



Consortium Application of Endophytic Bacteria and Fungi Improves Grain Yield and Physiological Attributes in Advanced Lines of Bread Wheat

Ghulam Muhae-Ud-Din¹, Muhammad Amjad Ali^{1,2*}, Muhammad Naveed³, Khalid Naveed¹, Amjad Abbas¹, Javed Anwar⁵, Muhammad Hammad Tanveer⁵

¹Department of Plant Pathology, University of Agriculture Faisalabad, Pakistan

²Centre of Agricultural Biochemistry & Biotechnology, University of Agriculture, Faisalabad, Pakistan

³Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad, Pakistan

⁴Department of Agronomy, University of Agriculture Faisalabad, Pakistan

⁵Wheat Research Institute, AARI, Jhang Road, Faisalabad, Pakistan

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*Corresponding Author:

E-mail: amjad.ali@uaf.edu.pk

ABSTRACT

Increasing human population places pressure on agriculture. To feed this population, two time increase in the current wheat production is needed. Today agriculture is becoming input intensive with more reliance on synthetic fertilizers and agrochemicals to fulfil the feed demand of the growing numbers. Use of synthetic fertilizer since last few years is impacting the soil quality. In this scenario, the use of beneficial endophytic microbes is an attractive strategy to overcome the use of synthetic products. To investigate the effect of consortium application of endophytic bacteria and fungus on plant growth, grain yield moisture status, a pot experiment was conducted in different wheat lines. It comprised four treatments like control, application of bacterial strain *Bacillus* sp. MN54, fungal strain *Trichoderma* sp. MN6, and their consortium (*Bacillus* sp. MN54 + *Trichoderma* sp. MN6). The effect of consortium application was more prominent and significantly different from the sole application of bacteria and fungus. The results showed that with a consortium application of endophytic bacteria and fungus, there was 28.6, 4.3, -6.3 and -3.7% increases in flag leaf area, chlorophyll content, relative membrane permeability and water content respectively. Consortia of endophytic microbes also resulted in the yield enhancement through the betterment of various yield attributes like number of spikelet's, grains per spike and grain yield per plant (32.2, 25.8 and 30.8%, respectively). So, consortia of endophytic microbes can greatly promote the progress of plants in dry land agriculture and increase the yield in an environmentally sustainable way.

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Introduction

Wheat (*Triticum aestivum* L.) is the major staple food crop in many countries of the world with special reference to Pakistan (Ali et al., 2010; Din et al., 2017). It is closely related with human nutrition, economic development and food security (Lawlor et al., 2013). More than one fifth of human population relies on wheat and its products for food. To meet the future food demand, two times increase in the current wheat production is needed (FAOSTAT, 2012). To achieve this goal, recent agriculture is becoming input intensive with more reliance on synthetic fertilizers and agrochemicals. Similarly, due to intensive use of synthetic fertilizers over the years has taken a toll on the health of soil as well as their quality (Verhulst et al., 2010). Increase in soil deterioration results in poor crop yields that is observed at several places. To maintain the health of soil it is the need of time to find out an

alternative method which is effective, cheap and environment friendly (Pandey et al., 2013).

Use of plant growth promoting endophytes (PGPEs) is a technique which act as a bio fertilizer that improves host capacity for nutrient uptake, enhanced production, reduce the cost of fertilizer and eco-safe nature due to reduction in nitrogen leaching (DaSilveria et al., 2016). These are asymptomatic microbes found in almost all living plant species (Firáková et al., 2007). They form colonies in living tissue of plants without causing any deleterious effect on plants (Bacon et al., 2000). They can also help plants for fixing atmospheric nitrogen and production of siderophores which can solubilize and sequester iron and mobilize it for plant use. Similarly, they produce many growth stimulating hormones like Indole acetic acid (IAA), gibberellic acid (GA) and abscisic acid (ABA) and

cytokinin which plays a very vital role in the plant growth promotion and alleviation of biotic stresses (Mayak et al., 2004; Perring et al., 2007; Arendt et al., 2013). Many researchers also reported that PGPEs protected the plants from many deleterious abiotic stresses like salt (Mayak et al., 2004), over irrigation i.e. flooding (Grichko et al., 2001), and drought (Mayak et al., 2004). They also increase directly or indirectly water use efficiency, fresh and dry weight and different physiological responses of the plants i.e. increase in proline concentration which act as an osmoregulation agent (Han and Lee, 2005).

Bacillus sp. are endophytic bacteria which improve plant growth directly or indirectly by production of many phytohormones and antibiotics which kill other plant pathogenic bacteria (Bais et al., 2004). Plants induce systematic resistant against different types of pathogens by the process of mobilization and solubilization of phosphate (Idris et al., 2004; Erturk et al., 2010). In some studies, it is reported that *Bacillus subtilis* bacteria enhance the root and shoot length of plant, number of grains and size of the grains, fresh and dry weight, flag leaf area and chlorophyll contents of the treated plants (Waheed et al., 2014; Aboud et al., 2014; Barutcular et al., 2015). Similarly, *Trichoderma* sp. is a plant growth promoting fungus which belongs to Ascomycetes. It is used as a biofertilizer, biocontrol agent and promotes the growth of the plants against various biotic and abiotic stresses (Mastouri et al., 2010; Murali et al., 2012). It also play an important role in nutrient absorption by colonizing the roots and enhance the chlorophyll content, fresh and dry weight of the plant leaf, increase flag leaf area, grain yield and plant height (Azarmi et al., 2011).

The potential of *Bacillus subtilis* has been exploited to control plant diseases under control and natural field conditions (Joseph et al., 2007). However, little information is available on the combined use of *Bacillus* sp. and *Trichoderma* sp. for improving plant growth and disease suppression in wheat (Mastouri et al., 2010; Murali et al., 2012; Adams et al., 2007; Harman et al., 2004; Yedidia et al., 2001). So, the present study was designed to evaluate the synergistic use of *Bacillus* sp. MN54 and *Trichoderma* sp. MN6 for enhancing growth and yield attributes and traits expressing the moisture status in different wheat lines and disease suppression in wheat.

Materials and Methods

Culture Preparation of *Bacillus* sp. MN54 and *Trichoderma* sp. MN6

The *Bacillus* sp. MN54 was previously evaluated for enhancing growth and physiological attributes of Quinoa under normal and salt-stressed conditions (Yang et al. 2016). The inoculum of selected bacterial strain MN54 was prepared using 10% Tryptic Soy Broth in 1000 mL Erlenmeyer flask incubated in a shaking incubator (Firstek Scientific, Tokyo, Japan) at 100 r.min⁻¹ for 72 h at 28±1°C. An optical density of the inoculum was adjusted to 0.5 measured at λ 600 nm using spectrophotometer to obtain a uniform population of bacteria (10⁸-10⁹ colony forming unit (CFU) mL⁻¹) in the broth at the time of inoculation.

The fungus inoculum was produced by potato dextrose broth in 1000 mL Erlenmeyer flask incubated in a shaking incubator (Firstek Scientific, Tokyo, Japan) at 100 r.min⁻¹ for 5 days at 28±1°C. The spores of the *Trichoderma* sp. were analyzed by using Hemocytometer to obtain a uniform population of fungus (10⁶-10⁷) spores mL⁻¹) in the media at the time of inoculation. The selected microorganisms *Bacillus* sp. MN54 and *Trichoderma* sp. MN6 were very kindly provided by Soil & Environmental Microbiology Laboratory, Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad, Pakistan.

Plant Cultivation and Strain Inoculation

Twenty wheat (*Triticum aestivum*) lines 126, 121, 135,128, 144, 123, 107, 127, 130, 120, 101, 118, 137,110,124, 104, 140,113, 142 and 106 were taken from Wheat Research Institute, Ayub Agriculture Research Institute (AARI), Faisalabad, Pakistan and were grown in pots with non-sterilized soil at the Department of Plant Pathology, University of Agriculture Faisalabad, Punjab, Pakistan during the year of 2015-2016. Ten seeds of each line were sown in each pot. Each line was repeated three times. Pots were irrigated after every 3 days. After germination, thinning was done and maintained three plants in each pot. Bacterial and fungal inoculation was performed by adding 6 mL of each bacterial and fungal culture on each pot with the help of pipette near the root zone of each plant. In the consortium interaction 3 mL bacteria and 3 mL fungus were applied near the root zone of each plant.

Determination of Morph-Physiological Characteristics

Flag leaf was cut at the base at anthesis stage early in the morning (at 8-9 am) when leaves were in turgid position and were placed on the plane page. Flag leaf area (FLA) was measured by the following formula; FLA=(length ×width) ×b, where 'b' is a constant (b=0.75) (Ahmad et al., 2015). The width was measured from three different places of the blade. After that, these leaves were oven dried at 74°C in the oven for 48 hours and determine its dry weight (DW) in grams (g) by using an electronic balance.

Flag leaves were used to measure relative membrane permeability and chlorophyll contents. The leaves were cut with scissors, pack into the zip bag and transferred to the laboratory. For the relative membrane permeability leaves were cut into small equal pieces and transfer into the test tube containing 20 mL of deionized distilled water. The test tubes were shaken with the stirrer for 10s and this solution was assessed for initial electrical conductivity (EC0). For the (EC1) these test tubes were kept at 4°C for 24 hours and then assayed. Then these samples were autoclaved at 121°C for 25 minutes for the determination of (EC2). Percent relative membrane permeability can be determined by the following formula given by (Yang et al., 1996).

$$\text{RMP (\%)} = \frac{\text{EC1}-\text{EC0}}{\text{EC2}-\text{EC0}} \times 100$$

To measure the chlorophyll contents, the chlorophyll meter was used (SPAD 502 plus, Minolta, Japan). Chlorophyll contents of flag leaves of each genotype in each treatment were measured at least from four different areas on the same leaf.

Flag leaves were used to determine the relative water content. Immediately after excision the leaf fresh weight (FW) was measured. After that, leaves were placed in the test tube containing distilled water for 24 hours at 4°C in darkness and after soaking the leaves, turgid weight (TW) was measured with the help of balance. Dry weight (DW) was measured after oven drying the leaf samples at 72°C for 48 hours. Relative water content was described by the equation given by the Teulat et al. (2003).

$$RWC (\%) = \frac{FW-DW}{TW-DW} \times 100$$

Relative dry weight can be calculated by using the formula used by Ali et al. (2009).

$$RDW = \frac{DW}{(TW-DW)}$$

Assessment of Growth and Yield Parameters

The randomly plants were collected from the pot for comparison of growth and yield parameters (from every line and treatment) and data were collected for plant height (cm), number of tillers per plant, number of spikelet's per spike, number of grains per spike, spike weight (g) and grain yield per plant. To measuring head weight (g), detached plants were placed in the sunlight for 5 days and their heads were weighed by using electric balance. The samples were thrashed for counting the number of grains per spike and grain yield per plant (g).

Statistical Analysis

The data were analyzed using statistical software (Statistix 8.1) under factorial design with three replications following Steel et al, (1997). The mean values of the data were tested with least significance difference (LSD) test at the probability level of 5% (P≤0.05).

Results

Analysis of Variance for Different Traits

Analysis of variance indicated that the wheat lines used in this study were significantly different from each other for various morph-physiological attributes like flag leaf area (FLA), chlorophyll contents (CC), relative membrane permeability (RMP), relative water content (RWC) and excised leaf weight loss (ELWL) (Table 1). Likewise, various treatments used as crop growth promoting strategies significantly affected the FLA, CC, RMP, RWC and ELWL of wheat line (Table 1). Similarly, analysis of variance indicates that plant height (PH), number of spikelet's per spike (SPS), number of grains per spike (GPS), spike weight (SW) and grain yield

per plant (GYPP) were significantly different among the various wheat lines; results being non-significant for number of tillers per plant TPP (Table 2). Likewise, various crop growth promoting strategies significantly affected the PH, TPP, SPS, GPS, SW and GYPP of wheat lines (Table 2). The interaction of wheat genotypes with crop growth promoting strategies was non-significant for PH, TPP, SPS, SW and GYPP but significant for GPS (Table 2). The interaction between wheat lines with crop growth promoting strategies was non-significant for FLA and RWC but significant for CC, RMP and ELWL), Table 1.

Performance of Morph-Physiological Attributes in Response to Endophytes

Graphical expression of mean values of various wheat lines under different environments like *Bacillus* sp. MN54, *Trichoderma* sp. MN6 and their consortium application revealed that increased moisture characters significantly were elucidated in consortium application for FLA and CC, while highest RMP was recorded by the control and *Bacillus* sp. MN54, RWC significantly increased by the treatment of *Trichoderma* sp.MN6 and maximum ELWL was recorded in the control, Figure 1.

An increase of 28.6, 19.0 and 11.7% was measured in FLA by the application of consortium, bacteria and fungus respectively. It is seen that there was -18.9, -14.1 and -4.9% decrease in the SFLA by the application of consortium, bacteria and fungus. There was 4.3, 2.9% increase in the CC by the application of consortium and bacteria respectively but there will be decrease -1.3% in CC by the application of fungus. There was -6.3, 0.0 and -12.5% decrease in the RMP by the application of consortium, bacteria and fungus respectively. There was, -3.7, -2.6 decreases in the RWC by the application of consortium and bacteria and 1.6% increase in the RWC by the application of fungus. There was 29, 22.6 and 12.9% increase in the RDW and 28.6, 14.3 and 0.0% increase in the SFLW by the application of consortium, bacteria and fungus. There was, 17.4% increase in the ELWL by the application of consortium and -42.1 and -27.4% decrease in the ELWL due to the application of bacteria and fungus respectively (Figure 3 B).

Performance of Growth and Yield Parameters in Response to Endophytes

Graphical expression of mean values of various wheat lines under different environments like *Bacillus* sp. MN54, *Trichoderma* sp. MN6 and their consortium application revealed that increased growth and yield characters significantly were elucidated in consortium application for PH, SPS, GPS, SW and GYPP while for TPP, maximum values were seen in the application of *Trichoderma* sp. MN6, Figure 2.

Table 1 Mean squares from analysis of variance by using statistical software (Statistix 8.1) for morph-physiological characters of wheat

SOV	FLA	CC	RMP	RWC	ELWL
L	167.81*	33.86*	0.057*	294.01*	91.97*
T	378.11*	128.93*	0.093*	136.18*	1146.94*
L×T	0.98 ^{NS}	33.65 ^{NS}	0.032*	18.32 ^{NS}	75.23*
Error	4.28	3.26	0.008	19.86	18.92

SOV= Source of Variation; L= Lines; T= Treatment; L×T= Lines ×Treatment, NS= non- significant and *= significant at 5% probability, FLA= Flag Leaf Area, CC= Chlorophyll Contents, RMP= Relative Membrane Permeability, RWC= Relative Water Contents, ELWL= Excised Leaf Weight Loss

Table 2 Mean square from ANOVA by using (Stastix 8.1) for grain and yield attributes of wheat

SOV	PH	TPP	SPS	GPS	SW	GYPP
L	920.16*	3.18 ^{NS}	18.26*	254.07*	2.07*	30.22*
T	868.63*	10.83*	188.18*	734.66*	9.57*	186.41*
L×T	3.820 ^{NS}	1.81 ^{NS}	2.78 ^{NS}	14.57*	0.01 ^{NS}	1.79 ^{NS}
Error	14.91	1.96	2.90	7.69	0.056	2.07

SOV= Source of Variation; L= Line= T= Treatment; L × T= Line×Treatment, NS= non- significant and *= significant at 5% probability, PH= Plant Height, TPP= number of tillers per Plant, SPS= number of Spikelet's Per Spike, GPS= number of grains per Spike, SW= Spike Weight and GYPP= Grain Yield per Plant.

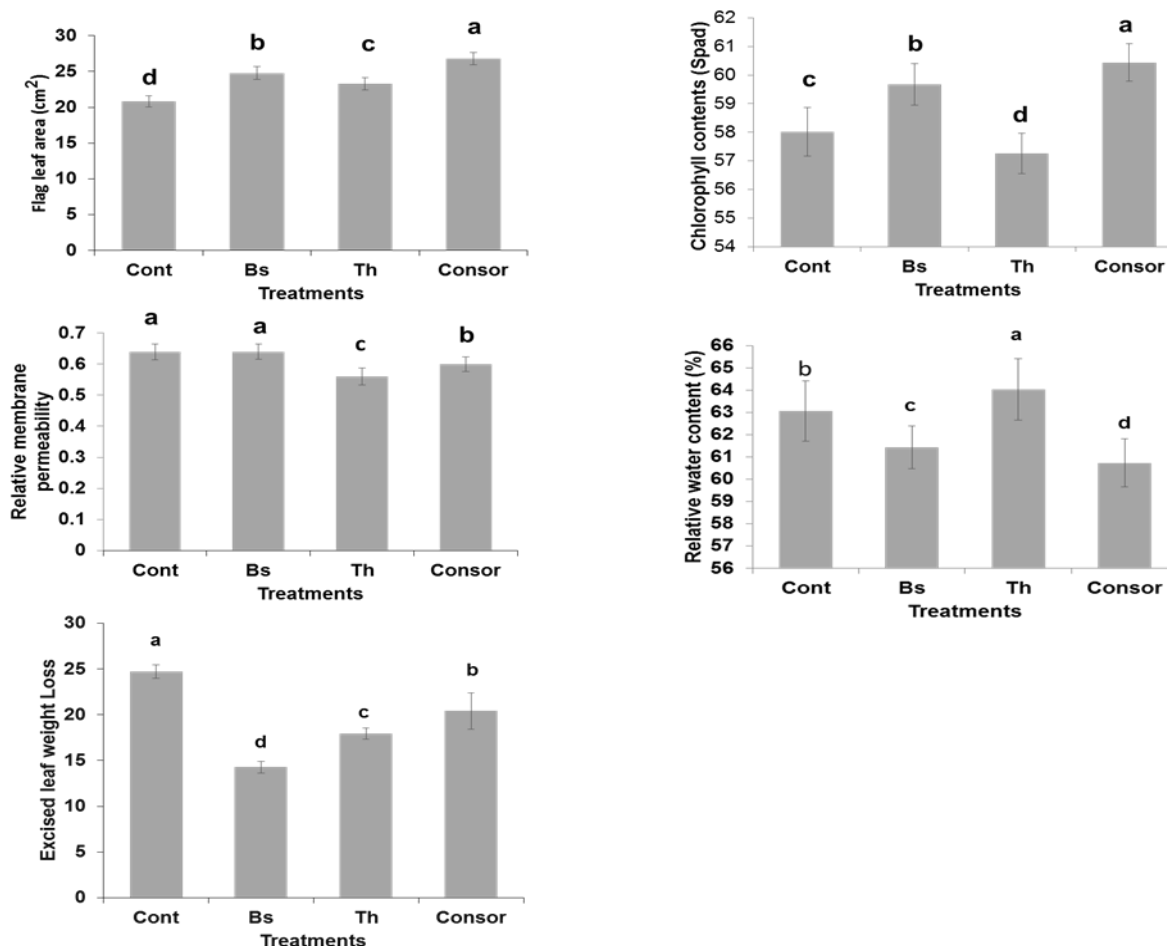


Figure 1 The statistical software (Statistix 8.1) was used for the mean comparison of three replicates by using factorial design for the morph- physiological characteristics of wheat.

Whereas Cont=control, Bs=*Bacillus* sp. MN54., Th=*Trichoderma* sp. MN6 and Consor=consortium (*Bacillus* sp. MN54 and *Trichoderma* sp. MN6). The means in the figures with same lettering are not statistically significant from each other according to the least significant difference (LSD) test at P= 0.05.

There was 37.1, 31.4 and 25.7% increase in the PH by the endophytic application of consortium, bacteria and fungus. It is observed that 23.7, 14.6, 7.7% increase in the PL by the application of consortium, bacteria and fungus. There was 56.0 31.9 and 14.0% increase in the IL by the application of consortium, bacteria and fungus. By the application of endophytes there was 41.1, 25.9, 12.2% increase in the SL. There was 8.2, 4.1 and 2.0% increase in the PH and PL by the consortium, bacteria and fungus respectively. Likewise, there was 32.2, 15.7 and 13.4% increase in the SPS and 25.8, 16.4 and 6.1% increase in the GPS by the application of consortium, bacteria and fungus respectively. There was 52.8, 26.7 and 13.1% increase in the SW by the application of consortium, bacteria and fungus. Similarly, increase of 30.8, 20.3 and 12.9% was observed in GYPP due to application of

consortium, bacteria and fungus respectively but in the case of TPP 12.5, 9.4 and 14.1% increase by the application of consortium, bacteria and fungus respectively, Figure 3A.

Performance of Various Wheat Advanced Lines Under Different Inoculation Regimes

Among the lines, Maximum FLA recorded in the line 120 (38.06 cm²) and minimum FLA was found in the line 104 (17.62 cm²). Similarly maximum CC was found in the line 107 (20.04), while minimum CC was in the line 101 (8.05). The RMP was maximum in the line 101 (50) and minimum in the line 144 (-41.18). Likewise highest RWC was recorded in the line 140 (5.21) and minimum in the line 123 (-14.04). The highest ELWL was recorded in the 104 (-53.71) and minimum ELWL was noted in the

line 127 (135). Among the wheat lines highest PH was recorded in lines 113 (24.03 cm) and minimum in line 130 (9.64). The highest TPP was recorded in the line 130 (53.40) and minimum TPP was noted in the line 106 (-14.29). Similarly maximum SPS recorded in the line 107 (53.05) but minimum SPS was recorded in the line 123 (8.74). The maximum GPS was observed in the line 127 (43.98) and minimum GPS was noted in the line 124 (-6.04). The highest SW was recorded in the line 140 (90.09) while minimum in the line 128 (34.68). However, the maximum GYPP was recorded in the line 150 (51.63) and Minimum GYPP was recorded in the line 104 (13.11). (Table 3).

Effect of Consortium Application on Correlation Matrices for Different Morph-Physiological and Yield Attributes

FLA depicted significantly positive association with ELWL at the control level. CC had significant correlation with PH at the control level. SPS was noted that it had significantly positive correlation with RWC and GPS at consortium and control level respectively. GPS showed positive significant correlation with the SPS and SW at consortium and control level respectively. SW showed variables at consortium level as it had significantly positive interrelation with SPS and GPS at consortium level. GYPP reported that it had solid positive correlation with the TPP at consortium level (Table 4).

Table 3 Performance of various wheat advanced lines under different inoculation regimes

Lines	FLA	CC	RMP	RWC	ELWL	PH	TPP	SPS	GPS	SW	GYPP
V-106	19.74	-8.00	6.06	-0.91	-37.00	20.66	-14.29	12.85	4.81	58.82	15.06
V-142	26.17	12.82	-6.02	-4.56	-36.32	11.74	34.56	50.04	37.32	43.46	33.65
V-113	35.55	-0.21	24.64	1.00	40.03	24.03	22.17	33.36	17.54	53.37	47.66
V-140	32.89	-2.72	-17.65	5.21	-17.42	9.92	12.60	23.79	12.10	90.09	32.20
V-104	17.62	2.18	-25.00	-5.91	-47.39	15.58	26.98	38.99	32.01	38.99	13.11
V-124	24.04	6.75	29.09	-12.76	-40.68	18.58	29.40	35.92	-6.06	50.54	43.20
V-110	31.63	12.09	19.15	3.66	0.64	15.46	0.00	17.80	29.01	47.03	17.10
V-137	25.11	3.12	24.44	3.04	-32.72	22.90	32.08	42.45	15.29	75.94	23.56
V-118	32.56	-3.09	25.00	-1.35	-27.93	17.15	-10.45	52.51	27.78	40.54	28.43
V-101	31.77	-8.05	50.00	0.84	-53.71	13.73	21.75	21.95	32.97	91.84	28.00
V-120	38.06	1.69	-26.03	-11.76	-32.74	13.07	9.40	19.46	25.03	77.97	31.73
V-130	19.61	17.33	0.00	-12.89	-28.20	9.64	53.40	23.36	28.84	45.96	51.63
V-127	37.65	-0.94	-5.17	9.00	135.00	15.31	50.94	24.36	43.98	45.19	35.68
V-107	34.18	20.04	-11.76	-6.01	52.20	14.14	5.87	53.05	35.81	58.16	31.94
V-123	33.82	-1.41	-15.38	-14.04	-14.88	17.02	-0.45	8.74	34.33	45.15	24.83
V-144	31.64	16.55	-41.18	2.71	-4.29	15.98	17.02	35.03	19.61	80.26	37.07
V-128	28.71	16.49	-22.86	-6.13	-23.08	13.14	9.40	40.00	24.98	34.68	27.60
V-135	27.96	1.03	-5.26	-8.94	-42.00	19.39	-9.57	42.07	32.23	38.39	29.02
V-121	24.80	-7.59	-24.29	-7.61	-41.03	12.43	-5.52	48.69	42.07	43.68	25.77
V-126	29.56	-0.77	-30.14	0.28	-43.90	23.14	-5.52	35.92	26.25	80.62	42.39

Note: The data is given in percentage increase/decrease of consortium application over control.

Table 4 Simple correlation matrices between different morph-physiological and yield attributes

	FLA	CC	RMP	RWC	ELWL	PH	TPP	SPS	GPS	SW	GYPP
FLA		0.02	-0.05	0.13	0.64	0.17	0.22	0.07	-0.14	-0.22	0.16
CC	0.12		-0.37	-0.14	0.03	0.51	0.19	0.05	0.13	0.08	0.18
RMP	-0.17	-0.02		0.27	0.04	0.09	-0.06	-0.05	-0.16	-0.14	-0.05
RWC	0.02	0.23	-0.19		-0.05	-0.15	0.11	0.08	-0.10	-0.12	-0.02
ELWL	-0.39	0.08	-0.07	0.23		-0.14	0.32	-0.09	-0.08	-0.13	0.11
PH	0.24	0.17	-0.22	0.13	0.44		0.09	0.37	0.39	0.00	0.19
TPP	0.15	0.21	0.25	-0.33	0.14	0.09		0.14	0.14	0.08	0.32
SPS	0.01	0.26	0.21	0.49	-0.07	0.35	0.02		0.77	0.32	0.26
GPS	0.06	0.00	-0.30	-0.24	0.19	0.34	-0.05	0.71		0.54	0.11
SW	0.42	0.17	-0.08	-0.29	0.11	0.09	0.19	0.45	0.45		0.01
GYPP	0.04	0.09	0.18	0.08	0.23	0.04	0.45	0.00	0.23	0.12	

Where, un-shaded upper parenthesis shows correlation coefficients for control and lower shaded area demonstrates correlation matrices from varieties/lines treated with consortium of bacteria and fungi.

Discussion

Endophytes live inside the plants without causing any deleterious effect on their health, but some endophytes provide directly or indirectly benefits to their host plant (Hardoim et al., 2008; Shores et al., 2010). These endophytes either as single strain or multi-strain inoculum have previously exhibited mutualistic behavior when added to other plant species including grasses, corn, rice,

Douglas-fir and a variety of crop plants (Xin et al., 2009; Knoth et al., 2013; Knoth et al., 2014; Khan et al., 2012; Khan et al., 2015; DaSilveria et al., 2016). Combined application of endophytes can result in larger effects than those possible with individual inoculations (Knoth et al., 2013; Knoth et al., 2014; Rogers et al., 2012).

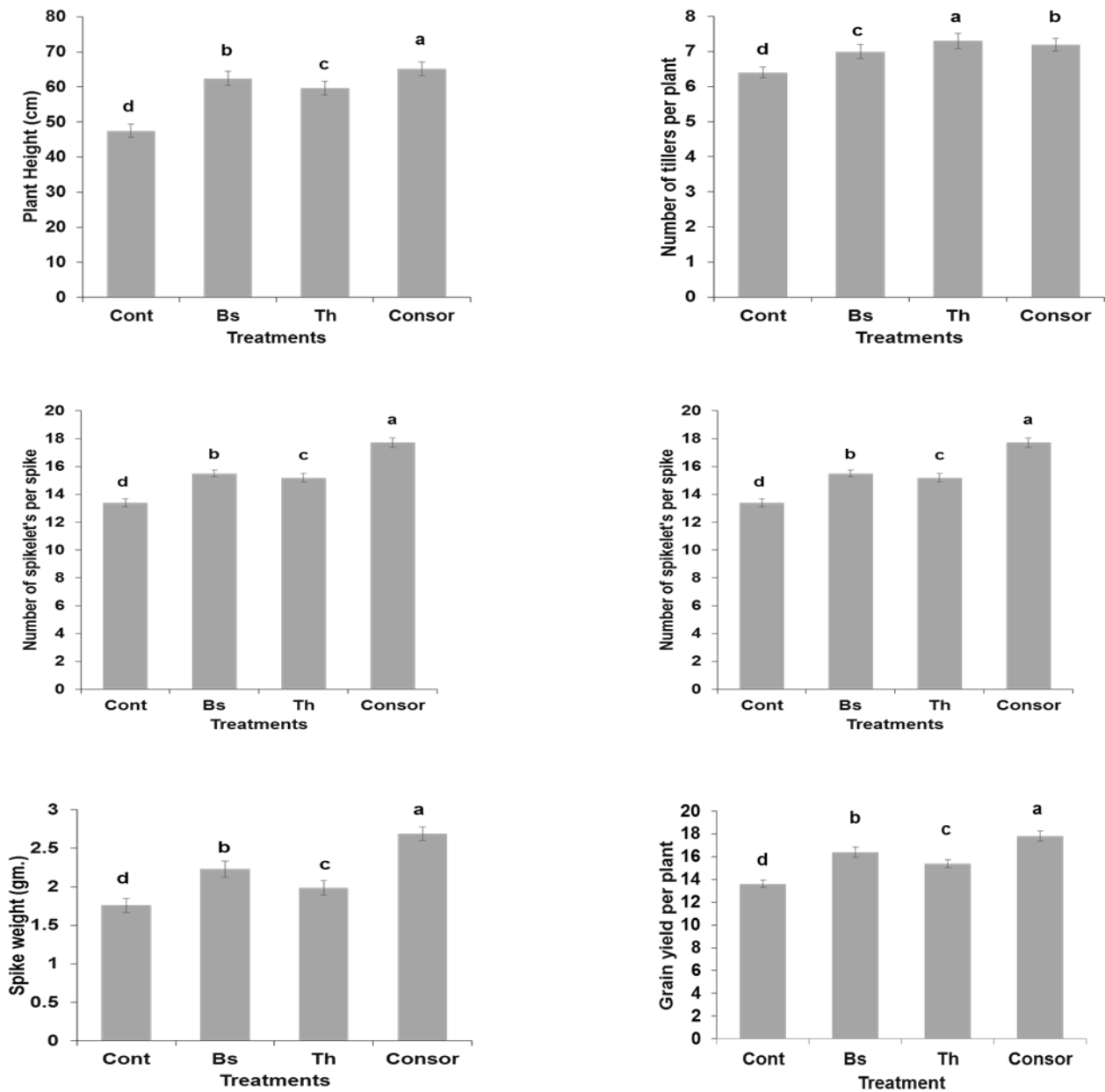


Figure 2 The statistical software (Statistix 8.1) was used for the mean comparison of three replicates by using factorial design for the growth and yield parameters of wheat.

Whereas Cont=control, Bs=*Bacillus* sp. MN54, Th=*Trichoderma* sp. MN6 and Consor=consortium (*Bacillus* sp. MN54 and *Trichoderma* MN6). The means in the figures with same lettering are not statistically significant from each other according to the least significant difference (LSD) test at P= 0.05

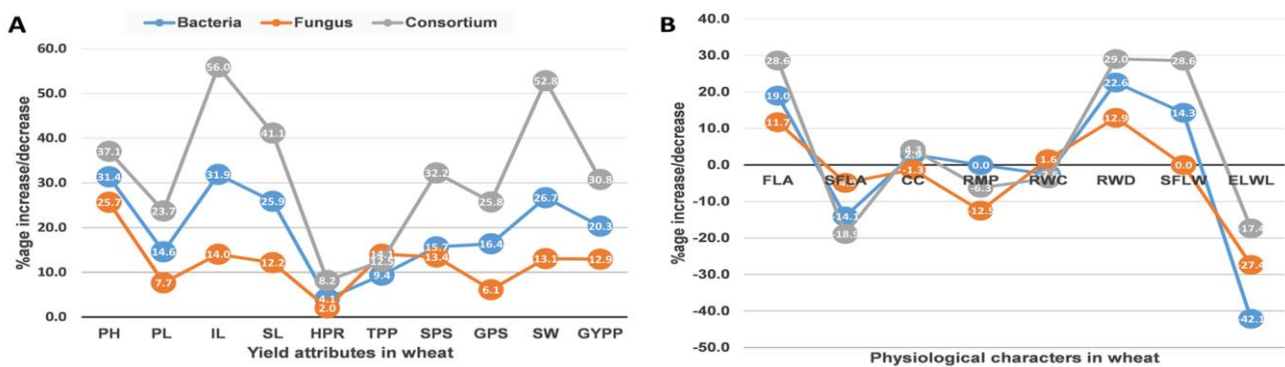


Figure 3 Percentage increase/decrease over control of morph-physiological attributes.

A) PH= Plant Height, PL= Peduncle Length, IL= Internode Length, SL= Spike Length, HPR= Plant height/ peduncle length Ratio, TPP= number of tillers per Plant, SPS= number of Spikelet's Per Spike, GPS= number of grains per Spike, SW= Spike Weight and GYPP= Grain Yield per Plant.
 B) FLA= Flag Leaf Area, SFLA= Specific Flag Leaf Area, CC= Chlorophyll Contents, RMP= Relative Membrane Permeability, RWC= Relative Water Contents, RDW= Relative Dry Weight, SFLW= Specific Flag Leaf Weight, ELWL= Excised Leaf Weight Loss

In this study, the bacterial and fungal strains of different endophytes (*Bacillus* sp. MN54 and *Trichoderma* sp. MN6) were applied alone and in combination to evaluate their effects on various morpho-physiological, growth and yield parameters of 20 different wheat lines in pot experiment. The statistically significant results were recorded by the application of consortium which resulted in improved physiology, growth and yield attributes of different wheat lines but in some cases only fungus or bacterial strain showed the best results.

The morpho-physiological traits such as flag leaf area, chlorophyll contents, relative membrane permeability, relative water content and excised leaf weight loss were assessed in response to various endophytes treatments. All of these traits showed improvement followed by the application of these endophytes in consortium. The increase in flag leaf area is possible due to the positive effects of endophytes on photosynthesis through production/stimulation of specific phytohormone(s) associated with leaf development (Zulini et al., 2007). This increase in leaf area would positively affect the productivity of wheat as FLA has been reported to be positively associated with grain yield (Ali et al., 2010). Other physiological aspects that might also be influencing the system are water-use efficiency related to photosynthesis, xylem hydraulic conductivity, and partitioning of photo assimilates. The delay of the decreasing photosynthetic activities by the inoculated endophytes corresponded with the delay of degradation of chlorophyll in the present study. Chlorophyll concentration is positively connected to photosynthetic activities of PSII, especially under water stressed conditions (Caudle et al., 2014). Similarly, the inoculated endophyte consortia may help the host plants to reserve chlorophyll molecules in the leaves by providing fixed nitrogen and phytohormones which eventually increase the host plants' adaptation to the stressed environment (Lawlor et al., 2009).

The grain yield and its attributes were also evaluated in response to various applications of endophytes. We were able to demonstrate that application of endophytic bacteria and fungus in combination resulted in 32.2, 25.8 and 30.8% increase in number of spikelets, grains per spike and grain yield per plant respectively. Buensanteai et al. (2014) reported that the interaction between *Glomus mosseae* and *Trichoderma harzianum* give significant increase in growth parameters of chilli plants such as shoot and root lengths, dry weight of shoot and root, number of leaves, number of branching. These are due to the ability of *Trichoderma* sp. to production of phytohormones such as auxin and gibberellin which enhance plant growth (Abood et al., 2008), increase nutrient uptake (Al- Kurtany et al., 2008) and mobilization of inorganic nutrients (Kaya et al., 2009; Lopez -Bucio et al., 2005). *Bacillus* sp. can also help plants for fixing atmospheric nitrogen and production of siderophores which can solubilize and sequester iron in the plants (Mayak et al., 2004). They produce many growth stimulating agents like Indole acetic acid (IAA), Gibberellic acid (GA) and Abscisic acid (ABA) and cytokinin which play a very important role in the plant growth and defense mechanism (Mayak et al., 2004; Perring et al., 2007; Arendt et al., 2013). Moreover, the

application of these endophytes increased concentrations (up to 5%) caused significant increase in grain yield per plant. The increase in yield of wheat was found similar to some previous findings (Nashwa et al., 2008; Sharma et al., 2012), where the formulation of *Trichoderma* sp. treatments enhanced green yield of bean plants compared to infected control. In this experiment the increase in yield can also be attributed to the application of *Trichoderma harzianum* (Th3) bio-formulation along with the Farm yard manure which helped increasing the colonies by providing nutrient to *Trichoderma* thereby increasing the plant growth and yield of wheat. Generally, fungi have been reported to possess greater ability to solubilize phosphate than bacteria, viz., *B. subtilis* and *B. megatarium* (Rajankar et al., 2007), this may explain the greater effectiveness of *T. harzianum* than *B. subtilis* in growth promotion and crop production. Thus, the use of these specific bio-agents in agriculture also helped in sustenance and growth promotion of plants.

Conclusions

In conclusion, we resulted that a consortia of endophytic bacteria and fungi significantly enhanced plant growth, yield and tolerance of water deficit stress of different wheat lines under pots. To produce stable high yield of wheat under stresses imposed by climate change, a plant-microbe interaction could be a key to adapting plants to a water limited environment. So, consortia of endophytic microbes can greatly promote the progress of plants into the fundamental mechanisms of symbiosis and increased the yield in an environmentally sustainable way.

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