The effect of plant-associated rhizobacteria on the resistance of white mustard to soil drought

Vitaliy Lebedev[1*], *Sofya* Kondrat[1], *Grigorii* Uraev[2], *Svetlana* Khuaz[3], and *Ruzana* Datkhuzheva[3]

[1]Herzen State Pedagogical University of Russia, 48, Moika Emb., Saint Petersburg, 191186, Russia
[2]Emperor Alexander I St. Petersburg State Transport University, 9, Moskovsky Ave., Saint Petersburg, 190031, Russia
[3]Saint-Petersburg State Agrarian University, 2, Peterburgskoye Highway, Pushkin, Saint Petersburg, 196601, Russia

Abstract. The paper discusses the results of a vegetation experiment with Plant Growth-Promoting Rhizobacteria (PGPR), namely such products as Agrofil, Mizorin, Flavobakterin, and Ekstrasol, used as organic fertilizers for white mustard of the Rapsodiya / Rhapsody variety (k-4278) grown in normal soil moisture and drought. The study aims to determine the role of plant-associated PGPR strains in physiological processes and productivity of white mustard grown in normal moisture and soil drought during the critical stages of plant development. The growth stimulators showed to increase plant productivity under normal moisture facilitating growth and an increase in the number of leaves and seeds. Insufficient soil moisture disrupts water balance in plants. The seeds treated with PGPR were more physiologically stable and retained more water under short-term drought facilitating sufficient seed yield. Mizorin and Flavobakterin proved to be most effective under normal moisture, while the seeds inoculated with Agrofil performed best—both biologically and economically—in drought.

1 Introuction

PGPR(plant-growth-promoting rhizobacteria) based bacterial products have been gaining popularity in farming. They stimulate plant growth, enhance plant resistance to stress and increase seed productivity [1]. The use of plant-associated PGPR to increase the resistance of plants to drought, especially during critical stages of plant development, holds promise. Microorganisms establish a strong connection with the plant which improves mineral nutrition and nitrogen fixation in plant roots and increases the production of phytohormones and resistance to stress, e.g., drought [2].

It is known [3] that PGPR will perform best if the chosen strain has the right sensitivity for a plant species and/or variety. White mustard (*Sinapis alba* L., 1753) is a minor crop [4], however, it is of value due its properties as a melliferous, medicinal, green manure and forage plant.

* Corresponding author: antares-80@yandex.ru

Hence, the study was underpinned by a practical interest in the role of plant-associated PGPR in the improvement of plant resistance to drought.

Drought is an abiotic stress factor that negatively affects plant growth and productivity. Insufficient soil moisture often means critically low crop yield and this may have serious economic consequences. Droughts are even more dangerous when they coincide with the most sensitive periods of plant development: flower-bud formation and initiation of flowering [5]. These stages of plant development are highly sensitive to insufficient soil moisture; hence, plants are more prone to stress. So far, this issue has been under-scrutinized [6].

2 Materials and methods

The study aims to determine the role of plant-associated PGPR strains in physiological processes and productivity of white mustard grown in normal moisture and soil drought during the critical stages of plant development.

Research objectives: 1) to identify plant-associated strains of rhizobacteria that have a stimulating effect on the growth processes of white mustard; 2) to investigate the change in some physiological processes that are criteria for drought resistance, depending on the biological product used in conditions of soil drought; 3) to study the features of the formation of productivity of white mustard when inoculated with bacterial preparations in conditions of normal moisture and soil drought.

The vegetation experiment with white mustard (*Sinapis alba* L.) of the Rapsodiya / Rhapsody variety (k-4278) were conducted at the biological research station of Herzen University in Vyritsa, an urban locality not far from St. Petersburg, Russia. The certified seeds for the experiment were provided by the N.I. Vavilov All-Russian Institute of Plant Genetic Resources (St. Petersburg, Russia). The study used a common methodology of planting and experimental research [7]. The soil used in the experiment is weakly acidic soddy/weak podzolic, sandy-loam soil with an average content of humus and available forms of phosphorus and potassium.

According to the guidelines in [8], before planting the seeds were inoculated with the following bacterial products: Agrofil, (*Agrobacterium radiobacter* (Beijerinck & van Delden, 1902) Conn, 1942, strain 10), Mizorin (*Arthrobacter mysorens* Conn & Dimmick, 1947, strain 7), Flavobakterin (*Flavobacterium sp*. Bergey, et al. 1923, strain 30) and Ekstrasol (*Pseudomonas fluorescens* Migula, 1895, strain PG-5). The strains were chosen after a preliminary laboratory screening. The bacterial products were provided by All-Russia Research Institute for Agricultural Microbiology, Pushkin, St. Petersburg, Russia.

The plants were grown under normal moisture conditions, i.e., 70% of the maximum water holding capacity. During the critical stage of plant development (flower-bud formation) some of the plants were drought-stressed. This part of the experiment lasted for ten days in the moisture conditions as low as 30% of water holding capacity. Once the critical stage was over, the plants were returned to normal moisture conditions.

The study explored only those physiological responses of plants that reliably indicate the level of drought resistance in plants [9]. At the full ripeness stage, following the vegetative phase, we analysed seed productivity of plants grown in different conditions [10]. The data was processed using dispersion analysis in the spreadsheet application Microsoft Excel.

The economic effect of inoculating plants of the Brassicaceae family with bacterial products was calculated as a difference in revenue relative to the control group. For more details on the evaluation of economic effects see [4]

3 Results

The results of the vegetation experiment showed drought-linked stunted growth. However, the inoculation of plants with bacterial products had a growth-stimulating effect. Plants treated with rhizobacteria strains before planting and then drought-stressed were 23-27% higher during the full flowering stage than the non-treated samples. As regards plant height, the study proved bacterial product Agrofil to be the most effective.

When used in drought conditions, rhizobacteria had a positive impact on the retention of leaves during the full flowering stage. In addition, lower leaves in rhizobacteria-treated plants showed a delayed die-off as compared to the control plants. This resulted in a longer retention of leaf function in experimental plants. Drought-stressed white mustard from the control group of plants had 8.5 leaves per plant. The plants treated with Mizorin and Flavobakterin had the biggest number of leaves: 14.5 and 13.2 leaves per plant respectively (Table 1).

Table 1. The number of leaves in plants inoculated with plant-associated rhizobacteria strains depending on soil moisture, leaves per plant.

Plant Type	NM*	SD*	SD/NM
Control group	17.6	8.5	0.48
Agrofil-treated	20.8	11.8	0.57
Mizorin-treated	21.6	14.5	0.67
Flavobakterin-treated	20.6	10.0	0.49
Ekstrasol-treated	21.1	13.2	0.63
LSD_{05}	3.0	2.6	-

Note: NM – normal moisture; LH – soil drought; *Source*: Compiled by the authors.

It is known that insufficient soil moisture disrupts water balance in plants and decreases the moister level in all organs and tissue. Plants with higher water retention capacity are more resistant to drought stress and show better productivity.

Flavobakterin and Mizorin performed best among the other bacterial products used in the experiment as regards the improvement of water-retention properties of white mustard in normal moisture conditions (Figure 1).

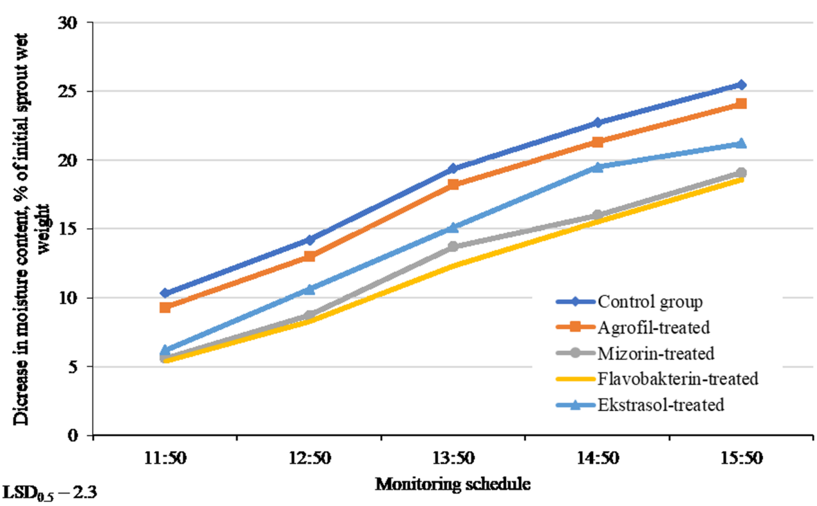

Fig. 1. The impact of plant-associated rhizobacteria on water-retention properties of white mustard in normal moisture conditions (flowering stage). *Source*: Compiled by the authors.

The plants that were not watered for ten days were marked by a lower moisture content. Under these conditions, bacterial product Agrofil had the highest effect. The plants inoculated with Agrofil showed higher water holding capacity throughout the experiment. Hence, the plants treated with Agrofil are more resistant to drought stress in the long term. This is yet another proof of the protective effect of agrobacteria in the conditions of insufficient moisture (Figure 2).

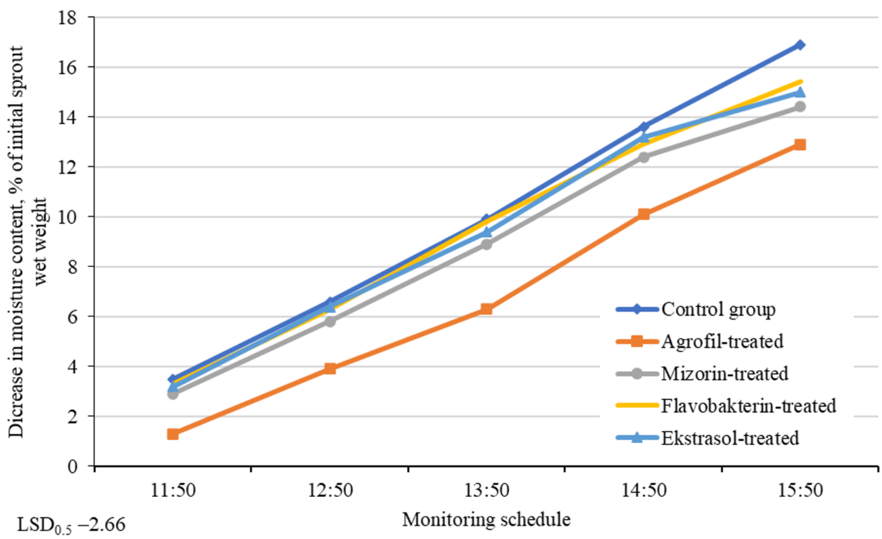

Fig. 2. The impact of plant-associated rhizobacteria on water-retention properties of white mustard in the conditions of drought (flowering stage). *Source*: Compiled by the authors.

Drought reduced the quantity and weight of white mustard seeds in all the experimental set-ups. However, the treatment of plants with plant-associated rhizobacteria improved the seed quantity and weight statistics (Table 2).

Table 2. Seed yield in plants depending on plant-associated rhizobacteria strains and soil moisture conditions.

Plant Type	Quantity of seeds (pieces/vial)			Seed weight (grams/vial)		
	NM*	**SD****	**SD/NM**	**NM**	**SD**	**SD/NM**
Control group	492	282	0.57	3.0	0.8	0.27
Agrofil-treated	604	548	0.91	4.8	1.7	0.35
Mizorin-treated	888	524	0.59	5.4	1.5	0.28
Flavobakterin-treated	832	488	0.59	5.0	1.5	0.30
Ekstrasol-treated	755	500	0.66	4.2	1.2	0.29
LSD$_{05}$	36.4	22.4	-	0.4	0.3	-

Note: NM – normal moisture; SD – soil drought; *Source*: Compiled by the authors.

To conclude, the inoculation of white mustard seeds with bacterial products neutralises the negative effect of short-term drought during the critical stage of plant development and enhances seed productivity.

Plant-associated rhizobacteria improve plant height growth, mineral nutrition, dry shoot mass, root size and mass. Deep roots increase drought resistance in plants providing them with more water from deeper layers of soil.

The above factors have economic consequences for a farming enterprise (Figure 3).

Inoculation of seeds has a pronounced positive impact on the revenue from seed sales even in soil drought—a 2.63-4.06 revenue increase.

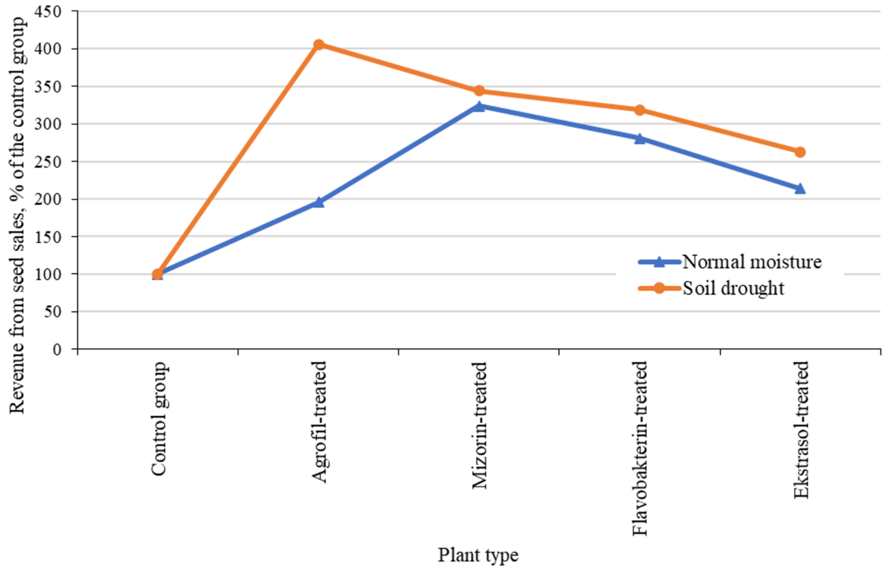

Fig. 3. Changes in the revenue from the sales of white mustard seeds in the conditions of normal moisture (NM) and soil drought (SD). *Source*: Compiled by the authors.

4 Discussion

The obtained results show that Mizorin performs best in normal soil moisture. These findings correlate with the results of our long-term study in annual Brassicaceae crops. However, the experiments showed that the best protective effect against drought stress is provided by Agrofil. The possible reason for it is that Agrofil agrobacteria are more likely to retain their physiological functions in arid soil increasing drought resistance in plants. In earlier studies [1], Agrofil never showed a high stimulating effect under normal moisture. In addition, studies on the use of bacterial preparations in conditions of soil drought have been poorly studied. It should be noted that the most effective Mizorin and Flavobakterin, which in the early works of the authors [4] were recommended for use on mustard do not have a stimulating effect in drought conditions. The stimulating effect of Agrophile in comparison with other preprates was noted for the first time in the conditions of drought. These findings are a point of departure for future research.

5 Conclusion

The vegetation experiments showed that the seeds of white mustard (*Sinapis alba* L.) inoculated with PGPR are physiologically more stable in short-term drought during flower-bud formation and initiation of flowering. Plant-associated bacteria facilitate plant growth, stability of dry root and shoot mass, improved mineral nutrition (nitrogen, phosphorus and potassium) as well as seed quantity and weight. The yield of inoculated plants, as compared to the control group, was 40-87% higher in normal moisture and 50-113% higher in soil drought.

The study showed that Mizorin (*Arthrobacter mysorens*, strain 7) proved to be most effective under normal moisture, while Agrofil (*Agrobacterium radiobacter*, strain 10) performed best in short-term plant drought stress.

The revenue of a farming enterprise producing white mustard seeds correlates with the soil moisture content and inoculation of seeds with bacterial products. Inoculated seeds of white mustard grown in normal soil moisture generate a 1.96-3.24 revenue increase. Inoculated seeds produce an even more substantial increase in revenue—a 2.63-4.06 times increase relative to the control group—when white mustard is subjected to drought.

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