Bacterial antagonists *Bacillus* sp. Q3 and *Pseudomonas chlororaphis* Q16 capable to control wheat powdery mildew in wheat

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**Abstract**

This paper outlines effects of the usage of two plant growth promoting (PGP) strains: *Bacillus* sp. strain Q3 and *Pseudomonas chlororaphis* strain Q16 trough vegetative experiments, performed in semi-controlled conditions on acid soils (Lessivated Cambisols). The studied parameters were chemical properties of soil and PGP effects of strains and their mixture on two wheat (Triticum aestivum L.) cultivars - NS 40S and CCB Ingenio. The seeds treatments with Q3 reduced the occurrence of *Blumeria graminis* f.sp. *tritici* natural infection only on CCB Ingenio (46.1%). The additional foliar treatment with Q3 decreased disease incidence (DI) 76.22% (CCB Ingenio) and 75.87% (NS 40S). Reduced initial DI of 77% caused Q16 solely or mixed with Q3 on cv. NS 40S and additional foliar treatment reduced DI to 16.30% (CCB Ingenio) and to 6.35% (NS 40S). The mixture of strains decreased DI to 14.69% (CCB Ingenio) and to 6.09% (NS 40S). Despite the effects of applied strains on wheat growth were affected by limited production capacity of soil and the extreme climatic conditions, SDW of inoculated cultivars were increased by 17-39% (NS 40S) and 35-43% (CCB Ingenio). The N increment ranged from 59% (Q3) to 152% (Q16) for cv. CCB Ingenio. Finding that the seeds treatments improved the plant biomass and N content (Q16) and decreased powdery mildew DI, we can recommended application of Q16 strain as biofertilizer for both wheat cultivars. Depending on wheat cultivar, the additional foliar treatments to prevent powdery mildew infection will be set up for further trials

**Keywords:** Lessivated Cambisols, chemical properties, *Bacillus* sp., *Pseudomonas chlororaphis*, *Triticum aestivum* L., *Blumeria graminis* f.sp. *tritici*

1. Introduction

Wheat (*Triticum aestivum* L., Poaceae) is a major cereal crop in many parts of the world and, after maize and rice, is the most cultivated cereal [1]. Besides human impact can improve wheat crop yield applying selected wheat cultivars, fertilizers and agricultural measures, environmental conditions remain to be the main factors affecting wheat productivity in many regions of the world [2]. Many unfavorable factors, as drought and heat stress, diseases, insects and weeds reduce yield potential [3]. The climate changes, particularly higher temperatures and changes on rainfall distribution and amount hinder increasing of wheat yield [4, 5].

The main task of every agricultural production is increasing and maintaining soil fertility. For normal soils maintaining and increasing of productive ability of soils is possible via
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optimal application of organic and mineral fertilizer. Soil acidity is a major limiting factor for crop production globally [6].

Plant growth-promoting bacteria (PGPB) are defined as free-living soil, rhizosphere, rhizoplane, and phyllosphere bacteria that are beneficial for plants under some conditions. The mechanisms employed by biocontrol PGPB to deter phytopathogens can be chemical, environmental (out competition and displacement of pathogens), or metabolic (induction of acquired or induced systemic resistance and modification of hormonal levels in plants). A large array of microbial substances is involved in the suppression of pathogenic growth and subsequent reduction in damage to plants. Application of PGPB in the field has yielded satisfactory results in controlled experiments, although results are less promising under agricultural conditions [7]. Pseudomonas and Bacillus are among the most widely reported PGPB. Selection of indigenous bacteria with PGP traits, applicable for improvement of plant growth and yield in Serbia, are already reported [8, 9,10,11]. Several strains from Bacillus and Pseudomonas species showed potential for biocontrol of several phytopathogens [12, 13, 14, 15, 16].

Wheat powdery mildew caused by Blumeria graminis f.sp. tritici (DC) Speef Emend Marchal is one of the economically most important wheat diseases worldwide. This pathogen is an important limiting factor in the production of wheat in Serbia reducing the yield and the quality of the grain. The mildew occurs every year with a varied intensity depending on the resistance of the local varieties, climate conditions and how virulent the pathogen is.

This disease can be controlled using fungicides and a race-specific host resistance. Fungicide treatment has been shown to contribute to a relatively large yield increase in spite of low levels of mildew infection in wheat [17]. However, the use of fungicides has been associated with some negative effects such as a reduced diversity in powdery mildew populations and increased resistance to fungicides [18], especially using triazoles for more than 10 years [19,20]. The European Union directive 2009/1287EC has established a framework to accomplish a sustainable use of pesticides by using integrated pest management strategies such as non-chemical alternatives to pesticides [21]. Biocontrol measures using bacterial and fungal antagonists offer alternatives to the use of synthetic pesticides to control pathogenic fungi and other microorganisms on crops. Many studies report that effective disease control of pathogens can be attained used antagonist microorganisms. Different combinations of the multi–strain mixtures, antagonists used alone in rotation are all being recommended. The non-chemical methods for powdery mildew control can be classified into two categories: 1. products of micro-organisms that protect the plant against powdery mildew infection by inducing host defense mechanisms and 2. microorganisms that are natural enemies of powdery mildews and attack their different structures through parasitism and/or antibiosis. The number biocontrol agents used against powdery mildew fungi has expanded tremendously over the last 30 years. This progress has resulted in the commercialization of fungal or bacterial – based products developed specifically for powdery mildew treatment, e.g. bio-fungicide Serenade, containing Bacillus subtilis [22]. However, as with any biocontrol agent, the results have been variable, and efforts to achieve consistency must be carried out to generate widespread acceptance of these products. From an ecological point of view, fungi are better suited than other phyllosphere inhabitants to attack powdery mildews [23]. According to the [24] found that all powdery mildews antagonists are fungi, but no bacterial antagonists have been reported to attack the pathogen naturally (except for the one notable example of B. subtilis). Until recently, interactions between fungal antagonists and a member of the Erysiphales were classified into two categories: parasitism and antibiosis [25]. A single study of the effect of a biocontrol mixture on powdery mildew, conducted in cucumber with the hyper-parasites Ampelomyces quisqualis and Trichoderma harzianum,
showed that the mixture efficacy was no better than *Ampelomyces* used alone [26]. Although antagonists appear to be successful at reducing the impacts of pathogens, they often result in sub-optimal or inconsistent control of plant diseases [27].

In this study we investigated the effects of bacterial antagonists on plant promoting effects and the incidence and severity of wheat powdery mildew. We tested two bacterial antagonists, belonging two bacterial genera- *Pseudomonas* and *Bacillus*, and whether the use of mixture offered a better approach to employing microbial diversity for plant protection. We compared these biocontrol treatments to two commercial wheat cultivars. The experiment was also aimed to define the main parameters for field trial and possible wider usage of tested bacterial strain in Lessivated Cambisols.

**2. Material and methods**

**Agro meteorological parameters**

Agro meteorological parameters were registered in the Meteorological Office in Belgrade during the experiment performed on wheat in semi-controlled-conditions in the experimental area of the Institute of Soil Science.

May 2013, was characterized by changeable weather and warmer than average with a surplus of precipitation. Lower temperatures were recorded twice, at the beginning of the second and larger part of the third decade of May. Rainfalls were almost a daily occurrence, and there were days with heavy rainfalls followed by extreme weather conditions and the ice storm which caused some damage to agricultural crops. During the almost whole month, the abundant rainfalls and high humidity created favorable conditions for the development of a number of plant diseases.

Jun 2013, was characterized by changeable weather with an average temperature conditions and the usual rainfalls. Precipitations, mainly local character, in June were almost a daily occurrence, except for the period from mid-month to mid-third week when the whole country had sunny and very warm weather. During the first half of June, frequent and abundant precipitation provided suitable conditions for the development of fungal and bacterial diseases. Activity of pests was more noticeable in the second half of the month.

July 2013, was characterized with warm weather, with the occasional occurrence of heavy rainfalls mainly in the first half of the month. Temperatures recorded in July were within average values for this month. Warm and dry weather during most of the month favored the development of plant pests. The table 1 below shows the basic meteorological and climatic parameters by decades of monitoring.

<table>
<thead>
<tr>
<th>Period of observation</th>
<th>Mean decade temperature (°C)</th>
<th>Isolation sums (h)</th>
<th>Sums ten-day rainfall ΣP (mm)</th>
<th>PET (mm)</th>
<th>Decade deviation P (mm)</th>
<th>Decade deviation PET (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-10 May 2013</td>
<td>22.6</td>
<td>82</td>
<td>9</td>
<td>57.7</td>
<td>6.1</td>
<td>-9.0</td>
</tr>
<tr>
<td>11-20 May 2013</td>
<td>20.4</td>
<td>87</td>
<td>8</td>
<td>53.5</td>
<td>2.6</td>
<td>-12.0</td>
</tr>
<tr>
<td>21-31 May 2013</td>
<td>16.4</td>
<td>63</td>
<td>87</td>
<td>40.5</td>
<td>-2.2</td>
<td>64.0</td>
</tr>
<tr>
<td>1-10 Jun 2013</td>
<td>18.1</td>
<td>48</td>
<td>12</td>
<td>37.5</td>
<td>-1.8</td>
<td>-33.0</td>
</tr>
<tr>
<td>11-20 Jun 2013</td>
<td>23.7</td>
<td>111</td>
<td>9</td>
<td>68.5</td>
<td>3.2</td>
<td>-2.7</td>
</tr>
<tr>
<td>21-30 Jun 2013</td>
<td>23.2</td>
<td>89</td>
<td>28</td>
<td>58.8</td>
<td>1.5</td>
<td>10</td>
</tr>
<tr>
<td>1-10 July 2013</td>
<td>23.0</td>
<td>112</td>
<td>1</td>
<td>64.4</td>
<td>0.9</td>
<td>-22.0</td>
</tr>
<tr>
<td>11-20 July 2013</td>
<td>23.1</td>
<td>116</td>
<td>0.2</td>
<td>66.3</td>
<td>0.5</td>
<td>-18.7</td>
</tr>
</tbody>
</table>

P-Precipitation; PET-Potential evapotranspiration (Penman Monteith)
**Soil Analysis**

Soils from all treatments and from three replications were analyzed for their soil chemical characteristics at the beginning of experiment. Determinations of particle size distribution were performed according to the method by sieving and sedimentation [28]. pH in water and 1M KCl was analyzed potentiometrically with glass electrode. Total N was analyzed on elemental CNS analyzer Vario EL III [29]. Available P₂O₅ and K₂O were analyzed by AL-method according to Egner-Riehm [30] where 0.1M lactate (pH = 3.7), was used as an extract. After the extraction, K was determined by flame emission photometry and P by spectrophotometer after color development with ammonium molybdate and SnCl₂. Ca and Mg were extracted by ammonium acetate followed by determination on atomic adsorption analyzer SensAA Dual (GBC Scientific Equipment Pty Ltd, Victoria, Australia) [31]. Determination of CEC was done by steam distillation method after a treatment of the samples with 1M ammonium acetate [32]. Exchangeable Al was determined by titration method after Sokolov: extraction with 1M KCl (1:2.5), followed by shaking for 1 h and titration with 0.01 M NaOH [33]. Microelements and heavy metals were determined with an ICAP 6300 ICP optical emission spectrometer, after the samples were digested with concentrated HNO₃ for extraction of total forms, and by DTPA for extraction of soluble forms of the elements [34]. The total content of CaCO₃ studied was determined using the “rapid titration method” by Piper, also called “acid neutralization method” [35].

**Greenhouse Experiment**

Wheat (*T. aestivum* L.) was chosen as an experimental crop due to its good response to neutralization of soil acidity. Two wheat varieties from different origins were chosen for investigation: NS 40S originating from Serbia (Institute of Field and Vegetable Crops, Novi Sad) and CCB Ingenio from France. Cultivars choice was based on its properties. NS 40S are recommended as medium early, resistant to lodging, tolerant to drought, resistant to powdery mildew and leaf rust. CCB Ingenio is also medium early and resistant to lodging, resistant to Fusarium willt and well adapted to the Serbian growing conditions.

Bacterial strain *Bacillus* sp. strain Q3 and *Pseudomonas chlororaphis* strain Q16 were chosen for this study on the basis of their biocontrol potential detected earlier for several phytopathogenic fungi [13,14,15,16]. Bacteria were grown in liquid King’s B medium (KB), their concentration were determined spectrophotometrically and diluted to 10⁸ CFU ml⁻¹. Wheat seed were placed in a bacterial suspension for 2h before sowing.

The experiment was undertaken with soil types from central Serbia region that have acid pH: Lessivated Cambisols from Mladenovac in plastic pots with 4 kg of homogenized soils. Four variants of experiment were set up in three replications: 1. Control; 2. *Bacillus* sp. Q3 treatment; 3. *P. chlororaphis* Q16 treatment; 4. mixed *Bacillus* sp. Q3 and *P. chlororaphis* Q16 treatment. The seeds were sown 16.05. and plants were grown until 17.07.2013 in pots on open air (in conditions outside). Each pot was thinned to the 12 juvenile plants.

The young plants at 4-leaf stage under conditions of natural infection with the powdery mildew were treated with culture solutions of *Bacillus* sp. Q3, *P. chlororaphis* Q16 and mix solutions Q3 and Q16 (concentration 10⁶ CFUml⁻¹). The powdery mildew populations on leaves were assessed before and one week after the application. The percentage of leaf area affected were estimated on lower leaves before application, and the upper (young) leaves after application according to the EPPO scale PP1/26 [36] and calculated used formula:
\[ P = \frac{\sum (n v)}{4N} \times 100 \]

(P = percentage of infection; n = number of leaf in each category; v = category of infection; N = total number of evaluated leaf)

The efficiency was calculated by the formula:

\[ E = \frac{DIC - DIT}{DIC} \times 100 \]

(DIC – Disease incidence in control; DIT – Disease incidence in treatment)

Plant parameters evaluated were: fresh and dry weight of plants, NO₃ and N content in wheat leaves. N content was measured using non-destructive methods of fresh leaves by Cardy Nitrate Meter 2305G, according the instruction of the manufacturer (Spectrum Technologies, Inc., USA).

Data were subjected to analysis of variance (ANOVA). When ANOVA showed treatment effects (P<0.05), the least significant difference test (LSD) was applied to make comparisons among the means (P<0.05). For analysis of data, STATISTICA 7 program was used.

3. Results and Discussion

According to soil and climatic conditions in Serbia, cultivation of wheat was based mainly on local varieties (e.g. Renesansa, Etida, NS 40S, Panonija- from IFVC Novi Sad). Some foreign varieties were introduced subsequently (CCB Ingenio, Illico, Bologna, SY Moisson- Syngenta). The use of PGPR may prove useful in developing strategies to facilitate growth of domestic and foreign wheat varieties in Lessivated Cambisols. Given that the PGPR inoculants are inexpensive, simple to use, and have no adverse effects on land, we tested the effects of two strains - *Bacillus* sp. Q3 and *P. chlororaphis* Q16, on plant health, biomass and nitrogen content in two wheat cvs. NS 40S and CCB Ingenio.

**Soil Properties**

Lessivated Cambisols belongs to the category of medium heavy soils, with a clear texture differentiation within the profile.

Humus-accumulative horizon is characterized with a favorable water-air regime, as result of optimal ratio of large, medium and fine pores. This type of soils is well drained and warm. In Table 2, the results of soil granulometric composition of plowed layer of the studied soil (Lessivated Cambisols) are presented. The results show that the soil is medium heavy by mechanical properties, with total technical clay fraction about 68.2 %, colloidal clay fraction of 31 % in Lessivated Cambisols.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Lessivated Cambisols Granulometric composition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bulky sand, 2-0.2mm</td>
<td>0.80</td>
</tr>
<tr>
<td>Miniature sand, 0.02-0.002mm</td>
<td>31.0</td>
</tr>
<tr>
<td>Dust, 0.02-0.002mm</td>
<td>37.2</td>
</tr>
<tr>
<td>Clay, &lt;0.002mm</td>
<td>31.0</td>
</tr>
<tr>
<td>Total sand,&gt;0.02mm</td>
<td>31.8</td>
</tr>
<tr>
<td>Dust+Clay, &lt;0.02mm</td>
<td>68.2</td>
</tr>
</tbody>
</table>
Bacterial antagonists *Bacillus* sp. Q3 and *Pseudomonas chlororaphis* Q16 capable to control wheat powdery mildew in wheat

The studied Lessivated Cambisols have acid reaction, with pH in 1M KCl 4.98. Soils with higher organic content show less pronounce response to metal input, although this tendency is not always consistent. Tested soil has medium content of humus, low content of soluble phosphorus and is well supplied with available potassium. The content of soluble calcium is average. The content of available Mg and microelements are generally within the range of optimal supply. The overall content of analyzed microelements and heavy metals has been falls within the usual content level in agricultural soils.

Lessivated Cambisol belongs to the soils with high ecological and production values, due to high dept, with relatively favorable mechanical and other physical composition and characteristics offering the plants deep development of root system and intensive growth of vegetation. Limits are within the climate factors, relatively high average temperatures and relatively low level of average participations.

Lessivated Cambisols belongs to the second quality class of agricultural soils and it is suitable for crop, vegetable, fruit and grape production. In Table 3 the results of soil chemical characteristics and elemental composition of plowed layer of the studied soil (Lessivated Cambisols) are presented.

<table>
<thead>
<tr>
<th>Property/composition</th>
<th>Lessivated Cambisols</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH in H₂O</td>
<td>5.96±0.03</td>
</tr>
<tr>
<td>pH in 1M KCl</td>
<td>4.98±0.03</td>
</tr>
<tr>
<td>The sum of bases - S (cmol kg⁻¹)</td>
<td>21.98±2.65</td>
</tr>
<tr>
<td>Potential acidity - Y'</td>
<td>11.50±2.10</td>
</tr>
<tr>
<td>Cation exchange capacity - CEC (cmol kg⁻¹)</td>
<td>29.46±4.21</td>
</tr>
<tr>
<td>Base saturation-V (%)</td>
<td>74.62±1.27</td>
</tr>
<tr>
<td>Total N (%)</td>
<td>0.28±0.01</td>
</tr>
<tr>
<td>Available P₂O₅ (mg 100g⁻¹)</td>
<td>7.98±0.62</td>
</tr>
<tr>
<td>Available K₂O (mg 100g⁻¹)</td>
<td>21.8±1.03</td>
</tr>
<tr>
<td>Available Ca (mg 100g⁻¹)</td>
<td>440±15</td>
</tr>
<tr>
<td>Available Mg (mg 100g⁻¹)</td>
<td>38±1.13</td>
</tr>
<tr>
<td>Available Fe (mg kg⁻¹)</td>
<td>63±0.6</td>
</tr>
<tr>
<td>Available Mn (mg kg⁻¹)</td>
<td>43±1</td>
</tr>
<tr>
<td>Available Zn (mg kg⁻¹)</td>
<td>2.2±0.4</td>
</tr>
<tr>
<td>Available Cu (mg kg⁻¹)</td>
<td>4.1±0.3</td>
</tr>
<tr>
<td>Available Co (mg kg⁻¹)</td>
<td>0.26±0.01</td>
</tr>
<tr>
<td>Available B (mg kg⁻¹)</td>
<td>0.73±0.04</td>
</tr>
</tbody>
</table>

The major factors controlling trace metal concentrations in soil are organic C content, pH, CEC and Fe, Al, Ca, Mg and P concentrations [37].

The optimum pH range for growth of most crops in soil is between 5.5 and 7.0, within which most plant nutritive are available [38]. In addition to the aforementioned growth limitations some trace elements may pose a toxicity threat if present at elevated levels as their availability and mobility increases under acidic conditions [39].

**Disease Incidence (DI)**

Observing occurrence of natural infection with the powdery mildew at 4-leaf stage wheat, it was detected that the seeds immersed in the bacterial suspension gave healthier plants. DI in the water control was higher than the DI of plants with the treated seeds, except for
Bacillus sp. Q3 applied on NS 40S (table 4; figure 1 and 2). By immersing the wheat seeds of the CCB Ingenio cultivar in the bacterial suspension DI dropped from 12.05% (Q3+Q16) to 46.1% (Q3). Cultivar NS 40S showed a drop in DI of 77% under effect of Q16 and Q3+Q16 application on seeds.

The foliar treatment with the Q3 strain with the starting disease incidence (DI) of 27.97% cv. CCB Ingenio and 50.5% cv. NS 40S, resulted in the DI of 2.01% for the cv. CCB Ingenio and 6.26% for the cv. NS 40S. The foliar treatment with the strain Q16, with the starting DI of 30.26% for the cv. CCB Ingenio and 16.77% for the cv. NS 40S resulted in the DI of 16.30% for the cv. CCB Ingenio and 6.35% for the cv. NS 40S. When the mixture of the two strains Q3 and Q16 were applied on the cultivars with the starting DI of 45.62% for the cv. CCB Ingenio and 16.76% for the cv. NS 40S the following DI was recorded: 14.69% for the cv. CCB Ingenio and 6.09% for the cv. NS 40S. Therefore, the highest and most uniform efficacy was observed with the strain Q3 -89.26% on the cv. CCB Ingenio and 76.22% on the cv. NS 40S. The efficacy of the strain Q16 and a mixture of Q3 and Q16 is very low on the cv. CCB Ingenio (12.98% and 21.56%) and satisfactory on the cv NS 40S (75.87 and 76.86%).

### Table 4- Disease incidence on wheat leaves before and one week after application of Bacillus sp. Q3 and Pseudomonas chlororaphis Q16 and their mixture. The seeds were previously incubated for 2h with the same bacterial suspension(s) before sowing.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Disease incidence$^\text{a}$</th>
<th>Efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before application (BA)</td>
<td>After application (AA)</td>
</tr>
<tr>
<td>Water control</td>
<td>51.87±11.40</td>
<td>50.42±7.13</td>
</tr>
<tr>
<td>Q3</td>
<td>27.97±6.76</td>
<td>50.50±8.70</td>
</tr>
<tr>
<td>Q16</td>
<td>30.26±12.09</td>
<td>16.77±7.28</td>
</tr>
<tr>
<td>Q3+Q16</td>
<td>45.62±9.36</td>
<td>16.76±4.01</td>
</tr>
</tbody>
</table>

$^\text{a}$ means ± standard deviation.

Fig. 1 DI of the wheat cultivar CCB Ingenio infection before and after foliar treatment (BA- before foliar application; AA- after foliar application). The seeds were previously incubated 2h with the same bacterial suspension(s) before sowing.
Untreated (control) leaves had a higher DI than when treated with the bacterial suspension. Disease suppression in Q3-treated plant’s leaf surfaces was very high and uniform on both treated cvs.- 89.26% (CCB Ingenio) and 76.22% (NS 40S). In addition to the initial disease suppression, foliar treatment with Q16 and a mixture of Q3+Q16 expressed a satisfactory efficacy only on the cv. NS 40S.

Our data are consistent with many of the studies that have investigated antagonist mixture effects on the suppression of foliar diseases. *Pseudomonas fluorescens* and a mixture of *Trichoderma* spp. isolates are reported as the most effective bio-control agents in controlling powdery and downy mildew, as well as the most effective in yields improving. (*Bacillus subtilis* is reported as a growth stimulant [40] and can induce ISR in some plants [41]. [42,43] found that PGPR applied individually were almost always as effective in controlling diseases as mixtures. [44,45] suggest that mixtures can lead to synergistic effects on disease control, outperforming the best biocontrol agents used singly. In contrast, it has been reported that such mixtures do not improve disease suppression, mainly due to incompatibility between the co-inoculants, such as when two biological agents inhibiting each other [46]. Our data are closer to the second group of results for cv. CCB Ingenio. All treatments had similar effectiveness for NS 40S wheat cultivar. Nevertheless, research should continue to include a greater number of varieties and antagonists in conjunction with the synthetic materials as recommended by [47].

**Effects of Bacillus sp. strain Q3 and P. chlororaphis strain Q16 on wheat growth**

The effects of applied strains on wheat growth were by limited by the poor production capacity of soil and extreme climatic conditions. The results obtained (Table 5 and 6) indicated that wheat SDW of cultivars NS 40S and CCB Ingenio were significantly higher after inoculation with *Bacillus* sp. Q3 and *P. chlororaphis* Q16 as well as their mixture compared to the control. There were no significant differences between SDW in inoculated treatments of cultivar CCB Ingenio (510 and 534 mg plant⁻¹), while the greatest SDW was recorded in cultivar NS 40S treated with strain Q3 (534 mg plant⁻¹) followed by strain Q16.
(467 mg plant\(^{-1}\)). The research results didn’t show the effect of tested strains and their mixture on root dry weight while some results indicated root increase in inoculated wheat with *Pseudomonas* strains [48].

SDW of inoculated cultivars NS 40S and CCB Ingenio were increased by 17-39% and 35-43% over control, respectively. The influence of *Bacillus* sp. Q3 on SDW increment of the both cultivars was similar (39 and 37%, respectively) in comparison to their controls while the influence of strain Q16 and mixed strains on SDW were much higher in cultivar CCB Ingenio, 43% and 35%, respectively. Results indicate that the effect of strains depend on wheat cultivar. In contrast to our results, [49] reported that wheat inoculation with a combination of *P. putida* and *B. lentus* increased the % seed germination, grain yield and 1000-seed weight, without statistically significant difference between two tested cultivars (Chamran and Pishtaz).

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Fresh weight (mg plant(^{-1}))</th>
<th>Dry weight (mg plant(^{-1}))</th>
<th>SDW increment (%)</th>
<th>NO(_3)-N (mg kg(^{-1}))</th>
<th>N (mg kg(^{-1}))</th>
<th>N increment (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ø</td>
<td>1521 ±351</td>
<td>273 ±62</td>
<td>384±67</td>
<td>132±31</td>
<td>100</td>
<td>3033 ±493</td>
</tr>
<tr>
<td>Q3</td>
<td>2401 ±708</td>
<td>386±106</td>
<td>534 ±100</td>
<td>170±53</td>
<td>139</td>
<td>2433 ±513</td>
</tr>
<tr>
<td>Q16</td>
<td>2428 ±602</td>
<td>319±65</td>
<td>467 ±85</td>
<td>136±28</td>
<td>121</td>
<td>5467 ±513</td>
</tr>
<tr>
<td>Q3+Q16</td>
<td>2239 ±292</td>
<td>378±61</td>
<td>433±54</td>
<td>158±22</td>
<td>117</td>
<td>2633 ±351</td>
</tr>
<tr>
<td>LSD .05</td>
<td>455.43</td>
<td>68.79</td>
<td>71.11</td>
<td>32.10</td>
<td>889</td>
<td>200</td>
</tr>
</tbody>
</table>

NSD indicates no significant difference at the P = 0.05 level of significance whereas *, ** and *** indicates statistical significant differences at the P<0.05, P<0.01 and P<0.001 levels, respectively. LSD indicates least significant differences. (means ± standard deviation).

The data of presented study showed that bacterial strains tested were able to promote growth of two wheat cultivars though increase SDW. Increase in biomass (19%) and in nitrogen yield (25%) compared to the control were reported with bacteria inoculation in wheat [50]. In pot and field experiments a significant increase in wheat straw yield was observed in plants inoculated with the most efficient P-solubilizing *Bacillus* [51, 52]. *Pseudomonas* spp. showed many plant growth promotion activities and among them the most important are: production of ACC deaminase, IAA like products as well as P solubilization. Increase in total N yield can be result of plant inoculation by PGPR which can possess many of mechanisms for PGP [53]. It is known that *Bacillus* and *Pseudomonas* species cause yield increases in wheat using the same mechanisms [54, 48]. Many results showed that bacterial strains of *Bacillus* and *Pseudomonas* are able to produce some phytohormones [55].
Dual bacterial treatments were applied in many experiments and sometimes a dual combination of P-solubilizing and N-fixing bacteria did not yield good outcome [56, 53]. The interaction of strains mixed culture can be synergistic or interaction of N fixing bacteria can inhibit N fixation or plant growth [53].

N uptake and distribution in plant involves many aspects of growth and development. Nitrogen content in the fresh leaf was measured using Cardy Nitrate (NO₃⁻) meter, as rapid and accurate screening methods of bacterial effectiveness. Cardy Nitrate (NO₃⁻) meter was used to determine nitrate-nitrogen concentrations in above ground biomass of winter cover crops [57]. It was also used for assessing soil nitrogen in the vegetable crops (cabbage, carrots and onions) grown on organic and mineral soils [58]. In this study, the highest N content was measured in NS 40S cultivar treated with *P. chlororaphis* Q16 strain (1237 mg kg⁻¹) and the lowest in untreated CCB Ingenio cultivar (197 mg kg⁻¹). *Bacillus* sp. Q3 adversely affected N content in cv. NS 40S regardless of the applied treatment. The greatest increase on N content between the treatments was observed for *P. chlororaphis* Q16 in both wheat cultivars. Although both cultivars showed SDW value in range 373 to 534 mg plant⁻¹, the N values for cv. CCB Ingenio (197-497 mg kg⁻¹) were lower than for cv. NS 40S (550-1237 mg kg⁻¹). The percentage of N increment ranged from 59% in *Bacillus* sp. Q3 treatment to 152% in *P. chlororaphis* Q16 treatment for cv. CCB Ingenio.

Many authors reported positive growth responses of wheat (*T. aestivum* L.) to inoculation with PGPB [52]. Inoculation of wheat with *Bacillus* sp. increased the mass of soil adhering to the roots which enhanced the stability of soil aggregates, stimulated plant growth and increased biomass and grain yield [59, 60, 61]. *Bacillus* species used as biofertilizers may have direct effects on plant growth through the synthesis of plant growth hormones, N₂-fixation and synthesis of the enzymes modulating the level of PGPR [62, 54, 63, 64, 52].

### 4. Small Conclusions

This experiment was performed on a limited production capacity soil type Lessivated Cambisol, under the extreme climatic conditions comparing to the long-term average regarding precipitation and relative humidity. Obtained results from the experiment on growing wheat cultivars are definitely affected by the above mentioned restrictions of soil and climatic conditions.

Seeds treatments with *Bacillus* sp. Q3 strain reduced the occurrence of powdery mildew infection on wheat cv. CCB Ingenio (46.1%) and increased SDW of the both cultivars (37-39%) in comparison to their controls, but decreased N content in cv. NS 40S. The additional foliar treatment with the *Bacillus* sp. Q3 strain provided high disease suppression efficacy in both wheat cultivars.

The best results were obtained in cv. NS 40S and its seeds treatments with *P. chlororaphis* Q16 strain - 77% of DI reduction and the highest N content (1237 mg kg⁻¹), as well as the foliar treatment which reduced DI to only 6.35% (or 6.09% if was applied as mixture). The mixture of Q3 and Q16 strains resulted in decreasing DI from 45.62% to 14.69% for cv. CCB Ingenio).

Further investigations will include application of *P. chlororaphis* Q16 strain as biofertilizer for both wheat cultivars. The additional foliar treatments with this strain (as a sole or mixture variant) for cv. NS 40S will be applied in a field experiment of powdery mildew prevention or DI reduction.
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References
Bacterial antagonists *Bacillus* sp. Q3 and *Pseudomonas chlororaphis* Q16 capable to control wheat powdery mildew in wheat


