Agronomical performance of potato (Solanum tuberosum L.) cv. ´Unica´ under inoculation with native rhizobacteria and application of acetyl salicylic acid

Desempenho agronômico da batata (Solanum tuberosum L.) cv. ´Unica´ sob inoculação com rhizobactérias nativas e aplicação de ácido acetil salicílico

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ABSTRACT

Peru is center of origin of potato and its wild relatives, so it is convenient to develop new technologies of agronomical management with less impact on the ecosystem, as in the use of pesticides and chemical fertilizers in potato is increasing in developing countries. The objective of the work was to evaluate the agronomic performance of potato cv. ´Unica´ under inoculation with PGPR rhizobacteria isolated from the rhizosphere of Andean potatoes and on the effect of the application of acetylsalicylic acid in field conditions of the Peruvian coast. Inoculation of four bacterial isolates were evaluated: Bacillus simplex B13, Bacillus amyloliquefaciens Bac 15Mb, Azotobacter sp. Azo 16M2 and Pantoea sp. DZ22, as well as a dose of 0.4 mM acetyl salicylic acid as promoter treatments for plant growth versus a control with no inoculation. A randomized complete block design was used in a sandy loam class soil, slightly alkaline, with low organic content and free of salts. There was a relative humidity between 54 and 71%, while the average air temperatures ranged between 13 and 21 °C in San Vicente de Cañete, Lima (coordinates UTM 18L3540788550372). Inoculated plants with bacterial strains at sowing time and control were evaluated at flowering in plots of 40 plants with three replications for several physiological and productive parameters. Means were separated statistically through Duncan’s multiple range test and processed with Infostat software. Inoculation with strain Azotobacter sp. Azo 16M2, showed significant differences (p<0.05) with respect to control without inoculation, increasing tuber yield and commercial tuber weight per hectare and with less damage by the leafminer fly (Liriomyza huidobrensis) vis-à-vis the control. Also application of acetyl salicylic acid showed higher percentage of inflorescences (p<0.05) compared to the control, while statistical differences were not found between bacterial strains for this character. It was concluded that the application of inoculant strain Azotobacter sp. Azo 16M2 improved the agronomic performance of potato cv. ´Unica’, under subtropical conditions of the valley of Cañete in terms of production of tubers and commercial quality, and less foliage damage by the leafminer fly compared to non-inoculated plants.

KEYWORDS: Azotobacter, agroecology, PGPR, potato-associated bacteria, plant-microbe interaction, salicylic acid.

RESUMO

Peru é o centro de origem da batata e seus parentes silvestres, por isso é conveniente desenvolver novas tecnologias de gestão agronômica com menor impacto no ecossistema, como o uso de pesticidas e fertilizantes químicos em batata está aumentando nos países em desenvolvimento. O objetivo do trabalho foi avaliar o desempenho agronômico da cv. ´Unica´ sob inoculação com rizobactérias PGPR isoladas da rizosfera de batata andina e pelo efeito da aplicação de ácido acetilsalicílico nas condições de campo da costa peruana. Avaliou-se a inoculação de quatro isolados bacterianos: Bacillus simplex B13, B. amyloliquefaciens Bac 15Mb, Azotobacter sp. Azo 16M2 e Pantoea sp. DZ22 bem como uma dose de 0,4 mM de ácido acetilsalicílico como promotores de tratamentos para o crescimento das plantas versus um controle sem inoculação. Utilizou-se um delineamento em blocos casualizados, em um solo franco-arenoso,

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ligeramente alcalino, de bajo contenido orgánico e libre de saí. O clima durante o ensaio apresentou uma umidade relativa entre 54 e 71%, temperaturas médias entre 13 e 21 °C em San Vicente de Cañete, Lima (coordenadas UTM 18L3540788550372). As plantas foram inoculadas com estirpes bacterianas no momento da semeadura e na floração e os tratamentos foram avaliados em parcelas de 40 plantas com três repetições para diversos parâmetros fisiológicos e produtivos. Os dados foram analisados estatisticamente através do teste de Duncan e processados com o software Infostat. A inoculação com a estirpe Azo 16M2, apresentou diferenças significativas (p<0,05) em relação ao controle sem inoculação, aumentando a produção de tubérculos e o peso comercial de tubérculos por hectare e com menor dano pela mosca minadora (Liriomyza huidobrensis) em relação ao controle. Também a aplicação de ácido acetilsalicílico apresentou maior porcentagem de inflorescências (p<0,05) em relação ao controle, enquanto que diferenças estatísticas não foram encontradas entre as cepas bacterianas para este caráter. Concluiu-se que a aplicação da estirpe inoculante Azotobacter sp. Azo 16M2 melhorou o desempenho agronômico da batata cv. 'Unica', em condições subtropicais do vale de Cañete, em termos de produção de tubérculos e qualidade comercial, e menor dano foliar por mosca minadora em comparação com plantas não inoculadas.

PALAVRAS-CHAVE: Azotobacter, agroecologia, PGPR, interação microbiana de plantas, bactérias associadas à batata, ácido salicílico.

INTRODUCTION

More than 80% of the agricultural area in Peru is under smallholders with less than 20 hectares. At present, potato is cultivated in more than 300,000 hectares nationwide (EGUREN 2012) with a national average tuber yield of 14.6 t ha⁻¹. It is known that the crop is very sensitive to a number of biotic and abiotic environmental stresses. These problems, coupled with the fact that the species is commercially propagated by vegetative parts, make crop health a considerable problem for the production of quality planting material and for commercial production (FAO 2008). In the Lima Region, around 6 266 potato hectares was grown during the agricultural season 2013-2014 with an average yield of 23.7 t ha⁻¹, according to the Bureau of Economic and Statistical Studies of the Ministry of Agriculture and Irrigation (MINAGRI 2013). In this potato production system, agricultural technology is used, as increased application of agricultural inputs (especially fertilizers and pesticides), mechanization, irrigation, phytosanitary control, technical assistance and other factors, needing to be weighed to assess whether they are used in a rational way and according to the standards of environmental, economic standards and social sustainability requirements.

In recent years, the interest for the use of rhizobacteria that promote plant growth (PGPR) has increased. The beneficial effects of these microorganisms involve the ability to act as regulators or bio fertilizers thereby increasing the yield of crops. In addition, PGPR can act as agents for bio control to produce antibiotics and trigger local or systemic resistance, preventing xenobiotic harmful effects of degradation pathogens, phenomenon that has been called phytoremediation (JACOBSEN 1997, SOMERS et al. 2004, VAN LOON 2007).

Domestication of plant species has substantially contributed to human civilization, but has also caused a strong decrease in the genetic diversity of modern crop cultivars that may have affected the ability of plants to establish beneficial associations with rhizosphere microbes (PÉREZ-JARAMILLO et al. 2016). Ages of mutualism between potato plants and soil bacteria in this region support the hypothesis that Andean soils harbor interesting plant growth-promoting bacteria (GHYSELINCK et al. 2013). In this context, systemic acquired resistance has a very interesting practical aspect. In agriculture, we could induce systemic resistance for infecting cultivars to be protected, using a strain such as PGPR, to respond to infection (VAN LOON 2007). Alternatively, farmers can spray the plants with either culture filtrates of Gram-positive or Gram-negative bacteria or even better, one of the chemicals identified as signals for induced resistance, i.e salicylic acid (SA). Since these substances are decomposed biologically and the spectrum of pathogens that can be considered is very wide, application in the release of the systemic response has good potential for plant protection. Salicylic acid is considered as a candidate for exogenous applications activating pathogenesis related proteins (PR) or related proteins, to induced resistance and acetylsalicylic acid in particular, has been selected as a low cost and nonphytotoxic product (RASKIN 1992). However, as considered by VALLAD & GOODMAN (2004), biological
limitations may hinder the practical use of chemical and biological elicitors, as the costs of fitness that plants incur when deploying these defenses, in terms of vegetative and reproductive growth.

ARCOS & ZÚÑIGA (2015) found that native strains of Bacillus sp. (Bac17M8 and Bac17M9) from highland regions of Peru and Bolivia, used to inoculate seedlings of traditional potato ‘Ccompis’ and ‘Andina’ had the ability to inhibit R. solani infection, possibly through some mechanism of antagonistic action or induced resistance. In another field-level study, it was shown that native potato cv. ‘Ccompis’ yield increased by 125.8% in plants inoculated with PGPR bacteria in comparison to control without inoculation (ARCOS & ZÚÑIGA 2016).

The overall objective of this research was to evaluate the effect of inoculation with biological inducers (native rhizobacteria) and chemical (acetylsalicylic acid) on the agronomic performance of improved potato cv. ‘Unica’ in the Cañete valley (Lima Region).

MATERIAL AND METHODS

- Materials: Four bacterial strains as inoculation treatments were provided by Laboratorio de Ecología Microbiana y Biotecnología of Universidad Nacional Agraria La Molina, which maintains a bank of bacterial strains for agricultural use. These strains were isolated from the rhizosphere of potato (VÉLEZ et al. 2008, CALVO & ZÚÑIGA 2010) and maca (Lepidium meyenii) in the Andean region.

Commercial acetylsalicylic acid at a dose of 0.4 mM (72 mg L⁻¹) was used as positive control. This dose was used taking into account available literature (RASKIN 1992, LÓPEZ et al. 2001, HAYAT & AHMAD 2007). These treatments were applied in inoculating tuber-seeds before planting as detailed below. Plant material used were tuber-seeds from in vitro plantlets of potato cv. ‘Unica’ (CIP number 392797.22) (GUTIÉRREZ-ROSALES et al. 2007) provided by the International Potato Center (CIP Genebank, Lima), and considering emergence of sprouts at inoculation time.

- Experimental site: A trial was carried out under field conditions during the spring time in the district of San Vicente de Cañete, located on the Central Coast of Peru between the coordinates: 13°06′34.21″ S, 76°20′46.67″ O, at an elevation 71 m above the sea level and about 150 km south of Lima. The soil was loam textural sandy class, pH 7.8, with low organic matter and free of salts; climate data showed a relative humidity between 54 and 71%, average temperatures between 13 and 21 °C and 3.2 hours of daily sunshine on average. NPK fertilization formula was 115-75-120 using only vermicompost equivalent to five t ha⁻¹ as a source of manure in a single dose at planting. Two applications of cypermethrin and one of cyromazine were performed to control Liriomyza huidobrensis infestation; the attack of Prodidotosis longifolia was also controlled with chlorpyrifos. No application of fungicides was carried out that could affect inoculation treatments.

- Procedures: AA dose of 30 ml of pure bacterial inoculum (density of ca. 10⁸ cfu l⁻¹) for each treatment was used approximately for a quantity of 120 potato tuber-seeds per bacterial strain for three replications and an additional 4 l of deionized water was used to make the immersion of the whole planting material, thus it bacterial inoculants in tubers were impregnated. Then, the planting material went immediately to field planting at a density of 30.000 plants per hectare. Control plots were treated only with deionized water for immersion tubers. The control + treatment was applied at a dose of 0.4 mM of previously prepared commercial acetylsalicylic acid, in immersion tubers. Later in the field after emergence of the plants, the same dose was applied with a frequency of 15 days by spraying the foliage. In all cases, the seed tuber inoculation lasted 5 minutes, after which they were sown in field plots. Biological treatments for inoculation with the bacterial strains were applied again to the plant foliage 45 days after planting at the dose of 30 ml of pure bacterial inoculum per
10 l of running water for each bacterial strain. Control plots without bacterial inoculants were applied to the foliage of plants with running water. Each plot included 40 plants and the total area per plot was 10.8 m².

- Traits evaluated: Agronomic characteristics were evaluated as listed below: number of emerged buds per plant after 15 days of planting, number of emerged plants 30 days after planting (data transformed to square root), plant height 60 days after planting (cm), percentage of surviving plants to harvest (%), percentage of damaged plants by leaf miner at 60 days, percentage of plants flowering 75 days after planting, dry weight of leaves per plot (data transformed to arc sin), foliage fresh weight per plot (data transformed to arc.sin). In addition, productive traits were evaluated, as follow: number of tubers per plant (kg) commercial tuber weight per plant (kg), noncommercial weight per plant (data transformed arc.sin), general appearance of the harvested plants measured in phenotypic scale from 1 (good) to 9 (bad), average weight of tubers per plant (kg), tuber yield projected to 30,000 plants ha⁻¹, total fresh weight of the biomass (tubers + foliage) projected per hectare.

- Experimental design and statistical analysis: A randomized complete block design (RCBD) with three replications was used. The size of experimental unit was 40 plants per treatment and 3 replicates per strain treatment. For hypothesis testing, a statistical significance level α = 5% was used. Analysis of variance for all the variables measured and the test "F" was used to determine the significance of the variance components (STEEL et al. 1997). Means for the inoculation treatments were compared using Duncan multiple range tests for each trait with a significance level α = 5%. Data was processed with InfoStat software (BALZARINI et al. 2014).

RESULTS

There were no statistical differences among treatments evaluated for: overall appearance of the harvest, tuber number per plant, noncommercial weight of tubers per plant, percentage of surviving plants in the field, plant height, foliage weight (fresh and dry), number of buds per plant emerged at 15 days after planting. Tests included in Tables 1 and 2 for productive and agronomical traits as evaluated in the experiment, noted significant differences for: total weight of the biomass per ha, tuber yield per ha, commercial weight of tubers per plant, fresh weight of tubers per plant. In addition, there were significant differences for flowering percentage, percentage of damaged plants by leafminer fly and number of emerged plants at 30 days after sowing.

Estimated total biomass per hectare under effect of the treatments was statistically above for *Azotobacter* sp. AZO16M2 in relation to control with no application. However, this effect was not significant as compared to other bacterial strains and acetylsalicylic acid (SA).

Inoculation with bacterial strain *Azotobacter* sp. AZO16M2, increased tuber yield ha⁻¹ when compared with control. Meanwhile no statistical differences were found between other bacterial strains and acetylsalicylic acid for this character.

Inoculation with the bacterial strain *Azotobacter* sp. AZO16M2 in potato increased the fresh weight of tubers per plant when compared to control, with no differences between bacterial strains and acetylsalicylic for this trait. There were differences for commercial weight of tubers (kg plant⁻¹) between *Azotobacter* sp. AZO16M2 and *B. amyloliquefaciens* Bac15MB in potato cv. ‘Unica’, showing increased commercial weight of tubers per plant when compared with control or treatment with acetylsalicylic acid (SA).

Acetylsalicylic acid at a dose of 0.4 mM significantly increased the number of inflorescences per plant compared to the control and bacterial strains except for *Pantoea* sp. DZ 22. However, there were no statistical differences between bacterial strains by comparing them with control.

In the case of percentage of damage to potato foliage by leafminer (*Liriomyza huidobrensis*), both control and inoculation of bacterial strain *Pantoea* sp. DZ22, significantly had more damage to foliage compared with the bacterial strain *Azotobacter* sp. AZO 16M2, which was affected to a lesser extent by pest damage.

A significantly higher number of plants emerged in field 30 days from planting after inoculation with bacterial strain *B. amyloliquefaciens* Bac15 MB compared to *Pantoea* sp. DZ22 and acetylsalicylic acid. There were no statistical differences among the remaining inoculated strains and the control.
Table 1. Productive traits of potato cv. ‘Unica’ under the effect of rhizobacteria and acetylsalicylic acid.

<table>
<thead>
<tr>
<th>Traits</th>
<th>Biomass</th>
<th>Yield ha⁻¹</th>
<th>Aspect</th>
<th>Com.W</th>
<th>NoCom.W</th>
<th>FWTuber</th>
<th>NTubers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>kg ha⁻¹</td>
<td>kg ha⁻¹</td>
<td>Scale</td>
<td>Arc.Sin</td>
<td>kg pl⁻¹</td>
<td>kg pl⁻¹</td>
<td># pl⁻¹</td>
</tr>
<tr>
<td>SA</td>
<td>14.725 ab</td>
<td>11.950 b</td>
<td>5.33 a</td>
<td>0.28 bc</td>
<td>0.39 a</td>
<td>0.43 ab</td>
<td>5.37 a</td>
</tr>
<tr>
<td>Azo16M2</td>
<td>18.675 a</td>
<td>16.550 a</td>
<td>4.67 a</td>
<td>0.51 a</td>
<td>0.29 a</td>
<td>0.59 a</td>
<td>6.63 a</td>
</tr>
<tr>
<td>B13</td>
<td>14.125 ab</td>
<td>12.275 ab</td>
<td>3.67 a</td>
<td>0.31 bc</td>
<td>0.35 a</td>
<td>0.43 ab</td>
<td>6.43 a</td>
</tr>
<tr>
<td>Bac15MB</td>
<td>17.050 ab</td>
<td>14.675 ab</td>
<td>4.00 a</td>
<td>0.47 a</td>
<td>0.25 a</td>
<td>0.54 ab</td>
<td>6.73 a</td>
</tr>
<tr>
<td>DZ22</td>
<td>14.425 ab</td>
<td>12.325 ab</td>
<td>5.67 a</td>
<td>0.39 ab</td>
<td>0.26 a</td>
<td>0.45 ab</td>
<td>5.23 a</td>
</tr>
<tr>
<td>Control</td>
<td>13.675 b</td>
<td>10.775 b</td>
<td>5.33 a</td>
<td>0.22 c</td>
<td>0.40 a</td>
<td>0.38 b</td>
<td>4.27 a</td>
</tr>
<tr>
<td>Standard Error</td>
<td>1 436.3</td>
<td>1 331.4</td>
<td>0.70</td>
<td>0.04</td>
<td>0.05</td>
<td>0.05</td>
<td>0.73</td>
</tr>
</tbody>
</table>

Means followed by the same letters in a column do not differ significantly by Duncan multiple range test (p>0.05).
NTub/pl = number of tubers per plant (kg)
Com.W = commercial weight of tubers per plant (kg)
NoCom.W = no commercial weight of tubers per plant (transformed arc.sin kg)
Aspect = overall appearance of the harvest measured in phenotypic scale (9 bad, 1 good).
FWTuber = average fresh weight of tubers per plant (kg)
Yield ha⁻¹ = projected yield of tubers for a density of 30.000 plants ha⁻¹.
Biomass = total weight of the biomass (tubers + foliage) projected per hectare (kg).

Table 2. Agronomic traits of potato cv. ‘Unica’ under the effect of rhizobacteria and acetylsalicylic acid

<table>
<thead>
<tr>
<th>Traits</th>
<th>%Surviv</th>
<th>%Flower</th>
<th>HPlant</th>
<th>DWFoliage</th>
<th>FWFoliage</th>
<th>%LMiner</th>
<th>NPPlants30d</th>
<th>NSprouts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Arc.Sin</td>
<td>Arc.Sin</td>
<td>sqroot (%)</td>
<td>sqroot (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>%</td>
<td>cm</td>
<td>kg pl⁻¹</td>
<td>kg pl⁻¹</td>
<td>%</td>
<td>#</td>
<td># pl⁻¹</td>
</tr>
<tr>
<td>SA</td>
<td>93.33 a</td>
<td>54.61 a</td>
<td>25.33 a</td>
<td>0.20 a</td>
<td>0.32 a</td>
<td>4.04 ab</td>
<td>4.65 b</td>
<td>1.88 a</td>
</tr>
<tr>
<td>Azo16M2</td>
<td>93.33 a</td>
<td>35.09 b</td>
<td>24.00 a</td>
<td>0.16 a</td>
<td>0.28 a</td>
<td>3.16 b</td>
<td>6.57 ab</td>
<td>1.87 a</td>
</tr>
<tr>
<td>B13</td>
<td>94.17 a</td>
<td>28.38 b</td>
<td>23.00 a</td>
<td>0.16 a</td>
<td>0.25 a</td>
<td>4.47 ab</td>
<td>6.06 ab</td>
<td>1.93 a</td>
</tr>
<tr>
<td>Bac15MB</td>
<td>90.83 a</td>
<td>28.43 b</td>
<td>24.00 a</td>
<td>0.17 a</td>
<td>0.30 a</td>
<td>3.93 ab</td>
<td>7.80 a</td>
<td>1.96 a</td>
</tr>
<tr>
<td>DZ22</td>
<td>90.00 a</td>
<td>38.05 ab</td>
<td>24.33 a</td>
<td>0.17 a</td>
<td>0.28 a</td>
<td>4.81 a</td>
<td>5.08 b</td>
<td>1.96 a</td>
</tr>
<tr>
<td>Control</td>
<td>95.00 a</td>
<td>24.38 b</td>
<td>22.33 a</td>
<td>0.21 a</td>
<td>0.32 a</td>
<td>5.14 a</td>
<td>5.66 ab</td>
<td>2.02 a</td>
</tr>
<tr>
<td>Standard error</td>
<td>2.32</td>
<td>5.29</td>
<td>1.95</td>
<td>0.02</td>
<td>0.03 a</td>
<td>0.41</td>
<td>0.73</td>
<td>0.07</td>
</tr>
</tbody>
</table>

Means followed by the same letters in a column do not differ significantly according to Duncan multiple range test (p>0.05).
NSprouts = number of buds per plant emerged at 15 days after planting.
NPPlants 30d = number of plants emerged at 30 days after planting (square root transformed data)
H.Plant = plant height at 60 days after sowing (cm)
%Surviv = Percentage of surviving plants per plot at harvest
%LMiner = percentage of damaged plants by leafminer fly at 60 days.
%Flower = Percentage of flowering plants on 75 days after sowing.
DWFoliage = dry weight of foliage per plot (transformed arc.sin kg)
FWFoliage = fresh weight of foliage per plot (transformed arc.sin kg)
DISCUSSION

*Azotobacter* sp. strain AZO16M2 and *Bacillus amyloliquefaciens* strain Bac15MB, inoculated in potato cv. ‘Unica’ increased the weight of tubers per plant biomass yield and tuber per hectare and commercial tuber yield, when compared to the control or the positive control with acetylsalicylic acid in the Cañete valley. This result would indicate that these bacterial strains have been favorable since the tuber yield components were significantly affected, although other strains inoculated had no effect relative to the control.

Application with acetylsalicylic acid significantly increased the number of inflorescences per plant compared to control with no application, however there were no differences between bacterial strains by comparing them with control. Hormonal effect of acetylsalicylic acid exceeded that of rhizobacteria inoculated on potato cv. ‘Unica’, which could be related to the effect on the delay of senescence that attribute to salicylic acid (ZHANG et al. 2013).

OSWALD et al. (2010) state that rhizobacteria of *Bacillus* and *Azotobacter* genera, as well as for some actinomycetes increased tuber yield significantly. Such tuber yields were comparable with those of different potato cultivars grown with inorganic fertilization, under greenhouse and field conditions in Peru, thus agreeing with the findings of our research. In addition, our results were similar to those of RICO (2009), who showed that strains of the genus *Azotobacter* sp. promoted plant growth by increasing production of potato tubers. As showed by ARCOS & ZÚÑIGA (2016), plant height and total yield of tubers were significantly higher in plots inoculated with native strains of *Bacillus amyloliquefaciens* compared to control, with the same strains used in this experiment, although in conditions different from those described in the present research. These results may suggest that native strains extracted from the rhizosphere of potato may differ in their effect according to environmental conditions (EGAMBERDIEVA 2012) and potato cultivars. Regarding this last point, traditional potato cultivars, unlike improved cultivars such cv. ‘Unica’, may interact differently with soil biota as a consequence of a long process of crop domestication (PÉREZ-JARAMILLO et al. 2016), thereby resulting in interactions with different bacterial strains, as found in our research.

CONCLUSION

In general, agronomic performance for potato cv. ‘Unica’ inoculated with *Azotobacter* sp. strain AZO 16M2, improved significantly in relation to weight of biomass, tuber yield per hectare, commercial tuber weight and lesser damage of potato foliage by leafminer (*Liriomyza huidobrensis*) under cropping conditions at the experimental site. It is, however, necessary to validate this information at field level in different environmental conditions of soil and climate and with different potato cultivars to determine the beneficial effect of these bacteria on the performance of *Solanum tuberosum*.

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