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ORIGINAL ARTICLE

Protective effect of *Pseudomonas* spp. isolates and zinc on seed germination and β-amylase activity in wheat cultivars under cadmium stress

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Abstract

Plant–bacteria interactions and plant nutrition have been exploited to abate the harmful effects of cadmium (Cd) on the germination of wheat cultivars. This study investigated the effects of *Pseudomonas* species and zinc (Zn) on the germination of wheat cultivars *Triticum aestivum* (bread wheat) and *Triticum turgidum* (durum wheat) under Cd stress. The application of bacteria (*Pseudomonas putida* inoculants, *Pseudomonas fluorescens* inoculants), Zn in three levels (0, 15 and 30 mg L^{-1}), Cd in five levels (0, 5, 15, 25 and 35 mg L^{-1}) and appropriate negative controls was evaluated in each wheat cultivar. β-Amylase activity was reduced with increasing Cd concentration. Durum wheat showed higher β-amylase enzyme activity than bread wheat after inoculation with *P. fluorescens*. Nevertheless, inoculated seeds of both wheat cultivars with *P. fluorescens* exhibited increased β-amylase activity and consequently increased germination speed. However, bacterial inoculation showed no effect on the increment of plumule and radicle dry weights of seedlings. Overall, the combined application of *Pseudomonas* species (especially *P. fluorescens*) and Zn was able to decrease the deleterious effects of Cd stress on β-amylase activity, and subsequently germination indices of wheat cultivars.

Keywords β-Amylase activity · Cadmium stress · Germination indices · *Pseudomonas* · Wheat · Zinc

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Introduction

Heavy metals such as cadmium (Cd) are being released into the environment not only by human activity, but also through natural processes (Tran and Popova 2013). The presence of excessive amounts of heavy metals has become a serious problem in the last few decades (Gratão et al. 2005), especially in the cultivation of important agricultural crops like wheat. Cd is a toxic element in plants with a critical leaf concentration of 5−10 μg g^{-1} dry weight (White and Brown 2010). Cd is not necessary for plant growth, but is easily taken up by roots and its accumulation in plant tissue causes cell death (De Maria et al. 2013; Souza et al. 2011). Although heavy metal pollution is increasing (Wu et al. 2013), there has been relatively little research carried out to investigate the effects of heavy metals on seed metabolism, germination and seed vigor, as well as effects on roots and shoots.

Germination is a complex physiological process which can be affected by heavy metal stress (Sethy and Ghosh 2013). Heavy metals could affect seed germination by preventing imbibition. Metal-induced genetic mutations, which

result from the actions of free radicals, could endanger germination and seedling establishment (Kranner and Colville 2011). The germination process can be affected by Cd through reduction of water uptake, and by oxidative stress which tends to interfere with other vital processes (Ahsan et al. 2007). Cd accumulation in a seed can result in delay or prevention of seed germination (Chugh and Sawhney 1996). Furthermore, Cd can negatively impact germination by interfering with the activities of key enzymes.

One of the critical enzymes required for germination is β-amylase, due to its role in starch degradation. Starch is an abundant storage compound in seeds and its degradation is initiated by α-amylase. α-Amylase produces soluble oligosaccharides from starch, which are then hydrolyzed by β-amylase to maltose (Yamasaki 2003). Therefore, β-amylase is an essential enzyme for germination. Moreover, it is suggested that β-amylase activity is a reliable indicator of the germination ability of rice seeds (Yamasaki 2003). It has also been demonstrated that β-amylase is more important than α-amylase during the early hours of the germination process in wheat (Yamasaki 2003). The activities of α-amylase and β-amylase have been shown to be substantially inhibited by Cd in rice and peas, respectively, leading to a restricted rate of starch hydrolysis and a reduction in the amount of energy available for germination and seedling growth (He et al. 2008). β-Amylase is more sensitive to heavy metals than α-amylase (Bilderback 1973). Significant declines in seed germination, seedling biomass, and root and shoot elongation in wheat with increasing Cd concentrations have been reported ($p < 0.01$) (Liu et al. 2007). Hence, it is essential to develop protocols to alleviate the harmful effects of Cd to seeds in soils.

Strategies for combating Cd toxicity include inoculation of soils with specific microorganisms and addition of zinc (Zn). Different microorganisms belonging to bacteria genera of *Bacillus*, *Pseudomonas* and *Streptomyces* have been successfully applied as absorbing agents to remove heavy metals (Vijayaraghavan and Yun 2008). *Pseudomonas* strains are among the most abundant phosphate solubilizers (Javadi Nobandegani et al. 2015) and have also been shown to be good candidates for absorbing Cu, Cd and Pb from solution (Johncy-Rani et al. 2010). *Pseudomonas* species are currently being applied in agricultural systems as plant growth promoting agents, as they have shown the ability to absorb heavy metals, especially Cd from the growth medium (Chellaiah 2018). The alleviation of heavy metal toxicity by microorganisms may be brought about by reduced metal absorption in plants or decreases in harmful amounts of ethylene produced under heavy metal stress (Madhaiyan et al. 2007). Since Zn and Cd are commonly absorbed by the same channels in the plants (Song et al. 2017), an increase in the concentration of Zn in the growth medium could inhibit Cd uptake by plants. Additionally, Zn could protect plants against Cd toxicity by improving the plant's defense systems against oxidative stress induced by Cd, or by competing with Cd for active sites of enzymes, proteins and lipid membranes (Köleli et al. 2004).

The objectives of this study were to determine the sole and aggregated effect of the application of *Pseudomonas* species and Zn on germination indices and β-amylase activity in wheat seeds under Cd stress. Our hypotheses were (I) any concentration of Cd would retard growth, (II) *Pseudomonas* species would lessen Cd toxicity in the wheat cultivars, and (III) Zn application would enable higher germination rates in the presence of Cd. Additionally, the combined application of bacteria and Zn may also profoundly reduce the detrimental effects of Cd on β-amylase activity, which would lead to higher germination rates.

Materials and methods

This experiment was conducted to investigate the effect of *Pseudomonas* species and Zn on the germination of wheat cultivars under Cd stress. The wheat cultivars used in this study are *Triticum aestivum* L. cv. Chamran (bread wheat) and *Triticum turgidum* L. var durum cv. MD87 (durum wheat), which are spring cultivars.

Bacterial species [*Pseudomonas putida* (P108), and *Pseudomonas. fluorescens* (P169)] with plant growth promoting characteristics were obtained from the Soil and Water Research Institute, Karaj, Iran. The strains *P. putida* (P108), and *P. fluorescens* (P169) were previously isolated from fields where wheat had been extensively cultivated (Asgharzadeh et al. 2011) and registered with the Global Catalogue of Microorganisms with a CCSM code (Culture Collection of Soil Microorganisms) at the Soil and Water Research Institute, Karaj, Iran. These two strains are notable for their ability to solubilize phosphates and to produce auxin, thereby promoting plant growth. However, an additional strain *P. fluorescens* (P193) had a significantly lower ability to produce auxin. Hence, *P. fluorescens* (P193) was used as a negative control (Asadi Rahmani et al. 2010; Asgharzadeh et al. 2011) in this experiment. Before the germination test, seeds were disinfected with sodium hypochlorite solution (4%) and then washed four times with distilled water.

Completely randomized experiments were performed with a factorial arrangement in four replicates for each wheat cultivar. In total, 240 experimental units were used for each wheat cultivar (bread wheat and durum wheat) with the following breakdown: bacterial treatment as one factor (*P. putida* inoculants (P108), *P. fluorescens* inoculants (P169), negative control (P193) and non-inoculated seeds), Zn element in three levels (0, 15 and 30 mg L^{-1}) and Cd concentration in five levels (0, 5, 15, 25 and 35 mg L^{-1}). The inoculated seeds were distributed over sterile filter paper in

Petri dishes with 9 cm inner diameter. Solutions containing different concentrations of Cd and Zn ions were prepared by dissolving measured masses of cadmium sulfate octahydrate (ACS grade, ≥ 98%) and zinc sulfate heptahydrate (ACS grade, 99.0–103.0%). Solutions with different concentrations of Cd (0, 5, 15, 25 and 35 mg L^{-1}) and Zn (0, 15 and 30 mg L^{-1}) ions were prepared and their pH values were adjusted to 6.5 before adding to Petri dishes. Optimum pH for Cd adsorption by bacteria species had been previously determined to be between 6 and 7 (Safari et al. 2013). Also, this range of pH is preferred for wheat cultivation. After adding these solutions to Petri dishes, the dishes were wrapped with parafilm and placed in the germinator at 20 °C. The total number of seeds in each Petri dish was 37, of which 25 were used for germination test and the rest for evaluating β-amylase activity. The number of germinated seeds after 7 days was counted. Lengths and weights of plumules and radicles were also measured. Germination indices were calculated according to the methodology of the International Seed Testing Association (ISTA).

The β-amylase activity in the presence of different Cd concentrations was ascertained with the 3,5-dinitrosalicylic acid method (Bernfeld 1955) at 12 h intervals within the first 48 h of the start of the experiment. Samples were collected from Petri dishes, placed in liquid $N_{2,}$ and, afterward, stored in a − 80 °C freezer until analysis.

The germination indices

Some characteristics like the lengths of plumules and radicles, length ratio of plumule to radicle, dry weight, germination percentage, germination speed, germination speed index, average time required for germination and seedling vigor index were analyzed using ISTA methods (ISTA 1999). The average lengths of plumules and radicles from ten randomly selected seedlings were measured by a ruler on the 7th day. Fresh weights of plumules and radicles were assessed by analytical balance. After putting them in a 70 °C oven for 48 h, their dry weights were also determined. The germination indices were evaluated using Eqs. 1–6 (see Table S1).

β-Amylase activity in seeds

A sample mass of 0.2 g of seeds was ground with 5 ml of sodium acetate buffer by mortar and pestle on ice, then poured into 15-ml Falcon centrifuge tubes, and the suspension was centrifuged at 12,000 *g* for 15 min at 4 °C. The supernatant was filtered through Whatman filter paper No. 42. and used as a crude extract for the assay of enzyme activity (Bernfeld 1955).

The preparation of the starch solution consisted of dissolving 1 g of starch into 70 ml of sodium acetate buffer in a hot water bath at 100 °C. After dissolving, the volume was raised to 100 ml with distilled water. The preparation of the color reagent consisted of taking 50 ml of distilled water and adding it to 1 g of 3,5-dinitrosalicylic acid in 100 ml beaker. The beaker with a magnet was placed on a stirring plate. Then 30 g of potassium sodium tartrate tetrahydrate was slowly added to the beaker, followed by adding 20 ml of 2N NaOH. The color of the solution changed from lemon yellow to orange. After dissolution, the volume was raised to 100 ml with distilled water in a volumetric flask and then filtered. This solution was stored in the refrigerator with the lid on and its maximum storage period was 1 week.

To perform β-amylase activity measurements, 0.5 ml of extract along with 0.5 ml of 1% starch and 1 ml DNS were mixed. The tubes were covered with lids and placed in a hot water bath at 100 °C for 5 min. The test tubes were cooled at room temperature and 10 ml of distilled water was added to each of them. After stirring, the absorbance of the solution was measured with the UV–Vis spectroscopy at 540 nm. Then, the β-amylase activity was obtained by using a standard curve to relate the optical density (OD) to actual amount of β-amylase. The β-amylase activity was expressed as μmol per milligram protein [μmol mg^{-1}(protein)].

Statistical analysis

This experiment was conducted in a completely randomized design with the factorial arrangement in four replicates. A three-way ANOVA was performed in SAS 9.1. Means comparisons were carried out using least significant difference (LSD) tests. Each wheat cultivar was evaluated separately.

Results

β-Amylase activity

The β-amylase activity was measured four times as a physiological indicator for examining wheat germination under Cd stress at 12-h intervals from the start of the test. The results of analysis of variance showed that all factors (wheat, bacteria, Zn and Cd) and their interactions significantly affected the β-amylase activity (Table 1) ($p < 0.01$).

Durum wheat showed 20–28% higher β-amylase activity when compared to bread wheat in four time intervals. *P. fluorescens* inoculants were most effective in increasing β-amylase activity in seedlings, followed by *P. putida* inoculants, negative control for auxin, and non-inoculated seeds (Table S2). An examination of the means showed that β-amylase activity had an increasing trend over time in each level of investigating factors (Table S2). *P. fluorescens* as an exception showed a decreasing trend initially, but after the 24th hour from the start of the test, β-amylase activity

Table 1 Analysis of variance of β-amylase activity in wheat cultivars in four time intervals

S.O.V	Mean square				
	d.f.	12 h	24 h	36 h	48 h
Wheat	1	499,686.76**	81,098,192**	1,343,431.47**	907,796.34**
Bacteria	3	2,771,969.28**	1,961,622.67**	1,320,581.67**	1,227,433.36**
Zn	2	669,054.39**	1,020,389.12**	1,999,101.28**	1,627,796.14**
Cd	4	275,442.24**	247,050.01**	304,049.75**	319,938.48**
Wheat × bacteria	3	33,733.30**	10,552.73**	4376.41**	80,970.63**
Wheat × Zn	2	20,999.51**	8235.71**	106,090.23**	152,479.53**
Wheat × Cd	4	20,999.51**	2616.95**	1640.18**	950.87**
Bacteria × Zn	6	43,216.25**	110,039.53**	77,659.52**	66,861.17**
Bacteria × Cd	12	7636.20**	4285.20**	7785.91**	6284.31**
Zn × Cd	8	786.23**	476.12**	2160.05**	2606.56**
Wheat × bacteria × Zn	6	46,517.28**	44,912.38**	42,588.41**	21,862.37**
Wheat × bacteria × Cd	12	1618.03**	1587.39**	5373.95**	5271.57**
Wheat × Zn × Cd	8	3532.28**	780.12**	772.82**	2647.99**
Bacteria × Zn × Cd	24	3710.73**	1499.56**	4157.47**	1063.18**
Wheat × bacteria × Zn × Cd	24	1930.83**	2205.75**	493.63**	927.71**
Error	360	2.67	2.67	2.67	6.00
Total	479				
C.V		0.469	0.447	0.378	0.525

**Significant at the 1% levels of probability

increased. The ability of bacterial treatments to increase β-amylase activity, when compared to non-inoculated seeds, reduced with the passage of time. For instance, in case of *P. fluorescens* application, which showed the highest amounts of β-amylase, decreasing trends in the increments of β-amylase activity (284, 231, 178, 165%) were observed when compared with non-inoculated seeds, respectively, in 12, 24, 36 and 48 h after the start of experiment. Similar trends with lower values were also observed with the two other strains.

Of all the applied concentrations of Zn, 30 mg L^{-1} of Zn had the largest effect on β-amylase activity in all investigating intervals (Table S2). Increases in Zn concentration in media induced more β-amylase activity, with 30 mg L^{-1} Zn treatment bringing about 1.5-fold increase in β-amylase activity when compared to 0 mg L^{-1} Zn. While increasing the Cd concentration in media resulted in a reduction of β-amylase activities, there was an exception at the 36th hour time point where the 5 mg L^{-1} of Cd had higher β-amylase activity when compared to control (Table S2).

An examination of the interactions of all factors in this experiment (Fig. 1a–d) showed that the inoculation of durum wheat seeds with *P. fluorescens* (P169) exhibited more β-amylase activity in all concentrations of Cd and Zn when compared to bread wheat. Increasing Cd concentration in three sets of time intervals (12, 24 and 48 h) resulted in reduction in β-amylase activities (maximum decreasing trend was also seen in durum wheat inoculated with *P. fluorescens* when no Zn was applied, Fig. 1b). Thirty-six hours after the start of the experiment, the increment of β-amylase activities in 5 mg L^{-1} Cd was higher than control in most cases.

The lengths of plumules and radicles, and length ratio of plumule to radicle

The analysis of variance showed that the main factors had significant effects on the lengths of plumules and radicles, and also on the length ratio of plumule to radicle ($p < 0.01$). The factor interactions such as wheat × bacteria × Zn, and bacteria × Zn × Cd significantly affected the lengths of plumules, and the length ratio of plumule to radicle ($p < 0.01$). However, the lengths of radicles were solely affected by the four-level interaction of wheat × bacteria × Zn × Cd (Table 2).

Durum wheat displayed larger plumules and radicles, but bread wheat showed higher length ratios of plumule to radicle. The longest of plumules and radicles were observed in the non-inoculated seed treatment, although the highest length ratio of plumule to radicle was obtained in the treatment with *P. fluorescens*. The lengths of plumules and radicles were longer when no Zn was applied. In spite of this, applying 15 mg L^{-1} Zn induced higher length ratio of plumule to radicle, 15 percent higher than both 0 and 30 mg L^{-1} (Table S3). Increasing the Cd concentration caused the reduction of the lengths of plumules and radicles. On the other hand, the length ratio of plumule to radicle was

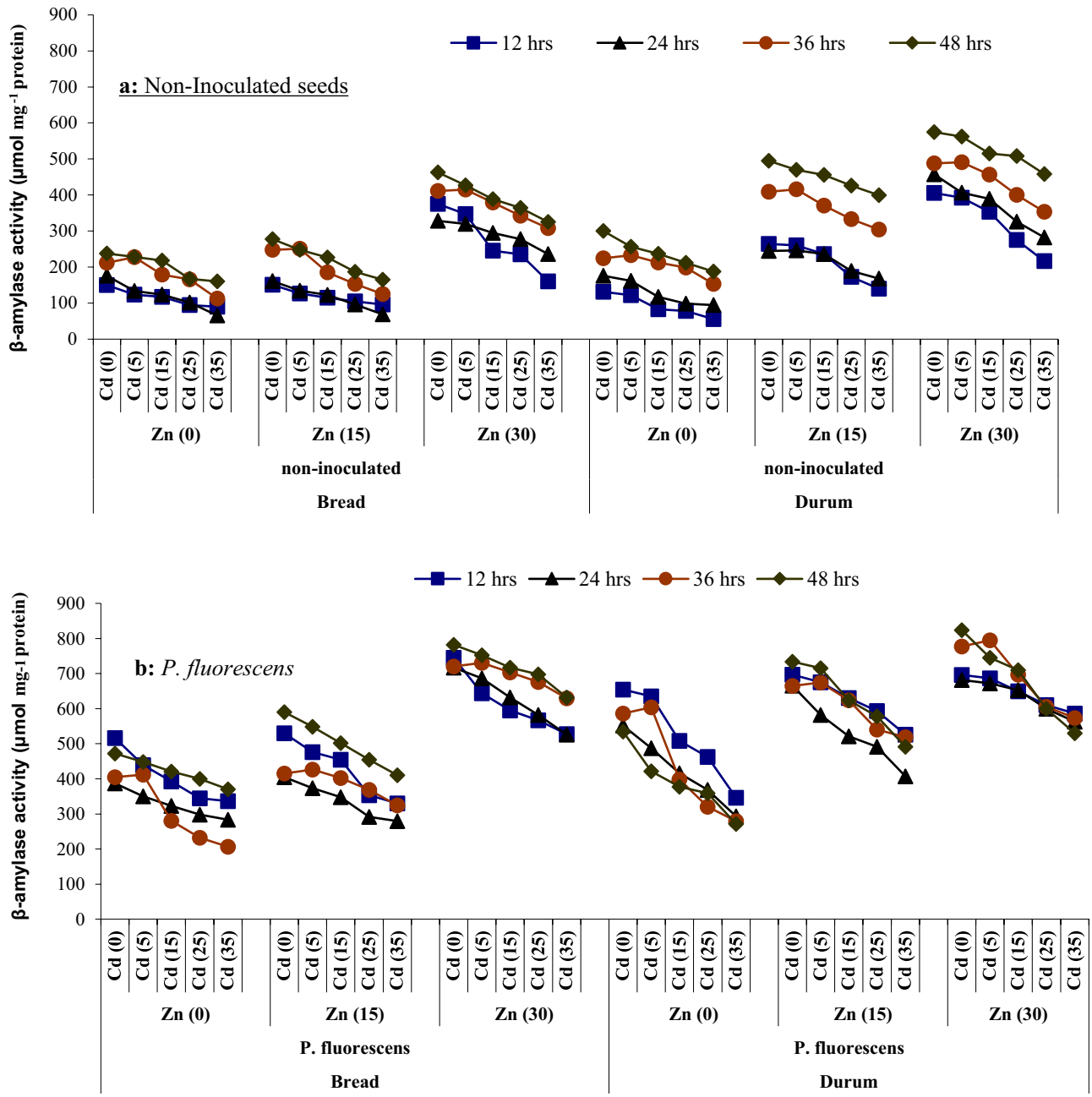

Fig. 1 Four level interaction of wheat × bacteria × zinc × cadmium on β-amylase activity in four investigating times. **a** Non-inoculated seeds. **b** Inoculated seeds with *P. fluorescens*. **c** Inoculated seeds with *P. putida.* **d** Inoculated seeds with *P. fluorescens,* negative control for auxin

increased (no significant differences observed at low levels of Cd).

Dry weights of plumules and radicles

The main factors investigated in this experiment had significant effects on dry weights of plumules and radicles ($p < 0.01$). The triple interaction effects of factors like wheat × bacteria × Zn and bacteria × Zn × Cd also had significant effects on dry weights of plumules. Dry weights of radicles were affected by wheat × bacteria × Zn × Cd interactions (Table 2) ($p < 0.01$).

Durum wheat had shown higher values of plumule and radicle dry weights when compared to bread wheat. The non-inoculated seed treatment had the highest values of plumule and radicle dry weights when compared to bacterial treatments. Next in rank, *P. fluorescens* had shown

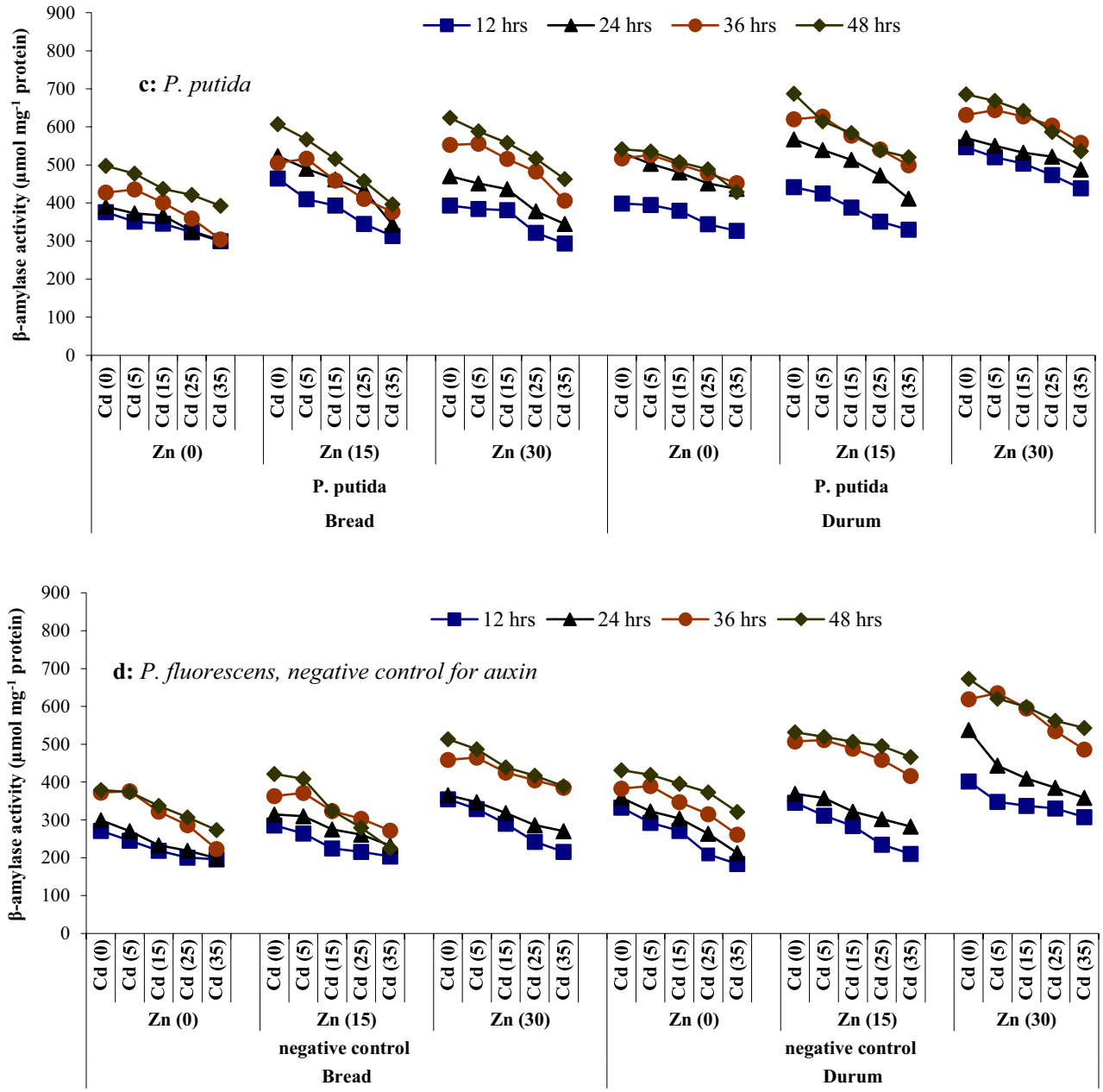

Fig. 1 (continued)

heavier plumules, and heavier radicles were seen in the negative control treatment (Table S3).

Application of Zn had no effect on plumule and radicle dry weights. A decreasing trend in the dry weight of plumules and radicles was observed with increasing Cd concentration. The lowest amounts were obtained at 35 mg L^{-1} Cd even though the differences between the levels of 0, 5 and 15 mg L^{-1} were not significant (Table S3).

The triple interaction effect of bacteria × Zn × Cd on plumule dry weights showed that the combined treatments of non-inoculated × 0 mg L^{-1} Zn × 5 mg L^{-1} Cd had the highest amount of plumule dry weights, which was not significantly different with non-inoculated × 0 mg L^{-1} Zn × 0 mg L^{-1} Cd treatments. Increasing Cd concentration to 5 mg L^{-1}, with no application of Zn, induced increases in plumule dry weights. The lowest amount of plumule dry weights in this experiment was observed in the negative control for auxin (P193) × 15 mg L^{-1} Zn × 35 mg L^{-1} Cd (data not shown).

The non-inoculated seeds of both wheat cultivar treatments had the highest radicle dry weights in all concentrations of Zn and Cd in contrast to other bacterial treatments.

Table 2 Analysis of variance of germination indices in wheat cultivars affected by treatments

S.O.V	*d.f.*	Mean square										
Treatments		Length of plumule	Length of radicle	Length ratio of plumule to radicle	Radicle dry weight	Plumule dry weight	Germination percentage	Germination speed	The average time required for germination	Germination speed index	Length seedling vigor index	Weight seedling vigor index
Wheat	1	4.56**	404.98**	7.28**	0.021**	0.0029**	0.041*	1613.06**	119.05**	4.92**	4,855,250.08**	411.25**
Bacteria	3	121.01**	432.61**	6.78**	0.010**	0.0092**	0.078**	12.37*	37.3**	0.064**	9,256,814.80**	368.78**
Zn	2	151.47**	216.14**	1.84**	0.024**	0.010**	0.021ns	447.39**	20.70**	0.681**	7,165,965.37**	619.86**
Cd	4	6.17**	55.01**	0.88**	0.002**	0.0001**	0.008ns	0.75ns	0.129ns	0.003ns	926,535.33**	34.005**
Wheat × bacteria	3	2.78**	45.05**	1.19**	0.001**	0.0002**	0.016ns	1.27ns	1.74**	0.009ns	680,512.30**	31.85**
Wheat × Zn	2	2.98**	16.91**	0.56**	0.002**	0.0001*	0.044*	43.52**	2.56**	0.185**	392,464.83**	30.42**
Wheat × Cd	4	0.23 ns	0.94ns	0.04ns	0.0003**	0.00004ns	0.028*	0.69ns	0.289ns	0.006ns	28,972.21ns	5.42*
Bacteria × Zn	6	42.62**	65.34**	1.56**	0.002**	0.003**	0.030**	121.08**	7.40**	0.208**	1,914,705.57**	86.21**
Bacteria × Cd	12	1.00**	3.33**	0.04ns	0.00006ns	0.00009**	0.011ns	1.78ns	0.459*	0.005ns	55,401.42**	2.40 ns
Zn × Cd	8	0.33ns	7.10**	0.31**	0.0004**	0.00004ns	0.013ns	1.59ns	0.293ns	0.003ns	105,096.00**	4.59**
Wheat × bacteria × Zn	6	3.81**	12.00**	1.23**	0.0008**	0.0001*	0.020ns	18.08**	1.74**	0.087**	280,674.28**	12.37**
Wheat × bacteria × Cd	12	0.41ns	1.36ns	0.12*	0.00009ns	0.00002ns	0.015ns	1.62ns	0.219 ns	0.003ns	37,148.56*	1.77ns
Wheat × Zn × Cd	8	0.23ns	1.24ns	0.12*	0.00008ns	0.00001ns	0.007ns	1.94ns	0.142ns	0.002ns	21,365.48 ns	1.43ns
Bacteria × Zn × Cd	24	0.88**	1.30*	0.11**	0.00006ns	0.00006*	0.027**	2.04ns	0.257ns	0.006ns	40,110.67**	1.58ns
Wheat × bacteria × Zn × Cd	24	0.58ns	2.03**	0.08ns	0.0001**	0.00003ns	0.016*	1.76ns	0.117ns	0.002ns	44,034.15**	2.28ns
Error	360	0.43	0.84	0.05	0.00005	0.00004	0.01	3.77	0.220	0.0057	20,426.20	1.72
C.V		13.81	16.68	23.56	16.19	13.12	6.77	10.07	12.56	11.11	14.16	1.124

*, **Significant at the 5% and 1% levels of probability, respectively

The negative control for auxin (P193) was next in rank (data not shown).

Germination indices

Analysis of variance showed that the type of wheat cultivar and bacterial treatment had significant effects on all germination indices. All germination indices except germination percentage were affected by Zn treatments. Cd treatments only had significant effects on the length and weight seedling vigor index ($p < 0.01$) (Table 2).

The triple interaction of factors for wheat × bacteria × Zn significantly affected germination speed, germination speed index, the average time required for germination and seedling vigor index ($p < 0.01$). The bacteria × Zn × Cd interaction had significant effects on germination percentage and length seedling vigor index. The interaction of all factors in the experiment had significant effects on length seedling vigor index ($p < 0.01$) and germination percentage ($p < 0.05$) (Table 2).

Durum wheat displayed more favorable germination indices than bread wheat such as higher germination percentages, germination speeds, the average time required for germination, and length and weight seedling vigor indices (Table S4). Inoculation with *P. fluorescens* (P169) accelerated seed germination. The other germination indices had shown the highest values in non-inoculated seeds (Table S4).

Zn application at 30 mg L^{-1} induced significant increases in germination percentage, germination speed, germination speed index and the length seedling vigor index (Table S4). Increasing Cd concentrations decreased seedling vigor index, even though significant differences were not observed between 5 mg L^{-1} and no Cd application but a decreasing trend was noticeable (Table S4). There were no significant differences observed at different Cd concentrations in the current experiment (0, 5, 15, 25 and 35 mg L^{-1}) on germination percentage, germination speed, germination speed index and the average time required for germination (Table S4).

Discussion

The impairment in amylase activity and seedling growth due to inhibitory effects of Cd has been previously reported (Kabata-Pendias and Pendias 1984; Liu et al. 2007). Our results show that there were overall decreasing trends in β-amylase activity with increasing Cd concentration. The results of a different study revealed that the total α-amylase and β-amylase activities significantly decreased at elevated concentrations of Cd (Liu et al. 2007). The application of *Pseudomonas* species and zinc ameliorated the negative impact of Cd on β-amylase activities in both wheat cultivars. It has been previously stated that some microorganisms including *Pseudomonas* sp. have the ability to produce β-amylase (Pandey et al. 2000). As shown in Fig. 1, the combination of *P. fluorescens* and zinc induced higher β-amylase activity. Thus, certain bacteria provide some protection to plants against the harmful effects of Cd.

The inhibitory effects of Cd on lengths of plumules and radicles were evident (Kabata-Pendias and Pendias 1984). Our results show that radicles were more negatively affected by Cd than plumules. It has been previously reported that with increasing Cd concentrations, root length appeared to be the most sensitive trait to Cd toxicity (Liu et al. 2007). Heavy metals such as Cd have noticeable impacts on germination and lengths of plumules and radicles; these impacts were exacerbated with increases in Cd concentration. Cd at low concentrations was a stimulator of the lengths of plumules and radicles in wheat, but at high concentrations caused reduction in the size of plumules and radicles (Ahmad et al. 2012).

Although there was no significant difference between 5 mg L^{-1} and no Cd application, the seedling vigor index had shown a decreasing trend with increasing Cd concentration. The seedling vigor index at low concentration (2 mg L^{-1}) increased, but decreased with increasing concentration (10 mg L^{-1}) (Rajjak Shaikh et al. 2013). Germination and the growth of plumules and radicles were induced by arsenic at low concentrations (0–1 mg kg^{-1}), but were suppressed at higher arsenic concentrations (5–20 mg kg^{-1}) (Liu et al. 2007).

The observation of no significant differences between Cd concentrations (0, 5, 15, 25 and 35 mg L^{-1}) in all germination indices except for seedling vigor index implied that Cd concentrations in current experiment were not high enough to have negative impacts on germination. Results of a different experiment showed that Cd concentrations exceeding 40 mg kg^{-1} affected germination negatively (Cheng and Zhou 2002). Similar results were observed in other studies with peas (Bansal et al. 2002; Mihoub et al. 2005).

Higher values of the length ratio of plumule to radicle in bread wheat could be ascribed to higher lengths of radicles in durum wheat seedlings in contrast to bread wheat. The enhanced performance of durum wheat in germination speed and germination speed index can be seen in the positive correlation between β-amylase activity and germination indices (Table S5). As mentioned earlier, this cultivar had shown higher β-amylase activity.

Our results reveal that increments in Zn concentration in the medium induced more β-amylase activity. This phenomenon has been reported elsewhere (Sarowar Jahan et al. 2012). The β-amylase activity in the presence of divalent cations like Zn^{2+} significantly increased, although high levels of heavy metals such as Cu^{2+}, Pb^{2+}, Sn^{2+}, and Hg^{2+} drastically reduced the β-amylase activity (Sarowar Jahan et al. 2012). The higher values for plumule and radicle dry

weights and lengths with no Zn application in comparison to Zn application showed that even though Zn had caused an increase in germination percentage and speed due to the induced increment in β-amylase activity, it had no effect on the lengths and dry weights of plumules and radicles (Table S2, S3 and S4).

The highest values of radicle dry weights were observed in non-inoculated seeds of both wheat cultivars, which was followed by the negative control for auxin (P193). This result is counter-intuitive, as we expected that the performance of inoculated strains whose auxin production was suppressed by Cd to be the same as the non-inoculated seeds. We do not have a good explanation for this observed trend.

Conclusions

This study indicates that Cd toxicity leads to a reduction in β-amylase activity, but can be offset by the application of both *Pseudomonas* species (especially *P. fluorescens*) and Zn. Cd stress reduced the β-amylase activity in wheat, but had no distinctive effect on germination indices at the end of the experiment. Durum wheat inoculated with *Pseudomonas fluorescens* in all Cd and Zn concentrations showed more β-amylase activity when compared to bread wheat. Inoculation with bacterial species alone was not influential on germination indices. We plan to continue this research by investigating auxin production in *Pseudomonas* species, which was probably affected by Cd.

Author contribution statement MS: executed the experiment and measured both morphological and physiological traits in seedlings. HKD: designed the experiments and analyzed the data, MS: also helped in data analysis and wrote the paper. UN and NAR: edited the manuscript for English and gave some valuable comments to improve the quality of work. All authors read and revised the manuscript, provided helpful discussions and approved its final version.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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