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THE INFLUENCE OF SOME BACTERIAL STRAINS ON THE GERMINATION OF GREEN SORREL SEEDS (*RUMEX ACETOSA* L.)

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Abstract. Sorrel is a versatile herbaceous plant, present in the spontaneous flora of Romania. It is valued for its leaves with a sour taste and is used in various culinary preparations. Currently, the species is appreciated for the production of "microgreens" type seedlings. Despite its recent popularity, sorrel is a difficult plant to grow, not because of its special nutritional requirements, but because of the limited ability of the seeds to germinate. The present study brings to attention the beneficial traits of seed treatments, with plant growth promoting bacteria (PGPB). The aim of such seed treatments was to improve the germination capacity of green sorrel seeds and establish the optimal inoculum concentration. The seed treatment was applied by immersion, in three different concentrations: 10^7, 10^8, and 10^9 cfu/ml respectively. Two bacterial strains were tested as inoculants, *Bacillus amyloliquefaciens* OS17 strain and *Phyllobacterium myrsinacearum* DSM5892 reference strain. Plants were analyzed four weeks after germination to determine fresh and dry weight, number of true leaves, and root length. Both tested bacterial strains, *B. amyloliquefaciens* OS17 and *P. myrsinacearum* DSM5892 improved sorrel seed germination and stimulated seedling growth. Best results were obtained using 10^9 cfu/ml inoculant. The seed treatment with *P. myrsinacearum* DSM5892, at 10^9 cfu/ml, registered the highest number of true leaves and roots length. The beneficial effects of this seed treatment were also highlighted by the increase in plant fresh and dry weight.

Key words: *bacteria, green sorrel, germination, growth promotion, seed inoculation*

INTRODUCTION

Seed germination is considered the most vulnerable and crucial stage in the life cycle of plants (Finch-Savage & Leubner-Metzger, 2006). This stage is influenced by edaphic factors such as the capacity to retain and drain water, temperature, the presence or absence of essential macro and micro elements, pH values, salinity, as well as and the presence of pathogenic microorganisms and pests. Improving seeds germination involves the optimization of the germination conditions to provide the right environmental requirements for the seeds to sprout successfully.

An effective method to improve seeds germination is to apply appropriate microbial bioinoculants. Beneficial microorganism can increase plant germination, seedling vigor, vegetal biomass, and can overcome some limitation factors, such as abiotic stress, both during and after emergence (Cardarelli el al., 2022). To ensure seed germination, recent studies have demonstrated that the application of bioinoculants, as seed treatment, improves germination by up to 80% when using selected *Bacillus* strains (Cendales et al., 2017). Moreover, the inoculation with certain plant probiotics, such as *Phyllobacterium* and/or *Rhizobium* strains, a significant increase in citric acid, vitamin C and epicatechin content was registered, while at

strawberry plants, an improved rooting was observed. These results showed that the inoculation with plant probiotic strains is a good agronomical practice, which improves the content of several bioactive compounds. Such advantages can overcome the chemical fertilization (Flores-Félix et al., 2018).

Considering these types of bacterial inoculants as seed germination improvers and plant growth promoters, their application as seed treatment could overcome the low germination capacity of certain plant species, such as sorrel.

Sorrel refers to several perennial herbs belonging to the genus *Rumex*, in the *Polygonaceae* family. It comprises nearly 200 species that are mostly perennials. The genus includes herbaceous plants, some having culinary and medicinal uses (Li et al., 2022). *Rumex* species are found in a variety of habitats, ranging from grasslands and meadows to disturbed areas and roadsides. They are adaptable and can thrive in different soil types, but they are more common on slightly acidic and low nutrient soils (Barbu et al., 2023).

One of the most common species is *Rumex acetosa* L., commonly known as garden sorrel or common sorrel, green sorrel, or simply sorrel. Often used in salads, soups, and sauces or with medicinal uses, sorrel could be cultivated as microgreens or as an ornamental plant. This is a leafy green vegetable with a tangy flavor a relatively easy to cultivate and can thrive in various climates, including that of Romania (Barbu et al., 2023). However, there are difficulties in obtaining sorrel plants due to the limited ability of the seeds to germinate. These is mostly due to the fact that sorrel is a deciduous plant, which means that female and male flowers are on separate plants. As female flowers aren't self-fertile and must be pollinated, the germination capacity is rarely higher than 85%, and can drastically decreased during storage (Stosik, 2007). The requisites for seed germination of many species of *Rumex* have been studied for several decades. One of the major constraints in successful establishment is its high degree of hard seed due to a waxy coat which must be broken before the seed will imbibe water and germinate (Shelton and Brewbaker, 1998). The low seed germination rate in sorrel seeds is caused also by long time physical dormancy (Omran, 2013). Little information exists on the factors affecting seed germination and seedling emergence of green sorrel (Yazdi et al., 2013).

The main objective of this study was to improve the germination rate and vegetative growth of sorrel by applying beneficial bacterial bioinoculants for plant growth. Finding the optimal concentration of bacterial inoculum was an additional aim of this study. For this aspect, two types of bacterial treatments were studied in three concentrations.

MATERIALS AND METHODS

The biological material

The biological material consisted of ecological and untreated seeds of sorrel (*Rumex acetosa* L.), Lord variety. The seeds were provided from the Plant Genetic Resources Bank for Vegetable, Floriculture, Aromatic and Medicinal Plants (BRGV), Buzau (Figure 1).

Before the test, seeds were stored for 10 days in paper bags in darkness at room temperature and protected from humidity prior to the germination experiments or the stratification treatment. The sorrel seeds were stratified at 4°C in darkness for 5 weeks prior to the tests. A number of 1430 seeds were tested for each experimental variant.

These seeds were superficially sterilized, in two steps, with 5% sodium hypochlorite (NaOCl) and 70 % ethanol respectively, followed by five rinses with distilled H_2O. The seeds were then kept for an hour in sterile distilled water for better hydration before being planted in the soil.

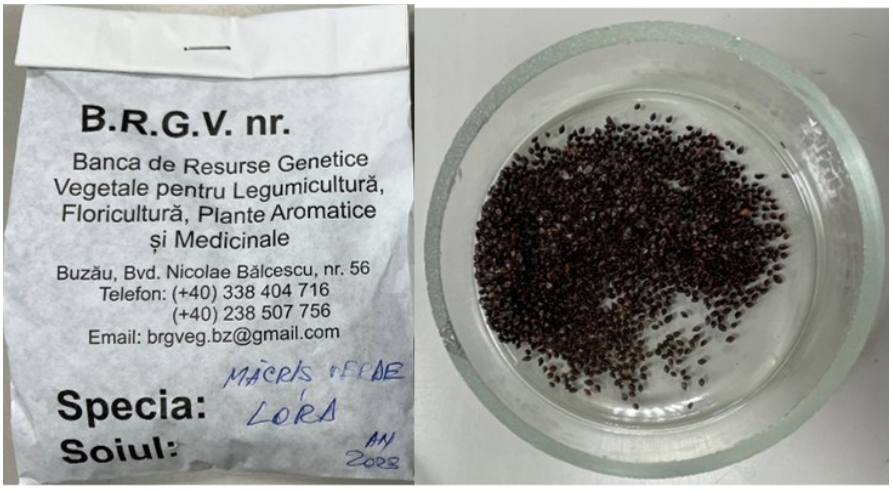

Figure 1. Sorrel seeds obtained in 2023 at BRGV Buzau

Two bacterial strains known for their plan growth promoting potential were chosen to be used in the experiments: a Romanian native strain of *Bacillus amyloliquefaciens*, isolated from rhizosphere, and a reference strain of *Phyllobacterium myrsinacearum* mentioned as plant probiotic (Table 1). The strains were formulated as concentrated suspensions, and were applied as seed treatments, in three different concentrations: 10^7, 10^8, and 10^9 cfu/ml, for each strain.

Table 1. Bacterial strains and cultivation

Strain	Source	Growth conditions
***Bacillus amyloliquefaciens* OS17 (reference number NCAIM B 001415)**	Romanian native strain from the Research-Development Institute for Plant Protection	The bacterial strains were grown in submersed conditions (150 rpm), under continuous shaking (150 rpm), in Luria Bertani broth, at 27°C for five days.
***Phyllobacterium myrsinacearum* DSM 5892**	Reference strain from the German Collection of Microorganisms and Cell Cultures (DSMZ)	

Bacterial inoculants were applied on superficially disinfected sorrel seeds, just before sowing. Treated seeds were immersed for 60 minutes in the bacterial suspension prepared in different concentrations. For the control, disinfected sorrel seeds were soaked in sterile distilled water (Fig.2.).

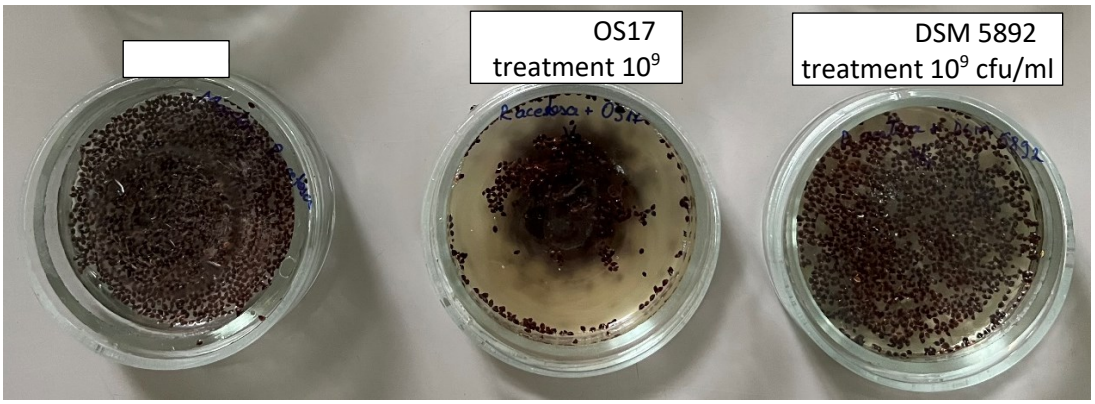

Figure 2. Sorrel during seed treatment

One control and six bacterial treated variants were prepared as follows:
V1 – Bacterial treatment with *Bacillus amyloliquefaciens* OS17, in three bacterial concentrations: *A* - 10^7 cfu/ml; *B* - 10^8cfu/ml; and *C* - 10^9 cfu/ml;
V2 – Bacterial treatment with *Phyllobacterium myrsinacearum* DSM 5892, in three bacterial concentrations: *A* - 10^7 cfu/ml; *B* - 10^8cfu/ml; and *C* - 10^9 cfu/ml;
Control – Untreated sorrel seeds, soaked in sterile water.

Sorrel was then seeded in garden substrate based on peat and sand, which was purchased from a local hardware store. Plants were grown in diurnal conditions, at 23±1°C during daylight, and 15±1°C during night. Soil moisture was maintained by sprinkling with tap water. Before harvest, plants were grown for 4 weeks after germination (Fig.3.).

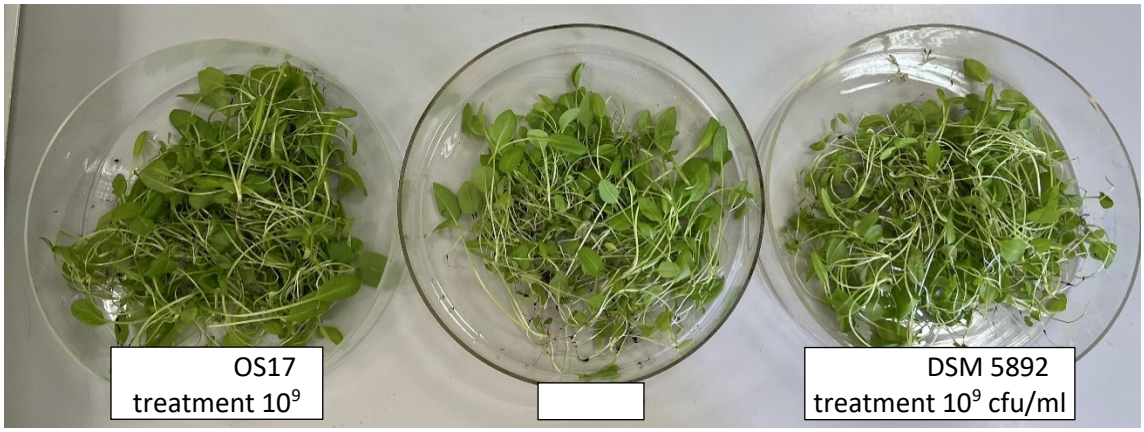

Figure 3. Sorrel plants four weeks after applying the seed treatment

The germination capacity (GC)

The germination capacity (GC) of sorrel seeds was determined by counting the relative number of germinated seeds compared to the total number of seeds used per experimental variant, based on the following formula (Fredrick et al., 2015):

$$GC(\%) = \frac{n_g}{n_t} \times 100$$

Where: n_g= the number of germinated seeds, n_t = the total number of seeds

Biometric measurements of sorrel plants

Four-week-old plants were used for different biometric measurements. A series of analysis was carried out for determining the number of true leaves, root length and fresh and dry weight.

The number of true leaves

True leaves are those that develop after cotyledonal leaves emergence, and often have the characteristic shape of the mature plant. In order to determine the number of true leaves per sorrel plant, the cotyledonal leaves were excluded from the total number of leaves. Counting the number of true leaves on a plant is a fundamental parameter in assessing its growth and development.

The root length

Measuring the root length of plants is an essential aspect of studying plant development, especially for evaluating root growth, nutrient uptake, and overall plant health. In order to perform this measurement, the sorrel plants were taken out from the substrate, and the root

system was gently washed in warm water, to remove the adhering soil particles, and enabling accurate measurements. A ruler was used to measure the length of each primary root, from the basal plant collar to the tip of the root.

Fresh and dry weight

These measurements provide insights into plant growth, water content, and biomass. The fresh weight of sorrel plants is the weight of the plant immediately after harvesting, without any drying process. The dry weight of sorrel plants is the weight of the plant material after it has been dried at 60°C to remove all water content. Both fresh and dry weights were measured using an analytical balance.

Statistical analysis

The measurements were carried out for each experimental variant, control and bacterial treated variants. The recorded data were expressed as mean values and standard deviation (SD).

RESULTS AND DISCUSSIONS

The germination capacity (GC) of sorrel seeds

The effect of bacterial seed treatment on the germination capacity is shown in Figure 4. The germination capacity of sorrel seed increased proportionally with the concentration of bacterial strain. It is observed that the highest average value of the germination capacity (76.43±2.96) was recorded in the case of the experimental variant that consisted of treatment with the *P. myrsinacearum* strain at 10^9 cfu/ml bacterial concentration.

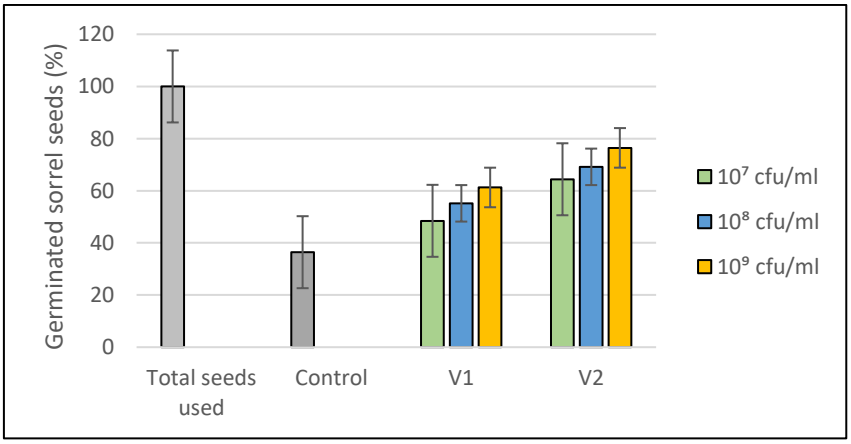

Figure 4. The percentage of germinated sorrel seeds

The number of true leaves

Regarding the bacterial seed treatment effect on the number of true leaves per plant, it was observed that the tested bacterial strains significantly promoted the growth of sorrel plants. The highest values were registered in bacterial treated variants, inoculated with 10^9 cfu/ml bacterial concentration (Figure 5). No significant differences were noticed between the two tested strains. The total number of leaves per plant was 3.569 ±0.53, when *B. amyloliquefaciens* OS17 strain was used, while whit *P. myrsinacearum* DSM 5892 strain an average of 3.502±0.47 leaves/plant was quantified.

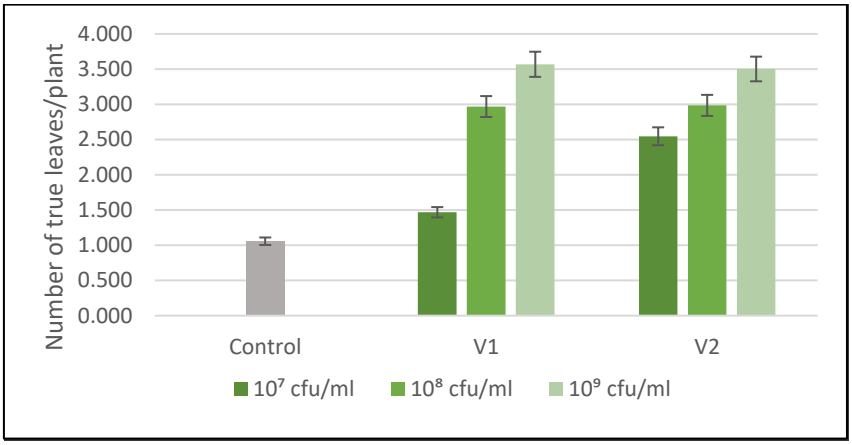

Figure 5. The number of true leaves of sorrel plants

The root length of sorrel plants

Among all experimental variants, the sorrel plants inoculated with *P. myrsinacearum* DSM 5892 as seed treatment, in 10^9 cfu/ml concentration, revealed the longest root length, 3.350 ±0.33 cm (Figure 6).

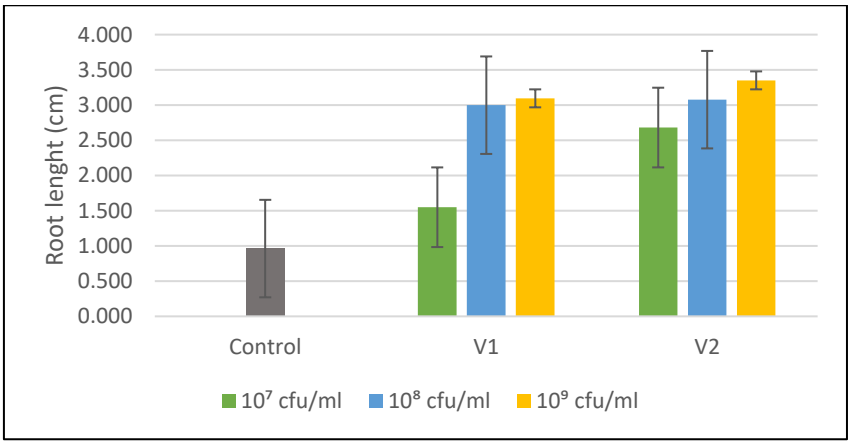

Figure 6. The root length of sorrel plants

The fresh and dry weight of sorrel plants

The beneficial effects of bacterial seed treatment were also highlighted by the increased fresh and dry weight of the sorrel plants compared to the control. Both tested bacterial strains were able to significantly increase sorrel biomass compared to the untreated control. Improved biomass production was seen at 10^9 cfu/ml bacterial concentration (Figure 7).

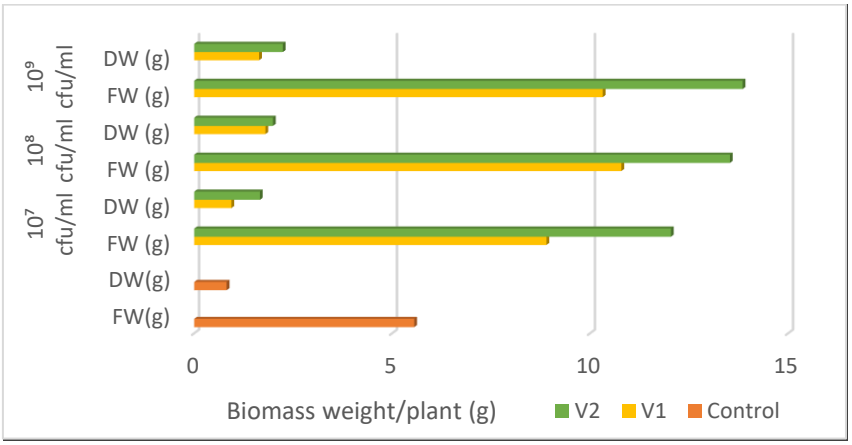

Figure.7. The fresh and dry weight of sorrel plants

Among all tested inoculation variants, the highest values of sorrel fresh (13.85±0.56 g) and dry weight (2.24±0.12 g) per plant were registered in the experimental variant treated with *P. myrsinacearum* DSM 5892 strain in 10^9cfu/ml concentration.

CONCLUSIONS

P. myrsinacearum DSM 5892 and *B. amyloliquefaciens* OS17 strains were able to improve seeds germination rate and sorrel plant growth parameters. However, to validate these results, field trials must be performed also.

Optimal inoculum concentration was established at 10^9 cfu/ml, when the bacterial treatments were applied on seeds.

Among the two tested bacterial strains, better results were obtained when the plant probiotic strain, *Phyllobacterium myrsinacearum* DSM5892, was used.

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