AgroLife Scientific Journal - Volume 10, Number 1, 2021
ISSN 2285-5718; ISSN CD-ROM 2285-5726; ISSN ONLINE 2286-0126; ISSN-L 2285-5718

THE INFLUENCE OF BACTERIAL INOCULATS ON PATHOGENS, YIELD AND QUALITY IN SOYBEAN CROP

Loredana SUCIU[1,2], Adina TĂRAU[1], Camelia URDĂ[1], Felicia CHEȚAN[1], Felicia MUREȘANU[1], Laura ȘOPTEREAN[1], Oana-Alina BOIU-SICUIA[3,4,], Lavinia-Diana-Nicoleta BARBU[3]

[1]Research and Development Station for Agriculture Turda, 27 Agriculturii Street, 401100, Turda, Romania
[2]University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, 3-5 Mănăștur Street, Cluj-Napoca, Romania
[3]Research and Development Institute for Plant Protection, 8 Ion Ionescu de la Brad Blvd, District 1, Bucharest, Romania
[4]University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Biotechnology, 59 Mărăști Blvd, District 1, Bucharest, Romania

Corresponding author email: camelia.urda@scdaturda.ro

Abstract

The paper aimed to present the influence of bacterial inoculation,applied to seeds, on the attack of pathogens, on yield, yield elements and quality in soybean crop.A two-year experiment was carried out at Research and Development Station for Agriculture Turda (RDSA Turda), in 2019-2020. Teo TD, an early soybean variety was used in the experiment. Seed stimulation was conducted with two nitrogen-fixing bacteria: Bradyrhizobium sp. JHI and Paenibacillus graminis FL400. The application of bacterial inoculants leads, in the second year of experiment, to an increase in soybean yield with 903 kg/ha, 39.8% higher compared to the control, when Bradyrhizobium + Paenibacillus was applied. Biostimulation also help the plant fight against pathogens with a positive impact on number of nodules, yield elements and quality parameters.

Key words: *soybean, inoculation, pathogens, yield, quality.*

INTRODUCTION

Soybean [*Glycine max* (L.) Merr.], is cultivated for a high content of protein and oil, it is often called the miracle crop due to its numerous uses. Soybean is an essential dominant source of proteins and oils with many uses in forage, food and industrial applications (Lee et al., 2007).

The soybean plant is well adapted to a number of environmental conditions, but certain abiotic and biotic stresses cause significant reductions in production. Salinity, temperature, drought and pH are the main constraints that limit plant productivity and cause losses, while among biotic stresses the attack of various pathogens are the main problem to be solved (Tewari et al., 2017). Under stressful conditions, the plant reduces its vegetative growth to preserve and redistribute the essential resources for the survival of the plant in severe conditions.

In the context of climate change and increasing population pressure, it is essential to increase crop productivity, even under stress. A number of techniques have been used to increase plant tolerance under conditions of ecological stress. Some of these techniques are based on the use of chemicals that can be toxic to the environment (Tewari et al., 2017).

As an alternative to chemicals is the natural growth of plants that promote rhizobacteria (PGPR). Data from the literature show the effectiveness of PGPR in improving plant growth and development (Glick, 2012; Paul and Nair, 2008). This population of ecological bacteria is effective in promoting crops production, but also in the management of diseases under normal and stressful conditions (Dimkpa et al., 2009; Tewari et al., 2017). Inoculation of legume seeds with fixing bacteria is a well-established practice and contributes significantly to obtaining high productions (Rodrigues et al., 2015).

The main ways we can prevent the attack of diseases and pests are seed treatments which, as it is known, have a period of effectiveness from two to three weeks, and their duration of activity is influenced by dose and climatic conditions (Dyer, 2012).

Recently, as a result of the widespread use of chemicals and inorder to find new safer environmentally technologies, in the control of plant diseases alternative methods are being developed.

MATERIALS AND METHODS

In order to observe the influence of bacterial inoculation, applied to seeds, on the attack of pathogens, and of some elements of productivity in the soybean crop, Teo TD soybean variety was used.

Two nitrogen-fixing bacteria were used in this study *Bradyrhizobium* sp. JHI and *Paenibacillus graminis* FL400. The JHI strain originates form the James Hutton Institute, UK and was kindly provided by dr. IoanToncea. As rhizobia, this strain presents *nif* genes encoding for enzymes involved in atmospheric nitrogen fixation into a nitrogen form available to plants, as well as nod genes, responsible for root nodulation in specific hosts of leguminous plants. The FL400 strain is a Romanian native bacteria isolated from bean roots. This strain is a diazotrophic bacteria that naturally possesses *nif* genes. Moreover, it was patented at OSIM (State Office for Inventions and Trademarks, Romania) for its potential to increase polyamine levels in crops and currently is deposited in NCAIM microbial collection as B001365. The bacterial based inoculum was prepared on agar plates. *Bradyrhizobium* sp. JHI strain was grown on Yeast-Manitol-Agar, and *P. graminis* FL400 on Luria Bertani agar. Bacterial biomass was harvested as concentrated suspension (minimum 109 CFU/ml) and applied as seed treatment.

Field observations:

- plant height;
- number of leaves/ plant;
- number of nodules/ plant;
- *Peronospora manshurica* (mildew) frequency and intensity.

Laboratory determinations:

- number of pods/plant;
- plant weight;
- number of seeds/plant;
- seeds weight/ plant;
- thousand kernel weight (TKW);
- quality parameters;
- yield.

RESULTS AND DISCUSSIONS

The plants height was influenced by the seed treatment, but also by the climatic conditions of the experimental years. In the background of climatic conditions of 2020, the plants weretaller, compared to 2019. Between developed, which are part of the experimental variants, there were differences but these were not statistically assured. In both experimental years in the variant in which the inoculation was made with *Bradyrhizobium* sp. the plants were the tallest (112.00 and 116.25 cm) (Table 1).

Table 1. The influence of bacterial inoculants on plantheight

No.	*Year*	*Experimental variant*	*Height (cm)*	*% to control*	*The difference to control (cm)*
1.	2019	Untreated	105.10	100.00	0.00
2.		*Bradyrhizobium* sp.	112.00	6.6	6.90
3.		*Bradyrhizobium* sp. + *Paenibacillus graminis*	107.48	2.3	2.38
4.	2020	Untreated	114.75	100,0	0.00
5.		*Bradyrhizobium* sp.	116.25	1.3	1.50
6.		*Bradyrhizobium* sp. + *Paenibacillus graminis*	110.25	-3.9	-4.50
LSD (p 5%)					13.65

The number of leaves is a genetically determined characteristic, specific to each genotype. From the data presented in Table 2 it can be seen that this parameter shows similar values, for all experimental variants, with the highest value for the variant to which *Bradyrhizobium* sp. in both experimental years.

Table 2. The influence of bacterial inoculants on number of leaves

No.	Year	Experimental variant	Number of leaves	% to control	The difference to control (no.)
1.	2019	Untreated	13.10	100.0	0.00
2.		*Bradyrhizobium* sp.	13.33	1.7	0.22
3.		*Bradyrhizobium* sp. + *Paenibacillus graminis*	12.45	-5.0	-0.65
4.	2020	Untreated	12.50	100.0	0.00
5.		*Bradyrhizobium* sp.	15.00	20.0	2.50
6.		*Bradyrhizobium* sp. + *Paenibacillus graminis*	14.00	12.0	1.50
LSD (p 5%)					4.35

The application of bacterial inoculum leads to an increase in the number of nodules (Rodrigues et al., 2015; Hungary et al., 2015). Our data confirm the data from the literature, the formation of nodules on the plant was influenced by the treatment applied to the seed. The application of any treatment product to the seed leads to the formation of a higher number of nodules, compared to the untreated variant. It is noticed the variant to which the mixed treatment *Bradyrhizobium* sp. + *Paenibacillus graminis*, with the highest number of nodules per plant (60) (Table 3).

Table 3. The influence of bacterial inoculants on number of nodules

No.	Year	Experimental variant	Nodule (no)	% to control	The difference to control (no.)
1.	2019	Untreated	39.50	100.0	0,00
2.		*Bradyrhizobium* sp.	43.75	10.8	4.25
3.		*Bradyrhizobium* sp. + *Paenibacillus graminis*	45.75	15.8	6.25
4.	2020	Untreated	35.75	100.0	0.00
5.		*Bradyrhizobium* sp.	39.75	11.2	4.00
6.		*Bradyrhizobium* sp. + *Paenibacillus graminis*	60.00	67.8	24.25*
LSD (p 5%)					22.14

Although the treatment of the seed has an efficacy of two to three weeks, the products used help the plant fight against diseases.

Despite the conditions of 2019, the mildew attack manifested itself with reduced frequency and intensity in comparison with the mildew attack in Figure 1. During the growing stages, observations were made regarding the attack of pathogens on soybeans. In 2019, in soybean crop, mildew and bacterial burn was manifested. Even though the values obtained are small, differences can be observed between the experimental variants regarding the degree of attack of *Peronospora manshurica*. In Figure 2, we can see the frequency, intensity and degree of attack of *Pseudomonas savastanoi* pv. *glycinea*.

The degree of attack of bacterial blight did not exceed 1%, with the highest value on the variation which had no treatment applied to the seed. The lowest values of frequency and intensity, and thus degree of attack, came from the variation to which *Bradyrhizobium* sp. + *Paenibacillus graminis* was applied.

In 2020, only the attack of mildew was manifested in soybean crop. From Figure 3 we can see that the highest degree of attack by *Peronospora manshurica* was on the variant which did not receive treatment to the seed (1.17%). The lowest degree of attack of mildew was on the variant where *Bradyrhizobium* sp. + *Paenibacillus graminis* was applied. These results can be put down to the fact that bacteria of the type *Paenibacillus graminis* are known for having biocontrol activity against bacteria, fungi and nematodes (Tiwari, 2019).

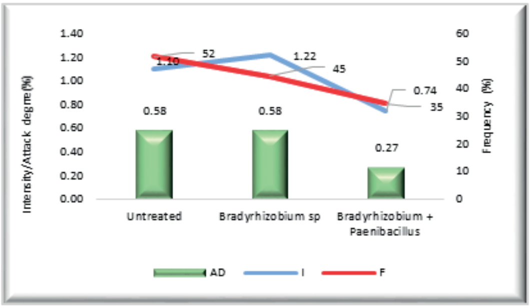

Figure 1. The attackdegree of *Peronospora manshurica* - 2019

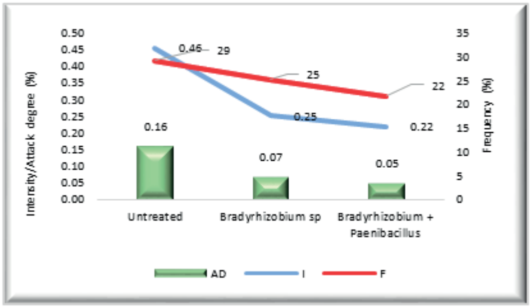

Figure 2. The attackdegree of *Pseudomonas savastanoi* pv. *glycinea* - 2019

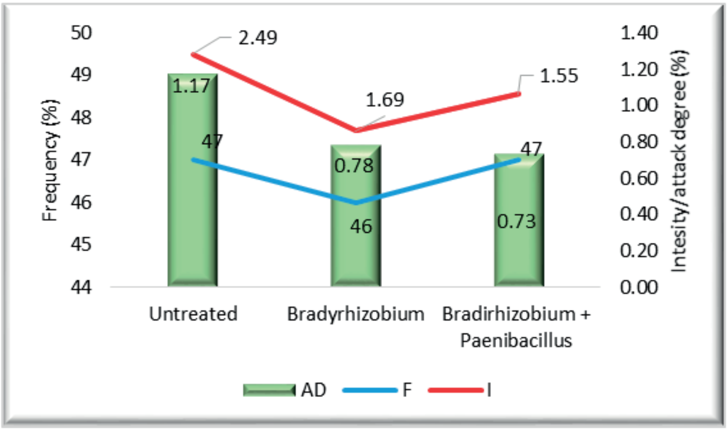

Figure 3. The attackdegree of *Peronospora manshurica* - 2020

Table 4. The influence of bacterial inoculants on seed number

	Year	*Grains number/10 pl (average)*	*% to control*	*The difference to control (no)*
1.	2019	57.00	100.0	0.00
2.	2020	48.42	-15.1	-8.58^{0}
LSD (p 5%)				5.25
	Treatment			
1.	Untreated	49.25	100.0	0.00
2.	*Bradyrhizobium* sp.	53.00	7.6	3.75
3.	*Bradyrhizobium* sp. + *Paenibacillus graminis*	55.88	13.5	6.63
LDS (p 5%)				9.24

The number of seeds per plant has been influence by the climate conditions of the 2 years of experiment, but also by the treatment applied to the seed. From the data presented in Table 4, we can observe that in 2020, the average number of grains (mean for 10 plants) was smaller compared to 2019, with a significant negative difference to the control.

The number of grains was larger on the variants which had a treatment on the seed, with the highest value on the variation to which *Bradyrhizobium* sp. + *Paenibacillus graminis* (55.88) was applied, but the difference compared to the control is not significant (Table 4).

The mass of a thousand kernel weight (TKW) was influenced by the climate conditions of the 2 years of the experiment, so that despite the climate conditions of 2020, the value of this parameter was larger, with a significant positive difference to the control (Table 5).

There are differences between the experimental variants regarding the weight of a thousand grains, but the differences were insignificant. The treatment *Bradyrhizobium* sp. positively influenced this parameter (Table 5).

Table 5. The influence of bacterial inoculants on mass of a thousand grains (TKW)

	Year	*TKW (g)*	*% to control*	*The difference to control (g)*
1.	2019	116.83	100.0	0.00
2.	2020	132.75	113.3	15.92*
LSD (p 5%)				9.64
	Treatment			
1.	Untreated	118.00	100.0	0.00
2.	*Bradyrhizobium* sp.	129.75	10.0	11.75
3.	*Bradyrhizobium* sp. + *Paenibacillus* sp.	126.63	7.3	8.63
LSD (p 5%)				41.29

The yield represents the results of interactions between the potential production of the genotype with the influence of the climatic conditions, to which the agro technical capacity of the genotype is also applied. The yield is one of the most complex characteristics, to which multitude of elements contribute, which in turn have a stable genetic determinism, which can be more or less influence on the climatic conditions (Racz, 2013). Harvest is determined by the genetic potential of each plant and the control factors (solar radiation, water, temperature, nutritive elements, weeds, pathogens) which influence the manifestation of the harvest to be larger or smaller. Farmers have plants with more and more genetically potential at their disposition, fruit of the improvement activity. Through technology, the cultivator is looking to create conditions that are favourable to the plants, to improve the control factors and to correct them when they are unfavourable, so that the harvest obtained is as close to the genetically potential obtained through improvement (Ion, 2020).

The yield obtained from the Teo TD variety was influenced by the application of treatment to the seed. Through the inoculation of soybean seeds with products of *Bradyrhizobium* we obtained an increase of production of 222 kg/ha (values obtained also by Hungria et al, 2015), however, with the inoculation with *Bradyrhizobium* + *Paenibacillus graminis* the increase of production was 903 kg/ha, the productivity input being 19.2% and 39.8% compared to the untreated control variant, during the two years of the experiment (Table 6).

Table 6. The influence of bacterial inoculants on yield

No.	*Year*	*Experimental variant*	*Yield (kg/ha)*	*% to control*	*The difference to control (kg/ha)*
1.		Untreated	2474	100.0	0.00
2.	2019	*Bradyrhizobium* sp.	2696	9.0	222
3.		*Bradyrhizobium* sp. + *Paenibacillus graminis*	2951	19.2	477*
4.		Untreated	2271	100.0	0.00
5.	2020	*Bradyrhizobium* sp.	2718	19.7	447*
6.		*Bradyrhizobium* sp. + *Paenibacillus graminis*	3176	39.8	903***

LSD (p 5%) 439;
LSD (p 1%) 616;
LSD (p 0.1%) 870

The quality of soybean seeds is based on the amount of protein and fat it contains. Similarly, essential fatty acids present in soybean seeds are the type of fate important in diets, with many benefits on human health.

Table 7 shows ranks for yield, yield elements and parameters of quality, analysed at the 3 experimental variables. Comparisons based on yield rank and productivity elements showed that the inoculation of seeds has positively influenced the behaviour of soybean variety Teo TD. For example, the experimental variant where products based on *Bradyrhizobium* and *Paenibacillus graminis* were used proved to benefit the most, being ranked first, both on the basis of the ranks obtained for each productivity element analysed (1) and on the basis of the total rank (5). A similar behaviour is observed following the analysis of quality indices. It would seem that by applying seed treatments, not only high yields are obtained but also superior in terms of quality.

Table 7. Production, productivity elements and quality parameters ranks (2020)

Elements of productivity	Untreated	*Bradyrhizobium*	*Bradyrhizobium + Paenibacillus graminis*
Number of pods/plant	3	2	1
Number of grains/plant	3	2	1
Grain mass/plant	3	2	1
TKW (g)	2	2	1
Yield (kg/ha)	3	2	1
Total	14	10	5
Grain quality	Untreated	*Bradyrhizobium*	*Bradyrhizobium + Paenibacillus graminis*
Stearic acid	1	1	1
Oleic acid	1	2	1
Linoleic acid	3	1	1
Linolenic acid	3	2	1
Fat	2	2	1
Protein	3	1	1
Total	13	9	6

CONCLUSIONS

In conclusion, we can say that the application to seed of products based on *Bradyrhizobium* sp. and *Paenibacillus graminis* influenced the formation of nodules (large number), reduced the frequency and intensity of mildew attack and bacterial blight, and was noted for best results obtained in all elements of productivity studied.

ACKNOWLEDGEMENTS

This research work was carried out with the support of the Romanian Ministry of Research and Innovation, CCCDI-UEFISCDI, project number PN-III-P1-1.2-PCCDI-2017-03-01/28PCCDI/2018, within PNCDI III.

REFERENCES

Dimkpa, C., Weinand, T., Asch, F. (2009). Plant-rhizobacteria interactions alleviate abiotic stress conditions. *Plant Cell Environ*, 32, 1682-169.

Dyer, A. (2012). Small Grain Seed Treatment Guide. MSU Extension Publications Mont Guide MT199608AG, 1-8 (http://msuextension,org/publications/AgandNaturalResources/MT199608AG, pdf).

Glick, B.R. (2012). Plant Growth-Promoting Bacteria: Mechanisms and Applications. *Hindawi Publishing Corporation Scientifica*, Article ID 96340, 15 (https://doi.org/10.6064/2012/963401).

Hungria, M., Nogueira, M.A., Araujo, R.S. (2015). Soybean Seed Co-Inoculation with *Bradyrhizobium* spp. And *Azospirillum brasilense*: A New Biotechnological Tool to Improve Yield and Sustainability. *American Journal of Plant Sciences*, 6, 811-817.

Ion, V. (2010). Fitotehnie. (http://www, horticultura-bucuresti.ro/images/ pdf/ Fitotehnie, pdf).

Lee, G.J., Wu, X., Shannon, J.G., Sleper, D.A., Nguyen, H.T. (2007). Genome Mappingand Molecular Breeding in Plants. Vol. 2, Oilseeds, Chapter 1,Soybean.

Paul, D., Nair, S. (2008). Stress adaptations in a Plant Growth Promoting Rhizobacterium (PGPR) within creasing salinity in the coastal agricultural soils. *Journal of Basic Microbiology*, 48, 378–384.

Racz, I. (2013). Cercetări privind formarea producţiei şi evaluarea unor însuşiri de calitate la unele cereale panificabile în Câmpia Transilvaniei. PhdThesis.

Rodrigues, A.C., Vendruscolo, C.T., Moreira, A., Vanilda, M., Santana, S., Oliveira, J., Bonifacio, A., Figueired, M.B. (2015). Rhizobium tropici exopolysaccharides as carriers improvet hesymbiosis of cowpea-*Bradyrhizobium, Paenibacillus*. *African Journal of Microbiology Research*, 9(37), 2037-2050.

Tewari, S., Naveen, K.A., Mohammad, M. (2016). Plant growth promoting rhizobacteria to alleviate soybean growthunder abiotic and biotic stresses. Abiotic and Biotic Stresses in Soybean Production, 1, 131-15.

Tiwari, S., Prasad, V., Lata, C. (2019). New and Future Developments in Microbial Biotechnology and Bioengineering, Microbial Biotechnology in Agro-Environmental Sustainability. Chapter 3 - Bacillus: Plant Growth Promoting Bacteria for Sustainable Agriculture and Environment.