INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
17–0946/2018/20–2–353–360
DOI: 10.17957/IJAB/15.0498
http://www.fspublishers.org

Full Length Article

Microbial Assisted Foliar Feeding of Micronutrients Enhance Growth, Yield and Biofortification of Wheat

Muhammad Yaseen[1], Tanveer Abbas[1*], Muhammad Zahir Aziz[1], Abdul Wakeel[1], Humaira Yasmeen[1], Wazir Ahmed[2], Aman Ullah[3] and Muhammad Naveed[1]

[1]*Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad 38080, Pakistan*
[2]*Department of Soil Sciences, Nawaz Sharif University of Agriculture Multan, Pakistan*
[3]*Department of Agronomy, University of Agriculture, Faisalabad 38080, Pakistan*
[*]For correspondence: tanveer.abbas52@yahoo.com

Abstract

Micronutrient malnutrition caused by inadequate dietary intake is a global nutritional problem of more than half of world's population. Fortification is expensive while biofotification is a natural approach and considered better than fortification. Biofotification of wheat with micronutrients especially zinc (Zn) and iron (Fe) is a relatively new public approach to control deficiencies of these nutrients in flour/diet in developing countries. This research study is comprised of results of field experiment to examine the effects of foliar feeding of micronutrients along with endophytic bacteria on growth, yield and nutrient uptake of wheat. Compared with control (without foliar feeding of micronutrients), foliar application on wheat with endophytic bacteria increased the plant height, leaf area, spike length and plant biomass by 13.53, 41.67, 55.07 and 42.50% respectively. It was observed that Zn and Fe concentration enhanced in grains up to 37.93 and 10.14% respectively with the combine application of micropower and endophytic bacteria. These results elucidate that foliar feeding of micronutrients effectively biofortified wheat grains. Furthermore, foliar feeding of micronutrients along with endophytic bacteria could be used as an easy, quick and cost-effective approach to enrich wheat flour with health essential micronutrients for correcting malnutrition.

Keywords: Micropower; Endophyte; *Bulkholderia phytofirmans* PsJN; *Enterobacter* MN17; Colonization

Introduction

Population of the world increasing day by day and has led to an increasing demand on food production and supply. Food security is becoming a major issue for the mankind. Humans need at least 50 different essential nutrients (including macro and micronutrients, vitamins, fatty acids, protein, water and energy) for sustaining human life. Zn and Fe among micronutrients and vitamin A among all the vitamins affect more than 2 billion people predominantly in developing countries (Stein, 2010). Balance diet is only way to combat with food security. Agriculture is considered as the foundation of all food systems and primary source of all the nutrients. Malnutrition develops if agriculture cannot supply all the essential nutrients required for good health. In fact, agriculture depends upon fertility status of soils. Of the total agricultural land about 40% is considered as degraded land (Global Environmental Facility, 2009). These adverse and cumulative changes reduce the soil capacity to support plant growth and animals feeding that impairs food security. Reduction in quality and quantity of food directly affect human health (Lal, 2009). Soil fertility can be enhanced through different methods of fertilizer application including soil application, foliar feeding, fertigation and microbial application.

Innovative fertilizers required for high yield and nutritional quality food consumed by the human population (Bindraban *et al*., 2015). Foliar feeding is used when soils or plants that limits some nutrients availability (Voogt *et al*., 2013). Foliar application of micronutrients rapidly diminish deficiency symptoms from the crops and chances of nutrients toxicity decreases due to less volume used compared to soil application. For sustainable agricultural production, foliar application is best method for micronutrients which employs the feeding of micronutrients directly on leaves and plant parts especially Fe, Mn, Cu and Zn (Fernandez and Brown, 2013). The nutrients applied as foliar can increase yield (Yaseen *et al*., 2004) and quality of produce in many crops, including vegetables, cereals and fruit trees especially under calcareous and alkaline conditions (He *et al*., 2013). Until now, the information about multi nutrient foliar application is rare (Rawashdeh and Florin, 2015). Combine application of Mn and Fe and Fe + Mn + Zn resulted in best yields in wheat (Zain *et al*.,

To cite this paper: Yaseen, M., T. Abbas, M.Z. Aziz, A. Wakeel, H. Yasmeen, W. Ahmed, A. Ullah and M. Naveed, 2018. Microbial assisted foliar feeding of micronutrients enhance growth, yield and biofortification of wheat. *Int. J. Agric. Biol*., 20: 353–360

2015). Similarly, combined multi nutrient foliar application of on *Lycopersicum* and *Beta vulgaris* shows the highest yields compared to individual nutrient (Gobarah *et al*., 2014).

Endophytic bacteria (living in internal tissues of plants) increased availability of these nutrients through releasing different enzymes and growth promoting substances. Unlike of pathogenic bacteria these neither show any disease symptom on plant nor change the morphology and internal structure of plants. Association between plants and endophytic bacteria is mediated by organic compounds released from both bacterial and host species (Brader *et al*., 2014). Endophytic bacteria affect the plant growth through biological nitrogen fixation (Compant *et al*., 2005a), decreasing plant ethylene level, increasing bioavailability of phosphorus and micronutrients as well and production of volatile organic compounds (Kai *et al*., 2009). Present study will test the hypothesis that using a combination of micronutrients with endophytic bacteria in foliar feeding could improve the growth, yield and nutrients uptake in wheat grain which is the major food crop of world.

Materials and Methods

Collection of Endophytic Bacterial Strains

Two pre-isolated endophytic bacterial strains *Enterobacter* sp. MN17 and *Burkholderia phytofirmans* strain PsJN, were collected from Environmental Science Laboratory Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad. Micropower, multinutrients containing potassium (2%), nitrogen (1%) Zn (4.7%), Mn (2%), Fe (2%), Cu (0.3%) and B (1%) was obtained from Soil Fertility and Plant Nutrition Laboratory Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad.

Culture Preparation of Bacterial Strains

The inoculum of selected bacterial strains was prepared in 250 mL Erlenmeyer flasks using 10% tryptic soya broth (TSB). Each flask containing 200 mL TSB broth was inoculated with selected bacterial strains and incubated in a shaking incubator (Firstek Scientific, Tokyo, Japan) at 180 rpm for 48 h at 28 ± 1°C. The optical density of the culture was measured at wavelength 600 nm using a spectrophotometer (Nicolet Evolution 300 LC, Cambridge, UK) and adjusted to 0.5 to obtain a uniform population of bacteria (10^8-10^9cfu mL^{-1}).

Field Trial

Soil was collected for experiment from experimental field having sandy clay loam texture, (Typic Haplocambid), pH 7.5, extract electrical conductivity (ECe) 1.5 dS m^{-1}, organic matter 0.77%, total N 0.036%, available P 8.8 mg kg^{-1}, and extractable K 158 mg kg^{-1}. Field experiment was conducted at the research farm of Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad, Pakistan to evaluate the effect of endophytic bacteria and micropower multinutrients applied through foliar spray on growth, yield and nutrients uptake of wheat. Four treatments were used in this experiment as Control (T_1), MN-17 (T_2), Strain PsJN (T_3), and Strain MN-17 + Strain PsJN (T_4) with (M+) and without (M-) micropower multinutrients. All the treatments were replicated thrice. Wheat variety (Faisalabad 2011) was sown on 16 November 2014 and harvested 21 April 2015. The recommended doses of NPK were applied @ 120: 90:60 kg ha^{-1}, respectively. The sources of N, P_2O_5 and K_2O were urea, diammonium phosphate (DAP) and sulphate of potash (SOP), respectively. Phosphorus and potassium were applied at the time of seed-bed preparation, while N was applied in three splits. One third dose of N was applied at the time of sowing, while remaining was applied with 2nd and 3rd irrigation. Both endophytic bacterial strains and micropower were applied in 5:1 respectively through foliar application. First spray was applied after 45 days after sowing (DAS) and second was applied after 15 days of first spray. Canal water was applied whenever needed and data was collected using standard procedures.

Agronomic and Physiological Parameters

Data regarding growth parameters like fresh leaf weight, plant height and flag leaf length was collected before harvesting. Leaf area (3rd leaf from top) of plants was recorded using LI-3100C Area Meter (Li-Cor, Inc., Lincoln, NE, USA). After harvesting number of spikelet/ plant, grain yield and number of tiller/ m^2 were measured. Physiological parameter like leaf chlorophyll contents was measured by using Chlorophyll Meter (SPAD 502 Plus, Minolta, Japan). Each leaf sample was measured in at least six different areas.

Chemical Parameters

At physiological maturity plant shoot and grain samples of wheat were collected from field for determination of nitrogen, extractable phosphorus, potassium and micronutrients. All the samples were ground and digested (Wolf, 1982). Total nitrogen was measured by using Kjeldhal ammonium distillation apparatus. Phosphorus was measured by adding 10 mL Barton reagent in 5 mL sample through spectrophotometer (ANA−720W, Tokyo Photo Electric Company Limited Japan). Actual concentration of phosphorus was measured following standard curve. Potassium simply measured by flame photometer (Jenway PFP−7, England) and its concentration was derived by using calibration curve. Micronutrients were measured by using atomic absorption spectrophotometer by following Yaseen *et al.* (2004).

Survival of Endophytic Bacteria in Micropower

Bacterial inocula was prepared and mixed with micropower at different bacterial to micropower ratio (4:9, 5:1 and 5:2) in sterilized falcon tubes at room temperature up to two months. Microbial survival was determined through Compant *et al*. (2005b).

Endophytic Colonization Assay of Shoot and Grain Tissues

For the isolation of endophyte, 3 and 5 g surface sterilized grain and shoot were homogenized in sodium chloride solution (15 mL of 0.85% solution w/v) by using sterile mortar and pestle. For determination of strains recovery (colonization) samples were crushed in 0.9% (w/v) NaCl solution, shaken with the pulsifier (Microgen Bioproduct Ltd, UK) for 30 sec and different dilutions were spread on TSA plates. Plates were kept for incubation at 28 ± 2^0C. Bacterial colonies were counted with colony counter (Colony counter 560 Suntex) after different time intervals (upto 60 days continuously) days of incubationand were expressed in cfu/mL.

Statistical Analysis

The experiment was arranged according to randomized complete block design (RCBD). The data regarding plant growth, chemical and physiological parameters were subjected to analyses of variance. The means were compared by least significant difference (LSD) test ($p<0.05$) to detect statistical significance among treatment (Steel *et al*., 1997). All the statistical analyses were conducted using SPSS software version 19 (IBM SPSS Statistics 19, USA).

Results

Foliar application of pre-isolated endophytic bacterial strains and micropower multinutrients significantly improve growth, yield and nutrient uptake of wheat as compared to control with different degree of efficacy.

Agronomic Physiological Parameters

Data regarding plant height show significant increase in plant height compared to control (Table 1). Maximum plant height (13.53%) was increased with the application of micropower along with both bacterial strains PsJN and MN-17 followed by separate application of bacterial strains with micropower compared to uninoculated control. Likewise flag leaf area was maximum observed with inoculation of bacterial strains micropower multinutrients. Combine application of both strains with micropower increased flag leaf area about 41.67% compared to control. Separate application of PsJN and MN-17 increased leaf area 33.39 and 31.60%, respectively compared to control. Spike length was increased with the application of micropower and endophytic bacterial strains as compared to control (Table 1). Inoculation with bacterial strains and separately along with micropower increased spike length but all the both sets of treatments were statistically non-significant, and highest spike length (55.07%) was observed in treatment containing combine bacterial strains with micropower source. Similarly yield in case of 1000 grain weight was maximum (22.22%) recorded with application of both bacterial strains with micropower multinutrients T_4 (MN-17+ PsJN micropower multinutrients) compared to uninoculated control. Separate application of both bacterial strains with micropower increased yield but they were statistically non-significant to each other. Inoculant deficient plants (control) decrease the total number of tillers compared to plants receiving alone micropower multinutrients. Maximum number of tillers (24.40%) was observed with combine application of both bacterial strains plus micropower followed by separate application of MN-17 and PsJN with micropower, which were not statistically significant to each other (Table 1). In case of fertile tillers combination of both strains with micronutrient source (micropower multinutrients) and separate application of MN-17 and PsJN with micropower results were non-significant to each other. Alone micropower increased fertile number of tillers only upto 11.58% whereas with both bacterial strains increased fertile tillers upto 21.25% as compared to uninoculated and micronutrient deficient control. All the treatments were statistically non-significant to each other but significantly increased number of fertile tillers compared to control. Combine application of both bacterial strainsMN-17 and PsJN with micropower multinutrients decreased unfertile tillers upto 57.14% compared to uninoculated and micropower deficient plants (Table 2). Similarly, maximum plant biomass (42.51%) was observed with both bacterial strains and micropower as compared to control (Table 2). Grain yield also recorded with respect to square meter and found that micropower alone increased yield only upto 18.17% compared to control. Maximum produced (38.86%) was recorded in case of micropower and both bacterial strains (MN-17 and PsJN) compared to uninoculated control (Table 2).

Chlorophyll contents significantly increased with the application of micropower multinutrients and endophytic bacteria compared to control. Separate application of endophytic bacteria MN-17 and PsJN without micropower increase chlorophyll contents 11.12 and 14.79% while with micropower chlorophyll contents enhanced up to 20 and 19.48% respectively compared to simple control (without micropower multinutrients). Maximum chlorophyll contents (27.69%) were observed with combine application of endophytic bacterial strains (MN-17 and PsJN) and micropower multinutrients compared to controls (Fig. 1).

Table 1: Effect of foliar feeding of micropower multinutrients and endophytic bacteria on plant height, flag leaf area, spikelet length, total number of tillers, total number of leaves and 1000 grain weight of wheat

Treatment	Plant height (cm)		Total no. of tillers (m^{-2})		Spike length (cm)		Flag leaf area (cm^2)		No. of leaves ($plant^{-1}$)		1000 grains weight (g)	
	MP (-)	MP (+)	MP (-)	MP (+)	MP (-)	MP (+)	MP (-)	MP (+)	MP (-)	MP (+)	MP (-)	MP (+)
Control	100.26e	105.53d	504.0e	546.0d	37.34f	42.16e	15.65e	18.46d	4.62d	5.00cd	34.43d	35.53d
NM-17	107.47c	110.67b	567.5cd	590.5bc	45.56d	49.14b	20.29c	22.45b	5.25bc	5.50abc	37.08c	39.09b
PsJN	108.41c	111.27b	569.0cd	601.2ab	46.76c	49.81b	20.58c	22.85b	5.37bc	5.75ab	37.16c	40.14b
NM-17 +PsJN	109.32bc	113.83a	600ab	627.0a	47.21c	52.90a	21bc	24.27a	5.25bc	6.00a	38.21bc	42.09a
LSD	2.48		1.34		0.58		19.9		1.47		29.61	

Note: All treatments except control contain recommended doses of N, P and K fertilizers as urea, DAP and SOP, respectively. Values followed by the same letter were not significantly different at the 5% level of significance. Control (T_1), Strain MN-17 (T_2), Strain PsJN (T_3), Strain PsJN + Strain FD17 (T_4) along with (MP +) and without (MP-) micropower multinutrients

Table 2: Effect of foliar feeding of micropower multinutrient and endophytic bacteria on number of fertile tillers, unfertile tillers, grain weight and plant biomass of wheat

Treatment	No. of fertile tillers (m^{-2})		No. of unfertile tillers (m^{-2})		Grain weight (gm^{-2})		Plant biomass (kg m^{-2})	
	MP (+)	MP (-)	MP (+)	MP (-)	MP (+)	MP (-)	MP (+)	MP (-)
Control	485.75c	542b	14a	8.75b	427.75e	505.5d	1.27e	1.40d
NM-17	565.5ab	582.5ab	7.75bc	6.75cd	536.5c	576ab	1.42d	1.54c
PsJN	571ab	589a	6.75cd	6.25d	563.5bc	583ab	1.43d	1.63b
NM-17 +PsJN	579ab	600a	6.25d	6d	570ab	594a	1.60ab	1.81a
LSD	0.27		1.054		8.99		2.056	

Note: All treatments except control contain recommended doses of N, P and K fertilizers as urea, DAP and SOP, respectively. Values followed by the same letter were not significantly different at the 5% level of significance. Control (T_1), Strain MN-17 (T_2), Strain PsJN (T_3), Strain PsJN + Strain FD17 (T_4) along with (MP +) and without (MP-) micropower multinutrients

Chemical Parameters

Macronutrients: Endophytic bacteria increased the N, P and K concentration in in grain as well as in wheat straw compared to uninoculated control. Both bacterial strains enhanced N contents in grain with micropower upto 38.02% while separate bacterial strains (MN-17 and PsJN) increased N concentration in grains about 22.53 and 31.45% respectively compared to uninoculated control. PsJN with micropower and MN-17 and PsJN with micropower were statistically non-significant to each other but both enhanced the N contents in grains of wheat as compared to control (Table 3). Similarly, N contents maximum (28.75%) observed with both endophytic bacterial strains and micropower in case of straw as compared to control. Data presented (Table 3) shows that maximum (62.50%) P contents in grain were observed with application of both bacterial strains and micropower compared to uninoculated and micronutrient deficient control. Separate application of both bacterial strains MN-17 and PsJN with micropower increased the P contents in grains upto 41.66 and 54.16% respectively compared to control. P contents in straw were increased less compared to grain. Only 4.5% P contents increased with alone micropower application while maximum P concentration were observed with both bacterial strains MN-17 and PsJN and micropower about 31.81% compared to uninoculated control. Separate application of both bacterial strains was not statistically different from each other. Similarly, K concentration both in grains and straw increased with micropower multi nutrients and bacterial inoculation compared to uninoculated and micronutrient deficient control. Maximum K contents about 60.89 and 62.29% were observed with both microbial strains MN-17 and PsJN and micropower in grain and straw respectively compared to control. Separate application of both bacterial strains with micropower remained non-significant in both cases (grain and straw) but improved K concentration as compared to control condition.

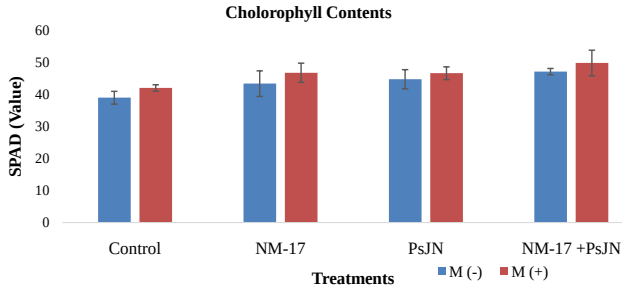

Fig. 1: Effect of foliar feeding of micropower multinutrient and endophytic bacteria on choorophyll contents of wheat
Note: All treatments except control contain recommended doses of N, P and K fertilizers as urea, DAP and SOP, respectively. Values followed by the same letter were not significantly different at the 5% level of significance. Control (T_1), Strain MN-17 (T_2), Strain PsJN (T_3), Strain PsJN + Strain FD17 (T_4) along with (MP +) and without (MP-) micropower multinutrients

Micronutrients: Application of micronutrient source (micropower multi nutrient) and endophytic bacteria improved the micronutrients concentration grain and straw compared to uninoculated control. In case of Fe concentration in grains separate application of bacterial strains MN-17 and PsJN increased 3.38 to 4.65%, while maximum increased was observed with both bacterial strains with micropower (T_4) upto 10.14% compared to control.

Table 3: Effect of foliar feeding of micropower multinutrient and endophytic bacteria on uptake of nitrogen, phosphorus and Potassium in wheat grain and straw

Treatment	N (%) in grains		N (%) in straw		P (%) in grains		P (%) in straw		K (%) in grains		K (%) in straw	
	MP (+)	MP (-)	MP (+)	MP (-)	MP (+)	MP (-)	MP (+)	MP (-)	MP (+)	MP (-)	MP (+)	MP (-)
Control	2.13e	1.53e	0.12c	0.22d	0.236c	0.141bc	1.68d	2.29d	1.56d	1.72cd	1.83e	2.23d
NM-17	2.61cd	1.76cd	0.150bc	0.246c	0.266bc	0.170b	1.85bc	2.71bc	1.82c	2.08b	2.26d	2.57bc
PsJN	2.80b	1.79c	0.153bc	0.25c	0.253c	0.185ab	1.90ab	2.82ab	1.90bc	2.10b	2.38cd	2.74b
NM-17 +PsJN	2.81bc	1.91ab	0.165bc	0.27b	0.29a	0.195a	1.97a	2.94a	1.98bc	2.51a	2.85ab	2.97a
LSD	0.27		0.11		0.035		0.03		0.219		0.237	

Note: All treatments except control contain recommended doses of N, P and K fertilizers as urea, DAP and SOP, respectively. Values followed by the same letter were not significantly different at the 5% level of significance. Control (T_1), Strain MN-17 (T_2), Strain PsJN (T_3), Strain PsJN + Strain FD17 (T_4) along with (MP +) and without (MP-) micropowermultinutrient

Table 4: Effect of foliar applocation of micropower multinutrients and endophytic bacteria on uptake of iron, mangenese nand copper in wheat grain and straw

Treatment	Fe (mg kg^{-1}) in grains		Fe (mg kg^{-1}) in straw		Mn (mg kg^{-1}) in grains		Mn (mg kg^{-1}) in straw		Cu (mg kg^{-1}) in grains		Cu (mg kg^{-1}) in straw	
	MP (+)	MP (-)	MP (+)	MP (-)	MP (+)	MP (-)	MP (+)	MP (-)	MP (+)	MP (-)	MP (+)	MP (-)
Control	206.70e	217.92c	172.66f	181.82cd	40.56f	49.27c	33.8f	41.10c	3.58d	4.82bc	2.63d	4.02b
NM-17	214.62d	221.25b	178.84e	184.37bc	45.25e	52.14b	37.71e	43.45b	4.12c	4.89ab	3.44c	4.08b
PsJN	216.32cd	222.40b	180.26de	185.33b	46.66d	52.86b	38.89d	44.04b	4.21c	5.12a	3.50c	4.27ab
NM-17 +PsJN	223.1b	227.66a	184.4bc	189.65a	50.51bc	55.49a	42.76bc	46.25a	5.21a	5.40a	4.10b	4.50a
LSD	2.26		2.65		0.78		0.70		0.50		0.35	

Note: All treatments except control contain recommended doses of N, P and K fertilizers as urea, DAP and SOP, respectively. Values followed by the same letter were not significantly different at the 5% level of significance. Control (T_1), Strain MN-17 (T_2), Strain PsJN (T_3), Strain PsJN + Strain FD17 (T_4) along with (MP +) and without (MP-) micropower multinutrients

Similarly, in straw maximum Fe concentration (9.84%) was found with combination of micropower multinutrients and both endophytic bacterial strains (MN-17 + PsJN). Maximum Mn contents were found in case of grain up to 36.80 mg/Kg with both bacterial strain and micropower compared to control while separate application of endophytic bacterial strains also improves Mn concentration in grains. Similar results were observed in case of straw. Data (Table 4) indicated that Cu concentration in straw of wheat increased with the application of micropower and bacterial strains. Application of separate bacterial strains MN-17 and PsJN increase the Cu concentration in grains upto 15.08 to 17.59%, respectively alone micropower enhanced Cu concentration 34.63% greater compared to uninoculated control. Maximum increase was recorded by application of both bacterial strains and micropower up to 50.83% compared to micronutrient deficient control. Cu concentration in straw was observed about 71.10% with the application of micropower and both bacterial strains, while other treatments also improved the Cu concentration in straw compared to control. Generally, Mn concentration was recorded greater in grains compared to wheat straw. Micropower alone significantly enhanced B concentration in grains upto 33.61% but maximum B contents were found in treatment T_4 (MN-17 + PsJN with micropower multinutrients) upto 46.86% compared to and uninoculated control. Separate application of bacterial strains MN-17 and PsJN with micropower with were not significantly different from each other but improve the B contents in grains as compared to control. As for concern B concentration in straw, it was observed that maximum (60.45%) concentration was found with application of both bacterial strains and micronutrient source (micropower multi strain), while only micropower increased 2.18% compared to control. In grains Zn concentration was enhanced with micropower and separate bacterial strain MN-17 and PsJN application up to 15.63 and 30.22%, respectively while maximum Zn contents was observed with combination of both strains and micropower (37.93%) compared to control. Zn concentration in straw was observed 33.52% only with micropower application while separately both bacterial strains increased less Zn contents compared to micropower. Maximum Zn concentration was found 55.39% with micropower and both bacterial strains MN-17 and PsJN in straw compared to control (Table 4).

Endophytic Bacterial Survival in Micropower

Both endophytic bacterial strains survival in micropower was determined by using different bacterial to micropower ratio with different time intervals. At bacteria and micropower ratio 4:9 initially bacterial population increased up to 45 days and then start decreasing. In case of PsJN strain maximum population (55×10^6 cfu mL^{-1}) was observed at 45 days of incubation after which population starts decreasing. Strain MN-17 performed best at 45 days of incubation (63×10^6 cfu mL^{-1}) as compared to PsJN strain (Fig. 2). Similar results were observed with varying bacterial to micropower ratio (5:1 and 5:2). In both cases, initially bacterial population increased up to 45 days of incubation and incubation towards 60 days starts decrease. Maximum PsJN strain population was observed about (57×10^6 cfu mL^{-1} and 83×10^6 cfu mL^{-1}) in 5:2 and 5:1,

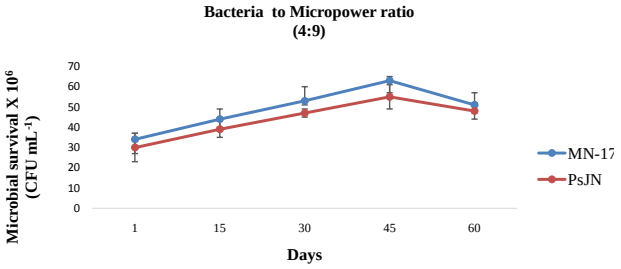

Fig. 2: Survival of endophytic bacteria in micropower multinutrients at different days' intervals with the concentration of bacteria and micropower (4:9)

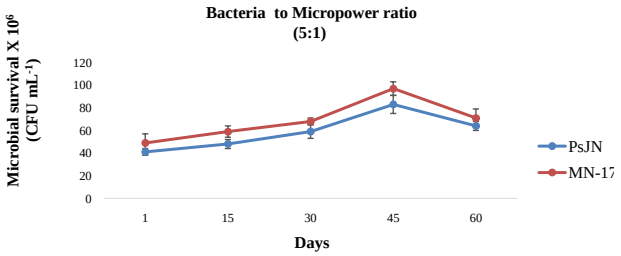

Fig. 3: Survival of endophytic bacteria in micropower multinutrients at different days' intervals with the concentration of bacteria and micropower (5:1)

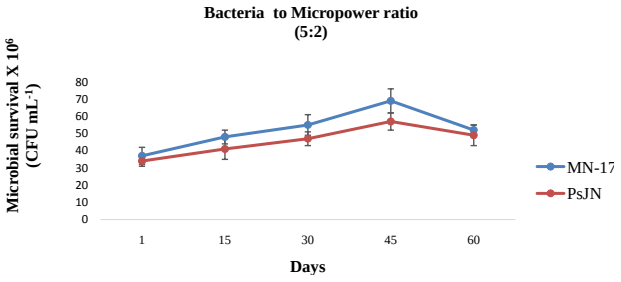

Fig. 4: Survival of endophytic bacteria in micropower multinutrients at different days' intervals with the concentration of bacteria and micropower (5:2)

respectively (Fig. 3 and 4).

At 45 days of incubation MN-17 population was observed 97×10^6 cfu mL^{-1} followed by 71×10^6 cfu mL^{-1} at 60 days of incubation in 5:1 bacteria to micropower ratio. Out of both strains excellent results were obtained by strain MN-17 at 45 days of incubation. In all three best results were obtained by 5:1 (bacteria: micropower multi nutrient) at which MN-17 population was more than PsJN.

Endophytic Bacterial Colonization

Endophytic bacteria efficiently colonized the interior of the plant. Strain MN-17 application wheat shoot have highest bacterial population 86×10^6 cfu mL^{-1} as compared to grains 91×10^3 cfu mL^{-1}. With the inoculation of simple PsJN strain less population was observed both in shoot and grains compared to other strain. Similar results were observed micropower application with inoculum (PsJN and MN-17) which shows maximum endophytic bacterial population in shoot compared to wheat grains. When both bacterial strains and micropower were applied together maximum population of both endophytic strains observed. In shoot 102×10^6 and 98×10^6 cfu mL^{-1} cfu mL^{-1} bacterial colonies were calculated with colony counter of MN-17 and PsJN respectively. Same pattern of results was obtained in case of endophytes that present in grains (Table 5 and Table 6).

Discussion

The present study was conducted to evaluate the effect of endophytic bacterial strains (MN-17 and PsJN) and foliar application of micropower multinutrients on growth, yield and nutrients uptake of wheat. Inoculation of endophytic bacterial strains with micropower multinutrients increase growth parameters including plant height, plant weight, root growth and yield parameters such as total number of tillers, number of fertile tillers and grain yield significantly compared to uninoculated control. *Burkhuldaria phytofirmans* (PsJN) stimulate the growth of many plant species including grapevine, tomato potato and many other plants. This is might be due to production of ACC deaminase, phytohormones including auxins and gibberellic acid, nutrients solublization, nitrogen fixation activity and organic acids production (Sessitsch *et al*., 2005). The hormone production might be considered possible mechanism behind root growth and development. Auxin is produced by bacteria from tryptophan because of root exudates, which is responsible for root development. Due to alteration in root architecture root surface area might be increased that consequently increased nutrient and water uptake which may positive effects on plant growth (Somers *et al*., 2004). Under stress condition plants produce ACC (precursor of ethylene), which reduce the plant growth. Applied endophytic bacteria produce ACC deaminase that stops the production of ethylene by degrading the ACC. Bhattacharjee *et al*. (2008) observed that production increase in plant biomass of rice cultivars due to ACC deaminase and IAA by plant growth promoting rhizobacteria upon inoculation. In this study both bacterial strains *Bulkholderia phytofirmans* (PsJN) and *Enterobacter* (MN17) produced ACC deaminase and IAA.

Data presented (Table 3) shows that with the application of endophytic bacteria and micropower multinutrients increased the concentration N and P in all the treatments compared to un inoculated control both in grain and shoot. P contents in grains increased upto 62.50% with inoculation of both endophytic strains and micropower multi nutrients. The increased P concentration may be attributed to organic acids and enzymes (phosphatases) produced by bacteria that solubilize the P thus yield increased (Yao, 2004). Nitrogen was observed about

Table 5: Effect of foliar application of micropower multinutrient and endophytic bacteria on boron and zinc uptake in wheat grain and straw

Treatment	B (mg/kg) in grains		B (mg/kg) in straw		Zn (mg kg^{-1}) in grains		Zn (mg kg^{-1}) in straw	
	MP (+)	MP (-)	MP (+)	MP (-)	MP (+)	MP (-)	MP (+)	MP (-)
Control	8.33e	11.13bc	11e	13.4d	8.70c	11.13ab	6.95d	8.28c
NM-17	10.80cd	10.06d	13.6d	15.46bc	10.66ab	10.06b	8.89bc	8.40c
PsJN	10.33cd	11.66ab	14.28cd	16.48ab	10.66ab	11.33ab	8.89bc	9.78ab
NM-17 +PsJN	11.25bc	12.23a	15.55bc	17.65a	11.50ab	12a	5.50ab	10.08a
LSD	1.41		1.42		1.34		0.95	

Note: All treatments except control contain recommended doses of N, P and K fertilizers as urea, DAP and SOP, respectively. Values followed by the same letter were not significantly different at the 5% level of significance. Control (T_1), Strain MN-17 (T_2), Strain PsJN (T_3), Strain PsJN + Strain FD17 (T_4) along with (MP +) and without (MP-) micropower multinutrients

Table 6: Colonization of MN-17 and PsJN strains in shoot and grains of wheat

Treatment	Bacteria in shoot10^6 cfu mL^{-1}		Bacteria in grains10^3 cfu mL^{-1}	
	MP (+)	MP (-)	MP (+)	MP (-)
MN-17	92	86	96	91
PsJN	87	73	79	67
MN-17+ PsJN	102	98	109	94

Note: All treatments except control contain recommended doses of N, P and K fertilizers as urea, DAP and SOP, respectively. Values followed by the same letter were not significantly different at the 5% level of significance. Control (T_1), Strain MN-17 (T_2), Strain PsJN (T_3), Strain PsJN + Strain FD17 (T_4) along with (MP +) and without (MP-) micropower multinutrients

38.02% in wheat grains compared to uninoculated control. This is might be due to nitrogen fixation by endophytic bacteria. Some free living bacterial genera including *Herbaspirillum* and *Azospirillum* colonize rhizosphere and increase the availability of nitrogen in the form of ammonia (Bashan and De-Bashan, 2010). In addition, numerous endophytic bacteria successfully colonize the different non-legumes plant such as rice, sugarcane, wheat and maize to form mutualistic interaction and fix nitrogen (Oliveira *et al*., 2009). Endophytic bacterial inocula increase organic matter that boosts the availability of nutrients such as nitrogen, phosphorus, potassium and iron (Leifheit *et al*., 2015). Potassium concentration in grains increased in grains (62.29%) due to endophyte application with micropower multinutrients. Bacteria applied in the form of inoculum lowering the pH by releasing H^+ and as result of acidolysis acids solubilize the potassium which taken up by the plants (Romheld and Kirkby, 2010).

In the present study iron concentration increased because of foliar application of micropower multinutrients with endophytic bacteria (MN-17 and PsJN) compared to uninoculated control. In most cases iron present in Fe^{+3} which is sparingly soluble and sidrophores have affinity to form complexes with ferric (Fe^{+3}) which reduced into ferrous Fe^{+2}. Later, cell membrane expels ferrous from sidrophores into cell plant body (Boukhalfa and Rumbliss, 2002). In this way plant get more iron (that present in unavailable form and converted into available form) and nutritional level of plants remain balance (Indiragandhi *et al*., 2008). It might be attributed on the basis that some bacterial species produce iron loving compounds called siderophore that convert insoluble iron to soluble form and concentration of bioavailable iron increased. Plant containing endophytic fungi released root exudates which have high iron reducing power and availability of iron increased with other nutrients.

For plant growth promotion and increment in yield survival and colonization of endophytic bacteria is necessary. In this study, maximum population (102×10^6) of MN-17 was observed in case of shoot. This may be due to biofilm formation, chemotaxis and aggregate stability (Danhorn and Fuqua, 2007). Exudates released from plant roots attract bacteria towards roots leading to root colonization. It is previously described that bacteria prefer nutrients rich areas (such as root hairs, root tips and lateral roots) for growth and survival. (Bais *et al*., 2006). In the present study, all the bacterial strains have common traits of aggregation and biofilm formation. Endophytic bacteria enter root system by producing different enzymes that degrade the cell walls of plants. It has been studied that bacterial strain B. phytofirmans (PsJN) secretes endoglucanase and endopolygalacturonase (Compant *et al*., 2005b), as well as endo-b-D-cellobiosidase and exo-b-1,4-glucanase (unpublished results), which may facilitate its entrance into the endorhiza. Following its entry inside roots, strain PsJN progressed from the rhizodermis to the exoderms, to the cortical cell layers, into xylem and finally enter into grains.

Conclusion

In the changing climate plant face different biotic and abiotic stresses such as nutrient stress, which is one of the most serious problems associated with plant growth, development and ultimately production. Therefore, nutrient use efficiency is most important for sustainable yield. Foliar application of micronutrients with endophytic bacterial inoculation improves the nutrient use efficiency. The

present study demonstrate that combine application of both endophytic bacterial strains with micropower multi nutrients improved all growth, yield and other attributes in wheat compared to separate bacterial strain application with micropower multi nutrients. The concentration micro nutrients increased with foliar application of micropower multi nutrients compared to control. The improved plant growth leads to enhanced crop yield and quality. The endophytic bacteria and micropower multi nutrients could be efficiently used to enhance agronomic and physiological parameter of wheat.

Acknowledgements

Authors are gratefully acknowledging the Higher Education Commission (HEC) of Pakistan for financial support.

References

Bashan, Y. and L.E. De-Bashan, 2010. Chapter two-how the plant growth-promoting bacterium *Azospirillum* promotes plant growth; a critical assessment. *Adv. Agron*., 108: 77–136

Bais, H.P., T.L. Weir, L.G. Perry, S. Gilroy and J.M. Vivanco, 2006. The role of root exudates in rhizosphere interactions with plants and other organisms. *Annu. Rev. Plant Biol*., 57: 233–266

Bhattacharjee, R.B., A. Singh and S.N. Mukhopadhyay, 2008. Use of nitrogen fixing bacteria as biofertiliser for nonlegumes: Prospects and challenges. *Appl. Microbiol. Biotechnol*., 80: 199–209

Bindraban, P.S., C.O. Dimkpa, L. Nagarajan, A.H. Roy and R. Rabbinge, 2015. Revisiting fertilizers and fertilization strategies for improved nutrient uptake by plants. *Biol. Fert. Soils*, 51: 897–911

Boukhalfa, H.C. and A.L. Rumbliss, 2002. Chemical aspects of siderophore mediated iron transport. *Biometals*, 4: 325–339

Brader, G., S. Compant, B. Mitter, F. Trognitz and A. Sessitsch, 2014. Metabolic potential of endophytic bacteria. *Curr. Opin. Biotechnol*., 27: 30–37

Compant, S., B. Reiter, A. Sessitsch, J. Nowak, C. Clement and E.A. Barka, 2005a. Endophytic colonization of (*Vitis vinifera* L.) by plant growth promoting bacterium, Burkholderia sp. strain PsJN. *Appl. Environ. Microbiol*., 71: 1685–1693

Compant, S., B. Duffy, J. Nowak, C. Clement and E.A. Barka, 2005b. Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action and prospects. *Appl. Environ. Microbiol*., 71: 4951–4959

Danhorn, T. and C. Fuqua, 2007. Biofilm formation by plant-associated bacteria. *Annu. Rev. Microbiol*., 61: 401–422

Fernandez, V. and P. Brown, 2013. From plant surface to plant metabolism: the uncertain fate of foliar applied nutrients. *Front. Plant Sci*., 4: 289–301

Gobarah, M.E., M.M. Tawfik, S.M. Zaghloul and G.A. Amin, 2014. Effect of combined application of different micronutrients on productivity and quality of sugar beet plants (*Beta vulgaris* L.). *Int. J. Plant Soil Sci*., 3: 589–598

Global Environmental Facility, 2009. *G.E.F. Annual Report, 2009*. GEF, Washington DC, USA

He, W., M.J.I. Shohag, Y. Wei, Y. Feng and X. Yang, 2013. Iron concentration, bioavailability and nutritional quality of polished rice affected by different forms of foliar iron fertilizer. *Food Chem*., 141: 4122–4126

Indiragandhi, P., R. Anandham and M.S.T. Madhaiyan, 2008. Characterization of plant growth promoting traits of bacteria isolated from larval guts of diamondback moth *Plutella xylostella* (Lepidoptera: *Plutellidae*). *Curr. Microbiol*., 4: 327–333

Kai, M., M. Haustein, F. Molina, A. Petri, B. Scholz and B. Piechull, 2009. Bacterial volatiles and their action potential. *Appl. Microbiol. Biotechnol*., 81: 1001–1012

Lal, R., 2009. Soil degradation as a reason for inadequate human nutrition. *Food Security*, 1: 45–57

Leifheit, E.F., E. Verbruggen and M.C. Rillig, 2015. Arbuscular mycorrhizal fungi reduce decomposition of woody plant litter while increasing soil aggregation. *Soil Biol. Biochem*., 81: 323–328

Oliveira, A.L.M., M. Stoffels, M. Schmid, V.M. Reis, J.I. Baldani and A. Hartmann, 2009. Colonization of sugarcane plantlets by mixed inoculations with diazotrophic bacteria. *Eur. J. Soil Biol*., 45: 106–113

Rawashdeh, H.M. and S. Florin, 2015. Foliar application with iron as a vital factor of wheat crop growth, yield quantity and quality: a review. *Int. J. Agric. Pol. Res*., 3: 368–376

Romheld, V. and E.A. Kirkby, 2010. Research on potassium in agriculture: needs and prospects. *Plant Soil*, 2: 155–180

Sessitsch, A., T. Coenye and A.V. Sturz, 2005. *Burkholderia phytofirmans* sp. nov. a novel plant-associated bacterium with plant beneficial properties. *Int. J. Syst. Evol. Microbiol*., 55: 1187–1192

Somers, E., V. Vanderleyden and M. Srinivasan, 2004. Rhizosphere bacterial signaling: a love parade beneath our feet. *Crit. Rev. Microbiol*., 30: 205–240

Steel, R.G.D., J.H. Torrie and D.A. Dicky, 1997. *Principles and Procedures of Statistics*, pp: 352–358. 3rd edition, McGraw Hill. Inc. Book Co. New York, USA

Stein, A.J., 2010. Global impacts of human mineral malnutrition. *Plant Soil*, 335: 133–154

Voogt, W., C. Block, B. Eveleens, L. Marcelis and P.S. Bindraban, 2013. *Foliar Fertilizer Application*. VFRC report 2013/2. Virtual fertilizer research Centre, Washington DC, USA

Wolf, B., 1982. The comprehensive system of leaf analysis and its use for diagnosing crop nutrient status. *Commun. Soil Sci. Plant Anal*., 13: 1035–1059

Yaseen, M., M. Nadeem and S. Hussain, 2004. Investigating the effectiveness of Micropower foliar spray on growth and yield of different crops. *Pak. J. Life Soc. Sci*., 2: 156–158

Yao, T., 2004. Associative nitrogen-fixing bacteria in the rhizosphere of *Avena sativain* an alpine region: III. Phosphate-solubilizing power and auxin production. *Acta Pratacult. Sin*., 13: 85–90

Zain, M., I. Khan, R.W.K. Qadri, U. Ashraf, S. Hussain, S. Minhas, A. Siddiquei, M.M. Jahangir and M. Bashir, 2015. Foliar application of micronutrients enhances wheat growth, yield and related attributes. *Amer. J. Plant Sci*., 6: 864–869

(Received 23 August 2017; Accepted 12 October 2017)