Effects of plant growth promoting rhizobacteria (PGPR) on *Citrus macrophylla* rootstock

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Abstract

Citrus is one of the largest fruit crops grown in Morocco. *Citrus* crops gain in importance due to the jobs generated during the production process of fresh or processed fruit. Intensive agriculture is characterized by the excessive use of inorganic fertilizers and pesticides. This production system has generated serious environmental contamination problems, thus, it is necessary to implement sustainable production strategies to reduce the use of synthetic chemicals and contribute to soil and water conservation. In this context, Seventy two Rhizobacterial isolates of fluorescent *Pseudomonas* were isolated from rhizosphere soil of *Citrus* in the Sapiama nursery. These isolates were tested on germination and growth of *Citrus macrophylla* rootstock. The results obtained showed that the isolate C11 significantly stimulated germination 16 days after seed inoculation. The C26, C6 and C24 isolates showed PGPR effects improving significantly the growth parameters of *C. macrophylla* rootstock. They significantly promoted plant height, collar diameter and root length. This study concluded that the *Pseudomonas* isolates could be potential alternative biofertilizers to chemical products and could be considered as a promising main component for sustainable agriculture development strategy in *Citrus* farming.

Keywords: *Citrus macrophylla*, *Pseudomonas*, PGPR

INTRODUCTION

Citrus is one of the most important fruit crops grown in Morocco. It represents an important element in the economy of the country, with an annual production of 1.5 to 2.0 million tons obtained from approximately 125,000 ha (ASPAM, 2019). Souss-Massa-Draa region is the main area of both production and exportation of fresh fruit from Morocco (Boubaker *et al.*, 2009). Given the economic importance of *Citrus* crops, producers became more and more dependent on agrochemicals as a reliable method to maintain soil productivity and thus to accelerate and improve *Citrus* production. However, the use of fungicides is increasingly becoming restricted owing to stringent regulation, high cost, environmental pollution and growing public concern about synthetic products (Mesnage and Antoniou, 2018). Therefore, the challenge is to develop healthy and effective strategies to enhance nutrition and growth of crops.

Over the last years, organic agricultural system has emerged as an effective alternative to improve crop growth and quality (Willer and Lernoud, 2017). The rhizosphere, narrow zone of soil that surrounds and get influenced by the roots of plant (Prashar *et al.*, 2014), is a highly favorable habitat for the proliferation of microorganisms and exerts a potential impact on plant health and soil fertility (Pathan *et al.,* 2019; Qessaoui *et al.,* 2019a). Bacterial species mostly associated with the plant rhizosphere known as "Plant growth promoting rhizobacteria" (PGPR) are non-pathogenic and reported to be beneficial for plant growth, yield and crop quality (O'Connell, 1992; Ahmad *et al.*, 2008; Esitken *et al.,* 2010; Olanrewaju *et al.*, 2017; Qessaoui *et al.*, 2019a). The success of some of these PGPR in laboratory studies and pilot tests conducted in the field have generated interest by several agrochemical companies in the development and commercialization of Bioproducts formulated with selected efficient PGPR. Several bacteria have been patented and evaluated for commercial use, of which BIOBOOST (*Delftia acidovorans*), BIOPLIN (*Azotobacter spp.*), BIOYIELD (*Bacillus spp.*), COMPETE (*Bacillus, Pseudomonas* and *Streptomyces spp.*) and KODIAK (*Bacillus subtilis*) are used as biofertilizers (Podile and Kishore, 2006).

The plant promoting effect of the PGPR is explained by various mechanisms including: (i) reduction of ethylene production (Glick *et al.*, 1995); (ii) production of plant hormones such as auxins (Egamberdiyeva, 2005), cytokinins (Garcia de Salamone *et al.*, 2001) and gibberellins (Gutierrez-Manero *et al.*, 2001); (iii) enhancement of the symbiotic N_2 fixation (Kim and Rees, 1994) and (iv) solubilization of nutrients (Jeon *et al.*, 2003; Glick, 1995). Besides, their role in plants growth promotion, PGPR also act as protectants of soil-borne pathogens (Howell and Stipanovic, 1978; Weller *et al.*, 2002; Guo *et al.*, 2004; Amkraz *et al.*, 2010). The production of siderophores, the synthesis of antibiotics, enzymes and/or fungidical compounds and competition for nutriments and space are the main mechanisms by which PGPR contribute to control plant bioagressors (Compant *et al.*, 2005; Haas and Defago, 2005 ; Qessaoui *et al.*, 2017; Qessaoui *et al.*, 2019a,b).

Among the diverse range of PGPR identified, *Pseudomonas* is a wide distributed bacteria, which is considered as one of the most extensively studied and used in organic production system. Within the genus, fluorescent *Pseudomonas* bacteria the most studied (Weller, 1988). They are reported to prevent proliferation of plant pathogens and stimulate plant growth by facilitating either uptake of nutrients from soil or producing certain plant growth promoting substances (Weller, 1988; Sutra *et al.*, 2000; Boudyach *et al.*, 2004; Qessaoui *et al.*, 2019a). Nevertheless, limited information is available on the promoting effect of fluorescent *Pseudomonas* on the growth of *Citrus* plants. Thus, the present research is aimed to (1) isolate fluorescent *Pseudomonas* from the rhizospheric soil of *Citrus* plants (*Citrus macrophylla*), (2) select efficient strains able to improve seed germination and growth of *Citrus* rootstocks.

MATERIAL AND METHODS

Fluorescent *Pseudomonas* isolation

Citrus root samples and soil adhering were collected from *Citrus* trees at Sapiama nursery, Taroudant, Morocco. The bacterial communities of the rhizosphere (RS) and the endorhizosphere (ER) were isolated as described by Dommergues and Mangenot (1970) and Amkraz (2010). One gram of rhizospheric soil, obtained by shaking roots, was added to 9 ml of sterile physiological water and the mixture was agitated for 15 min. Serial dilutions were prepared, and 0.1 ml of each dilution was dropped onto King B medium (King *et al.*, 1954), supplemented with 100 g ml^{-1} of cycloheximide to suppress fungi. Three replicates were incubated at 28 °C for 72 h. Results were expressed as colony forming units per gram (cfu g^{-1}) of rhizospheric soil. Fluorescent colonies on King B medium were sub-cultured twice before storage at 4 °C on yeast dextrose carbonate agar (YDC) (Jiménez *et al.,* 2004) and at -80 °C in 40% glycerol (Parke *et al.*, 1986). The isolates were identified in the plant protection laboratory at INRA Agadir (Qessaoui *et al,* 2019a).

Effect of fluorescent *Pseudomonas* on seeds germination of *C. macrophylla* rootstock

Seeds of *Citrus macrophylla* were surface disinfected with 30% sodium hypochlorite solution during 5 min and air-dried. Seeds were then treated with isolated *Pseudomonas* (10^8 cfu ml^{-1}) in Xantham gum 0.5% (Boubyach *et al.*, 2001). Control seeds were treated by Xantham gum in the same conditions. All treatments were performed in three replicates with 10 seeds of each treatment. The inoculated seeds were placed in Petri dishes covered with Whatman paper and were incubated at 25±2°C. The percent of germination was calculated form 14^{th} to 30^{th} days after treatment.

Effect of fluorescent *Pseudomonas* on *C. macrophylla* rootstock growth

During transplantation, *C. macrophylla* roots were inoculated with the *Pseudomonas* (10^8 cfu ml^{-1}) using dipping method for 20s. After inoculation, the seedlings were transplanted into the plastic pots contains a mixture of sand and peat in a ratio of 2:1 (v/v). All treatments were performed in three replicates with nine plants of each replicate. The plant height, number of leaves and collar diameter of seedlings were evaluated at the second month after transplantation.

Statistical analysis

The seed germination and PGPR parameters were calculated for each *Pseudomonas* isolate. The data were subjected to the analysis of variance test (ANOVA) using Statistica software (Version 6). Any difference mentioned is significant at $p< 0.01$ using Duncan's tests.

RESULTS

Fluorescent *Pseudomonas* isolation

The result shows the total number of bacteria per gram of soil and the percentage of fluorescent bacteria in both soils. Numbers of total bacteria were significantly higher in the rhizospheric soil compared to the endorhizosphere soil. However, the fluorescent *Pseudomonas* were significantly abundant in the endorhizosphere soil (Table 1). Seventy two (72) *Pseudomonas* isolates were selected in this work and assessed for their potential to promote seed germination and growth of *C. macrophylla* rootstock.

Effect of fluorescent *Pseudomonas* on seeds germination of *C. macrophylla* rootstock

Different bacterial effects on seeds germination were observed. Among 72 isolates, 30 have shown an inhibition effect on seed germination of *C. macrophylla* rootstock (these results were excluded from the current study). Among 42 isolates that showed a positive effect, only isolate C11 has improved seed germination percentage significantly from 16^{th} day after inoculation compared to the control (Figure 1).

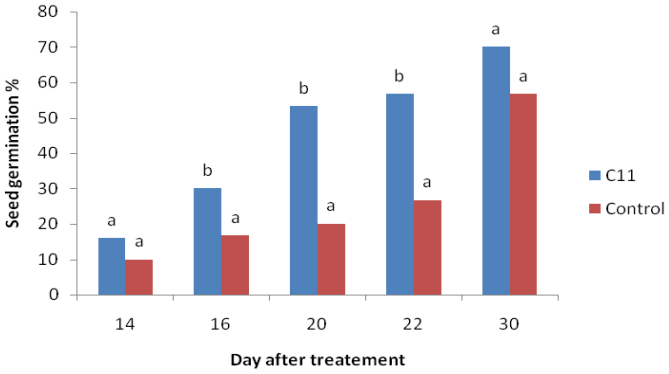

Figure 1: In vitro effect of fluorescent Pseudomonas isolate C11 on seed germination of C. macrophylla rootstock

Table 1: Total number and percentage of fluorescent and non-fluorescent bacteria in the rhizosphere of *Citrus* trees under orchard conditions of Taroudant region

Origin	Number of cfu /g of soil	Fluorescent bacteria (%)
Rhizospheric soil	1,87 10^9	58%
Endorhizosphere	6,25 10^8	80%

Effect of fluorescent *Pseudomonas* on *C. macrophylla* rootstock

Among 42 isolates tested on plant growth parameters, three (C6, C24 and C26) showed a significant effect on *C. macrophylla* rootstock growth at the 2nd month after transplantation (Table 2).

The two isolates, C6 and C24, increased significantly *C. macrophylla* rootstock height with 50.8 cm and 52.3 cm respectively resulting in a gain of 21 and 25 % respectively compared to control (Table 2). For the number of leaves, three isolates (C6, C24 and C26) showed no significant effect on this parameter (Table 2). However, the collar diameter as a plant vigor parameter of *C. macrophylla* rootstock was improved significantly by C6 and C26 isolates with a gain of 19 and 21% respectively (Table 2).

Isolates C24, C26 and C6 have shown a positive effect on height and collar diameter of *C. macrophylla* rootstock. They also had a significant effect on proliferation of the root system of *C. macrophylla* rootstock (Figure 2).These three isolates were identified as isolates of *Pseudomonas spp.*

DISCUSSION

*I*n this study, identified *Pseudomonas* isolates stimulated *C. macrophylla* rootstock growth in greenhouse conditions and improved its seed germination rate. Stimulating the growth of plants inoculated with each of these isolates could be explained by several mechanisms. These include (i) improving uptake of water and nutrients needed by plants and (ii) inhibition of pathogenic agents which can damage growth of rootstocks. Synthesized phytohormones and phosphate solubilization by rhizobacteria was reported to stimulate development of the root system and aerial part of plants. Some PGPR have the ability to synthesize indole-3-acetic acid (IAA), known for their beneficial effect on rooting and root development (Egamberdiyeva, 2005). Production of antibiotics and siderophores by bacteria inhibit pathogenic fungi and bacteria which increase plant competitiveness for nutrition and space, leading to more availability of nutrients and space. They consequently promote plant growth (Digat *et al.,*1993). The results of this study are consistent with numerous studies that have demonstrated the stimulation of plant growth after inoculation by bacteria. Indeed, Glick *et al*., (2007) reported that inoculation of plants with PGPR stimulates the growth and yield of plants, by the solubilization of phosphate, potassium and by stimulating the absorption of atmospheric nitrogen. The *Pseudomonas* act positively on the development of root system and stimulated significantly the length of the stem and collar diameter of plants (Satrani *et al*., 2009; Qessaoui *et al*., 2019a). Similarly, Esitken *et al*., (2010) showed that *Pseudomonas* BA-8 and *Bacillus* OSU-142 alone or in combination increase the nutrition, growth and yield of cherry plants. Concerning the observed effect on seed germination of *C. macrophylla*, only *Pseudomonas* C11 has shown significant potential to increase this parameter while *Pseudomonas* C24, C26 and C6 have potential to increase the growth and vigor of *Citrus* rootstock plants. These bacteria could be tool for bio-fertilizer formulation to improve rootstock *Citrus* production. Synergetic effect of the selected isolates and other eco-physiological investigations should be performed in the future to maximize benefit from these soil-microbial organisms in *Citrus* crops production.

Control C24

Figure 2: Effect of Pseudomonas isolate on growth of C. marcophylla rootstock

Table 2: Effect of three isolates of *Pseudomonas* on *C. macrophylla* rootstock growth

Isolate	Height		Leaf number		Collar diameter	
	cm	%	Number	%	mm	%
Control	41.9 a	-	21.9 a	-	3.86 a	-
C24	52.3 b	24.9 %	27.1 a	23.9 %	4.33 a	12.2 %
C6	50.7 b	21.1 %	25.4 a	16.3 %	4.67 b	21.0 %
C26	50.3 a	20.0 %	24.7 a	12.7 %	4.61 b	19.4 %

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