium sp.	$3^{\pm1}$	$3^{\pm2}$	5±0	$4^{\pm1}$	$5^{\pm1}$
	Aspergillus sp.	$1^{\pm1}$	$2^{\pm1}$	3±1	$3^{\pm0}$	$2^{\pm0}$
	Penicillium sp.	$1^{\pm0}$	$2^{\pm0}$	4±1	$3^{\pm1}$	$4^{\pm1}$
	Rhizopus sp.	$1^{\pm2}$	$1^{\pm0}$	2±0	$1^{\pm0}$	$3^{\pm0}$
	Rhizoctonia solani	$2^{\pm1}$	$2^{\pm1}$	3±0	$3^{\pm0}$	$2^{\pm1}$

Some pathogenic fungi that attack seeds while they are still on the plant during vegetation and this decreases seed viability. Fungi can be transmitted as contaminants that adhere to the seed coat, or infect the seed, which is considered the main mechanism of seed-mediated transmission. Recent research has shown that a total of 133 fungal strains were isolated from the surface and from the endosperm of beans, of which eighty-seven isolates caused symptoms and lethal necrosis (Marcenaro & Valkonen, 2016). *Fusarium* species can cause rot of the root, stem, and fruit or vascular wilt in a wide range of crop plants, and they survive as saprophytes (Silvestro et al., 2013). In most countries, the seed is not good for further planting if more than 5% of the seed is infected with one or more of the following fungi: *Alternaria*, *Aspergillus, Fusarium*, *Penicillium*, *Cladosporium*, *Curvularia*, *Macrophomina*, *Phoma* and *Rhizopus*. Also, seed-borne pathogens can cause heavy losses in the field (Amani Machiani et al., 2019; Rajput et al., 2020). In our research, the perecent of phytopathogenic fungi was the highest in the last year and could be related to the lowest total germination.

CONCLUSION

The effects of phytopathogenic fungi on green beans had a significant impact on reduced overall germination, and thus on seed quality and yield. The priority of future research is the application of biotreatments that will contribute to seed protection and improve overall germination, and thus the yield in the field

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