Cogent Food & Agriculture

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/oafa20

Cyanobacterial inoculation elicits plant defense response and enhanced Zn mobilization in maize hybrids

Radha Prasanna, Ngangom Bidyarani, Santosh Babu, Firoz Hossain, Yashbir Singh Shivay & Lata Nain |

To cite this article: Radha Prasanna, Ngangom Bidyarani, Santosh Babu, Firoz Hossain, Yashbir Singh Shivay & Lata Nain | (2015) Cyanobacterial inoculation elicits plant defense response and enhanced Zn mobilization in maize hybrids, Cogent Food & Agriculture, 1:1, 998507, DOI: 10.1080/23311932.2014.998507

To link to this article: https://doi.org/10.1080/23311932.2014.998507

Prasanna et al., *Cogent Food & Agriculture* (2015), 1: 998507
http://dx.doi.org/10.1080/23311932.2014.998507

cogent food & agriculture

SOIL & CROP SCIENCES | RESEARCH ARTICLE

Cyanobacterial inoculation elicits plant defense response and enhanced Zn mobilization in maize hybrids

Radha Prasanna[1]*, Ngangom Bidyarani[1], Santosh Babu[1], Firoz Hossain[2], Yashbir Singh Shivay[3] and Lata Nain[1]

Received: 26 September 2014
Accepted: 09 December 2014
Published: 09 January 2015

*Corresponding author: Radha Prasanna, Division of Microbiology, Indian Agricultural Research Institute (IARI), New Delhi 110012, India
E-mail: radhapr@gmail.com

Reviewing editor:
Manuel Tejada Moral, University of Seville, Spain

Additional information is available at the end of the article

Abstract: The present investigation evaluated the effect of inoculating different cyanobacterial formulations on a set of hybrids of maize, in terms of plant defense enzyme activity, soil health parameters, Zn concentration, and yields. Microbial inoculation showed significant effects on accumulation of Zn in flag leaf, with A4 (*Anabaena–Azotobacter* biofilm) recording the highest values. Analysis of variance (ANOVA) indicated that both the hybrids and cyanobacterial treatments brought about significant variation in terms of glomalin-related soil proteins and polysaccharides in soil and the activity of defense enzymes in roots and shoots of the plants. Cyanobacterial inoculants—A4 (*Anabaena–Azotobacter* biofilm) and A1 (*Anabaena* sp.– *Providencia* sp., CW1 + PW5) enhanced the activity of peroxidase, PAL and PPO in roots, which also showed a positive correlation with Zn concentration in the flag leaf. Grain yield ranged from 7.0 to 7.29 t/ha among the different inoculants. Comparative analyses of treatments showed that A3 (*Anabaena–Trichoderma*-biofilmed formulation) and hybrid B8 (Bio-9681) were superior in terms of parameters investigated. This represents the first report on the genotypic responses of maize hybrids to cyanobacteria-based inoculants. Future research should focus on dissecting the role of root exudates and cyanobacteria-mediated Zn mobilization pathway in maize.

ABOUT THE AUTHOR

The Indian Agricultural Research Institute is globally recognized for its outstanding contributions to the development of new and improved varieties of food crops and generation of technologies for diversification and economic development in India. The author's group has been involved for more than a decade in identifying promising bacterial/cyanobacterial strains and developing synergistically acting plant growth promoting and biocontrol formulations for the rice–wheat cropping sequence and other crops. This has helped to create awareness and widen the scope of cyanobacteria-based biofertilizers in crops other than rice. Further, our research efforts have illustrated their significance, not just as diazotrophs, but also in improving nutrient availability in soil, and as growth promoting and biocontrol agents for diverse crops. Such bio-inputs are being integrated into crop management schedules as environment-friendly agricultural technologies.

PUBLIC INTEREST STATEMENT

The work presented in the manuscript describes the promise of cyanobacteria-based (microbial) formulations as environment-friendly interventions for improving growth, yields, and zinc mobilization of maize hybrids. This also illustrates its utility for enhancing soil health and biofortification of plant tissues, especially as it is an important crop used as feed, fodder, and industrially important products. This is the first report on the promise of cyanobacteria-mediated nutrient mobilization for enhancing quality and yields of maize hybrids.

Subjects: Science; Technology; Medicine; Crop Science; Microbiology

Keywords: biofilms; biofortification; cyanobacteria; defense enzymes; yield

1. Introduction

Cyanobacterial inoculation is known to add organic matter, provide oxygen to the submerged rhizosphere, solubilize phosphate, increase the fertilizer use efficiency of crop plants, and enhance plant growth mediated through the liberation of amino acids, vitamins, and auxins (Mandal, Vlek, & Mandal, 1999; Nain et al., 2010; Prasanna, Rana, Chaudhary, Joshi, & Nain, 2012). These biofertilizers are important components of rice-based cropping systems for biological nitrogen fixation, which exhibit morphologically distinct forms having seasonal and crop-stage diversity (Nayak, Prasanna, Pabby, Dominic, & Singh, 2004; Prasanna, Babu, et al., 2013; Prasanna, Pattnaik, Sugitha, Nain, & Saxena, 2011; Prasanna et al., 2012). There is a growing awareness that micronutrient malnutrition is a serious global challenge, and the high cost-effectiveness of solving that problem using biological means is encouraging. Micronutrient malnutrition is widespread, especially in resource-poor populations across the globe, where daily calorie intake is confined mainly to staple cereals. Introduction of ecologically viable interventions to overcome the problem of micronutrient malnutrition can be considered to be among the best investments, which can also reap socioeconomic benefits (Welch, 1993; Welch & Graham, 2004).

Globally, maize is a major cereal crop for both human and livestock nutrition. With its high content of carbohydrates, fats, proteins, important vitamins, and minerals, maize is considered as the poor man's alternative for meeting his daily dietary requirements (Prasanna, Mazumdar, et al., 2011). The three major cereals—maize, rice, and wheat are known to provide about 30% of the calories to more than 4.5 billion people in 94 developing countries (Shiferaw, Prasanna, Hellin, & Bänziger, 2011). Maize is among the three most widely grown crops in 75 countries and is currently produced on nearly 100 million hectares in 125 developing countries. In India, ~25% of maize produce is used for human consumption, while ~61% is used for poultry and animal feed (Dass et al., 2010).

Among the various mineral elements, zinc (Zn) is one of the most common micronutrients that have been found deficient predominantly in cereal-based diets (Bouis, Hotz, McClafferty, Meenakshi, & Pfeiffer, 2011; Cakmak, Pfeiffer, & McClafferty, 2010; Pfeiffer & McClafferty, 2007). Deficiency of Zn leads to impaired growth and development, depression and psychosis besides affecting the immune system (Solomons, 2003). Fortification of food products, medical supplements, and dietary diversification represent the measures tried for decades to ameliorate the problems of micronutrient deficiency, but these have shown limited success (Pfeiffer & McClafferty, 2007). Biofortification is a process by which plants take up the minerals from the soil and immobilize them in the plant parts, including grains, so as to produce nutritionally rich grains; representing a sustainable and cost-effective approach (Banziger & Long, 2000).

Micro-organisms present in the rhizosphere are known to play important roles in ecological fitness of their plant hosts, through their involvement in growth promotion, colonization, geochemical cycling of nutrients, and mobilization for plant uptake. Such micro-organisms hold promise for further enhancement of micronutrients in maize plants, as they play a key role in mineralizing organic material as well as transforming inorganic nutrients (Rana, Joshi, Prasanna, Shivay, & Nain, 2012; Rana, Saharan, Nain, Prasanna, & Shivay, 2012). Micro-organisms can also influence nutrient availability by solubilization, chelation, and oxidation/reduction. They can also affect plant growth and nutrient uptake by the release of growth-stimulating or inhibiting substances that influence root physiology and root system architecture. Treatments with *Pseudomonas* and *Acinetobacter* strains resulted in enhanced uptake of Fe, Zn, Mg, Ca, K, and P by crop plants (Khan, 2005).

In earlier studies in wheat (Rana, Joshi, et al., 2012, Rana, Saharan, et al., 2012), enhancement in the uptake of N, P, and micronutrients by bacterial inoculants and cyanobacteria was supported by the PGPR traits possessed by these strains; this illustrated the positive involvement of such traits in

enhancement of yield, micronutrient concentration, and their uptake in wheat grains. Glomalin-related soil proteins (GRSPs) or Bradford-reactive soil proteins (BRSPs) are known to play a significant role in soil aggregation (Liu, Hamel, Hamilton, Ma, & Smith, 2000; Rillig, Wright, & Eviner, 2002). Inoculation of cyanobacteria in pathogen-challenged crops is also known to elicit enhanced activity of defense enzymes and promote plant growth, while these inoculants are known to improve soil fertility by increasing nitrogen, carbon (organic/microbial biomass), and polysaccharide content (Mandal et al., 1999; Prasanna et al., 2012; Prasanna, Babu, et al., 2013; Rana, Joshi, et al., 2012). The benefits of cyanobacteria as inoculants in rice, wheat, and selected vegetable crops are well recognized; however, their interaction with maize is less explored. The hypothesis underlying the present investigation was that maize hybrids will exhibit a differential response to cyanobacterial formulations, in terms of the activity of plant defense enzymes, Zn enrichment in plant, and soil fertility parameters. This can help to identify promising cyanobacterial inoculant–hybrid combinations showing effective nutrient acquisition by plants and enrichment of Zn.

2. Materials and methods

2.1. Organisms used in this study and their maintenance

The cyanobacterial strains—BF1 *Anabaena torulosa*; BF2 *Nostoc carneum*; BF3 *Nostoc piscinale;* BF4 *Anabaena doliolum, Anabaena* sp. (CW1)—and bacterial strains, viz. *Providencia* sp. (PW5), *Azotobacter chroococcum* (W5), were obtained from the germplasm of the Division of Microbiology, Indian Agricultural Research Institute, New Delhi. The characteristics of these strains are given elsewhere (Nain et al., 2010; Prasanna et al., 2008; Prasanna, Babu, et al., 2013; Prasanna, Chaudhary, et al., 2013; Rana, Joshi, et al., 2012). *Trichoderma viride* (ITCC 2211) was obtained from the Indian Type Culture Collection (ITCC), Division of Pathology, IARI, New Delhi, India. Cyanobacterial biofilms—*Anabaena -T. viride* and *Anabaena–Azotobacter* sp.—were developed and characterized as given in Prasanna, Pattnaik, et al. (2011) and Prasanna, Kumar, et al. (2013). The organisms used have been already evaluated in rice–wheat cropping system and in legumes (Nain et al., 2010; Prasanna et al., 2012; Prasanna, Babu, et al., 2013; Rana, Joshi, et al., 2012; Rana, Saharan, et al., 2012). The growth media used were Jensen's medium for *Azotobacter* sp., nutrient broth for *Providencia* sp., and potato dextrose broth for *T. viride*. The flasks were incubated at 30 ± 2 °C in a shaking incubator, except for *T. viride*, which was maintained as static culture at 30 °C. The cyanobacterial cultures were axenized using standard procedures (Prasanna et al., 2008) and grown in nitrogen-free BG11 medium (Stanier, Kunisawa, Mandel, & Cohen-Bazire, 1971), under a temperature of 27 ± 1 °C (light: dark cycles 16: 8) with white light (50–55 µmol photons m^{-2} s^{-1}) in Haffkine flasks.

2.2. Preparation of formulations

Paddy straw compost was used after amendment with vermiculite (1:1) as carrier, following the procedure given earlier (Prasanna, Babu, et al., 2013; Prasanna et al., 2008). For cyanobacterial cultures and their biofilms, the chlorophyll content was measured (MacKinney, 1941) and maintained as 100 µg g^{-1} carrier, with CFU ranging from 10^7 to 10^8 for the bacterial/fungal partners in the cyanobacterial biofilms. The water-holding capacity of the carrier was measured and maintained as 60% after amendment with cultures and suitable amount of water.

2.3. Experimental setup with maize crop

The field experiment was conducted at the research farm of Indian Agricultural Research Institute, New Delhi, during the rainy (Kharif) season of 2012. The geographical details are: latitude 28°40′ N and longitude 77°12′ E, altitude 228.6 m above the mean sea level (Arabian Sea). The mean annual rainfall of Delhi is 650 mm, out of which, more than 80% generally occurs during the southwest monsoon season (July–September), with mean annual evaporation of 850 mm. The soils of experimental field had 225 kg ha^{-1} alkaline permanganate oxidizable N, 16.0 kg ha^{-1} available P, 275 kg 1 N ammonium acetate exchangeable K, and 0.53% organic carbon as estimated by standard protocols (Olsen, Cole, Watanabe, & Dean, 1954; Prasad, Shivay, Kumar, & Sharma, 2006; Subbiah & Asija, 1956). The pH of soil was 7.5 (1: 2.5 soil-and-water ratio). The sources of N, P, and K fertilizers were prilled urea, single super phosphate, and muriate of potash, respectively. The experimental soil

of IARI is well-drained old alluvium soil, classified as a member of the coarse sandy loam, non-acidic, mixed hypothermic family of the type Haplustept. The recommended dose of fertilizers (120:80:60 NPK kg ha^{-1}) were based on soil test values and treated as control, with all the microbial inoculant treatments receiving 50% N + full dose of P and K fertilizers, as the microbial inoculants possessed the ability to fix atmospheric nitrogen. Nitrogen was given at the rate of 60 kg ha^{-1} in all the plots before sowing, while in the control (uninoculated) plots, additional three split doses of 20 kg N ha^{-1} were given during the progressive stages of crop. The details of inoculants used are—A1 *Anabaena* sp. – *Providencia* sp. (CW1 + PW5); A2 Cyanobacterial consortium (BF1- 4; BF1 *Anabaena torulosa*; BF2 *Nostoc carneum*; BF3 *Nostoc piscinale;* BF4 *Anabaena doliolum*); A3 *Anabaena* sp.–*Trichoderma* sp. biofilm; A4 *Anabaena* sp.– *Azotobacter* sp. biofilm, and A5 Control (no inoculation); and 11 hybrids of maize, viz. HQPM-4, Parkash, HM-4, HQPM-7, HQPM-1, PMH-3, DHM-117, Bio-9681, HM-8, PMH-1, and Vivek QPM-9 were selected. Details of the hybrids are given in Supplementary Table 1. The experiment was conducted using split plot design with three replications. Different microbial inoculations were applied in larger plot size (main plots), while maize hybrids were grown in smaller plot size (subplots) of one row each. Each of the hybrids was grown in 3 m row having 20 cm plant-to-plant and 75 cm row-to-row distance.

2.4. Soil-related parameters at flowering stage

The concentration of GRSPs or BRSPs was estimated by the procedure of Wright and Upadhyaya (1996) and assayed by adding 20 mM citrate buffer (pH 7.0) at 121 °C for 90 min to the soil samples. The supernatant was removed by centrifugation at 3000 rpm for 15 min. The extracted GRSPs were again diluted to 100 times. Bradford dye (0.5 ml) was mixed with 2 ml of the above solution and absorbance was recorded at 590 nm. The GRSP content was expressed in µg/g soil. Total polysaccharides were assayed in soil (0.5 g) suspended in 4 ml H_2SO_4 (12 N), following the method of Lin (2005). This solution was diluted to 0.5 N by adding distilled water, autoclaved, and then filtered through Whatman 42. To 1 ml of filtrate, 1 ml of 5% phenol solution + 5 ml H_2SO_4 was added and OD recorded at 490 nm. The polysaccharide content was expressed in mg g^{-1} soil, based on comparison with known standard solutions of glucose.

2.5. Activity of defense enzymes in shoots and roots at flowering stage

The fresh shoots and roots were washed in running tap water after sampling and homogenized with 5 ml of 50 mM Tris HCl buffer in a mortar and pestle. The tissue extracts were centrifuged at 12,000 rpm for 20 min at 4 °C and the supernatant was transferred to vials and stored at −20 °C. Peroxidase (PO) activity was also measured using guaiacol (molar extinction coefficient 26.6 mM^{-1} cm^{-1}) as the hydrogen donor. The procedure was modified from the method of Jennings, Brannaman, and Zoheille (1969). Aliquots of 1 ml were taken in 1.5 ml cuvette containing 1% (v/v) guaiacol in 0.01 M sodium phosphate buffer (pH 6.0) and 0.1 M H_2O_2. The enzyme extract was added at the end to initiate the reaction. The changes in absorbance at 470 nm were recorded at 30 s intervals for 3 min. One unit of enzyme is defined as the change in absorbance of 0.01 unit min^{-1}.

Polyphenol oxidase (PPO) activity was measured using catechol, which serves as the substrate. The procedure was modified from the method given by Jennings et al. (1969). Aliquots of 500 µl were taken in 2 ml cuvette containing 0.02 M citrate phosphate buffer (pH 6.0), proline (5 mg ml^{-1}), catechol (2 mg ml^{-1}), and 500 µl of enzyme extract. The mixture was aerated before addition of catechol, which serves as the substrate. The enzyme activity was determined spectrophotometrically at 546 nm. The changes in absorbance at 546 nm were recorded at 30 s intervals for 3 min. One unit of enzyme is defined as the change in absorbance of 0.01 IU min^{-1}. Phenylalanine ammonia-lyase (PAL) activity was assayed in leaf and root extracts (100 µl) using 2.5 ml of 0.2% L-phenylalanine in 50 mM Tris-HCl (pH 5–6) buffer solution. The reaction mixture was incubated at 40 °C for 60 min as described (Beaudoin-Eagan & Thorpe, 1985). The amount of trans-cinnamic acid formed from L-phenylalanine was measured spectrophotometrically at a wavelength of 290 nm against the blank. In blank, 0.1 ml of distilled water was used in place of test sample. One unit of enzyme (IU) is defined as change in absorbance min^{-1} g^{-1} fresh weight.

2.6. Zinc concentration at flowering stage

A small portion of flag leaf from five randomly selected plants per row was collected for estimation of Zn concentration. Leaves were washed in deionized water to remove any remaining nutrient solution. Tissue was dried to constant weight in a forced air oven at 60 °C. The tissues were wet digested using diacid mixture (concentrated HNO_3 and $HClO_4$; 10:3). Mineral concentration was determined using an atomic absorption spectrophotometer (Optima, 3000, Perkin-Elmer) by standard procedure (Prasad, Shivay, Kumar, & Sharma, 2006)

2.7. Yield-related observations

The ear height and yield (tonnes ha^{-1}) were recorded at the time of harvest. Other biometrical parameters were also measured (data submitted elsewhere).

2.8. Statistical analyses

The data for the various parameters were analyzed by ANOVA using WINDOWSTAT 8.0 statistical package. The mean performance of inoculants (A1-A4) over uninoculated treatment (control A5) in all hybrids (B1–B11) was calculated and denoted as microbial inoculation efficacy for each hybrid. Pearson's correlation analyses were undertaken using Microsoft Excel package.

3. Results

3.1. Variability in soil and plant parameters

Analysis of variance (ANOVA) indicated significant variation among the cyanobacterial inoculants in terms of GRSPs and polysaccharides in soil and activity of defense enzymes in roots and shoots of the plants (Table 1). Hybrids were significantly different for the polysaccharide and GRSP content in soil, besides defense enzyme activity of both roots and shoots. Inoculant × hybrid interactions were significant for majority of traits except for PAL activity of roots, which was significant for hybrids alone in terms of Zn content in leaves and grain and plant parameters—ear height and yield (Table 2). The proportion of sum of square over total sum of square for inoculants × hybrids was 60.39% for Zn content of grains, while it was 53.33% for polysaccharide content in soil. The proportion of sum of square over total sum of square for inoculants for GRSP content was 59.3% and 45.45% for PAL activity of shoots. The proportion of sum of square over total sum of square for inoculants × hybrids was 80.72% for shoot peroxidase activity. A similar trend of preponderance of interaction effects was also observed for soil parameters and PPO activity of roots and shoots.

3.2. Soil parameters

Polysaccharide values ranged from 164.97 to 200.59 mg g^{-1} soil (Table 3). The highest values were recorded in A1 (CW1 + PW5) followed by A5 (control) and A3 (*An–Tr*), respectively. Statistical analyses of the combined influence of all the inoculants (A1–A4) against control, in all the 11 varieties,

Table 1. ANOVA for plant defense and soil parameters in maize crop, as influenced by cyanobacterial inoculants

Source of variance	df	Mean square							
		GRSPs	Polysaccharide	Peroxidase activity		PAL activity		PPO activity	
				Shoot	Roots	Roots	Shoots	Roots	Shoots
Replicates	2	4333.36	2.52	0.31	37.48	0.129	0.00028	0.0053	0.00350
A Treatment	4	559062.44**	7011.86**	12.98**	20026.69**	0.236	0.254**	1.52**	0.0102
Error A	8	3512.06	66.54	0.79	139.13	0.384	0.00026	0.023	0.0031
B treatment	10	41918.20**	2040.63**	89.26**	3987.52**	0.408*	0.022**	0.280**	0.0025**
A*B	40	24382.00**	1586.65**	107.08**	2670.56**	0.150	0.024**	0.213**	0.0017**
Error B	100	1044.20	59.03	0.71	131.56	0.177	0.00006	0.011	0.00009
Total	164	22974.97	717.32	32.351	1470.42	0.195	0.0136	0.115	0.0010

*significant at 5%.

**significant at 1%.

Table 2. ANOVA for plant yield and micronutrient concentration in maize crop

Source of variance	df	Zinc in leaves	Ear height	Yield
Replicates	2	148.41	0.055	31.72
A Treatment	4	4474.67**	54.99	0.51
Error A	8	35.50	22.38	9.53
B treatment	10	504.36**	427.47**	15.89**
A*B	40	436.50**	38.68**	1.90
Error B	100	34.54	14.27	1.87
Total	164	270.95	46.63	3.44

*significant at 5%.

**significant at 1%.

Table 3. Mean performance of microbial inoculants on the activity of plant defense enzyme and soil parameters in maize crop

Treatment	GRSPs µg g^{-1} soil	Polysaccharides mg g^{-1} soil	Peroxidase activity IU g^{-1} fresh weight		PAL activity IU g^{-1} fresh weight		PPO activity IU g^{-1} fresh weight	
			Shoots	Roots	Roots	Shoots	Roots	Shoots
A1 (*Anabaena* sp. CW1 + *Providencia* sp. PW5 formulation)	349.54	200.59	15.21	223.47	0.85	0.23	1.54	0.06
A2 (BF1–4; *Anabaena–Nostoc* spp. consortial formulation)	341.93	164.97	14.46	156.18	0.66	0.20	1.55	0.07
A3 (*Anabaena–Trichoderma*-biofilmed formulation)	359.13	171.78	14.91	187.40	0.69	0.31	1.50	0.08
A4 (*Anabaena–Azotobacter*-biofilmed formulation)	425.65	165.74	16.07	197.96	0.83	0.18	1.47	0.08
A5 Control (uninoculated)	87.67	174.99	15.65	180.11	0.77	0.07	1.03	0.11
CD 5%	33.64	4.63	0.51	6.69	0.35	0.009	0.086	0.032
CV %	10.13	4.37	5.51	6.06	55.33	3.84	7.40	11.86

generated values ranging from 0.91 to 1.11. The highest values were recorded in B7 (DHM-117), followed by the varieties—B2 (Parkash) and B3 (HM-4). Polysaccharide content of soil and Zn concentration in leaf exhibited a positive correlation (0.60; $p < 0.05$).

In our study, the values of GRSPs ranged from 87.67 to 425.65 µg g^{-1} soil. The highest GRSP content was recorded in A4 (*An–Az*), followed by A3 (*An–Tr*) and A1 (CW1 + PW5), which were 3–4 folds higher than control (A5). Mean performance of inoculants over all hybrids revealed that microbial inoculation efficacy ranges from 2.41 to 15.15. The highest values were recorded in hybrid B5, followed by B7 and B6. GRSP content of soil and Zn concentration in leaf exhibited a positive correlation (0.67; $p < 0.05$).

3.3. Plant-related physiological parameters

Plant defense enzyme activity, in terms of peroxidase, polyphenol oxidase, and phenylalanine ammonia-lyase (PAL) enzymes, was analyzed in the root and shoot samples of plants. Peroxidase activity in shoot ranged from 14.46 to 16.07 IU g^{-1} fresh tissues. The highest peroxidase activity was recorded in cyanobacterial inoculant A4 (*An–Az*), which was statistically at par with A5 (Control); other inocula exhibited significantly lower values. Peroxidase activity in root ranged from 156.18 to 223.47 IU g^{-1} fresh tissue. The highest values were recorded in A1 (CW1 + PW5) followed by A4 (*An–Az*) and A3 (*An–Tr*), which were significantly higher as compared to control A5 (180.11 IU g^{-1} fresh tissue). Peroxidase activity was higher in roots, as compared to shoots. Microbial inoculation efficiency, measured in terms of shoot peroxidase activity revealed values ranging from 0.69 to 1.28. The highest mean performance over A1–A4 were recorded in B8 (Bio-9681) with a value 1.28

followed by B9 (HM-8) and B1 (HQPM-4), whose values were 1.25 and 1.09. In terms of microbial inoculant efficiency of root peroxidase activity, the values ranged from 0.87 to 1.41. The highest activity was recorded in B7 (DHM-117), with a value 1.41, followed by B4 (HQPM-7) and B9 (HM-8), with values of 1.30 and 1.14 (data not shown). Peroxidase activity of shoots and polysaccharide content of soil exhibited a significantly high positive correlation (0.72; $p < 0.05$).

Polyphenol oxidase (PPO) activity in shoot ranged from 0.06 to 0.11 IU g^{-1} fresh tissue. The highest activity of 0.11 IU g^{-1} fresh tissues was recorded in uninoculated A5 (control), but it was not significantly different from the inoculated treatments. Polyphenol oxidase activity in roots ranged from 1.03 to 1.55 IU g^{-1} fresh tissue. The highest activity were recorded in A2 (BF1 + 2 + 3 + 4), followed by A1 (CW1 + PW5) and A3 (*An–Tr*), whose values were statistically at par with control A5. In general, the activity of this enzyme was higher in roots, as compared to shoots. Microbial inoculation efficiency, measured in terms of polyphenol oxidase activity in shoot, revealed values ranging from 0.45 to 0.93. The highest mean performance over A1–A4 was recorded in B3 (HM-4). In terms of microbial inoculant efficiency of root polyphenol oxidase activity, the values ranged from 1.12 to 1.85. The highest activity was recorded in B11 (Vivek QPM-9), followed by B9 (HM-8) and B3 (HM-4).

Phenylalanine ammonia-lyase activity in shoot ranged from 0.07 to 0.31 IU g^{-1} fresh tissue. The highest activity was recorded in A3 (*An–Tr*), followed by A1 (CW1 + PW5) and A2 (BF1 + 2 + 3 + 4). The activity in roots ranged from 0.66 to 0.85 IU g^{-1} fresh tissue. The highest activity was recorded in microbial inoculation A1 (CW1 + PW5), followed by A4 (*An–Az*) and A5 (control). A1 and A5 were found be statistically at par with control A5 (0.77 IU g^{-1} fresh tissues). Phenylalanine ammonia-lyase activity was in general higher in roots as compared to shoots. The efficiency of microbial inoculation, measured in terms of phenylalanine ammonia-lyase activity in shoots revealed values ranging from 2.14 to 9.33. The highest mean performance over A1–A4 was recorded in B4 (HQPM-7), followed by B6 (PMH-3) and B8 (Bio-9681). In terms of microbial inoculation, efficiency of root activity ranged from 0.73 to 1.73. The highest activity was recorded in B8 (Bio-9681), followed by B4 (HM-7) and B10 (PMH-1). The peroxidase activity of roots and PAL activity of shoots exhibited a very high positive correlation (0.76; $p < 0.001$). Peroxidase activity in roots and PAL activity of shoots were also positively correlated with dehydrogenase activity in soil (0.72 and 0.83, respectively; $p < 0.001$).

3.4. Zn concentration in flag leaf

Zn values of flag leaf ranged from 77.84 to 107.01 µg g^{-1} (Table 4). Microbial inoculation showed significant effects on accumulation of Zn in flag leaf with A4 (*An–Az*) recording the highest value, followed by A2 (BF1–4) and A3 (*An–Tr*). Microbial inoculation efficiency ranged from 1.09 to 1.35, with the highest values being recorded in B1 (HQPM-4), followed by B7 (DHM-117) and B8 (Bio-9681). Zn concentration showed a positive correlation with peroxidase activity in roots (0.68; $p < 0.05$) and shoots (0.61; $p < 0.05$), and PAL activity of shoots (0.62; $p < 0.05$).

3.5. Plant biometric and yield parameters

Ear height was recorded as an index of plant growth, with values ranging from 35.67 to 38.75 cm (Table 4); however, no significant differences among the microbial inoculants were recorded. Microbial inoculation efficacy among 11 hybrids ranged from 0.86 to 1.52 cm, with B4 (HQPM-7) and B9 (HM-8) ranking highest. Grain yield ranged from 7.0 to 7.29 t ha^{-1} among the different inoculants, with significant differences with control, which received 60 kg N ha^{-1} additionally as split doses. Hybrids brought about a significant variation in grain yield. Ear height was found to show a positive correlation with SPAD values, and days to 50% male and female flowering (data not shown).

Comparative analysis of performance of the different hybrid–inoculant combinations for the parameters analyzed (Table 5) revealed that among the hybrids, B8 (Bio-9681) was the top performing (appearing in nine top-ranked treatments), followed by B1 (HQPM-4). In terms of microbial inoculants, A3 (*Anabaena–Trichoderma*-biofilmed formulation) was present 15 times among the top five ranks among all the treatments, for all the attributes analyzed.

Table 4. Mean performance of microbial inoculants on plant physiological parameters, plant yield, and Zn in leaves of maize crop

Treatment	Zinc in leaves (μg g^{-1})	Ear height (cm)	Yield (tonnes ha^{-1})
A1 (*Anabaena* sp. CW1 + *Providencia* sp. PW5 formulation)	81.98	38.64	7.00
A2 (BF1–4; *Anabaena–Nostoc* spp. consortial formulation)	94.97	38.43	7.29
A3 (*Anabaena–Trichoderma*-biofilmed formulation)	87.90	38.30	7.11
A4 (*Anabaena–Azotobacter*-biofilmed formulation)	107.01	38.75	7.21
A5 Control (uninoculated)	77.48	35.67	7.01
CD 5%	3.38	2.68	1.75
CV %	6.53	9.95	19.23

Table 5. Comparative performance, in term of top five ranking treatments for all the parameters evaluated

Parameters	Top ranking treatments				
	I	II	III	IV	V
Zinc in leaves	A4B8	A4B7	A4B9	A4B10	A2B7
Ear height	A4B4	A3B4	A2B4	A3B9	A2B9
GRSPs	A4B1	A4B8	A1B1	A2B8	A3B5
Polysaccharides	A1B2	A1B4	A1B10	A3B3	A1B1
Peroxidase activity (shoots)	A4B8	A2B9	A1B10	A3B3	A3B4
Peroxidase activity (roots)	A3B4	A4B8	A1B7	A1B6	A4B6
PAL Root	A2B8	A3B3	A1B8	A1B6	A5B1
PAL Shoot	A3B1	A3B6	A3B3	A3B8	A3B7
Yield	A4B1	A3B9	A3B11	A5B6	A1B9

A1, *Anabaena* sp. – *Providencia* sp. (CW1 + PW5); A2, Cyanobacterial consortium (BF1- 4); A3, *Anabaena* sp.–*Trichoderma* sp. biofilm (*An-Tr*); A4, *Anabaena* sp.– *Azotobacter* sp. biofilm (*An-Az*), and A5, Control (no inoculation) and hybrids of maize, viz.—B1, HQPM-4; B2, Parkash, B3, HM-4; B4, HQPM-7; B5, HQPM-1; B6, PMH-3; B7, DHM-117; B8, Bio-9681; B9, HM-8; B10, PMH-1; B11, Vivek QPM-9.

4. Discussion

Plant growth-promoting rhizobacteria, including cyanobacteria, represent a heterogeneous assemblage of soil bacteria, which stimulate the growth of their hosts through various direct and indirect mechanisms, including facilitation and increase in the availability, uptake, and enrichment of nutrients in the plant (Khan, 2005). Cyanobacteria represent one of the largest subgroups of Gram-negative photosynthetic prokaryotes, which exhibit relatedness to plants in terms of their photosynthetic machinery, while possessing the advantages of relatively short generation time and capability of being easily handled and cultured under controlled conditions. They have tremendous potential as a source of several bioactive compounds, restriction enzymes, and pigments, besides their extensive utilization as a biofertilizer in agriculture (Mandal et al., 1999; Nain et al., 2010; Prasanna et al., 2012). Although pathogen–host interaction and induction of defense and PR enzymes by micro-organisms have been studied by different investigators, little is known about the ability of PGPR, especially cyanobacteria to induce higher levels of defense enzymes and related compounds, and improve nutrient mobilization in soil and plant in maize. The ability of cyanobacteria to sequester CO_2 and N_2 from the atmosphere has immense implications in the light of current scenario of nutrient depletion in soils worldwide, and the need for sustainable technologies for maintaining crop productivity and soil health (Prasanna et al., 2012; Prasanna, Babu, et al., 2013; Rana, Joshi, et al., 2012).

Quantities of micronutrients in grains are influenced by numerous complex factors, including genotypes, soil properties, environmental conditions, and nutrient interactions (Agarwal et al., 2012; Cakmak et al., 2010; Pfeiffer & McClafferty, 2007; Welch, 1993; Welch & Graham, 2004). It is well known that micronutrient deficiency in plants greatly increases their susceptibility to diseases, especially fungal root diseases of the major food crops (Welch & Graham, 2004). Maize (*Zea mays* L.) is among the oldest and important crops cultivated in many areas of the world, which has been investigated as a model system for biofortification, as it represents a major source of calories for human consumption, besides being exploited as a promising source of fodder and renewable bioenergy (Menkir, 2008; Welch, 1993). Zn in human body is required for biosynthesis of numerous proteins, having both a catalytic and a structural role. The present study revealed that Zn concentration in flag leaf showed a significant increase as compared to control, differentially in all the maize hybrids receiving microbial inoculants, thereby suggesting that microbial associations show differential relations with the roots of maize hybrids, with some combinations exhibiting synergistic interactions on Zn mobilization.

A survey of published literature reveals that crop plants vary in their ability to take up Zn, particularly under conditions of limited availability to roots. Halvorson and Lindsay (1977) found that in grasses, Zn influx into the root symplasm may occur as free Zn^{2+} ions, mediated by Zn complexes with non-protein amino acids known as phytosiderophores (Khan, 2005; Mullins & Sommers, 1986; Pfeiffer & McClafferty, 2007) or phytometallophores (Welch & Graham, 2004). Mullins and Sommers (1986) observed concentration-dependent uptake of free Zn^{2+} ions, which is saturable in several plant species, including maize, and this ionic uptake in grasses is mediated through a carrier-mediated system, with conserved transport systems for Zn^{2+}. Although differences have been observed for the accumulation and utilization of nutrients in dry-matter production among corn (*Zea mays* L.) cultivars ('Pioneer Hybrid 3369A' and 'Conico Composite'), in the present study, no indications of either cultivar being superior in Zn absorption by root systems or in translocation of absorbed Zn to shoots was recorded (Peaslee, Isarangkura, & Leggett, 1981). A large number of rhizobacteria and fungi release iron chelators (siderophores), which possess high binding affinity and specificity for micronutrients; this may facilitate this process; which can explain the significant differences in Zn concentration recorded in the treatments receiving microbial inoculation in the present study. A similar trend was recorded in an earlier study with wheat (Rana, Joshi, et al., 2012; Rana, Saharan, et al., 2012), in which biofortification was mediated by inoculation of bacteria and cyanobacteria–bacteria consortia.

Among the proteins induced during host plant defense, soluble class III plant peroxidases are well known to participate directly or indirectly in a broad range of physiological processes, such as lignin and suberin formation, crosslinking of cell wall components, and synthesis of phytoalexins, or participate in the metabolism of active oxygen species, switching on the hypersensitive response, a form of programmed cell death at the infection site associated with limited pathogen development (Jennings et al., 1969; Khan, 2005). In an earlier study, cyanobacterial formulations were found to trigger high levels of plant defense enzyme activity in tomato plants, which was found to be among the underlying bioprotection mechanisms against *Fusarium* wilt (Prasanna, Chaudhary, et al, 2013). It is well known that large amounts of H_2O_2 are produced due to cellular processes, as a response to stress factors or to external sources to plant–pathogen interactions and micro-organisms can elicit enhanced activity of these enzymes. Plant peroxidases are associated with several cellular processes related to plant development and stress responses (Mika, Boenisch, Hopff, & Luthje, 2010) and represent versatile biocatalysts with an ever-increasing number of applications, as they can detoxify or generate reactive oxygen species, polymerize cell wall compounds, and regulate H_2O_2 levels (Passardi, Cosio, Penel, & Dunand, 2005). In the present investigation, A4 (*Anabaena* – *Azotobacter*-biofilmed formulation) and A1 (CW1 + PW5) significantly enhanced peroxidase activity in roots and shoots, revealing their strong interaction with the plant. A strong positive correlation (0.72) was recorded between dehydrogenase activity and root peroxidase activity in inoculated treatments

(A1–A4) vis a vis a negative correlation (−0.54) in uninoculated treatment (A5) (data submitted elsewhere). It is of immense significance that the peroxidise activity of both roots and shoots had a positive correlation with Zn concentration in leaves. In the present study, the positive correlation among the defense enzymes, especially with PAL reveals an intricate networking of plant enzymes elicited by the inoculation of cyanobacterial consortia or biofilms. This illustrates the significance of microbial inoculation and its positive interactions with eliciting plant defense and micronutrient mobilization from soil and its translocation in plants.

GRSPs or BRSPs are known to contribute towards the aggregation and sequestration of organic matter, however, their origin and chemical composition has been a controversial area of microbiology (Rillig et al., 2002). Although they were considered solely of AMF (Arbuscular Mycorrhizal Fungi) origin, recent proteomic studies have revealed that this fraction may contain large amounts of other soil-related heat-stable proteins, mainly of non-mycorrhizal origin (Gillespie et al., 2011). As it contains carbon, it is closely related to net primary productivity and constitutes a non-trivial portion of the terrestrial carbon pool, and its concentration in soil is mainly influenced by management practices. Liu et al. (2000) reported an increase in acquisition of Fe, Zn, Cu, and Mn by mycorrhizal maize, which was associated with higher N rates, but the reason for the higher N was attributed to mechanisms other than biological N fixation. The excretion of nitrogenous compounds by microbes, among other mechanisms, explains the contribution of microbial activity to micronutrients' acquisition by plants. Cyanobacterial strains used in this study are diazotrophs, which exhibit levels of nitrogenous compounds and GRSPs-like proteins ranging from 2,400–3,900 μg g^{-1} dry biomass of the organism 200–300 μg g^{-1} wet biomass (pers. commun.). Hence, their inoculation in soil can increase the GRSP's content significantly by 4–5 folds over control. Such an enhancement in GRSP content can play an important role as carbon stock, and contribute to net primary productivity (Wright & Upadhyaya, 1996). This illustrates their benefits in improving soil quality and enhancing microbe-mediated Zn translocation in the plant from soil. However, there are no reports on the mechanisms underlying enhancement of GRSPs through cyanobacterial inoculation; this can be an interesting offshoot from the present study, which needs to be investigated in future.

The micronutrient-mediated interactions between soils, plants, and microbes impact plant growth health and productivity, hence of major agronomic interest. The main advantage of using PGPR or biofilms is the beneficial effect in plant growth promotion and biofortification due to the synergistic interactions among the microbial partners. It is well recognized that biofilms possess the capacity to maintain the metabolic activity under adverse environmental conditions and exhibit increased survival in a competitive environment, such as rhizosphere of different crops grown in different agro-ecologies. Cyanobacteria represent a valuable system for developing biofilms, due to the presence of mucilage, which is known to be a reservoir of nutrients. The cyanobacterium *Anabaena torulosa* used in the present study as the matrix, is also known to be nutritionally facultative and can provide additional benefits of accretion of nitrogen, carbon, phosphorus, besides enhancing plant growth through the production of phytohormones and bioactive metabolites (Prasanna et al., 2012; Prasanna, Babu, et al., 2013; Prasanna, Chaudhary, et al., 2013), besides GRSP content of soil. This study illustrated the positive interaction of *Anabaena* with *Azotobacter* sp., a PGP agent for several crops, including wheat, cotton, and vegetables, and with *Trichoderma* sp., a well-established biocontrol agent and plant growth-promoting agent suitable for a diverse range of crops.

Peaslee et al. (1981) had shown that within each cultivar, the trend in labeled Zn uptake follows an order related to the proximity of plant part to source, suggesting that Zn translocation is not rapid in new tissues. Differential responses to nutrient sequestration from soil by plant cultivars, though not clearly understood, are generally believed to be genetically controlled (Prasanna, Kumar, et al., 2013); however, the present investigation reveals the promise of cyanobacterial inoculation in enhancing the mobilization and translocation to the leaves from soil. Thus, increased mineral

concentration in the flag leaf could be attributed to the enhancement of the root growth and development due to positive influence of the microbial activities in soil, as shown earlier by Ryu and co-workers (2005).

The results of our study concluded that inoculation with such promising cyanobacteria, bacterial strains, and biofilms can lead to significant enhancement in micronutrient concentration in the rhizosphere region, besides exhibiting significant differences with respect to control (which received 60 kg N ha^{-1} additionally as split doses) in terms of grain yield, which ranged from 7.0 to 7.29 t ha^{-1} among the different inoculants. This represents a first report on the promise of cyanobacterial inoculation on biofortification in different hybrids of maize.

5. Conclusions

Our study depicted that with 50% less application of N fertilizer, at par values of grain yield with the control that received 100% recommended dose of N fertilizer can be possible; and interestingly, grain yield and microbial inoculation were positively correlated. Thus, application of microbial inoculation thereby provides twofold benefits (i) increase in the Zn concentration in the flag leaf and (ii) reduction in the need for N fertilizer by half, thereby reducing the cost of the cultivation. Future research needs to be directed towards understanding the further translocation of Zn from leaf to kernels in the maize hybrids, as was observed in wheat, in our previous experiments (Rana, Joshi, et al., 2012; Rana, Saharan, et al., 2012). Molecular tools can be employed to decipher the mechanisms involved in micronutrient mobilization by cyanobacteria in promising hybrids.

Supplementary material
Supplemental material for this article can be accessed here http://dx.doi.org/10.1080/23311932.2014.998507

Acknowledgements
The members of the Maize Genetics Unit are acknowledged for facilitating the conduct of field experiment at the research field of Division of Genetics, IARI. The authors gratefully acknowledge the Division of Genetics, Agronomy and Microbiology, IARI, New Delhi, for providing necessary facilities to undertake this study.

Funding
The authors are thankful to the DBT and ICAR-AMAAS projects for funding the research work.

Author details
Radha Prasanna[1]
E-mail: radhapr@gmail.com
Ngangom Bidyarani[1]
E-mail: bidyamicro@gmail.com
Santosh Babu[1]
E-mail: Santoshbabu1908@gmail.com
Firoz Hossain[2]
E-mail: fh_gpb@yahoo.com
Yashbir Singh Shivay[3]
E-mail: ysshivay@hotmail.com
Lata Nain[1]
E-mail: latarajat@yahoo.co.in
[1] Division of Microbiology, Indian Agricultural Research Institute (IARI), New Delhi 110012, India.
[2] Division of Genetics, Indian Agricultural Research Institute (IARI), New Delhi 110012, India.
[3] Division of Agronomy, Indian Agricultural Research Institute (IARI), New Delhi 110012, India.

Citation information
Cite this article as: Cyanobacterial inoculation elicits plant defense response and enhanced Zn mobilization in maize hybrids, R. Prasanna, N. Bidyarani, S. Babu, F. Hossain, Y.S. Shivay & L. Nain, *Cogent Food & Agriculture* (2015), 1: 998507.

References

Agarwal, P. K., Jaiswal, S. K., Prasanna, B. M., Hossain, F., Saha, S., Guleria, S. K., & Gupta, H. S. (2012). Genetic variability and stability for kernel iron and zinc concentration in maize (*Zea mays* L.) genotype. *Indian Journal of Genetics and Plant Breeding, 74*, 421–428.
Banziger, M., & Long, J. (2000). The potential for increasing the iron and zinc density of maize through plant- breeding. *Food Nutrition Bull, 21*, 397–400.
Beaudoin-Eagan, L. D., & Thorpe, T. A. (1985). Tyrosine and phenylalanine ammonia lyase activities during shoot initiation in tobacco callus cultures. *Plant Physiology, 78*, 438–441.
Bouis, H. E., Hotz, C., McClafferty, B., Meenakshi, J. V., & Pfeiffer, W. H. (2011). Biofortification: A new tool to reduce micronutrient malnutrition. *Food Nutrition Bull, 32*, S31–S40.
Cakmak, I., Pfeiffer, W. H., & McClafferty, B. (2010). Biofortification of durum wheat with zinc and iron. *Cereal Chemistry, 87*, 10–20.
Dass S., Manivannan A., Kaul J., Singode A., Sekhar J. C., Chikkappa G. K. (2010). *Inbred-hybrid technology in maize* (Technical Bulletin No. 2010/1). DMR Technical Bulletin. New Delhi: Directorate of Maize Research, Pusa Campus. 52 pp.
Gillespie, A. W., Farrell, R. E., Walley, F. L., Ross, A. R. S., Leinweber, P., Eckhardt, K.-U., Regier, T. Z., & Blyth, R. I. R. (2011). Glomalin-related soil protein contains non-mycorrhizal-related heat-stable proteins, lipids and humic materials. *Soil Biology and Biochemistry, 43*, 766–777.
Halvorson, A. D., & Lindsay, W. L. (1977). The critical Zn2+ concentration for corn and the nonabsorption of chelated Zinc1. *Soil Science Society of America Journal, 41*, 531–534.
Jennings, P. H., Brannaman, B. L., & Zoheille, F. P. (1969). Peroxidase and polyphenoloxidase activity and associated with *Helminthosporium* leaf spot of maize. *Phytopathol, 59*, 963–967.
Khan, A. G. (2005). Role of soil microbes in the rhizospheres of plants growing on trace metal contaminated soils in phytoremediation. *Journal of Trace Elements in Medicine and Biology, 18*, 355–364.

Lin, A. (2005). Effect of cover crops on soil aggregate stability, total organic carbon and polysaccharide. *Soil Science Society of America Journal, 69*, 2091–2098.
Liu, A., Hamel, C., Hamilton, R. I., Ma, B. L., & Smith, D. L. (2000). Acquisition of Cu, Zn, Mn, and Fe by mycorrhizal maize (*Zea mays* L.) grown in soil at different P and micronutrient levels. *Mycorrhiza, 9*, 331–336.
MacKinney, G. (1941). Absorption of light by chlorophyll solutions. *Journal of Biological Chemistry, 140*, 315–322.
Mandal, B. P. L., Vlek, P. L. G., & Mandal, L. N. (1999). Beneficial effects of blue-green algae and *Azolla*, excluding supplying nitrogen, on wetland rice fields: A review. *Biology and Fertility of Soils, 28*, 329–342.
Menkir, A. (2008). Genetic variation for grain mineral content in tropical- adapted maize inbred lines. *Food Chemistry, 110*, 454–464.
Mika, A., Boenisch, M. J., Hopff, D., & Luthje, S. (2010). Membrane-bound guaiacol peroxidases from maize (*Zea mays* L.) roots are regulated by methyl jasmonate, salicylic acid, and pathogen elicitors. *Journal of Experimental Botany, 61*, 831–841.
Mullins, G. L., & Sommers, L. E. (1986). Cadmium and zinc influx characteristics by intact corn (*Zea mays* L.) seedlings. *Plant Soil, 96*, 153–164.
Nain, L., Rana, A., Joshi, M., Jadhav, S. D., Kumar, D., Shivay, Y. S., Paul, S., & Prasanna, R. (2010). Evaluation of synergistic effects of bacterial and cyanobacterial strains as biofertilizers for wheat. *Plant Soil, 331*, 217–230.
Nayak, S., Prasanna, R., Pabby, A., Dominic, T. K., & Singh, P. K. (2004). Effect of urea, blue green algae and *Azolla* on nitrogen fixation and chlorophyll accumulation in soil under rice. *Biology and Fertility of Soils, 40*, 67–72.
Olsen, S. R., Cole, C. V., Watanabe, F. S., & Dean, L. A. (1954). *Estimation of available phosphorus in soil by extraction with sodium carbonate*. Washington, DC: USDA.
Passardi, F., Cosio, C., Penel, C., & Dunand, C. (2005). Peroxidases have more functions than a Swiss army knife. *Plant Cell Reports, 24*, 255–265.
Peaslee, D. E., Isarangkura, R., & Leggett, J. E. (1981). Accumulation and translocation of Zinc by two corn cultivars. *Agronomy Journal, 73*, 729–732.
Pfeiffer, W. H., & McClafferty, B. (2007). HarvestPlus: Breeding crops for better nutrition. *Crop Science, 47*, S88–S105.
Prasad, R., Shivay, Y. S., Kumar, D., Sharma, S. N. (2006). *Learning by doing exercise in soil fertility (A practice manual for soil fertility)* (p. 68). New Delhi: Division of Agronomy, Indian Agricultural Research Institute.
Prasanna, B. M., Mazumdar, S., Chakraborti, M., Hossain, F., Manjaiah, K. M., Agarwal, P. K., Guleria, S. K., & Gupta, H. S. (2011). Genetic variability and genotype X environment interactions for kernel iron and zinc concentrations in maize (*Zea mays* L.) genotypes. *Indian Journal of Agricultural Sciences, 81*, 704–711.
Prasanna, R., Babu, S., Rana, A., Kabi, S. R., Chaudhary, V., Gupta, V., Kumar, A., Shivay, Y. S., Nain, L., & Pal, R. K. (2013). Evaluating the establishment and agronomic proficiency of cyanobacterial consortia as organic options in wheat–rice cropping sequence. *Experimental Agriculture, 49*, 416–434.
Prasanna, R., Chaudhary, V., Gupta, V., Babu, S., Kumar, A., Singh, R., & Shivay, Y. S. (2013). Cyanobacteria mediated plant growth promotion and bioprotection against Fusarium wilt in tomato. *European Journal of Plant Pathology, 136*, 337–353.
Prasanna, R., Kumar, A., Babu, S., Chawla, G., Chaudhary, V., Singh, S., Gupta, V., Nain, L., & Saxena, A. K. (2013). Deciphering the biochemical spectrum of novel cyanobacterium based biofilms for use as inoculants. *Biological Agriculture & Horticulture, 29*, 145–158.
Prasanna, R., Nain, L., Tripathi, R., Gupta, V., Chaudhary, V., Middha, S., Joshi, M., Ancha, R., & Kaushik, B. D. (2008). Evaluation of fungicidal activity of extracellular filtrates of cyanobacteria-possible role of hydrolytic enzymes. *Journal of Basic Microbiology, 48*, 186–194.
Prasanna, R., Pattnaik, S., Sugitha, T. C. K., Nain, L., & Saxena, A. K. (2011). Development of cyanobacterium based biofilms and their *in vitro* evaluation for agriculturally useful traits. *Folia Microbiologica, 56*, 49–58.
Prasanna, R., Rana, A., Chaudhary, V., Joshi, M., & Nain, L. (2012). Cyanobacteria-PGPR interactions for effective nutrient and pest management strategies in agriculture. In T. Satyanarayana, B. N. Johri, & A. Prakash (Eds.), *Microorganisms in sustainable agriculture and biotechnology* (pp. 173–195). Dordrecht: Springer.
Rana, A., Joshi, M., Prasanna, R., Shivay, Y. S., & Nain, L. (2012). Biofortification of wheat through inoculation of plant growth promoting rhizobacteria and cyanobacteria. *European Journal of Soil Biology, 50*, 118–126.
Rana, A., Saharan, B., Nain, L., Prasanna, R., & Shivay, Y. S. (2012). Enhancing micronutrient uptake and yield of wheat through bacterial PGPR consortia. *Soil Science and Plant Nutrition, 58*, 573–582.
Rillig, M. C., Wright, S. F., & Eviner, V. T. (2002). The role of arbuscular mycorrhizal fungi and glomalin in soil aggregation: Comparing effects of five plant species. *Plant and Soil, 238*, 325–333.
Ryu, C. M., Hu, C. H., Locy, R. D., & Kloepper, J. W. (2005). Study of mechanisms for plant growth promotion elicited by rhizobacteria in *Arabidopsis thaliana*. *Plant and Soil, 268*, 285–292.
Shiferaw, B., Prasanna, B. M., Hellin, J., & Bänziger, M. (2011). Crops that feed the world 6. Past successes and future challenges to the role played by maize in global food security. *Food Security, 3*, 307–327.
Solomons, N. W. (2003). Zinc deficiency. In B. Caballero (Ed.), *Encyclopedia of Food Sciences and Nutrition* (2nd ed.). (pp. 6277–6283). Oxford: Elsevier Science.
Stanier, R. Y., Kunisawa, R., Mandel, M., & Cohen-Bazire, G. (1971). Purification and properties of unicellular blue green algae (order: Chroococcales). *Bacteriological Reviews, 35*, 171–305.
Subbiah, B. V., & Asija, G. L. (1956). A rapid procedure for the determination of available nitrogen in soils. *Current Science, 25*, 259–260.
Welch, R. M. (1993). Zinc concentrations and forms in plants for humans and animals. In A. D. Robson (Ed.), *Zinc in Soils and Plants* (pp. 183–195). Dordrecht: Kluwer Academic.
Welch, R. M., & Graham, R. D. (2004). Breeding for micronutrients in staple food crops from a human nutrition perspective. *Journal of Experimental Botany, 55*, 353–364.
Wright, S. F., & Upadhyaya, A. (1996). Extraction of an abundant and unusual protein from soil and comparison with hyphal protein of arbuscular mycorrhizal fungi. *Soil Science, 161*, 575–586.

Cogent Food & Agriculture **(ISSN: 2331-1932) is published by Cogent OA, part of Taylor & Francis Group.**
Publishing with Cogent OA ensures:

- Immediate, universal access to your article on publication
- High visibility and discoverability via the Cogent OA website as well as Taylor & Francis Online
- Download and citation statistics for your article
- Rapid online publication
- Input from, and dialog with, expert editors and editorial boards
- Retention of full copyright of your article
- Guaranteed legacy preservation of your article
- Discounts and waivers for authors in developing regions

Submit your manuscript to a Cogent OA journal at www.CogentOA.com