

# PGPR mediated Zinc biofertilization of soil and its impact on growth and productivity of wheat

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## Abstract

**Objectives:** This study describes Zinc biofortification of wheat using a rhizospheric isolate, *Pseudomonas fluorescens* strain Psd.

**Methods:** The strain was used as a bio-ionculant in soil deficient in zinc and the plant-growth promoting potential and biofortification was assessed using a number of physical and biochemical parameters. The enzymatic studies indicated towards the zinc supply mediated by the strain

**Result:** This plant-growth-promoting strain, apart from the Zn accumulation potential, has the ability to solubilise Zn and was also able to leach out Zn from ore-tailings. The application of a Zn-laden biomass of the strain in soil resulted in increased growth and productivity of wheat crop as demonstrated by pot experiments. The beneficial effect was also reflected in increased activities of some enzymes. In addition, grain Zn<sup>2+</sup> content was enhanced by ~85% in comparison to wheat grown in Zn<sup>2+</sup>-deficient soil.

**Keywords:** Biofortification, Zinc, plant-growth-promoting rhizobacteria, biofertilization

## 1. Introduction

Zinc is a crucial micronutrient for all life forms. In plants, it is involved in a wide array of metabolic processes like carbohydrate, lipid, protein and nucleic acid synthesis and degradation. Zinc is structurally bound to some important plant enzymes, like carbonic anhydrase and superoxide dismutase, the activities of which are negatively affected under zinc-deficient conditions (Hacisalihoglu *et al.* 2003). Zinc deficiency is prominently encountered

in plants growing in calcareous and sodic soils due to the formation of insoluble zinc carbonate which decreases the availability of this metal (Hafeez *et al.* 2013). Major crops affected by zinc deficiency include wheat, rice, maize, cotton and citrus (Rashid and Ryan 2008).

Plant growth promoting rhizobacteria (PGPR) have been known for long to be involved in plant growth promotion via mechanisms such as phytohormone

production, suppression of plant diseases and improved nutrient acquisition by nitrogen fixation, phosphate solubilization and metal accumulation (Martínez-Viveros *et al.* 2010, Upadhyay and Srivastava 2010). Apart from this, PGPR are also known to be key players in bioremediation of contaminated soils due to their ability to accumulate metals (Zhuang *et al.* 2007). Thus, the metal-laden PGP rhizobacterial biomass aids in the establishment of plants in metal-contaminated soils. Also, this biomass can serve as a bioinoculant in metal-deficient soils, sufficing for the requirement of essential metals as shown for Zinc by Whiting *et al.* (2001) and for nickel by Abou-Shanab *et al.* (2003).

*Pseudomonas fluorescens* strain Psd has been earlier characterized to possess multiple plant-growth promoting and biocontrol properties (Upadhyay and Srivastava 2008, 2010). We have also characterized zinc biosorption by this strain (Upadhyay and Srivastava 2014). In the present communication, we demonstrate the zinc biofertilization potential of this strain and its impact on the growth and development of wheat crop.

## 2. Materials and Methods

### 2.1. Organism, culture conditions and metal source

The PGPR strain used in this study is *Pseudomonas fluorescens* strain Psd which was originally isolated from the roots of *Vigna mungo* and characterised by Upadhyay and Srivastava (2008). For plant experiments, a variant of strain Psd tagged with *lacZ* through a mini-Tn5, namely, PsdLacZ was used in order to ease the selection of the desired strain from a heterogenous soil population. Bacterial suspension was prepared by raising the culture of PsdLacZ in

Gluconate Minimal medium (Gilotra and Srivasatva, 1997) in a Controlled Environment Shaker Incubator (Kühner, Switzerland) at 30 °C and 200 rpm for the required period of time.

The  $Zn^{2+}$  sources used for the experiments were autoclaved metal salt solution of  $ZnSO_4 \cdot 7H_2O$  and ore tailings procured from Regional Research Laboratory, Bhubaneswar, India. For *in vitro* solubilization assay, ZnO was used. Cells were grown in the presence of different sources of Zinc for different times as per the requirement of the experiment.

### 2.2. In vitro assessment of Zinc solubilization

#### 2.2.1. Plate assay

Qualitative assay for ZnO solubilization was performed as per the protocol of Fasim *et al.* (2002). Ten microlitre culture of *P. fluorescens* strain Psd was spotted on Tris minimal agar plates substituted with 14mM ZnO and containing glucose as the carbon source. The plates were incubated at 30 °C for 14 days to see the solubilization efficiency. The control consisted of the same medium supplemented with 14mM ZnO with gluconate as carbon source.

#### 2.2.2. Broth assay

Broth assay for ZnO solubilization was done according to the protocol of Saravanan *et al.* (2003). Solubilization was monitored for 4 weeks by measuring the amount of  $Zn^{2+}$  in the cell free supernatant.

#### 2.2.3. Zinc leaching from low-grade ore tailings

Low grade ore tailings (20% pulp density) were suspended in distilled water for 24h followed by

addition of a carbon source (gluconate 0.4% w/v). Ten millilitre and twenty millilitre culture biomass of strain Psd (corresponding to approx. 6mg and 12mg dry weight, respectively) was added to this suspension and Zn removal from ore suspension was monitored till 10 days.

#### 2.2.4. Estimation of $Zn^{2+}$

Samples for  $Zn^{2+}$  analysis were acid-digested using a mixture of  $HNO_3$  and  $HClO_4$  in 6:1 ratio.  $Zn^{2+}$  was estimated from appropriate dilutions of the digested samples using the Atomic Absorption Spectrophotometer (PerkinElmer model AAnalyst 400) at 219.86nm.

### 2.3. Plant experiments

#### 2.3.1. Experimental setup

In order to look at the Zn biofertilization ability of *P. fluorescens* strain Psd, *Triticum aestivum* var. HD2851 was taken as an experimental crop. The seeds were procured from Indian Agricultural Research Institute (IARI), New Delhi, India. Soil used in this study was low-zinc soil ( $Zn^{2+}$  ~3ppm) obtained from a wheat field in Sonapat, Haryana, India, which falls under most zinc deficient areas in India. The experiment was performed in 8-inch earthen pots in two different sets. In the first set, 500g soil was added to the pots and in second set, pots were filled with the mixture of soil and ore-tailings in the ratio 4:1 (Table 1).

Wheat Seeds were surface sterilized with 0.1%  $HgCl_2$  for 5min followed by 3-4 times washing with autoclaved distilled water. Sterilized seed were

inoculated with strain Psd grown in presence of 2mM  $ZnSO_4 \cdot 7H_2O$  for 24h for the first set. For the second set, wheat seeds were inoculated with the bacterial cells grown in GMM basal medium for 24h.

A talc based carrier formulation was prepared by modification of the protocol suggested by Vidhyasekaran and Muthamilan (1995). For preparation of 80g carrier formulation, 50g of talc was mixed with 20mL bacterial suspension ( $\sim 10^8$  cfu/mL) followed by addition of 1mL Carboxymethyl cellulose (0.1% w/v) and 1mL Glycerol (50% v/v).

#### 2.3.2. Strain survivability *in vivo*

Periodic assessment for survival of *P. fluorescens* strain Psd in soil under environmental conditions was performed during the months of November, 2012 to February, 2013 using enumeration by plating (cfu/mL) the soil suspension on LB agar medium containing Kanamycin (50 $\mu$ g/mL), Chloramphenicol (50 $\mu$ g/mL), Cycloheximide (50 $\mu$ g/mL) and X-gal (40  $\mu$ g/mL). Blue colonies obtained upon overnight incubation at 30 °C were taken into account as strain Psd.

#### 2.3.3. Growth parameters

Three plants were uprooted during 3<sup>rd</sup> and 6<sup>th</sup> weeks after sowing for measurement of physical parameters such as root and shoot length along with their respective fresh and dry weights. Root and shoot of the plants were separated by cutting with scissor and weighed individually for their fresh weight and drying after drying at 70 °C for 72 h.

**Table 1.** Scheme of the experimental setup

Set 1		Set 2	
Set code	Experimental details (Low-Zn soil filled in pots)	Set code	Experimental details (Mixture of Low-Zn soil and Zn ore in 4:1 ratio)
1a	Seeds inoculated with strain PsdLacZ grown in 2mM Zn	2a	Seeds bacterized with strain PsdLacZ grown in minimal medium without zinc
1b	Seeds coated with carrier containing PsdLacZ grown in 2mM Zn	2b	Seeds coated with carrier having Strain PsdLacZ
1c	Carrier along with PsdLacZ grown in 2mM Zn mixed with soil (10% w/w)	2c	Carrier mixed with soil (10% w/w)
C1	Uninoculated soil	C2	Uninoculated soil and Zn ore (mixed in 4:1 ratio)

#### 2.3.4. Biochemical studies

Biochemical assays were performed at 3-week intervals till 9<sup>th</sup> week after sowing of seeds. Total Chlorophyll content was estimated in fresh leaves according to the protocol of Arnon (1949). Nitrate reductase (NR) assay was performed using leaf discs as per the protocol given by Jaworski (1971). For enzyme assays, 100 mg leaf samples were weighed and homogenised in pre-chilled extraction buffer containing sodium phosphate buffer (0.1 M), EDTA (0.4mM), PMSF (1mM), DTT (2mM), glycerol (10%), MgCl<sub>2</sub> (5mM), HEPES (50mM, pH 7.5) and PVP (1%). The homogenate was transferred to 2 ml Eppendorf tubes and centrifuged at 10,000 rpm for 20 min at 4 °C. Supernatant was transferred to fresh tubes and the extract was used for different enzyme assays. Protein Content in the extracts was determined using BCA kit (Sigma Aldrich, USA). The presence of certain Zn-containing enzymes, namely,

Carbonic anhydrase (CA), Superoxide dismutase (SOD) and Alkaline phosphatase (AP) was assessed using the protocols of Smith and Ferry (2000), Kakkar *et al.* (1984) and Gugi *et al.* (1991), respectively. Similarly, the activities of anti-oxidant enzymes, Glutathione reductase (GR) and Peroxidase (POD), were determined according to the protocols of Rao *et al.* (1996) and Reddy (1998).

#### 2.3.5. Zinc analysis of soil and plant parts

Periodic assessment of available soil zinc in both the sets was done by preparing a soil suspension containing 1g soil in 1mL deionised water followed by filtration to remove soil particles. The resulting suspension was then analysed for Zn<sup>2+</sup> using AAS. For determination of Zn<sup>2+</sup> content in plant parts, the root (50mg dry weight) and shoot (50mg dry weight) were separately acid-digested and were taken forward for Zn<sup>2+</sup> estimation.

### 2.3.6. Seed analysis

After completion of the life-cycle of plant, seeds were harvested and carried forward for quantitative and qualitative analysis. Biomass of the seeds was obtained by weighing 10 seeds/pot. The biochemical parameters such as  $Zn^{2+}$  content, total soluble protein and reducing carbohydrate levels were also analysed. In addition, the elemental analysis in terms of carbon, hydrogen and nitrogen content of seeds was performed using the CHN Analyser (Vario El Cube, Elementar, Germany).

### 2.3.7. Statistical analysis

Values reported in this paper are presented as the mean of three replicates. Data were tested at a significance level of  $P < 0.05$  using one-way ANOVA followed by Dunnett's t test and expressed as mean  $\pm$  SD.

## 3. Results

### 3.1. Zinc solubilization in vitro

Zinc solubilization potential of *P. fluorescens* strain Psd was indicated by a clear zone around the bacterial growth on plate substituted with 14mM ZnO (Figure 1A). The solubilization was further confirmed by broth assay, wherein, a consecutive increase in available  $Zn^{2+}$  in the medium substituted with 12mM ZnO was observed till the 4<sup>th</sup> week in the presence of glucose as the carbon source (Figure 1B). This process was also accompanied by a drop in pH to  $\sim 5.0$  in the medium containing glucose. The pH of uninoculated control and gluconate-containing medium, however, was maintained at 7.0 and 8.0, respectively.

### 3.2. Zinc leaching from low-grade ore tailings

Leaching of Zn from ore tailings was observed by a drop in  $Zn^{2+}$  after 4<sup>th</sup> day of bacterial inoculation. Since, no acid-digestion was used to catalyse metal removal, the increase in solubilized Zn till 4<sup>th</sup> day was obtained due to spontaneous Zn release and any accumulation by the cells during this period was not significant. As indicated in Figure 1C, 10 mL culture biomass (corresponding to  $\sim 8$ mg dry weight) of *P. fluorescens* strain Psd led to  $\sim 27\%$  decrease in  $Zn^{2+}$  content of the ore suspension after 10 days of exposure.

### 3.3. Plant experiments

#### 3.3.1. Survival of strain in external environment

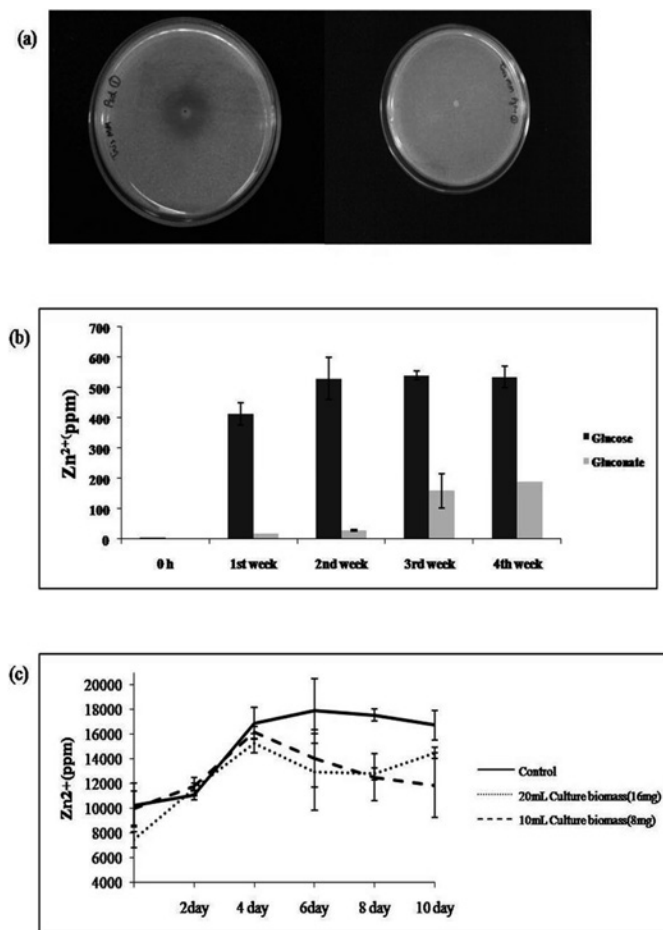
The survivability of the *P. fluorescens* strain Psd was followed for 10 weeks after inoculation in soil. In the presence of bacterial carrier formulation,  $\sim 40\%$  survival was obtained till 10<sup>th</sup> week as compared to  $\sim 23\%$  survival in case of direct bacterial inoculation in soil (Table 1).

#### 3.3.2. Effect on growth of wheat crop

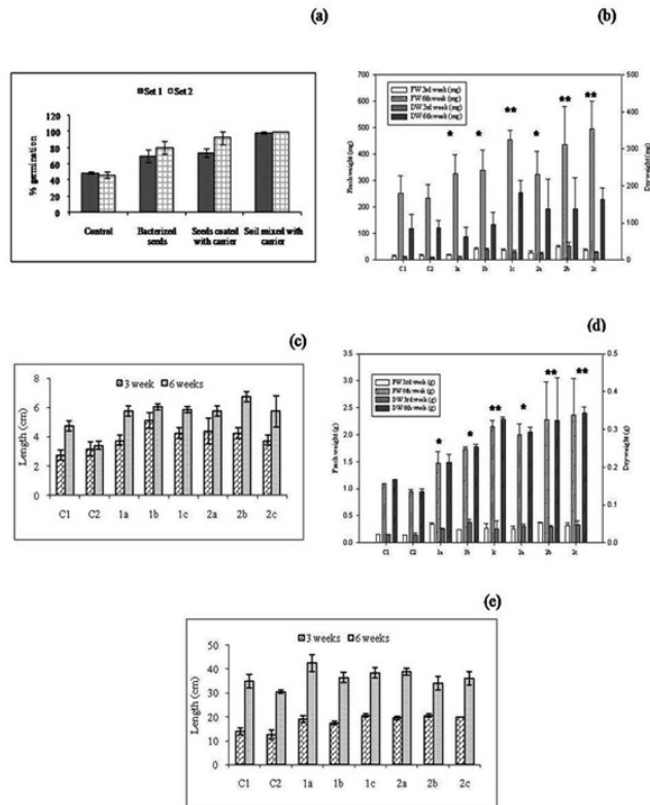
The effect of Zn biofertilization of soil by strain Psd on growth and development of wheat was studied by assessing various parameters. Initially, seed germination was observed after 6 days of sowing. The deficiency of Zn in soil affected seed germination which was only 42-45% in untreated control. On the other hand, fortification of soil by Zn-grown *P. fluorescens* strain Psd (Psd-Zn) led to increased percent germination, which varied within the experimental sets. As is indicated, 100%

seed germination was obtained in soil mixed with bacterial carrier formulation. This was followed by 80-90% germination of seeds coated with the carrier and approximately 70-80% germination in case of seeds inoculated with strain Psd-Zn (Figure 2A). In addition, in controls, slow emergence of seedling was also observed compared to inoculated seeds. Comparative analysis of morphological parameters,

such as length of root and shoot and their respective biomasses, also indicated towards a better growth of plants growing in soil supplemented with zinc-laden Psd biomass (Figure 2 B-E & Figure 3 A-B). Apart from this, tillering was observed at 6<sup>th</sup> week in the plants growing in soil supplemented with Psd-Zn while no tillers were found in untreated control plants at this stage (Figure 3B).



**Figure 1.** *In vitro* zinc oxide solubilization and Zinc ore leaching by *P. fluorescens* strain Psd. (A) Plate assay showing a clear zone near the inoculated region (image on the right is negative control) (B) Quantification of zinc solubilization indicated by increase in zinc over time (C) Zinc status of ore suspension inoculated with Psd indicative of leaching of zinc by the bacterial strain



**Figure 2:** Assessment of growth and biomass of plants (A) percent seed germination; (B) root biomass; (C) root length; (D) shoot biomass and (E) shoot length. {\*\*\* indicates  $P < 0.001$ , \*\* indicates  $P < 0.01$  & \* indicates  $P < 0.05$  versus controls (C1 and C2)}

### 3.4 . Effect on total Chlorophyll and protein content

No significant difference in total chlorophyll and protein content was observed between control and treated sets (data not shown). However, an age-dependent increase in total chlorophyll was found in all the plants while no such variation was observed in the case of protein levels.

### 3.5. Quantification of Nitrate reductase (NR) activity

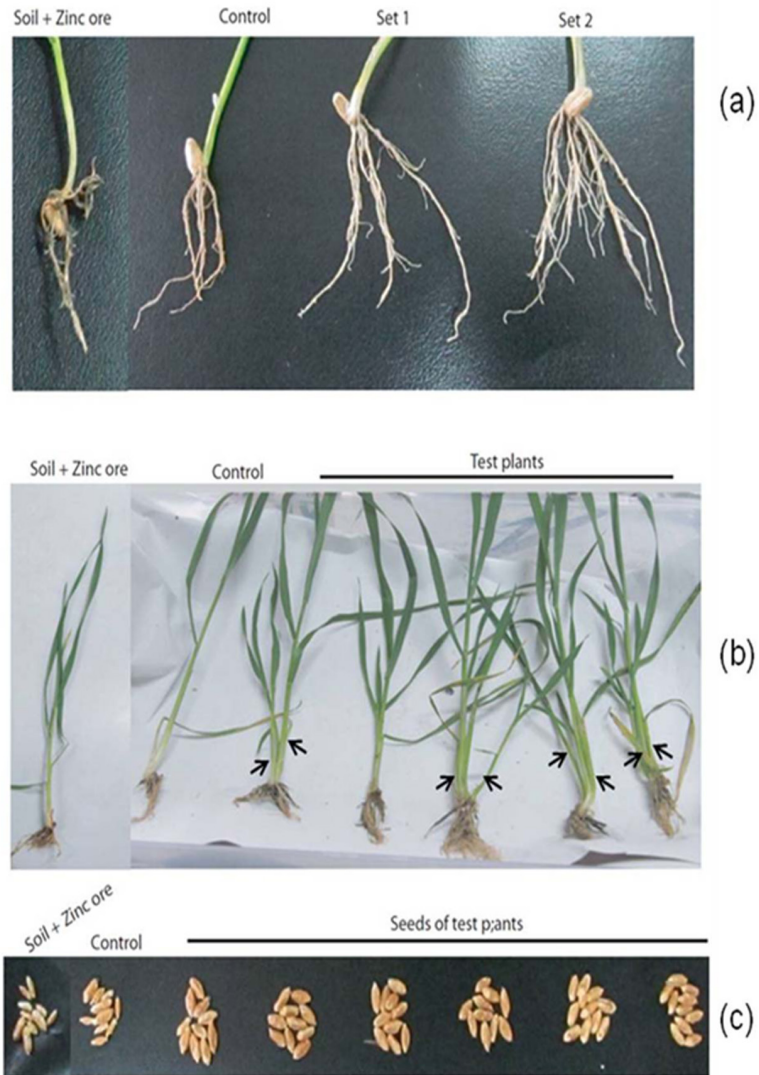
Comparison of the nitrate reductase activity between Psd-Zn fortified plants and controls showed that

$Zn^{2+}$  supply led to a drop in NR activity in wheat, the activity being maximum in control plants. Apart from this, a decline in activity was obtained with time (Figure 4 A).

### 3.6. Estimation of anti-oxidant enzymes

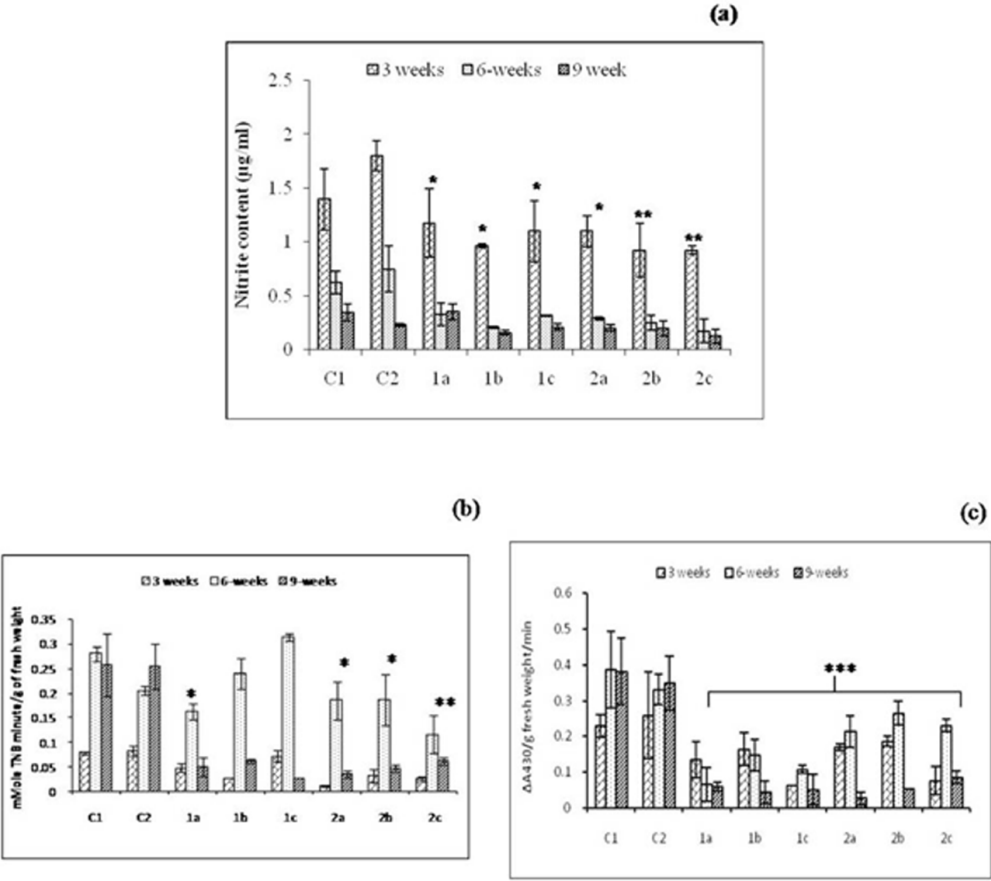
The activities of anti-oxidant enzymes, Glutathione reductase (GR) and Peroxidase (POD), were determined in Psd treated sets as well as controls. Different levels of these enzymes were detected as the plants progressed from 3<sup>rd</sup> to 9<sup>th</sup> week (Figure 4 B&C).





**Figure 3.** Morphological study of plants. (A) root phenotype (B) tillering status (shown by arrows) (C) seed morphology





**Figure 4:** Assessment of (A) Nitrate reductase; (B) Glutathione reductase and (C) Peroxidase activities {\*\*\* indicates  $P < 0.001$ , \*\* indicates  $P < 0.01$  & \* indicates  $P < 0.05$  versus controls (C1 and C2)}

3.7. Activity of enzymes with Zn as co-factor

A progressive increase in CA activity was obtained in all sets. But the activity was prominently higher for plants growing in Psd-inoculated soil as compared to plants growing in uninoculated soil (Figure 5A). Low levels

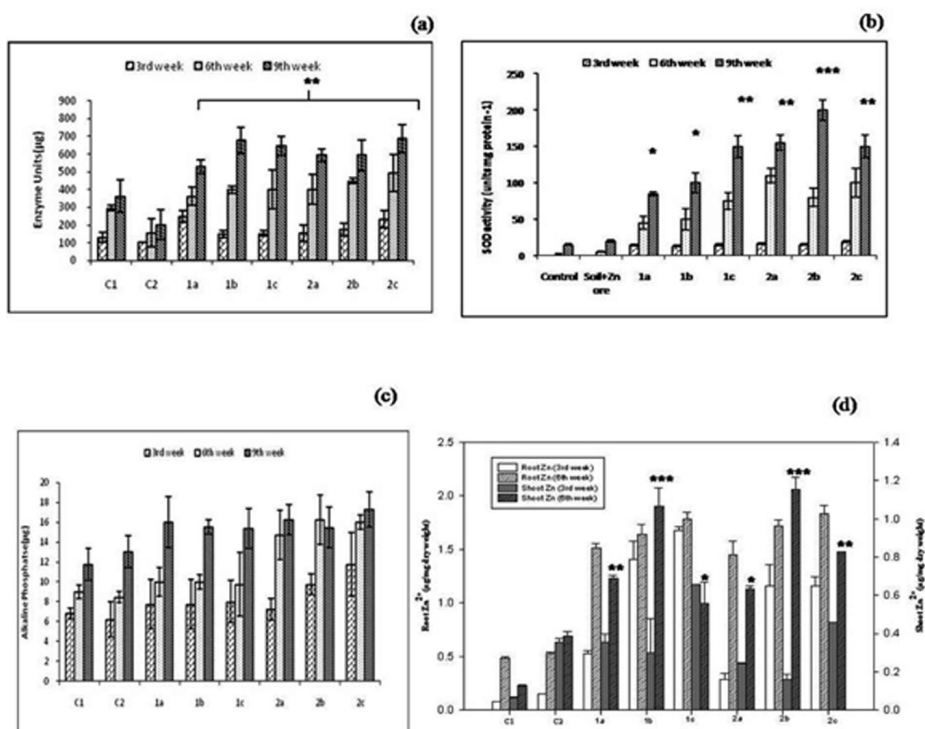
of superoxide dismutase activity, measured in terms of inhibition in reduction of NBT, were obtained in control sets. On the other hand, increase in SOD activity of upto 20-folds in 6<sup>th</sup> week was detected in Psd-treated sets (Figure 5B). In addition alkaline phosphatase was also estimated containing zinc as co-factor and the activity

of this enzyme was also obtained higher in treated plants compared to untreated controls (Figure 5C).

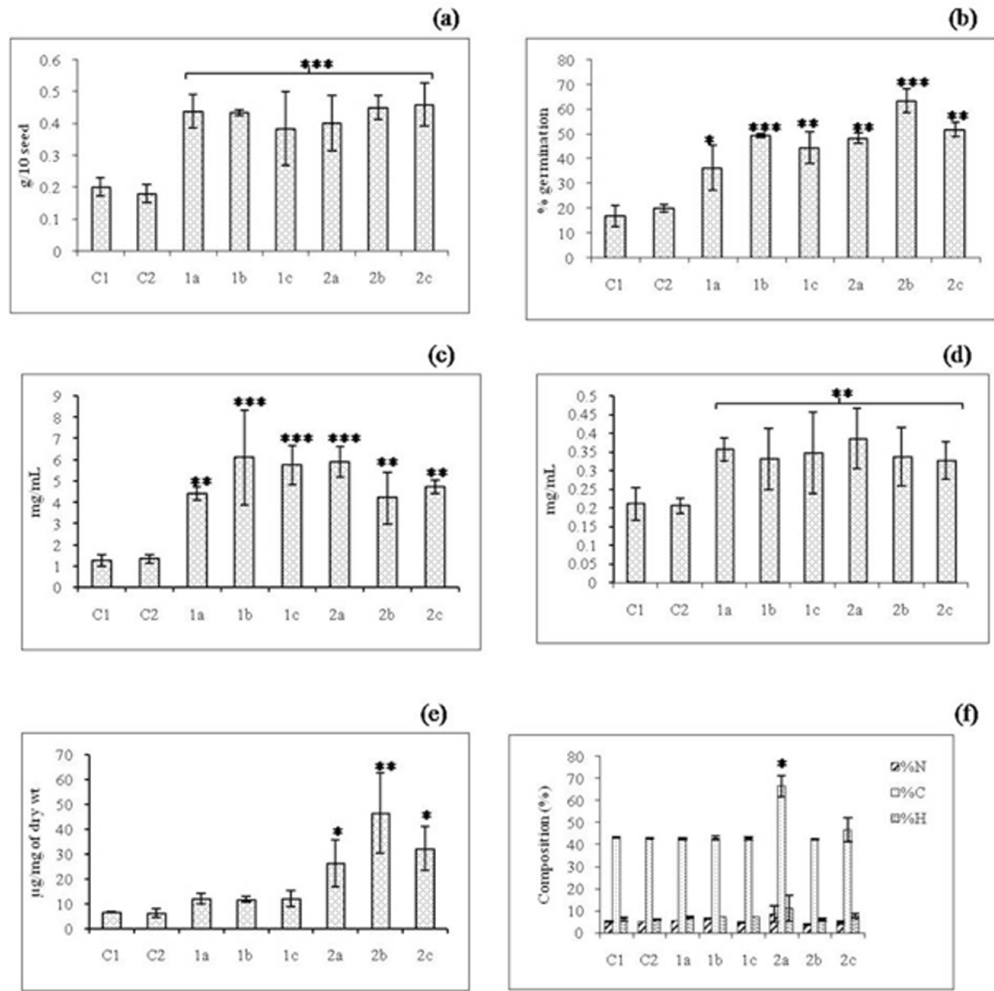
### 3.8. Zinc analysis of soil and plant parts

Estimation of Zinc released in soil for set 1 and solubilization of Zn ore in soil for set 2 by *P. fluorescens* strain Psd was confirmed by analysing the  $Zn^{2+}$  status of soil suspension till 6-weeks after inoculation (data not shown). Since the soil used for the experiments was low in  $Zn^{2+}$  content and the  $Zn^{2+}$  from ore-tailings was in an unavailable form, the control sets showed negligible levels

of the metal. The treated sets, however, displayed approximately 100-fold increase in  $Zn^{2+}$  content (data not shown). This was more prominent in set 2 soil, since the  $Zn^{2+}$ -source in this set was a low-grade ore tailing and any resultant increase in  $Zn^{2+}$  content is indicative of solubilization mediated by the strain. Apart from monitoring the soil suspension,  $Zn^{2+}$  levels were also followed in the uprooted plant parts like leaf and root till 6 weeks (Figure 5D). The application of Zn-laden bacterial biomass also aided in enhancing the  $Zn^{2+}$  content of plant parts, the increase in root  $Zn^{2+}$  being more apparent than shoot.



**Figure 5.** Quantification of the activity of enzymes containing zinc as co-factor. (A) Carbonic anhydrase; (B) Super oxide dismutase and (C) alkaline phosphatase (D)  $Zn^{2+}$  status of roots and shoots {\*\*\* indicates  $P < 0.001$ , \*\* indicates  $P < 0.01$  & \* indicates  $P < 0.05$  versus controls (C1 and C2)}



**Figure 6.** Quantitative analysis of seeds harvested from control and treated plants. (A) Seed biomass; (B) percent seed germination; (C) total protein content; (D) carbohydrate content; (E) Zinc content and (F) elemental analysis (C,H and N) {\*\*\* indicates  $P < 0.001$ , \*\* indicates  $P < 0.01$  & \* indicates  $P < 0.05$  versus controls (C1 and C2)}

### 3.9. Seed analysis

Apart from various plant growth and development parameters, the effect of strain Psd inoculation in soil was also monitored in the harvested grains in terms of their yield and nutritional value. Increased seed biomass, indicative of good crop yield, was obtained in all Psd-treated sets (Figure 6A). Also

seeds from treated plants appeared healthier and fuller compared to controls, based on grain morphology. As is indicated, the deficiency of Zn in soil affected the seed germination which was only 17% in untreated control. On the other hand, Zn fortification by *P. fluorescens* Psd led to increased percent germination, ranging from 35–65% in different sets (Figure 6B). In addition, plants growing in soil inoculated with strain

Psd showed higher seed protein and carbohydrate content compared to control plants (Figure 6C & D). Higher seed  $Zn^{2+}$  was also detected in the treated sets (Figure 6E). The  $Zn^{2+}$  levels were found higher in case of set 2 plants, where Zn source was industrial ore tailings. The composition of Carbon, Hydrogen, and Nitrogen in seeds was found to be largely unaffected by the treatments. However, an abundance of carbon was found in all the seeds (45-65%) whereas, the percent composition of Nitrogen and Hydrogen ranged between 5-10% (Figure 6F). Overall, PGPR inoculation in soil did not induce significant differences in the CHN levels between treated and control plants.

#### 4. Discussion

PGPR, including *Pseudomonas fluorescens*, have substantial effects on plant growth, development and physiology by a combination of direct and indirect mechanisms (Upadhyay and Srivastava 2008). In the present investigation, we report the  $Zn^{2+}$  biofertilization of soil mediated by *P. fluorescens* strain Psd and its effect on growth and yield of *T. aestivum* var. HD2851. The strain was capable of solubilising ZnO in medium which made it a suitable candidate for application in contaminated environments where  $Zn^{2+}$  may be present in bound and unavailable forms. *In vitro*  $Zn^{2+}$  solubilisation ability of other bacteria, including *Pseudomonas* and *Bacillus* species have been reported by Saravanan *et al* (2003). Additionally, inoculation of other PGPR species of *Burkholderia* and *Acinetobacter* led to improved growth and yield of rice by zinc solubilisation and IAA production (Vaid *et al.* 2014). Solubilisation process was also followed by a drop in pH, signifying the production of organic acids since glucose was provided as the carbon source

to the bacterium. This was in consonance to the results obtained by Di Simine *et al.* (1998), where zinc phosphate solubilization by *P. fluorescens* was accompanied by production of gluconic acid and 2-keto gluconic acid. Present study also corroborated the fact that strain Psd acts as a potent bioleaching agent in metal recovery from ore-tailings. Although widely-used candidates for bioleaching processes are chemoautotrophic acidophilic bacteria, there are some reports that indicate towards the efficacy of *Pseudomonas sp.* in bioleaching processes (Pradhan and Kumar 2012).

Practical implication of PGPR inoculation in soil is based on the maintenance of effective cell population in rhizosphere (Viveros *et al.* 2010). During this study, application of *P. fluorescens* strain Psd in the form of a talc-based carrier formulation aided in better survival of the strain. Strain was able to maintain the cell density of approximately  $10^8$  cfu/mL for 4 weeks, though a decline of  $10^4$  fold was observed after 10<sup>th</sup> week. This fluctuation might be the result of competition of *P. fluorescens* strain Psd with other soil microflora and the physical environmental conditions, since the pots were exposed to external environment. Micronutrient availability plays a crucial role in ascertaining better seedling growth. Lower levels of supplemented zinc (upto  $25\text{mgL}^{-1}$ ) resulted in increased percent seed germination in *Cyamopsis tetragonoloba* but further increase in zinc concentration led to drop in germination (Manivasagaperumal *et al.* 2011). In *Zea mays*, concentrations of zinc upto 12ppm in the medium did not have any effect on seed germination and further growth of seedling (Mahmood *et al.* 2005). In the present study, zinc concentration in soil was found to be conducive for plant growth and, moreover, the presence of a plant growth promoting rhizobacterial strain aided in better seedling growth.

PGPR, in general, are known to have a stimulatory effect on chlorophyll and protein content of leaves (Stefan *et al.* 2013) and other parameters like growth of roots and shoots (Mahmood *et al.* 2005). Present report shows an enhancement in growth of plants was detected in all the treated sets though we have not found any increase in the levels of protein and chlorophyll content. This can be attributed to the fact that the presence of heavy metals may prove detrimental for these parameters as was shown by Bonnet *et al.* (2012) in ryegrass. However, the effect was possibly nullified by the presence of strain Psd and hence, no significant differences were observed.

In this study, increased activities of Zinc-containing enzymes like superoxide dismutase (SOD), carbonic anhydrase (CA), alkaline phosphatase were obtained in wheat leaves after bacterial zinc supplementation of the soil. Similar results were reported by Kleckerova *et al.* (2011). Apart from the activities of enzymes where Zn is directly involved, the antioxidant enzymes namely glutathione reductase and peroxidase were also quantified. Activity levels of these enzymes were found to be low in all the treated sets when compared to uninoculated controls. Increased production of these anti-oxidant enzymes in control sets may be one of strategies by which plants combat the stress posed by Zn-deficiency in soil. However, within the sets, an increase in the enzyme activities till 6<sup>th</sup> week was followed by a decrease at 9<sup>th</sup> week. This may be explained by the fact that these antioxidant enzymes carry out H<sub>2</sub>O<sub>2</sub> detoxification during periods of high metabolic activity in plants. Similar results were reported in *Phaseolus coccineus* L. plants treated with *Bacillus* strains (Stefan *et al.*, 2013).

An important determinant in biofertilization of cereal crops is the mobilization of micronutrient to edible parts of the plant (Palmgren *et al.* 2008). When grown

in Zn-deficient soils, Zn levels in wheat have been reported between 5-12 mg/kg (Erdal *et al.* 2002). In the present report, Zn biofortification mediated by *P. fluorescens* strain Psd resulted in ~85% increase in grain Zn<sup>2+</sup> concentrations. In addition, beneficial effect of zinc-laden biomass of *P. fluorescens* strain Psd was also reflected in grain yield and nutritional quality. Higher biomass, carbohydrate and protein content were obtained in case of seeds from the plants harvested from strain Psd inoculated soil.

## 5. Conclusion

Our study demonstrates the efficacy of zinc-laden biomass of plant growth promoting rhizobacterial strain *P. fluorescens* Psd in biofertilization of wheat. The plant-microbe interactions were affected by the micronutrient availability in soil, thus aiding in the zinc-fortification of wheat crop.

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