RESEARCH ARTICLE

Indole-3-acetic acid (IAA) producing *Pseudomonas* isolates inhibit seed germination and α-amylase activity in durum wheat (*Triticum turgidum* L.)

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Abstract

The role of plant-associated bacteria in plant physiology and metabolism is well documented, but little has been known about the roles played by *Pseudomonas* in durum wheat (*Triticum turgidum* L. var *durum*) growth and development. An *in vitro* experiment was conducted to observe the effect of the inoculation of four indole-3-acetic acid (IAA) -producing *Pseudomonas* isolates and exogenous IAA on seed germination traits and α-amylase activity of durum wheat. The results showed inoculation with all bacterial isolates led to a decrease in the germination percent, although the extent of the depression varied with the isolate. A significant relationship between concentrations of bacterial IAA and the germination inhibition percent in durum wheat seeds by different bacteria strains was observed. The results of this assay showed the effect of bacterial isolates on α-amylase activity after six and 8 days of inoculation was significant, while effect of these isolates on α-amylase activity after two and 4 days of inoculation was not meaningful. In addition, the exogenously applied IAA displayed a concentration-dependent effect on seed germination attributes and α-amylase activity, consistent with the possibility that the inhibitory effect of bacterial inoculation on seed germination was in consequence of bacteria-produced IAA. Therefore, it may suggested that the inhibitory role of IAA in seed germination and α-amylase activity should be taken into account during the screening of IAA-producing *Pseudomonas* isolates for durum wheat growth promoting agents.

Additional key words: exogenous IAA; germination inhibition percentage; *Pseudomonas*; seed germination traits; plant growth promoting rhizobacteria

Introduction

A wide range of microorganisms, varying from pathogenic to beneficial, interact continuously with higher plants in the soil ecosystem, influencing the growth, development and functions of plants (Taghavi *et al*., 2009). Bacteria that are able to colonize plant root systems and promote plant growth are referred to as plant growth promoting rhizobacteria (PGPR). PGPR can affect plant growth either indirectly or directly. The indirect promotion of plant growth occurs when PGPR lessen or prevent the deleterious effects of one or more phytopathogenic organisms. The direct promotion of plant growth by PGPR involves either providing the plants with certain bacterial-synthesized compounds or facilitating the uptake of certain nutrients from the environment (Glick, 1995; Lugtenberg & Kamilova, 2009). On the other hand, deleterious rhizosphere bacteria (DRB) are defined as rhizobacteria that inhibit plant growth without causing disease symptoms (Brime-

combe et al., 2007). Several mechanisms for growth inhibition by this undesirable group of rhizobacteria have been proposed, the most likely being the production of phytotoxins such as cyanide and other volatile and non-volatile compounds, as yet unidentified. An alternative mechanism by which DRB may inhibit plant growth is through the production of phytohormones (Brimecombe et al., 2007). Indole-3-acetic acid (IAA) produced by DRB has been shown to inhibit root growth in sugar beet and blackcurrant (Brimecombe et al., 2007). DRB may also compete with the plant and beneficial rhizobacteria for nutrients, contributing to the decreased plant growth and, therefore, lowered yields (Brimecombe et al., 2007). Further, DRB may indirectly reduce growth by counteracting the effects of nitrogen-fixing rhizobacteria (Brimecombe et al., 2007).

Auxins are a group of plant growth regulators that stimulate cell division and elongation. IAA is the principal auxin of higher plants, to which the amino acid L-tryptophan (L-Trp) plays a precursory role. Microbial synthesis of IAA has been known for a long time. This property is best documented for bacteria that interact with plants. IAA production by PGPR is one of the most studied and, perhaps, the most effective mechanism of plant growth promotion by these bacteria (Patten & Glick, 1996; Arshad et al., 2010). Plant roots secrete signalling chemicals (L-Trp, a precursor for IAA, as well as other amino acids and small molecules) into the rhizosphere soil, promoting the binding of bacteria to the root surface (Simons et al., 1997). The root-bound bacteria may use the L-Trp in the soil to produce IAA (Patten & Glick, 1996; Mirza et al., 2001; Gravel et al., 2007). The IAA is subsequently secreted from the PGPR and absorbed by the plant and used primarily to increase cell growth or proliferation (Glick et al., 1998). Therefore, it seems that IAA has a concentration-dependent dual role (Arshad & Frankenberger, 1992; Chauhan et al., 2009) with optimal IAA levels in plant roots being several orders of magnitude lower than its optimal levels in the shoots.

The genus Pseudomonas comprises a group of ubiquitous bacteria that have frequently been reported to play a dual role in plant health and growth (Meyer et al., 2008). For example, some of the bacteria in this group are known to be pathogenic (Hirano & Upper, 2000), while others are known to be involved in disease suppression (Halgren et al., 2011). In addition, some members of this group of bacteria appear to increase seed germination (Selvakumar et al., 2009), while others exert inhibitory effects on seed germination (McPhail et al., 2010; Lee et al., 2013). Germination arrest factors (GAFs) have been the subject of increased research in recent years. GAFs are defined as microbial-derived compounds that lead to irreversible arrest of seed germination in a wide range of graminaceous species (Banowetz et al., 2008). For example, the compounds 4-formylaminooxyvinylglycine (FVG) (McPhail et al., 2010) and L-2-amino-4-methoxy-trans-3-butenoic acid (AMB) (Lee et al., 2013) are two GAFs produced by Pseudomonas species. However, there is a gap in knowledge regarding the impact of GAFs on wheat seed germination, in general, and on durum wheat (Triticum turgidum L.) seed, in particular. Durum wheat represents 10% of the wheat grown globally, occupying about 11 million hectares in the countries around the Mediterranean Basin. Durum wheat in Iran is cultivated across diverse environments, ranging from warm lowlands to cold highlands. The success of durum wheat in Iran, as a food security crop, is largely due to its good ability and capacity to produce well under drought-prone environments and marginal and poor management conditions where other crops would fail (Mohammadi et al., 2010).

Therefore, the aim of the present experiments was to evaluate the effect of some IAA–producing Pseudomonas isolates on seed germination, α-amylase activity, and seedling growth of durum wheat. In addition, because of concentration-dependent effects of IAA on plant growth, potential role of the exogenous IAA in germination behaviour of durum wheat seeds was also examined.

Material and methods

Bacteria strains and growth conditions

The Pseudomonas isolates UW3 (Pseudomonas sp.), UW4 (Pseudomonas putida) (Glick, 1995; Duan et al., 2013) have been described previously and isolates 550 (Pseudomonas fluorescens) and 57 (Pseudomonas sp.) were obtained from the Department of Soil Sciences, University of Tehran, Iran. The isolates were grown on nutrient agar (NA) (2 g yeast extract, 1 g meat extract, 5 g peptone, 5 g sodium chloride, 20 g agar-agar, 1000 mL distilled water) or nutrient broth (NB) for routine use and maintained in NB with 20% glycerol at – 80 °C for long-term storage. For preparing the bacterial cultures, single colony of each bacterial isolate was grown in 250-mL flasks containing 100 mL NB medium and incubated for 24 h at 28 ± 2 °C on a rotary shaker (KS 130 basic, IKA, Germany) at 120 rpm. After incubation, the cell suspension was centrifuged at 5,000 × g for 5 min at 4 °C and washed twice with sterile distilled water. The final pellet was resuspended in sterilized distilled water and the bacterial cultures were standardized to 10^8 colony-forming units (CFU)/mL and used, immediately, for seed germination experiments.

IAA production assay

The production of IAA by the isolates was determined as described by Glickman & Dessaux (1995). The isolates (10^8 cells/mL) were grown in 100 mL flasks containing 50 mL NB supplemented with L-Trp (100 μg/mL) for 48 h on a rotary shaker at 120 rpm. Then, cultures were centrifuged at 8,000 g for 10 min and the supernatants collected. Two mL of Salkowsky reagent (1 mL of 0.5 M $FeCl_3$ in 50 mL of 35% $HClO_4$) with 1 mL of the supernatant was allowed to react at 28 ± 2°C for 20 min at room temperature. Pink color developed indicating the presence of IAA was determined by measuring the absorbance in a spectrophotometer (HITACHI U1800) at 535 nm at the end of the incubation (Patten & Glick, 2002). A standard curve was plotted with IAA and Salkowsky reagent dissolved in NB medium to quantify the IAA (μg /mL) present in the culture supernatant. Concentration of IAA produced was estimated against standard curve of IAA in the range of 0–100 μg/mL.

Effect of bacterial strains on durum wheat seed germination traits

To evaluate the effect of *Pseudomonas* isolates on germination and emergence traits of durum wheat seeds (*Triticum turgidum* L. var *durum*), a laboratory experiment was conducted as a completely randomized design (CRD) with four replicates in Department of Agronomy and Plant Breeding, Isfahan University of Technology, Iran. Five treatments were made as follows: 1) seeds treated with isolate UW3; 2) seeds treated with isolate UW4; 3) seeds treated with isolate 550; 4) seeds treated with isolate 57; 5) uninoculated control. Seeds of durum wheat were obtained from the seed bank of Isfahan University of Technology. Four replicates of 25 seeds per treatment were used. Seeds were surface disinfected in a 2% (v/v) solution of sodium hypochlorite for 15 min and rinsed four times with sterile distilled water, and air-dried before being used in the germination experiments. All further manipulations were carried out under sterile conditions. The surface-sterilized seeds were immersed into individual bacterial suspensions for 30 min, shaking at 120 rpm. Twenty-five seeds were sown into sterile plastic 9-cm-diameter Petri dishes containing filter paper (Whatman No. 1) and watered with 7 mL of sterile distilled water. The Petri dishes were transferred into a dark growth chamber at 25 ± 2°C for 10 days, and the germinated seeds were counted on a two-day-interval basis in a certain time. A seed was considered as germinated when its radicle emerged by about 2 mm

in length. Final germination percentage and rate were measured at day 10 after incubation. The germination rate was estimated according to the Eq. [1], modification of Timson's index (Khan & Ungar, 1984). The root and shoot lengths were measured and seedling vigour index was determined according to the Eq. [2] (Abdul-Baki & Anderson, 1973).

$$\text{Germination rate} = \sum (G/t) \qquad [1]$$

where, G is the percentage of seeds germinated at two-day-intervals, and t is the total germination interval period.

$$\text{Vigour index} = (\text{Mean root length} + \text{Mean shoot length}) \times \text{Germination percentage} \qquad [2]$$

Assay of α-amylase activity

For determination of α-amylase activity, durum wheat seeds were harvested at two, four, six, and 8 days after incubation with bacterial isolates as described above. To prepare the enzyme extract, five germinating seeds per Petri dish were weighed, frozen, ground to a fine powder in a pre-chilled mortar in liquid N_2 using a pestle, homogenized with 5 mL of a 0.1 M sodium acetate buffer (pH 4.8), and filtered through Whatman filter paper to remove large particles. The extract was centrifuged at $12,000 \times g$ for 20 min (5810R, Eppendorf refrigerated centrifuge, Germany). All of the preparations were carried out at 4°C. The supernatant was served as the crude enzyme extract for the α-amylase assay.

For the enzyme assay, the reaction medium (3 mL) contained 1 mL of the 0.1 M sodium acetate buffer, pH 4.8, 0.5 mL of enzyme extract diluted to 1 mL using acetate buffer, and 1 mL of 0.1% soluble starch (Merck #31231373) solution. The enzyme extract was diluted to obtain an absorbance change of less than one during the enzyme assay. The reaction medium was incubated for 10 min at room temperature (22 ± 2 °C), then the reaction was terminated by adding 1 mL of a 0.1% iodine reagent (6 g potassium iodide, 0.6 g iodine in 1 L of 0.05 N HCl) and 3 mL of 0.05 N HCl. The absorbance was measured at 620 nm and the decrease in the absorbance relative to the blank was considered as α-amylase activity (Beri & Gupta, 2007).

Effect of exogenous IAA on seed germination and seedling growth

To evaluate the effect of exogenous IAA on germination and emergence traits of durum wheat seeds,

an *in vitro* experiment was conducted as a CRD with four replicates as well. Five treatments were made as follows: 1) seeds treated with 5 µg/mL IAA; 2) seeds treated with 10 µg/mL IAA; 3) seeds treated with 15 µg/mL IAA; 4) seeds treated with 20 µg/mL IAA; 5) untreated control. Briefly, seeds were placed in 9-cm diameter Petri dishes and, after subjecting to 7 mL of IAA (0, 5, 10, 15 , 20 µg/mL), incubated at $25 \pm 2°C$ for 10 days, and finally the effect of the exogenous IAA levels on germination percent, germination rate, α-amylase activity, and seedling vigour index were measured and recorded as described above.

Statistical analysis

Analysis of variances (ANOVA) was conducted on the data and, when F values were significant ($p \leq 0.05$), mean comparisons were conducted using least significant differences (LSD, 0.05) procedure. Data were reported as means ± the standard error of the mean (SEM).

Results

Effect of bacterial isolates on rate and percentage of seed germination

The results indicated that bacterial isolates had a significant effect ($p<0.001$) on seed germination percentage (Table 1). All bacterial strains (except for isolate UW4) led to significant decreases in seed germination percent as compared to control (Fig. 1). The extent of the decrease in the seed germination percent was greater for isolate 57 compared to other isolates. The highest and the lowest inhibition percentages were detected in isolate 57 (26.3%) and isolate UW4 (1.7%) respectively. The germination rate was not signifi-

cantly ($p<0.05$) different between inoculated and control seeds (Fig. 1).

Potential of bacterial IAA production and its relationship with seed germination attributes

As shown in Fig. 2, isolates 57 and UW4 produced the highest (16.6 µg/mL) and lowest (7.1 µg/mL) concentrations of IAA, respectively. In addition, these two isolates also showed the highest and lowest germination inhibition percent respectively (Fig. 2). A significant relationship between concentrations of bacterial IAA and the germination inhibition (%) in durum wheat seeds by different bacteria strains was observed (r =0.84, $p<0.01$).
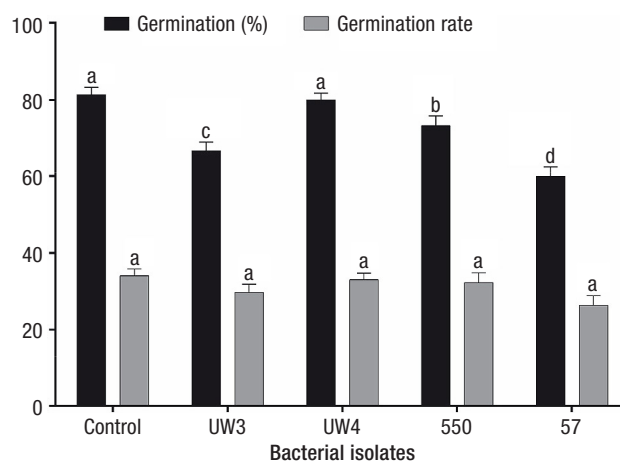

Figure 1. Response of durum wheat seeds to inoculation with *Pseudomonas* isolates: changes in germination (%) and germination rate developed in the Petri dishes containing 25 seeds. Error bars indicate the standard error of the mean (n = 4). Means with the same letter(s) are not significantly different from each other (according to mean comparison based on LSD, $p<0.05$). Control, bacterium-free; UW3, *Pseudomonas* sp.; UW4, *P. putida*; 550, *P. fluorescens*; 57, *Pseudomonas* sp.

Table 1. Analyses of variance (S.O.V) for the effect of inoculation with *Pseudomonas* isolates and exogenous IAA on durum wheat seed germination percent (G%), germination rate (GR), α-amylase activity at 2, 4, 6 and 8 days after inoculation, and seedling vigour index (VI).

S.O.V	df	Mean squares						
		G%	GR	Amylase 2	Amylase 4	Amylase 6	Amylase 8	VI
Bacterial strains	4	244.32***	56.091[ns]	0.0078[ns]	0.0596[ns]	0.1618**	0.075***	599528.945***
Error	15	5.037	22.586	0.0073	0.0501	0.0108	0.0002	12075.420
IAA	4	310.75***	54.59[ns]	0.00442[ns]	0.0882**	0.01426[ns]	0.01428**	1233143.37***
Error	15	9.252	27.158	0.00275	0.0149	0.04381	0.00012	29005.98

Four replicates were analyzed for each treatment level. *,**,***,[ns] indicate significance at $p \leq 0.05$, 0.01, 0.001 or non-significant effect, respectively.

Effect of bacterial isolates on α-amylase activity

Changes in α-amylase activity in durum wheat seeds in two-day intervals after inoculation with bacterial strains were also studied (Fig. 3). It was found that bacterial inoculation had a day-dependent effect on seed germination. The results of this assay showed the effect of bacterial isolates on α-amylase activity after six and 8 days of inoculation was significant, while the effect of these isolates on α-amylase activity after 2 and 4 days of inoculation was not meaningful.

Effect of bacterial isolates on seedling vigour index

Bacterial inoculation affected significantly ($p<0.001$) the seedling vigour index (Table 1). All of the bacterial isolates (except for isolate 57) led to the increase of the vigour index of durum wheat seedlings. The highest and the lowest increases were detected in isolates UW4 and UW3, and isolate 57 compared to the control respectively (Fig. 4).

Effect of exogenous IAA on rate and percentage of seed germination

Treating seeds with exogenous IAA led to a significant ($p<0.001$) effect on their germination percentage (Table 1). The exogenously applied IAA appeared to leave adverse effects on germination (%), *i.e.* the higher the IAA concentration in the medium the greater the inhibition (Fig. 5A). However, the germination rate was not significantly ($p<0.05$) different between inoculated and control seeds (Fig. 5A).

Effect of exogenous IAA on seedling vigour index

Treating durum wheat seeds with exogenous IAA led to a significant effect ($p<0.001$) on the seedling vigour
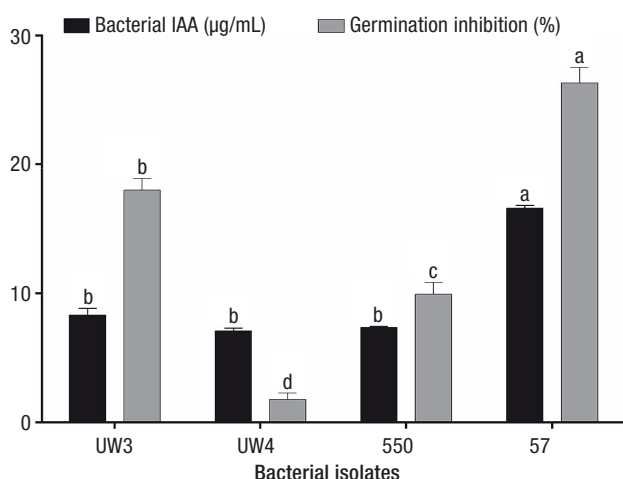

Figure 2. The IAA production of different *Pseudomonas* isolates and germination inhibition (%) in durum wheat seeds by different bacterial isolates. Germination inhibition (%) = [(Germination percentage in control - Germination percentage in inoculated seeds) / Germination percentage in control) × 100]). Error bars indicate the standard error of the mean (n = 4). Means with the same letter(s) are not significantly different from each other (according to mean comparison based on LSD; $p<0.05$). UW3, *Pseudomonas* sp.; UW4, *P. putida*; 550, *P. fluorescens*; 57, *Pseudomonas* sp.
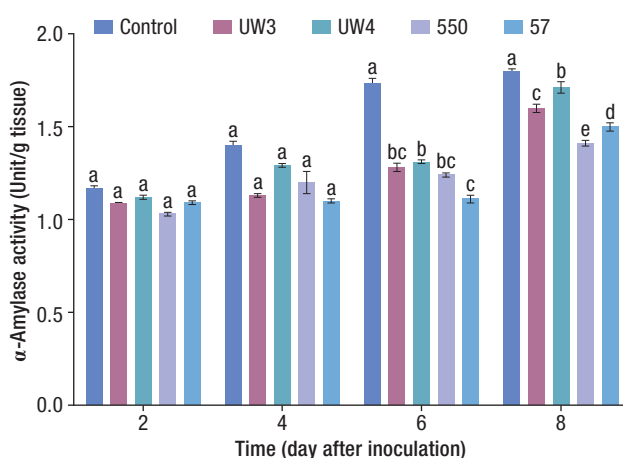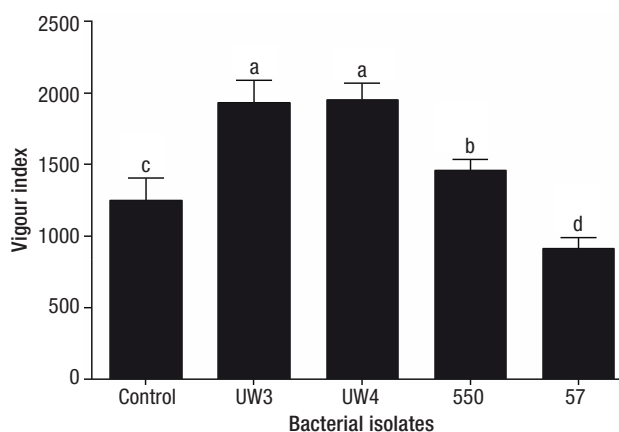

Figure 3. Time-course of α-amylase activity of durum wheat seeds in response to inoculation with *Pseudomonas* isolates. Error bars indicate the standard error of the mean (n = 4). Control, bacterium-free; UW3, *Pseudomonas* sp.; UW4, *P. putida*; 550, *P. fluorescens*; 57, *Pseudomonas* sp.

Figure 4. Effect of different bacterial strains on vigour index of durum wheat seedlings. Error bars indicate the standard error of the mean (n = 4). Means with the same letter(s) are not significantly different from each other (according to mean comparison based on LSD; $p<0.05$). Control, bacterium-free; UW3, *Pseudomonas* sp.; UW4, *P. putida*; 550, *P. fluorescens*; 57, *Pseudomonas* sp.
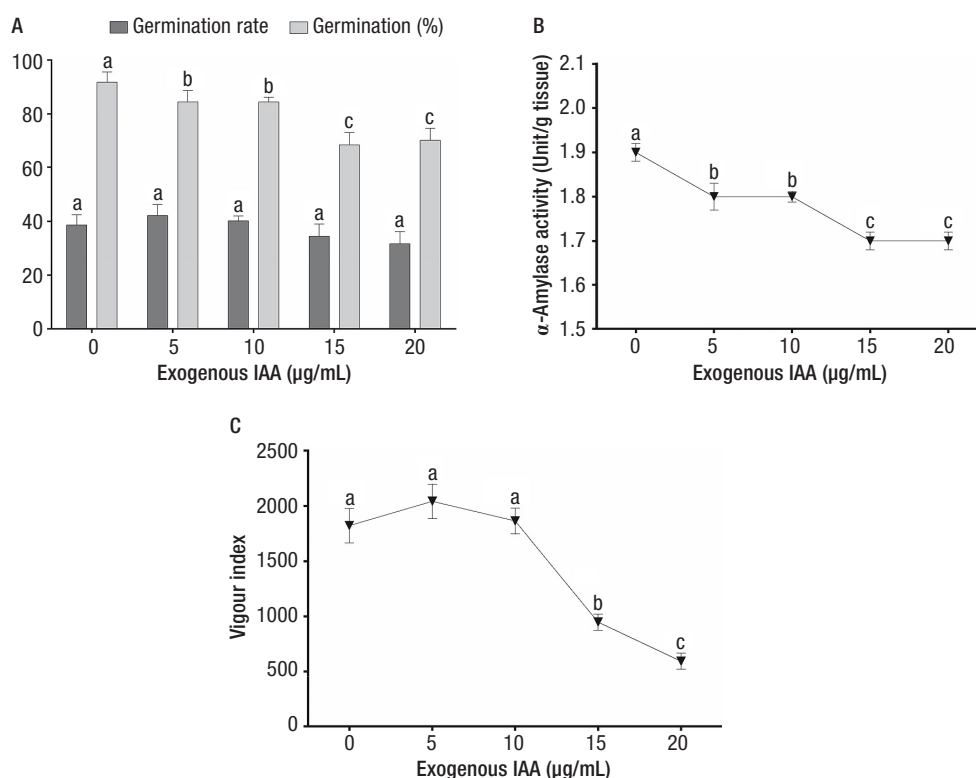
Figure 5. Response of durum wheat seeds to exogenous IAA: changes in germination percentage (A), germination rate (A), α-amylase activity (B), and vigour index (C) developed in the Petri dishes containing 25 seeds. Error bars indicate standard error of the means (n = 4). Means with the same letter(s) are not significantly different from each other (according to mean comparison based on LSD, p <0.05).

index (Table 1). When IAA was applied at low concentrations (*i.e.* up to 10 µg/mL), it had no effect on the seedling vigour index, however, when applied at high concentrations (*i.e.* greater than 10 µg/mL), it left significant negative effects on this attribute (Fig. 5C).

Effect of exogenous IAA on α-amylase activity

When applied at concentrations greater than 5 µg/mL, the exogenously applied IAA decreased the activity of α-amylase in durum wheat seeds (Fig. 5B). The two concentration–response curves (Figs. 5A and 5B) indicated that the seed germination percent is positively related with the activity of α-amylase in durum wheat seeds. The relationship between germination percent and α-amylase activity in durum wheat seems to be polynomial (Fig. 6), with a regression coefficient of 0.95 (*p*<0.01).

Discussion

This study provided an initial assessment of the potential of some IAA-producing *Pseudomonas* isolates on durum wheat seed germination traits and
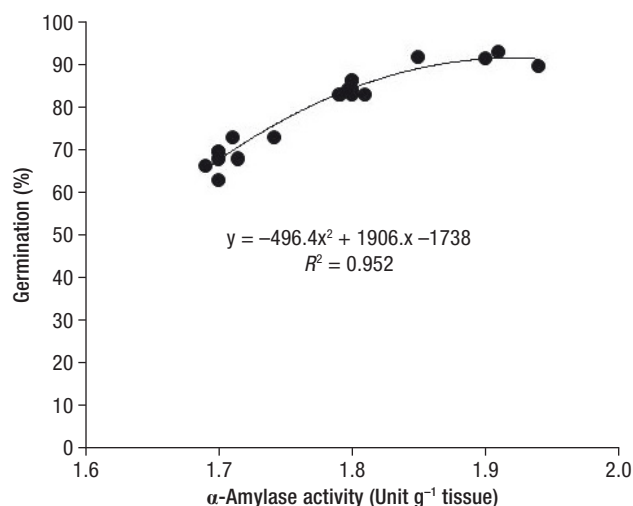
Figure 6. Relationship between the α-amylase activity and the germination percentage of durum wheat seeds. The data points are derived from the data in Figs. 5A and 5B.

α-amylase activity under *in vitro* conditions. The results of this experiment indicate that the germination process is slowed down due to the presence of bacteria in the *in vitro* conditions and this suppressing role is likely in consequence of a bacteria-induced increase in IAA level of the medium. There are some contradic-

tory reports on the effect of PGPRs, in general, and *Pseudomonas* species, in particular, on seed germination. In some reports, inoculation with bacteria has promoted seed germination and rate (Ashrafuzzaman *et al*., 2009; Selvakumar *et al*., 2009; Zarrin *et al*., 2009; Noumavo *et al*., 2013), while in some other reports it has been found to decrease seed germination (Banowetz *et al*., 2008; McPhail *et al*., 2010). For example, Selvakuma *et al*. (2009) reported that *P. fragi* CS11RH1, an IAA-producing strain, significantly increased the germination percentage and rate, plant biomass and nutrient uptake of wheat seedlings. However, according to Banowetz *et al*. (2008), *P. fluorescens* WH6 suppressed germination of *Poa annua* seeds and some other graminaceous species due, presumably, to the production of 4-formylaminooxyvinylglycine.

The inhibitory action of bacteria on seed germination could be due to the elevated levels of IAA or some unknown metabolites produced by the bacteria or the stress induced by PGPR. In order to address these possibilities, the effects of different concentrations of exogenous IAA on the percentage and rate of seed germination were also examined. During germination, plant seeds accelerate their respiratory metabolism to produce metabolic energy and biosynthetic precursors (Perata *et al*., 1997). To maintain respiratory metabolism crucial to germination, readily available respiratory carbohydrates and soluble sugars must be supplied constantly. However, the amount of readily utilizable soluble sugars in plant seeds is usually very limited, with starch being the main reserve carbohydrate (Guglielminetti *et al*., 2000). The hydrolytic enzyme α-amylase is known to play a major role in degradation of reserve carbohydrates (*i.e*. starch) to soluble sugars during germination (Perata *et al*., 1997). Thus, the induction of α-amylase is essential to maintain an active respiratory metabolism and, therefore, seed germination in durum wheat seeds.

During seed germination in wheat, barley and other graminaceous species, gibberellic acid (GA) is formed in the embryo and transferred to the aleuronic layer, where it induces the synthesis of α-amylase (Beri & Gupta, 2007). Measurement of α-amylase activity in germinating seeds is a classical bioassay for determining the GA level (Beri & Gupta, 2007). In the present study, low concentrations of IAA (*i.e*., 5 and 10 µg/mL) promoted the induction of α-amylase in germinating durum wheat seeds, perhaps, because the exogenous IAA stimulated GA biosynthesis. This possibility is consistent with previous reports (Anitha, 2010; Li *et al*., 2012). Paulsen & Auld (2004) concluded that IAA inhibited the germination of wheat seeds and acted in concert with GA and cytokinins to regulate the germination process. The observation that L-Trp, a precursor of IAA, sup-

pressed the sprouting of resistant wheat cultivars supports a role for IAA in controlling seed germination (Morris *et al*., 1988). Evidence has been accumulated (Chauhan *et al*., 2009; Roychowdhury *et al*., 2012) in support of a concentration-dependent role for IAA in seed germination, that is, low concentrations of exogenous IAA can promote, whereas high concentrations can inhibit seed germination. The regulatory role of bacterium-produced IAA in seed germination is supported by the evident biphasic response of germination of durum wheat seeds to IAA concentration (Fig. 2).

From the present results, it is concluded that IAA impaired the induction of α-amylase activity and, thus, germination in durum wheat seeds. There was a tendency for seed germination to increase with increasing the activity of α-amylase in the seeds. The bacterial strains used in the present study inhibited seed germination of durum wheat seeds due, presumably, to biosynthesis and accumulation of IAA in the inoculated seeds (Fig. 2).

The finding that high levels of IAA produced by bacteria-inoculated plants was probably the main cause of the inhibition of germination in durum wheat seeds contradicts some previous reports (Ashrafuzzaman *et al*., 2009; Zarrin *et al*., 2009). Zarrin *et al*. (2009) observed that coating of wheat seeds with IAA-producing PGPR strains (19.4-30.2 µg/mL) positively influenced their germination percentage and rate. Ashrafuzzaman *et al*. (2009) also reported that rice seed germination increased when seeds were pre-treated with IAA-secreting PGPR isolates and that the high-IAA producing isolates, PGB4 and PGG2, were proven to enhance the germination of the rice seeds. Our findings, however, agree with argument made by Glick *et al*. (1998). They have argued that plant roots may produce either optimal or suboptimal IAA levels endogenously. Then, the bacterial-produced IAA may enhance or inhibit plant growth, depending on whether the total IAA (that endogenous to the plant plus that produced by bacteria) is at optimal or supra-optimal levels, respectively.

The α-amylase enzyme activity in inoculated and non-inoculated seeds appeared to be low at the beginning, whereas it increased as germination progressed, the extent of the increase was much greater in non-inoculated, relative to the inoculated durum wheat seeds. The latter difference showed that the starch was metabolized faster in control, relative to the inoculated seeds. Thus, the bacterial strains used in the present study exerted inhibitory effects on the induction of α-amylase activity and, consequently, the germination of durum wheat seeds.

Despite the promoting effects exerted by the low concentrations of IAA (5-10 µg/mL) on the seedling vigour index, it seems that, in agreement with observa-

tions made by Chauhan *et al*. (2009), high concentrations (15-20 µg/mL) of this phytohormone negatively affect the seedling vigour index of durum wheat. The latter researchers reported a concentration-dependent response to IAA of seedling growth in black gram and horse gram plants. It is well known that auxins induce vascular differentiation in germinating embryos (Lovisolo *et al*., 2002; Pereyra *et al*., 2012). During seed germination and the early stages of seedling growth, the developing roots release some exudates, including L-Trp. Some rhizobacteria use the L-Trp in the soil to produce IAA. This positive interaction between plant root and bacterial IAA can, in turn, stimulate root development (Lambrecht *et al*., 2000).

Auxin, particularly at high concentrations, tends to exert inhibitory impacts on certain biological systems (*e.g*., root growth). This inhibitory effect has, almost, invariably been shown to be associated with the auxin-induced biosynthesis of ethylene (Davies, 2010). Although IAA is known as a prominent plant growth promoter, it can also stimulate transcription of 1-aminocyclopropane-1-carboxylic acid (ACC) synthase. The latter enzyme facilitates a key step in ACC oxidase-mediated ethylene biosynthesis (Mayak *et al*., 1999). The IAA-derived ethylene is believed to participate in the disruption of the normal growth (*i.e*. germination and seedling growth) of the host plant.

It is known that microbial functions such as nitrogen assimilation, siderophore secretion, and phosphorous mobilization occur only when deficiencies are present in the soil (Okon & Labandera-Gonzalez, 1994). According to Davies (2010), stem elongation (*i.e*. seedling height) is governed both by IAA and GA_1. O'Neill & Ross (2002) reported that IAA may promote the biosynthesis of the active GA_1 in shoots of pea seedlings. Therefore, even moderate changes in IAA supply can lead to physiologically significant changes in GA_1 content and growth attributes of seedlings. IAA-producing isolates used in our study may have enhanced plant growth due to the increased production of fine roots (Saharan & Nehra, 2011). The latter findings are in concert with other reports (Sheng *et al*., 2008; Sinha & Mukherjee, 2008). It is seemed the performance of bacterial strains in terms of enhancing the seedling vigour index is greater in nutrient-deficient conditions, than in nutrient-sufficient conditions. In our study, no attempt was made to measure the bacterial-induced IAA production of inoculated seeds under nutrient-sufficient and deficient conditions. Instead, we measured the *Pseudomonas* potential for IAA production before inoculating the seeds. Therefore, the assumption that bacteria are able to produce and secret higher amounts of IAA in nutrient-deficient conditions than in sufficient conditions needs to be validated in future studies.

Based on the results obtained, *Pseudomonas* strains have, evidently, the capacity to suppress durum wheat seed germination. IAA-induced suppression of α-amylase activity seems to be a major contributing factor to the lowered germination percent. Despite the negative effect of moderate IAA levels on seed germination, production of comparable amounts of IAA by rhizobacteria appeared to exert promoting effects on the seedling growth. Since isolate 57 behaved as a high-IAA-producing bacterium and, therefore, induced certain adverse influences on both germination and seedling growth of durum wheat, its mechanism of action needs to be further investigated in the future.

Acknowledgments

References

Abdul-Baki AA, Anderson JP, 1973. Vigour determination in soybean seed by multiple criteria. Crop Sci 13: 630-633. http://dx.doi.org/10.2135/cropsci1973.0011183X001300060013x

Anitha KG, 2010. Enhancing seed germination of mono and dicotyledons through IAA production of PPFM Trends. Soil Sci Plant Nutr 1: 14-18.

Arshad M, Frankenberger WTJr, 1992. Microbial production of plant growth regulators. In: Soil microbial ecology applications in agricultural and environmental management, 1st edn; Metting FBJr (ed). pp 27-32. CRC Press, New York.

Arshad M, Khalid A, Shahzad SM, Mahmood T, 2010. Role of ethylene and bacterial ACC deaminase in nodulation of legumes. In: Microbes for legume improvement; Khan MS, Zaidi A, Musarrat J (ed). pp 103-122. SpringerWien, New York. http://dx.doi.org/10.1007/978-3-211-99753-6_5

Ashrafuzzaman M, Akhtar Hossen F, Razi Ismail M, Ana-mul Hoque Md, Zahurul Islam M, Shahidullah SM, Meon S, 2009. Efficiency of plant growth-promoting rhizobacteria (PGPR) for the enhancement of rice growth. Afr J Biotechnol 8: 1247-1252.

Banowetz GM, Azevedo MD, Armstrong DJ, Halgren AB, Mills DI, 2008. Germination-Arrest Factor (GAF): Biological properties of a novel, naturally-occurring herbicide produced by selected isolates of rhizosphere bacteria. Biol Control 46: 380-390. http://dx.doi.org/10.1016/j.biocontrol.2008.04.016

Beri V, Gupta R, 2007. Acetyl cholinesterase inhibitors neostigmine and physostigmine inhibit induction of alpha-amylase activity during seed germination in barley,

Hordeum vulgare var. Jyoti. Life Sci 80: 2386-2388. http://dx.doi.org/10.1016/j.lfs.2007.02.018

Brimecombe MJ, De Leij FAAM, Lynch JM, 2007. Rhizodeposition and microbial populations. In: The rhizosphere biochemistry and organic substances at the soil-plant interface, 2nd edn; Pinton R, Varanini Z, Nannipieri P (eds). pp. 74-98. CRC Press, New York.

Chauhan JS, Tomar YK, Indrakumar Singh N, Seema A, Debarati A, 2009. Effect of growth hormones on seed germination and seedling growth of black gram and horse gram. J Am Sci 5: 79-84.

Davies PJ, 2010. Plant hormones, biosynthesis, signal transduction, action, rev. 3rd edn. Springer.

Duan J, Jiang W, Cheng Z, Heikkila JJ, Glick BR, 2013. The complete genome sequence of the plant growth-promoting bacterium *Pseudomonas putida* UW4. PLoS ONE 8: e58640. http://dx.doi.org/10.1371/journal.pone.0058640

Glick BR, 1995 .The enhancement of plant growth by free-living bacteria. Can J Microbiol 41: 109-117. http://dx.doi.org/10.1139/m95-015

Glick BR, Penrose DM, Li J, 1998. A model for the lowering of plant ethylene concentrations by plant growth promoting bacteria. J Theor Biol 190: 63-68. http://dx.doi.org/10.1006/jtbi.1997.0532

Glickman E, Dessaux Y, 1995. A critical examination of the specificity of the Salkowski reagent for indolic compounds produced by phytopathogenic bacteria. Appl Environ Microb 61: 793-796.

Gravel V, Antoun H, Tweddell RJ, 2007. Growth stimulation and fruit yield of greenhouse tomato plants by inoculation with *Pseudomonas putida* or *Trichoderma atroviride:* Possible role of indole acetic acid (IAA). Soil Biol Biochem 39: 1968-1977. http://dx.doi.org/10.1016/j.soilbio.2007.02.015

Guglielminetti L, Busilacchi HA, Alpi A, 2000. Effect of anoxia on α-amylase induction in maize caryopsis. J Plant Res 113: 185-92. http://dx.doi.org/10.1007/PL00013929

Halgren A, Azevedo M, Mills D, Armstrong D, Thimmaiah M, McPhail K, Banowetz G, 2011. Selective inhibition of *Erwinia amylovora* by the herbicidally active germination-arrest factor (GAF) produced by *Pseudomonas* bacteria. J Appl Microbiol 111: 949-959. http://dx.doi.org/10.1111/j.1365-2672.2011.05098.x

Hirano SS, Upper CD, 2000. Bacteria in the leaf ecosystem with emphasis on *Pseudomonas syringae* a pathogen, ice nucleus, and epiphyte. Microbiol Mol Biol Res 64: 624-653 http://dx.doi.org/10.1128/MMBR.64.3.624-653.2000

Khan MA, Ungar IA, 1984. The effect of salinity and temperature on the germination of polymorphic seeds and growth of *Atriplex triangularis* Willd. Am J Bot 71: 481-489. http://dx.doi.org/10.2307/2443323

Lambrecht M, Okon Y, Vande Broek A, 2000. Indole-3-acetic acid: a reciprocal signalling molecule in bacteria-plant interactions. Trends Microbiol 8: 298-300. http://dx.doi.org/10.1016/S0966-842X(00)01732-7

Lee X, Azevedo MD, Armstrong DJ, Banowetz GM, Reimmann C, 2013. The *Pseudomonas aeruginosa* antimetabolite L-2-amino-4-methoxy-trans-3-butenoic acid inhibits growth of *Erwinia amylovora* and acts as a seed germination-arrest factor. Environ Microbiol Rep 5: 83-89. http://dx.doi.org/10.1111/j.1758-2229.2012.00395.x

Li F, Zhou L, Shi J, Gao S, 2012. Promotion of IAA, NAA on seed germination of *Jacaranda mimosifolia*. J Ag Sci Tech B 2: 1184-1189. http://www.davidpublishing.com.

Lovisolo C, Schubert A, Sorce C, 2002. Are xylem radial development and hydraulic conductivity in downwardly-growing grapevine shoots influenced by perturbed auxin metabolism? New Phytol 156:65-74. http://dx.doi.org/10.1046/j.1469-8137.2002.00492.x

Lugtenberg B, Kamilova F, 2009. Plant-growth-promoting rhizobacteria. Annu Rev Microbiol 63: 541-556. http://dx.doi.org/10.1146/annurev.micro.62.081307.162918

Mayak S, Tivosh T, Glick BR, 1999. Effect of wild type and mutant plant growth-promoting rhizobacteria on the rooting of mung bean cuttings. J Plant Growth Regul 18: 49-53. http://dx.doi.org/10.1007/PL00007047

McPhail KL, Armstrong DJ, Azevedo MD, Banowetz GM, Mills DI, 2010. 4-Formylaminooxyvinylglycine, an herbicidal germination-arrest factor from *Pseudomonas* rhizosphere bacteria. J Nat Prod 73: 1853-1857. http://dx.doi.org/10.1021/np1004856

Meyer JM, Gruffaz C, Fischer-LeSaux M, 2008. Siderotyping, a straightforward tool to identify soil and plant-related pseudomonads. In: Molecular mechanisms of plant and microbe coexistence; Nautiyal CS, Dion P (eds). pp 369-382. Springer, Verlage Berlin Heidelberg. http://dx.doi.org/10.1007/978-3-540-75575-3_15

Mirza MS, Ahmad W, Latif F, Haurat J, Bally R, Normand P, Malik KA, 2001. Isolation, partial characterization, and the effect of plant growth-promoting bacteria (PGPB) on micro-propagated sugarcane in vitro. Plant Soil 237: 47-54. http://dx.doi.org/10.1023/A:1013388619231

Mohammadi R, Haghparast R, Amri A, Ceccarelli S, 2010. Yield stability of rainfed durum wheat and GGE biplot analysis of multi-environment trials. Crop Pasture Sci 61: 92-101. http://dx.doi.org/10.1071/CP09151

Morris CF, Mueller DD, Faubion GM, Paulsen GM, 1988. Identification of L-tryptophan as an endogenous inhibitor of embryo germination in white wheat. Plant Physiol 88: 435-440. http://dx.doi.org/10.1104/pp.88.2.435

Noumavo PA, Kochoni E, Didagbé YO, Adjanohoun A, Allagbé M, Sikirou R, Gachomo EW, Kotchoni SO, Baba-Moussa L, 2013. Effect of different plant growth promoting rhizobacteria on maize seed germination and seedling development. Am J Plant Sci 4: 1013-1021. http://dx.doi.org/10.4236/ajps.2013.45125

O'Neill DP, Ross JJ, 2002. Auxin regulation of the gibberellin pathway in pea. Plant Physiol 130: 1974-1982. http://dx.doi.org/10.1104/pp.010587

Okon Y, Labandera-Gonzalez C, 1994. Agronomic applications of *Azospirillum:* An evaluation of 20 years worldwide field inoculation. Soil Biol Biochem 26: 1591-1601. http://dx.doi.org/10.1016/0038-0717(94)90311-5

Patten C, Glick BR, 1996. Bacterial biosynthesis of indole-3-acetic acid. Can J Microbiol 42: 207-220. http://dx.doi.org/10.1139/m96-032

Patten CL, Glick BR, 2002. Role of *Pseudomonas putida* indole acetic acid in development of host plant root sys-

tem. Appl Environ Microbiol 48: 3795-3801. http://dx.doi.org/10.1128/AEM.68.8.3795-3801.2002

Paulsen GM, Auld AS, 2004. Preharvest sprouting of cereals. In: Handbook of seed physiology applications to agriculture; Benech-Arnold RL, Sánchez RA (eds). Food Products Press, The Haworth Reference Press and imprints of The Haworth Press, New York, London and Oxford.

Perata P, Guglielminetti L, Alpi A, 1997. Mobilization of endosperm reserves in cereal seeds under anoxia. Ann Bot 79: 49-56. http://dx.doi.org/10.1093/oxfordjournals.aob.a010306

Pereyra MA, García P, Colabelli MN, Barassi CA, Creus CM, 2012. A better water status in wheat seedlings induced by *Azospirillum* under osmotic stress is related to morphological changes in xylem vessels of the coleoptile. Appl Soil Ecol 53: 94-97. http://dx.doi.org/10.1016/j.apsoil.2011.11.007

Roychowdhury R, Mamgain A, Ray S, Tah J, 2012. Effect of gibberellic acid, kinetin and indole 3-acetic acid on seed germination performance of *Dianthus caryophyllus* (Carnation). Agr Conspectus Sci 77: 157-160.

Saharan BS, Nehra V, 2011. Plant growth promoting rhizobacteria: A critical review. Life Sci Med Res 21: 1-30.

Selvakumar G, Joshi P, Nazim S, Mishra PK, Bisht JK, Gupta HS, 2009. Phosphate solubilization and growth promotion by *Pseudomonas fragi* CS11RH1 (MTCC 8984), a psychrotolerant bacterium isolated from a high altitude Himalayan rhizosphere. Biologia 64: 239-245. http://dx.doi.org/10.2478/s11756-009-0041-7

Sheng XF, Xia JJ, Jiang CY, He LY, Qian M, 2008. Characterization of heavy metal-resistant endophytic bacteria from rape *(Brassica napus)* roots and their potential in promoting the growth and lead accumulation of rape. Environ Pollut 156: 1164-70. http://dx.doi.org/10.1016/j.envpol.2008.04.007

Simons M, Permentier HP, Weger LA, Wijffelman CA, Lugtenberg BJJ, 1997. Amino acid synthesis is necessary for tomato root colonization by *Pseudomonas fluorescens* strain WCS365. Mol Plant Microbe In 10: 102-106. http://dx.doi.org/10.1094/MPMI.1997.10.1.102

Sinha S, Mukherjee SK, 2008. Cadmium-induced siderophore production by a high Cd-resistant bacterial strain relieved Cd toxicity in plants through root colonization. Curr Microbiol 56: 55-60. http://dx.doi.org/10.1007/s00284-007-9038-z

Taghavi S, Garafola C, Monchy S, Newman L, Hoffman A, Weyens N, Barac T, Vangronsveld J, Lelie D, 2009. Genome survey and characterization of endophytic bacteria exhibiting a beneficial effect on growth and development of poplar trees. Appl Environ Microbiol 75: 748-757. http://dx.doi.org/10.1128/AEM.02239-08

Zarrin F, Saleemi M, Zia M, Sultan T, Aslam M, Rehman R, Fayyaz Chaudhary M, 2009. Antifungal activity of plant growth-promoting rhizobacteria isolates against *Rhizoctonia solani* in wheat. Afr J Biotechnol 8: 219-225.