

The Response of Potato Minitubers to the Use of Plant Growth Promoting Bacteria in Field Conditions

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Abstract

In recent decades, Plant Growth Promoting Bacteria (PGPRs) have been widely utilized as an environmentally friendly achievement that enhances soil fertility without causing pollution, ultimately increasing plant production. This experiment aimed to investigate the effect of growth-promoting bacteria on minitubers of three potato cultivars in a factorial design based on RCBD (completely randomized block design), using two factors in three replications. These factors included minitubers of potato cultivars (Agria, Caesar, Banba) and PGPRs (without PGPRs and PGPRs inoculation including *Pseudomonas putida*, *Azospirillum lipoferum*, and *Azotobacter chroococcum*). Experiment results indicated that the application of *A. lipoferum* significantly impacted the number of tubers in cv. Agria and Banba, while *P. putida* had a notable effect on the number of tubers in cv. Banba. The highest tuber weight was achieved in cv. Agria and Caesar and the highest dry weight of tubers were observed in the cv. Agria with the application of *A. lipoferum*. The application of *A. chroococcum* had the most significant influence on the Fresh weight of shoots in the cv. Agria. Based on the findings of this study, the utilization of *Azospirillum lipoferum* bacteria in potato cultivation could lead to an increase in potato yield.

Keywords

Minitubers, *Azospirillum lipoferum*, *Azotobacter chroococcum*, *Pseudomonas putida*, Potato

1. Introduction

Potato (*Solanum tuberosum* L.) is recognized as one of the foremost, cost-effective, and most valuable food commodities worldwide. In 130 countries, where approximately three-fourths of the global population resides, potato consumption is prevalent, encompassing over one billion individuals worldwide [1]. In the past, potatoes were reproduced by tubers. At the present time, potato seed production is achieved through microtubers and minitubers, which is a significant advancement. It not only reduces the production and revenue cycle, but also by providing quality seeds for farmers, it leads to yield increase [2]. The issue of food safety constitutes a substantial global challenge, while a significant objective within the realm of agriculture is the ongoing advancement of high-quality agricultural output alongside their processing and preservation [3]. To augment agricultural performance, the judicious utilization of mineral fertilizers, guided by optimal standards for enhancing soil fertility and inducing productive crop yields, is imperative [4]. In contemporary agricultural practices, the utilization of biofertilizers has acquired pronounced importance due to their role in bolstering crop production, preserving soil fertility, and fostering sustainable agricultural practices [5]. Plant growth-promoting bacteria, through the synthesis of auxins, zeatin, gibberellins, nitrogen fixation, and iron solubilization, play a pivotal role in fostering plant growth and facilitating the equitable distribution of essential nutrients, a function they execute within the

rhizosphere [6]. These microorganisms have effectively ameliorated organic carbon content, water composition, soil acidity, alkalinity, and soil porosity [7]. In order to maximize the impact of biofertilizers on plant growth and yield, the judicious application of appropriate microorganism strains, characterized by heightened efficiency, assumes paramount significance [8]. In the context of this research, three bacterial types, namely *Azospirillum*, *Azotobacter*, and *Pseudomonas*, were employed. Multiple reports have elucidated the efficacy of these aforementioned bacteria in influencing the performance of potato plants. Notably, the *Azospirillum* genus, distinguished among growth-promoting bacteria, holds the capacity to significantly amplify the growth and development of microcolonies within potatoes, particularly under controlled laboratory conditions [9]. Moreover, a substantial impact resulting from the application of *Azotobacter chroococcum*, concomitant with phosphate-solubilizing bacteria such as *Pseudomonas*, on potato plant performance has been extensively documented [10]. This study aims to investigate the impact of growth-promoting bacteria on the growth and performance of three potato cultivars under field conditions.

2. Methods and materials

This study was conducted during 2021–2022 at the Ardabil Agricultural Center and Natural Resources. The place of the experiment has a semi-arid and cold climate. During the winter, the temperature is usually below zero. The altitude is 1,350 meters above sea level, and the longitude and latitude are 20°48' and 15°38', respectively. The average minimum and maximum annual temperature and the absolute maximum temperature are 1.98, 15.8, and 21.58 Celsius, respectively, and the average annual rainfall is 291 mm [11]. The experiment was conducted in a factorial design based on a complete randomized block arrangement with three replications and two factors. The first factor consisted of mini-tubers of three cultivars: *Agria*, *Caesar*, and *Banba* (Table 1), and the second factor had four levels, which were the absence and application of growth-promoting bacteria, including *Azospirillum lipoferum* of strain, *Pseudomonas putida* 169 strain, and *Azotobacter chroococcum* 5 strain. The minitubers were individually submerged for 20 minutes in a bacterial suspension solution for separate bacterial inoculation and then transferred to the primary planting substrate. Fertilizer application was as follows: 25% potassium sulfate fertilizer in one instance, 15% ammonium phosphate fertilizer in two instances, and 30% ammonium nitrate fertilizer in three instances, based on soil analysis (Table 2). This arrangement involved mixing 25% ammonium nitrate, 50% ammonium phosphate, and the entire potassium sulfate together, placing them in the created trench, and covering them with a 5 cm layer of field soil. Subsequently, the mini-tubers were placed on the soil bed and covered with soil to a depth of about 5 cm. The planting date was the twentieth day of April. The planting and field preparation was conducted following the method established by Farn et al. [12]. The traits under study in the field included the number and weight of tubers per plant, dry weight of tubers, plant height, and Fresh weight of shoots per plant. These attributes were randomly selected and measured during the growth period and after harvesting. Variance analysis of the data resulting from the experiments was carried out using the SAS software, and mean comparisons based on the LSD test were performed using the MSTATC software.

Table 1. Specifications of potato cultivars

Cultivars	Specifications						
	Tuber form	Skin color	Flesh color	Eye depth	Maturation	Harvest rate	Dry matter
<i>Agria</i>	oval	creamy	sharp yellow	shallow	late	high	medium to high
<i>Banba</i>	oval	yellow	light yellow	shallow	medium early	very high	medium
<i>Caesar</i>	oval	creamy	light yellow	very shallow	medium	high	medium to high

Table 2. The results of soil analysis of the field experiment

Soil characteristics										
Mg	Ca	P	K	SP	O.C	TNV	Total nitrogen	pH	EC	soil texture
(ppm)			(%)					(S/m)		
<i>6/59</i>	<i>6/68</i>	<i>17/9</i>	<i>433</i>	<i>51</i>	<i>1/2</i>	<i>8/95</i>	<i>0/14</i>	<i>7/80</i>	<i>1/084</i>	Silty clay loam

3. Results and discussion

Table 3. Variance analysis of the effect of potato cultivars and PGPRs

S.O.V.	df	Number of tubers	Weight of tubers	MS	
				dry Weight of tubers	Fresh weight of shoots
Replication	2	<i>0194^{ns}</i>	<i>2655/188^{**}</i>	112/363 ^{**}	<i>581/021^{ns}</i>
Cultivar(A)	2	3/241 ^{**}	173027/896 ^{**}	7386/783 ^{**}	29543/063 ^{**}
PGPRs (B)	3	3/111 [*]	23706/063 ^{**}	2169/145 ^{**}	2169/145 ^{**}
A*B	6	4/796 ^{**}	18208/646 ^{**}	747/435 ^{**}	747/435 ^{**}
Error	24	0/285	209/551	8/686	<i>7243</i>
C.V. (%)	-	<i>18/14</i>	<i>2/41</i>	<i>2/37</i>	<i>2/09</i>

ns, * and ** are insignificant and significant at the 5% and 1% probability levels, respective

4. The number of tubers

Analysis of variance showed that the effect of the interaction of cultivar and PGPRs on the number of tubers in m² was significant with $P < 0.01$ (Table 3). Based on the mean comparison results, the cv. Agria and Banba, when treated with *A. lipoferum*, exhibited the highest number of tubers per m² with means of 24.89 and 24, respectively. Similarly, the cv. Banba treated with *P. putida* produced the highest number of tubers per m² with a mean of 24.70, surpassing the numbers from cultivars inoculated with *Azotobacter chroococcum* bacteria and the control (Figure 1). The auxin hormone produced by microorganisms stimulates germination, accelerates root growth, alters root system structure, and promotes root development [13]. External application of auxin has been confirmed to enhance tuber formation and increase gene expression levels in auxin biosynthesis during the potato tuber development phase [14]. Reported data indicate an up to 45% increase in the number of potato tubers after inoculation with *Azospirillum* SPP bacteria [15]. *Pseudomonas* SPP bacteria have been highlighted as some of the most potent solubilizers of insoluble phosphate in soil [16]. Considering the vital role of phosphorus in regulating potato tuber numbers, especially during early growth stages and tuber maturation [17]. It is likely that *Pseudomonas putida* bacteria have exerted their most significant influence by accelerating plant access to phosphorus, solely leading to an increase in tuber numbers without affecting their weight in this experiment. In another trial, the application of *Pseudomonas putida* bacteria resulted in increased potato yield [15].

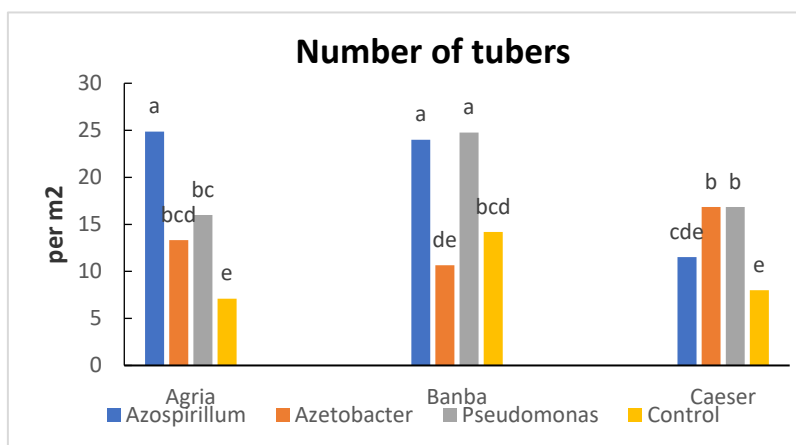


Figure 1. The interaction of cultivar and bacteria in the tuber number.

The means of every column that shares in at least one letter do not have a significant difference at the 5% probability in LSD.

5. The weight of the tuber

The interaction between Cultivar and PGPRs in the attribute of tuber weight was significant, with $P < 0.01$ (Table 3). Examination of the means indicated that the utilization of *A. lipoferum* had a significant impact on increasing tuber weight in the plant compared to the application of *P. putida*, *A. chroococcum*, and the control. The cv. Agria achieved the highest tuber weight with 800 g, followed by cv. Caesar with 790 g under this treatment (Figure 2). Two characteristics defined

for *Azospirillum* spp bacteria are nitrogen fixation and phytohormone production [19]. Elevated nitrogen levels contribute to the growth, development, and translocation of photosynthetic products from the source to the sink, resulting in increased performance [20]. Inoculation with *Azospirillum* spp primarily enhances root growth, water absorption, and mineral uptake, with the translocation of growth-promoting substances, especially auxins, responsible for these effects. In addition to auxins, other hormones such as cytokinin and gibberellins are also translocated by bacteria of this genus. Furthermore, the production and release of nitric oxide by these bacteria are essential factors in stimulating lateral root growth in plants [21]. In a field experiment, potato inoculation with *Azospirillum* spp bacteria resulted in a reported 30% increase in tuber weight compared to the control [22, 23].

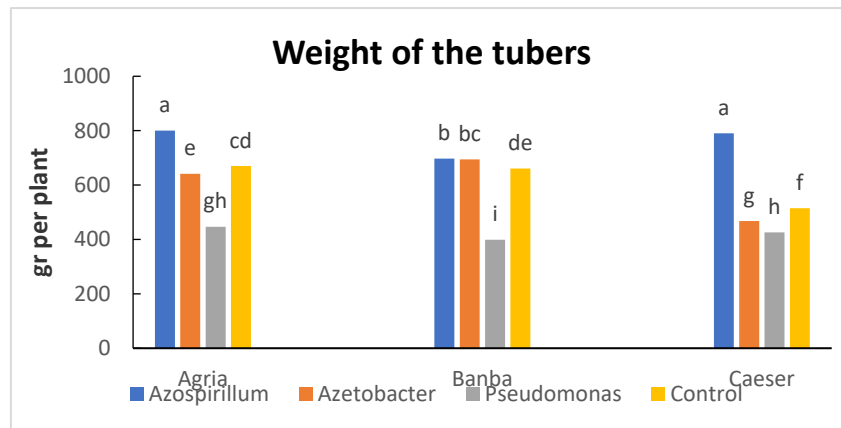


Figure 2. The interaction of cultivar and bacteria in tuber weight.

The means of every column that share in at least one letter do not have a significant difference at the 5% of probability in LSD.

6. The dry weight of tubers

There was a statistically significant interaction effect between Cultivar and PGPRs on the dry weight of tuber g per plant at the 1% probability level (Table 3). The comparison of means demonstrated that the application of *A. lipoferum* bacteria had a significant impact on increasing the dry weight of tubers compared to the bacteria *P. putida*, *A. chroococcum*, and the control. The highest g dry weight of tubers was achieved in cv. Agria with an average of 169.9, obtained through the aforementioned treatment (Figure 3). The production of abscisic acid is mentioned as one of the capabilities of *Azospirillum lipoferum* [24]. It has been reported that abscisic acid hormone enhances transportation to the destination. Levels of abscisic acid in the destination during periods of material accumulation through photosynthesis increase, suggesting that abscisic acid and auxin might influence the relative destination uptake ability by regulating relative sucrose uptake [25]. Furthermore, the enhancement of starch synthesis and accumulation in the pith, as well as disruptions in these processes in the cortex, occur due to changes in abscisic acid content. An increase in potato dry weight has been reported through the application of *Azospirillum* spp strains under field and in Under laboratory conditions, also known as in vitro conditions [23].

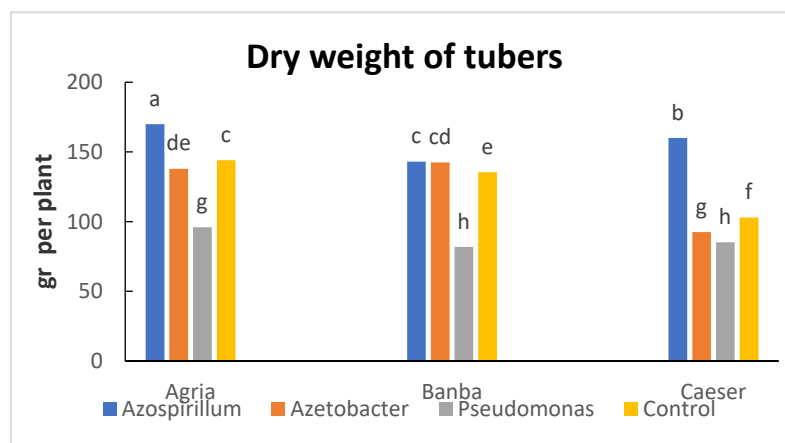


Figure 3. The interaction of cultivar and bacteria in tuber dry weight.

The means of every column that share in at least one letter do not have a significant difference at the 5% of probability in LSD.

7. Fresh weight of shoots

The interaction effect of cultivar and PGPRs was significant at the 1% probability level in the Fresh weight of shoots (Table 3). The comparison of the Fresh weight of shoots (Figure 4) shows that the use of *A. chroococcum* has a positive effect on increasing the weight of the leaves of cv. Agria and the average is 569 g per plant for this variety, but this procedure is the same for cv. Banba and Caesar. The treatment is not correct; therefore, the lowest fresh weight of the shoots is related to cv. Caesar with *A. chroococcum* treatment with an average of 289 g per plant. Cultivars affected by *A. lipoferum* generally had more shoots weight than the control cultivars. In this group, cv. Agria was at the top with an average of 536 g, and all the cultivars with *P. putida* treatment had lower shoot weights compared to the control cultivars. In addition to nitrogen fixation, *Azotobacter* spp enhances plant growth and performance by producing vitamins such as thiamine and riboflavin, as well as releasing growth-promoting substances like indole-3-acetic acid (IAA), gibberellins, and cytokinins [27]. Alongside notable changes in plant growth and development following seed treatment with *Azotobacter* spp, it was acknowledged that hormonal properties cannot solely be attributed to accelerated growth due to limitations in plant hormone synthesis during the stationary phase. Thus, it was suggested that other important indicators present in *Azotobacter* spp should be considered, and overall growth progress should be attributed to all available mechanisms of this bacterium [28]. Considering this, and the effect of the bacterium on increased shoot growth, it is likely due to nitrogen fixation. Some studies have proposed an excellent state for *Azotobacter* spp in terms of nitrogen fixation [29]. Concerning the role of nitrogen, it has been stated that low nitrogen levels not only lead to lower performance but also result in reduced leaf area and subsequently diminished tuber size. On the other hand, an excessive amount of nitrogen leads to increased dry matter production in plant sections other than the tubers [30].

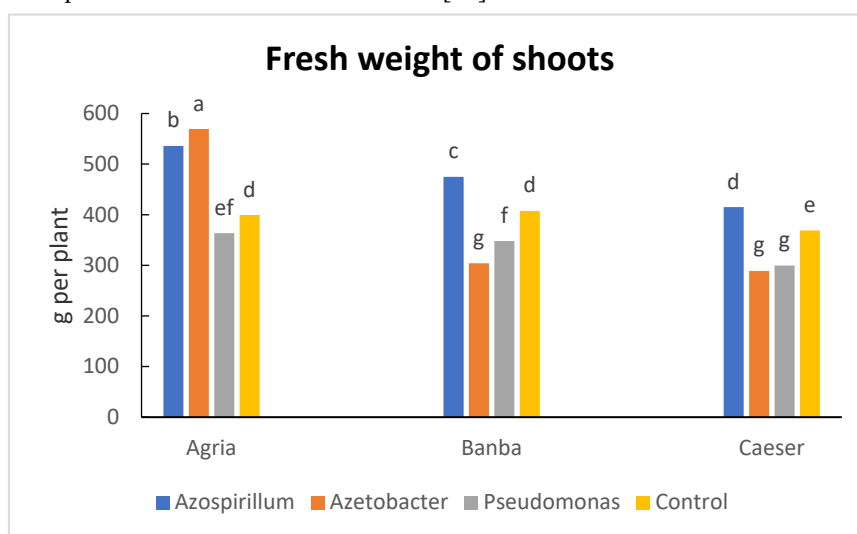


Figure 4. The interaction of cultivar and bacteria in Fresh weight of shoots.

The means of every column that share in at least one letter do not have a significant difference at the 5% of probability in LSD.

8. Conclusion

Among the available tools for achieving sustainability in agriculture, bio-organic fertilizers play a significant role. This is because they possess all the desired characteristics of soils and have beneficial effects on the physical, chemical, and biochemical properties of soils. Substituting bio-organic fertilizers for mineral and organic fertilizers leads to a considerable increase in dry matter production. Moreover, the application of these fertilizers in infertile regions also results in an increase in soil organic matter and enhances the uptake of micro-nutrients in these soils. The application of these fertilizers can lead to improved agricultural yield and quality without the need for extensive capital investment and labor. Additionally, these bio-organic fertilizers can contribute to environmental remediation by reducing pollutants. According to the findings of this research, the bacterium *A. lipoferum* OF had the greatest impact on increasing the number and weight of tubers, while the bacterium *A. chroococcum* demonstrated the most significant effect on increasing shoot weight in the Agria cultivar.

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