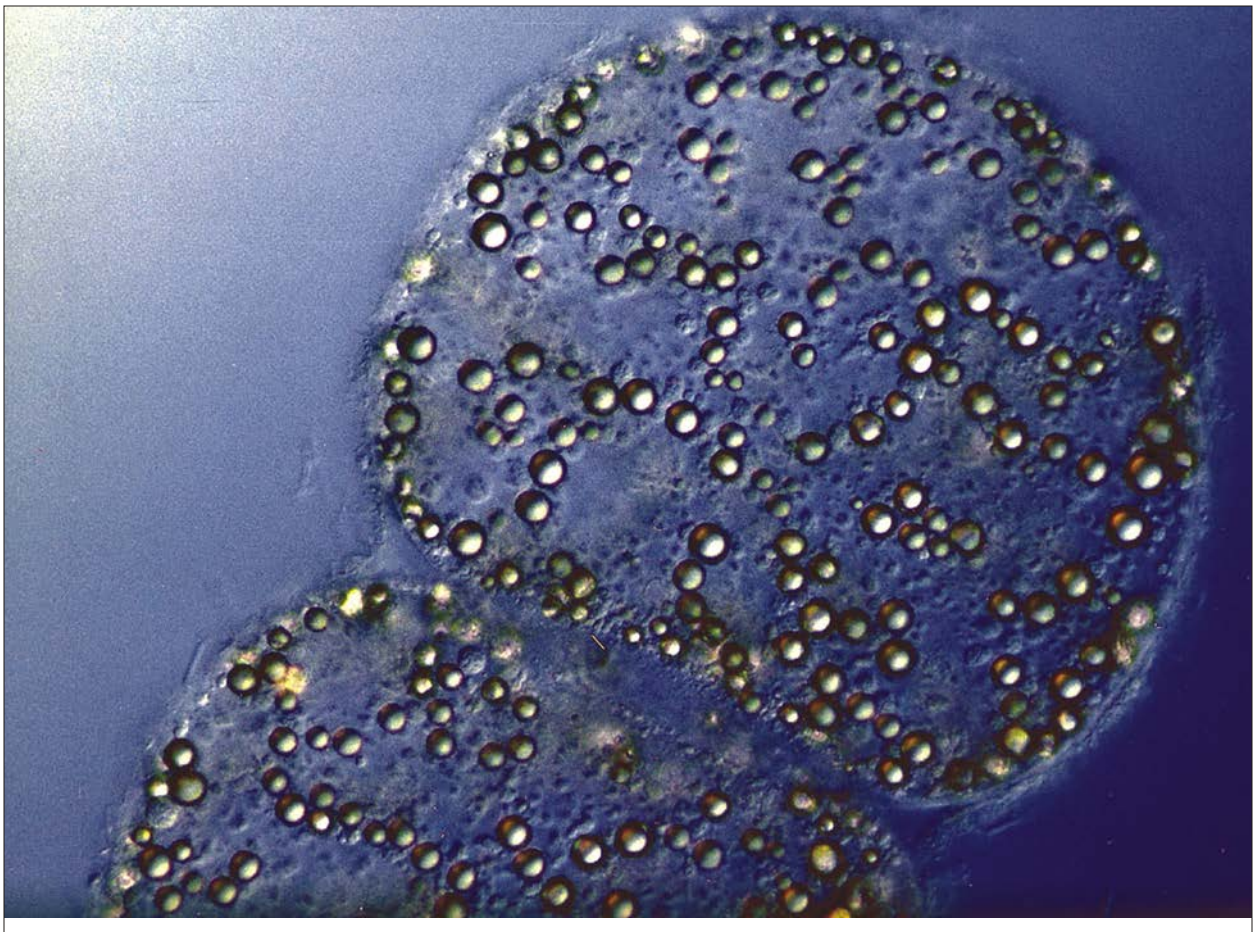

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Improvement of Wheat (*Triticum aestivum* L.) Yield under Field Conditions by Inoculation of Microbial Strains

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ABSTRACT

The effect of inoculation of wheat (*Triticum aestivum* L.) with different microbial strains was studied under experimental field conditions. Seeds of two wheat varieties viz. WH711 and Raj3765 were inoculated either singly with microbial strains, namely *Azotobacter chroococcum* strain Mac27, *Trichoderma viride*, *Pseudomonas* strain P20, *Bacillus* strain SYB101 or seeds were co-inoculated with biomixture of *Pseudomonas* and *Bacillus* strains. Inoculation with *Bacillus* strain SYB101 showed maximum seed germination in both the varieties of wheat. Seed treatment of wheat with *Bacillus* strain SYB101 or their co-inoculation with *Pseudomonas* strain CP56 and *Bacillus* strain SB155 produced longer root and shoot length and also resulted in higher vigour index values over untreated control. Under field conditions, inoculation of the wheat variety WH711 with *Bacillus* strain SYB101 caused 32.6% increase in seed yield whereas inoculation of this strain on the other wheat variety Raj3765, caused 23.1% increase in seed yield in comparison to uninoculated control treatment. Inoculation with *Azotobacter chroococcum* showed only 7.4% increase in the variety Raj3765. Co-inoculation of *Pseudomonas* strain CP56 and *Bacillus* strain SB155 showed 23.3 and 19.4% increase in variety WH711 and variety Raj3765, respectively. Maximum enhancement in seed yield was obtained with *Bacillus* strain SYB101 and, therefore, this strain could be exploited for use as biofertilizer for improvement of wheat growth and seed yield under field conditions.

Key words: Microbial strains, wheat, seed germination, seedling growth, seed yield

INTRODUCTION

Soil health and productive capacity has declined recently due to use of high input technologies and intensification of agriculture for increasing the production of farm commodities to feed the surging population. To obtain improved plant growth and yield of crops, essential nutrients especially nitrogen, phosphorus and potassium are usually provided to plants in the form of chemical fertilizers (Marschner, 1995) and pesticides are applied for protection of plants from insect pests, plant diseases and weeds (Oerke *et al*., 1995). These soil amendments are not only costly but also have resulted in stagnating yields. Moreover, their negative impact on the environment and soil health emphasized the use of an alternative technology for eco-friendly farming which includes the integration of biological, cultural and natural inputs along with integrated nutrient and disease management practices for sustainable agriculture (Doran and Zeiss, 2000).

Among biological inputs, microorganisms found in the soil or rhizospheres are major contributors to the biogeochemical cycles (Benizri *et al*., 2001; Smalla *et al*., 2006; Franche *et al*., 2009). Some rhizosphere microorganisms may be pathogenic or deleterious in regard to plant growth, whereas other microbes have been found to stimulate the plant growth (Miller *et al*., 1989; Sindhu *et al*., 1997). Plant growth promoting rhizobacteria (PGPR) (Kloepper *et al*., 1980) can result in significant promotion of root and shoot biomass, increase in yield of crops, reduce pathogen infection as well as biotic and abiotic plant stresses (Ahmad *et al*., 2008; Weyens *et al*., 2009; Tiwari *et al*., 2011). The beneficial effects of PGPR have been correlated with increased recycling, solubilization and uptake of mineral nutrients (Lifshitz *et al*., 1987; Sindhu *et al*., 2010), synthesis of vitamins, amino acids, auxins and gibberellins (Ahmad *et al*., 2008; Malik and Sindhu, 2011) and by antagonism of potential plant pathogens by production of antibiotics, siderophores, cyanide and hydrolytic enzymes (Weller, 2007; Sindhu *et al*., 2009). These beneficial microorganisms maintain the fertility status and physical characteristics of the soil, which is essential for improving biomass production and for enhancing crop productivity (Sindhu *et al*., 2010). These supplementary technologies, such as application of biofertilizers and biocontrol agents, may partially compensate the need of chemical fertilizers or pesticides in sustainable agriculture (Lucy *et al*., 2004; Karlidag *et al*., 2007; Lugtenberg and Kamilova, 2009). Experimental and field applications of PGPR have resulted in significant enhancement of plant growth, as observed in terms of emergence, vigor, biomass, development of root systems and increase in the yield of different crop species, such as wheat (Carlier *et al*., 2008; Shaharoona *et al*., 2008), corn (Marques *et al*., 2010), soybean (Dashti *et al*., 1998), chickpea (Sindhu *et al*., 2002; Wani *et al*., 2007), cluster bean (Khandelwal and Sindhu, 2012), green gram (Sahu and Sindhu, 2011) and rice (Keyeo *et al*., 2011).

Wheat (*Triticum aestivum* L.) is the second most important grain crop and is a source of staple food in many countries of the world. Though the production of wheat has increased after green revolution in India, the attack of various diseases like powdery mildew, root rots, rusts, smuts, take-all and Karnal bunt of wheat has greatly affected its yield and quality (Ryder *et al*., 1998; Rush *et al*., 2005). Therefore, production of good quality seed and maintenance of high germination is of utmost importance in seed production programme in agriculture. The pre-sowing inoculation of seeds with beneficial microorganisms usually result in enhanced germination, plant growth and yield as well as minimize the incidence of seed borne diseases. Therefore, the present study was conducted to assess the effect of inoculation of microorganisms on plant growth promotion and yield of wheat under field conditions.

MATERIALS AND METHODS

The experiment was performed under field conditions in the farm area of the Department of Seed Science and Technology, CCS Haryana Agricultural University, Hisar during the months of November-April in the year 2009 and 2010.

Characteristics of microbial strains used for inoculation: The characteristics of different bacterial and fungal cultures used for inoculation are listed in Table 1. Cultures of rhizobacterial strains i.e., *Pseudomonas/Bacillus* were grown on Luria Bertani (LB) medium slants for 2 days and maintained by periodic transfer on LB agar slants (Sambrook *et al*., 1989). *Trichoderma viride* was maintained on potato dextrose agar (PDA) medium slants. *Azotobacter chroococcum* strain Mac27 was grown and maintained on Jensen's medium (Sindhu *et al*., 1994). These microbial cultures were stored at 4°C in refrigerator for further use.

Table 1: Characteristics of microbial strains used for inoculation of wheat

Microbial strain	Characteristics	References
Azotobacter chroococcum strain Mac27	N_2 fixation, ammonia excretion, methyl ammonium chloride resistant mutant of strain A103, PGPR	Lakshminarayana *et al.* (2000)
Pseudomonas strain CP56	Siderophore production, antifungal activity, PGPR	Sahu and Sindhu (2011)
Pseudomonas strain PS31	Siderophore production, antifungal activity, PGPR	Sahu and Sindhu (2011)
Bacillus strain SB155	Antifungal activity, PGPR	Sivaramaiah *et al.* (2007)
Bacillus strain SYB101	IAA production, antifungal activity, PGPR	This study
Pseudomonas strain P20	PGPR	This study
Trichoderma viride	Antifungal activity	This study

Determination of microbial count: Soil samples collected from random locations of the wheat grown field were thoroughly mixed to form composite samples for microbiological analysis. Samples were serially diluted in 9 mL water blanks up to 10^{-7}. One milliliter of different diluted samples i.e., 10^{-5}-10^{-7} was pour plated on nutrient agar medium plates under aseptic conditions in front of laminar flow chamber. The plates were incubated for 2-5 days at 28±2°C in a BOD incubator. Numbers of microbial colonies appeared on medium plates were counted.

Effect of microbial strains inoculation on seed germination and seedling growth: Seeds of wheat (*Triticum aestivum* L.) cv. WH711 and Raj3765 were obtained from the Department of Seed Science and Technology, CCS Haryana Agricultural University, Hisar. Healthy seeds of wheat var. WH711 and Raj3765 were surface sterilized with acidic alcohol (H_2SO_4: ethanol, 7:3, v/v) for 3 min followed by thorough washing with repeated changes of sterilized distilled water (Sindhu *et al.*, 1999). Different *Pseudomonas/Bacillus* strains were grown in 500 mL capacity flasks containing LB broth and the fungus *Trichoderma viride* was grown in potato dextrose agar broth. The growth suspension of different microbial cultures (5.2×10^8 cells mL^{-1}) was used for seed treatment of wheat.

The surface sterilized seeds were inoculated with broth culture of different microorganisms and allowed to be adsorbed for 45 min. Inoculated seeds were germinated on water agar plates (8 g agar L^{-1} distilled water) at 28±1°C in a BOD incubator. Uninoculated seeds treated with LB broth alone were sown as control. For germination test, random samples of 100 seeds were tested from each treatment. For seedling growth measurements, the root and shoot lengths were measured at 7 days after sowing.

Determination of vigour index of inoculated seeds: Seed vigour index (standard germination×seedling length) was computed as described by Abdul-Baki and Anderson (1973). Seed quality parameters and vigour index were studied after inoculation with different microbial strains. The data were analyzed statistically using completely randomized block design (CRBD).

Inoculation of wheat under field conditions: Field experiment was conducted in the research farm area of CCS Haryana Agricultural University, Hisar (situated at longitude 75°46'E and latitude 29°10'N), India. The farm soil had a pH 8.0, organic carbon content of 0.28%, available nitrogen of 145 kg ha^{-1}, available phosphorus of 16 kg ha^{-1} and available potassium of 320 kg ha^{-1}. The cultures of selected *Pseudomonas* and *Bacillus* isolates were grown in 500 mL capacity flasks containing LB broth. The bacterial growth was vortexed on rotary shaker to get uniform suspension. Growth of *Trichoderma viride* (4 days old) was harvested from PDA plates

with the help of an inoculation needle and then sterilized saline water was added to get fungal growth suspension. The 100 mL fungal growth suspension was used in treatment T_1.

The seeds of wheat (*Triticum aestivum* L.) variety WH711 as well as variety Raj3765 were inoculated with broth culture of different bacterial strains. The viable count in the broth was kept 10^8-10^9 cells mL^{-1} and 1 kg seeds were inoculated with 50 mL of bacterial growth suspension (Sindhu *et al.*, 1999). There were three replications for each treatment and the different treatments were sown in plots (size, 5×4 m^2) using the randomized complete block design. The NPK fertilizers were applied to soil at 100% of the recommended doses i.e., N_{150}-P_{60}-K_{30} ha^{-1} in the form of urea, single super phosphate and muriate of potash, respectively. The different treatments applied were as follows:

- **T_0 :** Soil (control, uninoculated)
- **T_1 :** Soil+*Trichoderma viride*
- **T_2 :** Soil+*Pseudomonas* strain P20
- **T_3 :** Soil+*Azotobacter* strain Mac27
- **T_4 :** Soil+Co-inoculation of *Pseudomonas* strain CP56+*Bacillus* strain SB155
- **T_5 :** Soil+Co-inoculation of *Pseudomonas* strain PS31+*Bacillus* strain SYB101
- **T_6 :** Soil+*Bacillus* strain SYB101

Plants were irrigated with good quality canal water. Observations were recorded for soil microbiota (at initial sowing period, tillering and flowering stage) and seed yield (q ha^{-1}) data of wheat were collected at harvesting (160 days after seeding).

Statistical analysis: All measurements regarding seed quality parameters, vigour index and seed yield were carried out in triplicate. Statistical analysis for determination of critical difference (CD) and SEM value was done by using SPSS software program (OPSTAT).

RESULTS

Microbial population was determined at different stages of plant growth. At the time of sowing, bacterial population in the two varieties of wheat varied from 1.8 to 9.2×10^6 g^{-1} rhizosphere soil (Table 2) and population of actinomycetes ranged from 1.1 to 2.3×10^5 g^{-1} soil whereas population of fungi in different treatments varied from 2.8 to 7.2×10^5 g^{-1} rhizosphere soil. At tillering stage, bacterial population increased as compared to initial count (at the time of sowing) in both the varieties of wheat and bacterial count varied from 8.2×10^6-1.2×10^8 g^{-1} rhizosphere soil. Actinomycetes population ranged from 2.2×10^5- 2.4×10^6 g^{-1} rhizosphere soil and the population of fungi in different treatments varied from 6.9×10^5-5.4×10^6 g^{-1} rhizosphere soil.

Table 2: Microbial population in the wheat rhizosphere at different stages of plant growth

	Population (CFU g^{-1} soil)		
Microorganism	Sowing	Tillering	Harvest
Bacteria	1.8-9.2×10^6	8.2×10^6-1.2×10^8	3.6×10^6-1.3×10^7
Actinomycetes	1.1-2.3×10^5	2.2×10^5-2.4×10^6	1.3-3.1×10^5
Fungi	2.8-7.2×10^5	6.9×10^5-5.4×10^6	3.5×10^5-1.0×10^6

The counts have been determined by serial dilution technique of the composite samples collected from different treatments. CFU indicates the variation in population of microbes in different samples

Bacterial population in the wheat rhizosphere soil varied from 3.6×10^6-1.3×10^7 g^{-1} soil at maturity stage of the crop and actinomycetes population ranged from 1.3-3.1×10^5 g^{-1} rhizosphere soil. The population of fungi in different treatments varied from 3.5×10^5 to 1.0×10^6 g^{-1} rhizosphere soil at maturity stage.

In the wheat variety WH711, seeds treated with *Bacillus* strain SYB101 recorded higher germination (97%) followed by seeds treated with biomixture of strains *Pseudomonas* strain CP56 and *Bacillus* strain SB155 (Table 3). Inoculation with *Trichoderma viride* and *Pseudomonas* strain P20 resulted in higher germination when compared to control non-treated seeds (95% vs. 93%). Maximum 17.3% increase in seedling length was observed on inoculation with *Bacillus* strain SYB101 followed by 16.6% increase by co-inoculation of biomixture consisting of *Pseudomonas* strain CP56 and SB155. Inoculation with *Pseudomonas* strain P20 resulted in only 9.6% increase in comparison to uninoculated controls. Inoculation with *Bacillus* strain SYB101 caused 20.3% increase in vigour index followed by 19.9% increase on co-inoculation of *Pseudomonas* strain CP56 and *Bacillus* strain SB155 over to those of uninoculated plants. The lowest vigour index of 2656.76 was observed in the control non-treated seeds. Treatment of seeds with *Bacillus* strain SYB101 also showed 22.5% increase in seedling dry weight followed by 14.2% increase on co-inoculation with *Pseudomonas* strain CP56 and *Bacillus* strain SB155. Interestingly, 32.6% increase in seed yield was obtained on inoculation with *Bacillus* strain SYB101 whereas co-inoculation of *Pseudomonas* strain CP56 and *Bacillus* strain SB155 showed 23.3% increase in comparison to uninoculated control treatment. Inoculation with *Trichoderma viride* and *Pseudomonas* strain P20 resulted in 14.1 and 9.3% increase as compared to control untreated plants.

In the wheat variety Raj3765, seed treatment with *Bacillus* strain SYB101 or co-inoculation with *Pseudomonas* strain CP56 and *Bacillus* strain SB155 produced longer root and shoot length, and also resulted in higher vigour index values over untreated control (Table 4). Maximum 45.6% increase in seedling dry weight was observed on inoculation with *Bacillus* strain SYB101. *Azotobacter chroococcum* inoculation showed 23.0% increase whereas 21.8% increase was observed on co-inoculation of *Pseudomonas* strain CP56 and *Bacillus* strain SB155. Inoculation with *Bacillus* strain SYB101 also showed 23.1% increase in seed yield as compared to untreated control plants. On the other hand, co-inoculation with *Pseudomonas* strain CP56 and *Bacillus* strain SB155 caused 19.4% increase whereas inoculation with *Azotobacter chroococcum* resulted in only 7.4% increase in comparison to control untreated plants. Thus, seeds treated with *Bacillus* strain SYB101 and co-inoculated with *Pseudomonas* strain CP56 and *Bacillus* strain SB155 were found effective in enhancing seed quality parameters.

Table 3: Effect of microbial inoculation on quality parameters in wheat var. WH711

Treatment	Germination (%)	Seedling length (cm)	Seedling dry wt. (mg)	Seed vigour index	1000-seed weight (g)	Seed yield (q ha^{-1})
T_0	93.00	28.26	190.00	2656.76	44.25	35.83
T_1	95.00	29.93	197.00	2843.10	41.10	40.83
T_2	95.00	30.96	198.00	2941.13	42.20	39.16
T_3	94.66	29.76	198.00	2767.40	40.49	35.00
T_4	94.00	28.66	195.00	2693.70	44.09	37.50
T_5	95.33	32.96	217.00	3184.66	44.48	44.16
T_6	97.00	33.16	232.66	3197.16	44.52	47.50
CD (0.05%)	1.19	3.14	4.02	298.51	2.69	NS
SE (m)	0.38	1.00	1.29	95.81	1.22	3.84

Values are mean of two years data, For germination test, 100 seeds were tested after inoculation with particular microbial strain, For seedling growth measurements, the root and shoot lengths were measured at 7 days after sowing. Seed yield (q ha^{-1}) data was collected at harvesting

Table 4: Effect of microbial inoculation on seed quality parameters in wheat var. Raj3765

Treatment	Germination (%)	Seedling length (cm)	Seedling dry wt. (mg)	Seed vigour index	1000-seed weight (g)	Seed yield (q ha^{-1})
T_0	94.00	29.13	168.00	2796.10	35.95	42.50
T_1	95.33	30.93	188.00	2907.03	38.91	45.83
T_2	95.00	30.70	181.33	2916.83	38.61	45.66
T_3	95.00	30.93	206.66	2937.96	36.53	43.33
T_4	94.00	29.96	189.33	2817.26	38.33	45.00
T_5	95.00	31.76	204.66	3058.96	38.07	50.75
T_6	97.33	32.20	244.66	3112.40	40.16	52.33
CD (0.05%)	1.01	N.S.	6.14	190.53	NS	NS
SE (m)	0.32	0.65	1.97	61.16	0.83	3.03

Values are mean of two years data, For germination test, 100 seeds were tested after inoculation with particular microbial strain, For seedling growth measurements, the root and shoot lengths were measured at 7 days after sowing. Seed yield (q ha^{-1}) data was collected at harvesting

DISCUSSION

Soil and rhizosphere of crop plants supports a conglomerate of microorganisms with a high degree of diversity (Miller *et al*., 1989; Doran and Zeiss, 2000). The interactions between microorganisms or with the plant result into symbiotic, associative, neutralist or antagonistic effects (Schippers *et al*., 1987; Benizri *et al*., 2001). These effects can be either pathogenic, saprophytic and/or plant growth promoter. Generally, many rhizosphere microorganisms have been characterized for their capacity to confer plant growth promotion (Vessey, 2003). However, these beneficial microorganisms often fail to confer these beneficial effects when applied in the field, which is often due to insufficient rhizosphere colonization.

Bacterial population on the two varieties of wheat varied from 1.8 to 9.2×10^6 g^{-1} rhizosphere soil at the time of sowing and population of fungi in different treatments varied from 2.8 to 7.2×10^5 g^{-1} rhizosphere soil. Such large populations of microorganisms present in the soil along with abiotic stress conditions create a hostile environment for the introduced microorganisms (Van Veen *et al*., 1997; Sindhu and Dadarwal, 2000). Therefore, success of seed-applied bacterial or fungal strains depends on their ability to colonize the soil and compete with the indigenous microbial strains (Goel *et al*., 1997). Moreover, intrinsic characteristics of the inoculant strains and their responses to environmental variables contribute to the ability of the introduced microorganisms to occupy the particular ecological niche and perform the beneficial activity/function for the benefit of the host plant. Enhancement of seed yield in some of the treatments in this study indicated that inoculated microbial strains are competent enough to displace the resident microbial population leading to improvement in growth and yield of wheat under field conditions.

In the wheat variety WH711, inoculation of seeds with *Bacillus* strain SYB101 caused 17.3% increase in seedling length and 22.5% increase in seedling dry weight. Co-inoculation of biomixture consisting of *Pseudomonas* strain CP56 and *Bacillus* strain SB155 resulted in 16.6% increase in seedling length. Similarly, in the wheat variety Raj3765, seed treatment with *Bacillus* strain SYB101 or their co-inoculation with *Pseudomonas* strain CP56 and *Bacillus* strain SB155 produced longer root and shoot length over untreated control. Maximum 45.6% seedling dry weight increase was observed on inoculation with *Bacillus* strain SYB101. Similar effect on stimulation of root elongation has been reported in different plants by inoculation of *Pseudomonas putida* strain GR12-2 (Jacobson *et al*., 1994). In cluster bean, inoculation with ACC utilizing

Bradyrhizobium/Rhizobium isolates showed stimulation of root growth on water agar plates at five days and significant stimulation of shoot growth was observed at 10 days of growth (Khandelwal and Sindhu, 2012). On the other hand, stunting/retardation effect has been reported on seedling growth of chickpea by inoculation of IAA producing *Pseudomonas* isolates (Malik and Sindhu, 2011).

Inoculation of *Bacillus* strain SYB101 caused 32.6% increase in seed yield of the wheat variety WH711 whereas its co-inoculation with *Pseudomonas* strain CP56 and *Bacillus* strain SB155 showed 23.3% increase in comparison to uninoculated control treatment. Seed treatment with *Trichoderma viride* and *Pseudomonas* strain P20 caused 14.1 and 9.3% increase as compared to control untreated plants. In another wheat variety Raj3765, maximum 23.1% increase in seed yield was obtained on inoculation with *Bacillus* strain SYB101 as compared to untreated control plants. Co-inoculation with *Pseudomonas* strain CP56 and *Bacillus* strain SB155 resulted in 19.4% increase whereas inoculation with *Azotobacter chroococcum* showed only 7.4% increase in comparison to control uninoculated plants. Thus, seeds either treated with *Bacillus* strain SYB101 or co-inoculated with *Pseudomonas* strain CP56 and *Bacillus* strain SB155 were found more effective in enhancing the seed yield of the two wheat varieties under field conditions.

Similar increases in crop yield have been reported by seed inoculation of wheat with different rhizosphere bacteria (Rai and Gaur, 1988). Pandey and Kumar (1989) summarized the results of several field experiments and concluded that *Azotobacter* inoculation increased the yields of wheat, rice, maize, pearl millet and sorghum by 0 to 72% over to the uninoculated controls without any amendment and reported 8 to 43% increase over control when farm yard manure and fertilizers were added. Lakshminarayana *et al.* (2000) reported that inoculation of wheat variety WH291 with *Azotobacter chroococcum* strain A103 increased the grain yield by 16.3% and wheat inoculation with different analogue resistant mutants of *Azotobacter* i.e., Msx1, Msx27, Mal27, Mal30, Mac19 and Mac27 resulted in increased grain yield varying from 10 to 30% under field conditions. Shaharoona *et al.* (2008) found that inoculation of wheat with *P. fluorescens* biotype F (ACC_{73}) caused maximum increase in grain yield (35%) over uninoculated control under unfertilized conditions in pot trials. Similarly, 6% increase in weight of 1,000 grains, 13% in number of spikes per plant and 30% in number of grains per spike was observed by inoculation of wheat with the rhizobacterium *Pseudomonas chlororaphis* subsp. *aurantiaca* strain SR1 at an experimental field without fertilization (Carlier *et al.*, 2008). Dua and Sindhu (2012) showed that single inoculation of rhizobacterial isolate WPS3 resulted in 131% increase of plant dry weight and its co-inoculation with *Rhizoctonia solani* (root rot disease causing fungi) enhanced 115% plant dry weight as compared to uninoculated control plants under pot house studies at 90 days of plant growth.

In view of the potential application of these rhizosphere microbes to enhance the availability of nutrients leading to plant growth-promoting effects, the inoculation with *Bacillus* strains SYB101 and SB155 are routinely used for inoculation as biofertilizers under field conditions. Thus, characterization of beneficial microorganisms is a promising area of research to achieve maximum benefits for improvement of crop productivity.

CONCLUSION

Inoculation with plant growth promoting microbial strains enhanced the seed yield of two wheat varieties viz. WH711 and Raj3765 under experimental field conditions. Seed treatment of wheat with *Bacillus* strain SYB101 or their co-inoculation with *Pseudomonas* and *Bacillus* strains promoted seedling growth and also resulted in higher vigour index values in comparison to

uninoculated control. Inoculation of the wheat variety WH711 with *Bacillus* strain SYB101 caused 32.6% increase in seed yield whereas inoculation of this strain in another wheat variety Raj3765, caused 23.1% increase in seed yield to those of uninoculated treatment. Results of this study suggested that inoculation with different microbial strains could enhance the seed yield over to those of currently applied 100% recommended doses of fertilizers. Thus, more microbial strains could be characterized for inoculation on different crops in an integrated approach along with fertilization to improve crop productivity under field applications in sustainable agriculture.

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