Research Article
THE INTERNATIONAL JOURNAL OF BIOLOGICAL RESEARCH (TIJOBR)
ISSN Online: 2618-1444
Vol. 3(1) Jan-March. 2020., 56-64; 2020
http://www.rndjournals.com

Effect of multi-strain inoculation on NPK contents grain and straw of rice under field conditions

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Abstract

A field trial was conducted to evaluate the effect of multi-strain inoculation on growth and yield of rice under field conditions at the research area of the Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad, during Kharif season 2017. At maturity, data regarding paddy yield (Kg m^{-2}), protein contents of grain, NPK contents of grain and straw was recorded and analyzed by standard statistical procedures. The results showed that multi-strain inoculation of PsJN, LSI-29 and 6K increased grain N contents, straw N contents and grain protein contents by 33, 26 and 33% respectively, co-inoculation of PsJN and 6K increased grain N contents, straw N contents and grain protein contents by 26, 21 and 26% respectively, co-inoculation of PsJN and LSI-29 increased the same by 18, 19 and 22% respectively, and sole inoculation of PsJN and 6K increased grain N contents by 14 and 12% respectively. Multi-strain inoculation of LSI-29, 6K and PsJN significantly increased grain and straw phosphorus contents up to 39 and 38% respectively over control. Co-inoculation treatments i.e., PsJN + 6K, PsJN + LSI-29 and LSI-29 + 6K significantly increased grain phosphorus contents up to 28, 25 and 22% respectively and straw phosphorus contents up to 29, 28 and 25% respectively over un-inoculated control. Single strain inoculation treatments also significantly increased grain and straw phosphorus contents. The increase in paddy yield caused by multi-strain inoculation of LSI-29, 6K and PsJN was significantly higher than all the co-inoculation treatments (i.e., LSI-29 + 6K, PsJN + 6K and PsJN + LSI-29), all the sole inoculation treatments (i.e., LSI-29, PsJN and 6K) and un-inoculated control. Multi-strain combination of LSI-29, 6K and PsJN significantly increased the grain protein contents over co-inoculation of LSI-29 and 6K, all the sole inoculation treatments (i.e., LSI-29, 6K and PsJN), and un-inoculated control.

Key word: Rice, PGPR strains, NPK

Introduction

It is an important cereal crop of Pakistan and is a major export commodity. Rice crop serves as food for almost half of the world's population (Ladha *et al.,* 1997). Rice grains carry 21, 14 and 2% of global energy, protein and fat supply, respectively (FAO, 1999). Rice is a staple food crop in Pakistan after wheat and it contributes a major share in forex earnings now-a-days due to high quality aroma. Its production fulfills the domestic as well as export demands of the country. The main objective of agricultural research is to enhance the crop yield and quality of the produce. The theme behind all the techniques used is either to overcome the factors which hamper plant growth or to invigorate the factors which improve plant growth. Our farmers employ expensive physical and chemical approaches to manage the factors which influence crop growth negatively. Using biological techniques, although less common among farmers, are the only promising potential sources for sustainable agriculture. The manipulation of soil microbial population in order to increase the crop yield has now been established in many studies (Cook, 2002). The potential benefits of this technique to farmers and environment are very high due to reduced usage of the expensive chemical inputs. These benefits have been proved evidently in many legumes and non-legumes.

Plant Growth Promoting Rhizobacteria (PGPR) have achieved worldwide fame for their agricultural benefits. These are the potential trend for the future research as well as tools for sustainable agriculture (Podile and Kishore, 2006). Their use as biofertilizers is now potentially capable of improving plant productivity (Vessey, 2003). Biofertilizers were defined as the substances containing alive microbes which when applied to seed, soil or plant colonize it and promote its growth by enhancing nutrient availability (Vessey, 2003). Many researchers have reported rhizosphere-associated nitrogen fixers and PSB as potential inoculum for non-legume crop species such as rice, wheat, corn and sugarcane (Döbereiner, 1997; Schilling *et al.,* 1998). Their mechanisms of action includes associative and symbiotic nitrogen fixation, mineralization and solubilization of nutrients, production of growth substances e.g. hormones, production of ACC-deaminase to reduce the elevated ethylene levels in plant roots thus enhancing root length and density, production of siderophores like *ß*-1-3-glucanase, chitinases, antibiotics, fluorescent pigment and cyanide against pathogens and enhanced resistance to drought and oxidative stresses by producing water soluble vitamins niacin, thiamine, riboflavin, biotin and pantothenic acid. Indirectly, PGPR enhance plant growth through suppression of soil borne plant pathogenic microorganisms by their substrate competition, antibiotic production, and induced systemic resistance in the host (Lugtenberg and Kamilova, 2009; Vessey, 2003; Marulanda *et al.,* 2010). The present research was conducted to investigate the combined effect of *Rhizobium, Pseudomonas* and endophytic strain PsJN on rice seedlings.

Materials and methods

A field trial was conducted to evaluate the effect of multi-strain inoculation on growth and yield of rice under field conditions at the research area of the Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad, during Kharif season 2017. At maturity, data regarding paddy yield (Kg m^{-2}), protein contents of grain, NPK contents of grain and straw was recorded and analyzed by standard statistical procedures. Three pre-isolated PGPR strains were collected from the Soil Microbiology and Biochemistry Laboratory of the Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad. Fresh inocula of the selected pre-isolated strains were prepared by taking 100 mL of sterilized Luria Bertani (LB) media broth in four conical flasks having 250 mL volume and sterilized at 15 PSI (pounds per square inch) pressure and 121 ºC temperature for 20 minutes. Each sterilized conical flask was inoculated with a strain (along with an un-inoculated control) and incubated at 28 ± 1 ºC in a shaking incubator at 100 rpm. After gaining proper population (10^7-10^8 CFU mL^{-1}) of each strain in the flasks, all possible combinations were prepared by mixing respective inoculum in equal proportion (10 mL each) in separate sterilized conical flasks. Fresh seedlings were obtained from the research area of the Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad. The roots of seedlings were inoculated before transplanting by dipping the roots (Gutierrez-Zamora and Martinez-Romero, 2001) in freshly prepared inoculum of each strain and their combinations for two hours. In case of un-inoculated control seedlings were transplanted after dipping in the sterilized broth for two hours. Seedlings inoculated with different strains alone and their all possible combinations were transplanted in the field. Each treatment was replicated three times using Randomized Complete Block Design (RCBD). All agronomic practices were performed during field trial. Plants from one square meter area of each plot were harvested at physiological maturity stage and data regarding growth and yield parameters was collected. Grain and straw samples were collected from each plot, digested according to the method described by Wolf (1982) and analyzed chemically for potassium (K^+) concentration by flame photometer, nitrogen (N) by micro-kjeldahal apparatus and phosphorus (P) by spectrophotometer as described by Ryan *et al.* (2001). Protein contents were also determined by multiplying the nitrogen contents with 6.25 (Jones, 1931

Results and discussion

Paddy yield (Kg m^{-2})

The data regarding the paddy yield showed that multi-strain inoculation caused maximum increase in the paddy yield over co-inoculation, single strain inoculation and un-inoculated control. The increase in paddy yield caused by multi-strain inoculation of LSI-29, 6K and PsJN was significantly higher than all the co-inoculation treatments (i.e., LSI-29 + 6K, PsJN + 6K and PsJN + LSI-29), all the sole inoculation treatments (i.e., LSI-29, PsJN and 6K) and un-inoculated control. After that, co-inoculation of PsJN and 6K significantly increased the paddy yield over single strain inoculation of LSI-29 and 6K which was statistically at par with co-inoculation of PsJN and LSI-29, co-inoculation of LSI-29 and 6K and sole inoculation of PsJN. All the sole inoculation treatments (i.e., LSI-29, PsJN and 6K) caused significant increase in paddy yield over control. The maximum increase in paddy yield was obtained by multi-strain inoculation of 6K, LSI-29 and PsJN which was 21% higher than control, followed by 16, 14, 13, 8, 6 and 5% increase caused by co-inoculation of PsJN and 6K, co-inoculation of PsJN and LSI-29, co-inoculation of LSI-29 and 6K, sole inoculation of PsJN, sole inoculation of 6K and sole inoculation of LSI-29 over un-inoculated control under field conditio

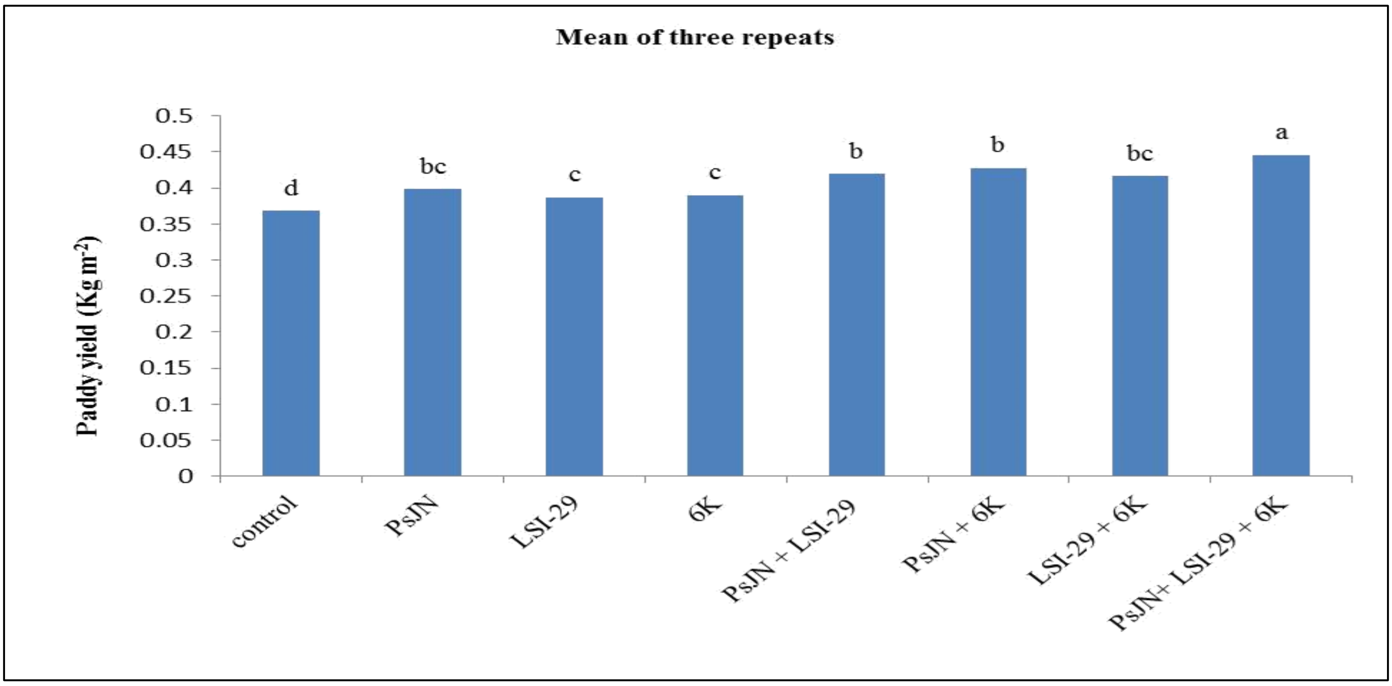

Figure 1. The effect of multi-strain inoculation on paddy yield of rice crop under field conditions.

Grain nitrogen (%)

The results regarding the data collected on grain nitrogen contents showed that multi-strain inoculation of LSI-29, 6K and PsJN significantly increased it over co-inoculation of LSI-29 and 6K, sole inoculation of PsJN, sole inoculation of 6K, sole inoculation of LSI-29 and un-inoculated. Multi-strain inoculation of LSI-29, 6K and PsJN caused a non-significant increase in grain nitrogen contents over co-inoculation of PsJN and 6K, and co-inoculation of LSI-29 and PsJN. The multi-strain combination caused maximum increase in the parameter (33%), followed by co-inoculation of PsJN and 6K (26%), co-inoculation of LSI-29 and PsJN (22%), co-inoculation of LSI-29 and 6K (18%), sole inoculation of PsJN (14%), sole inoculation of 6K (12%), and sole inoculation of LSI-29 (7.5%) over un-inoculated control under field conditions.

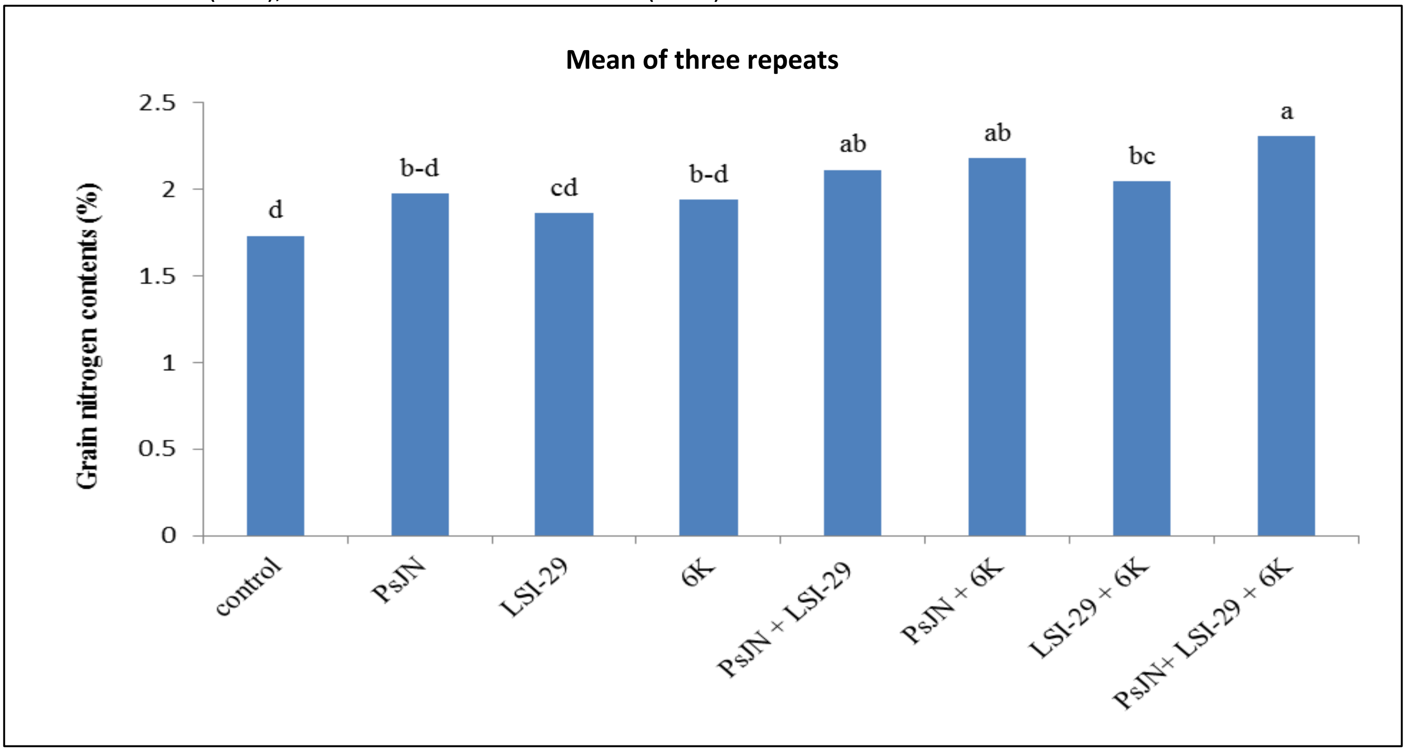

Figure 2. The effect of multi-strain inoculation on grain nitrogen contents of rice crop under field conditions.

Grain phosphorus contents (%)

Multi-strain inoculation of 6K, LSI-29 and PsJN caused maximum increase in grain phosphorus contents (39%), followed by co-inoculation of PsJN and 6K (28%), co-inoculation of LSI-29 and PsJN (25%), co-inoculation of LSI-29 and 6K (22%), sole inoculation of PsJN (18%), sole inoculation of 6K (17%), and sole inoculation of LSI-29 (12%) over un-inoculated control under field conditions. Multi-strain combination of LSI-29, 6K and PsJN significantly increased the grain phosphorus contents over co-inoculation of LSI-29 and PsJN, co-inoculation of LSI-29 and 6K, sole inoculation of PsJN, sole inoculation of 6K, sole inoculation of LSI-29 and un-inoculated control while non-significant increase was observed over co-inoculation of PsJN and 6K. Co-inoculation of PsJN and 6K prominently increased the grain phosphorus contents over sole inoculation of 6K, sole inoculation of LSI-29 and un-inoculated control while the increase was non-significant over co-inoculation of LSI-29 and PsJN, co-inoculation of LSI-29 and 6K and sole inoculation of PsJN. Co-inoculation of LSI-29 and PsJN also significantly increased the grain phosphorus contents over sole inoculation of sole inoculation of 6K, sole inoculation of LSI-29 and control while the increase was non-significant over co-inoculation of LSI-29 and 6K, sole inoculation of 6K and sole inoculation of LSI-29. Similarly, co-inoculation of LSI-29 and 6K significantly enhanced grain phosphorus contents over sole inoculation of LSI-29 and control while it was statistically similar to sole inoculation of PsJN and sole inoculation of 6K. Sole inoculation of PsJN and sole inoculation of 6K also significantly increased grain phosphorus contents over un-inoculated control but it non-significantly increased the parameter over sole inoculation of LSI-29. Single strain inoculation of LSI-29 also increased the grain phosphorus contents over control but the change was non-significant statistically.

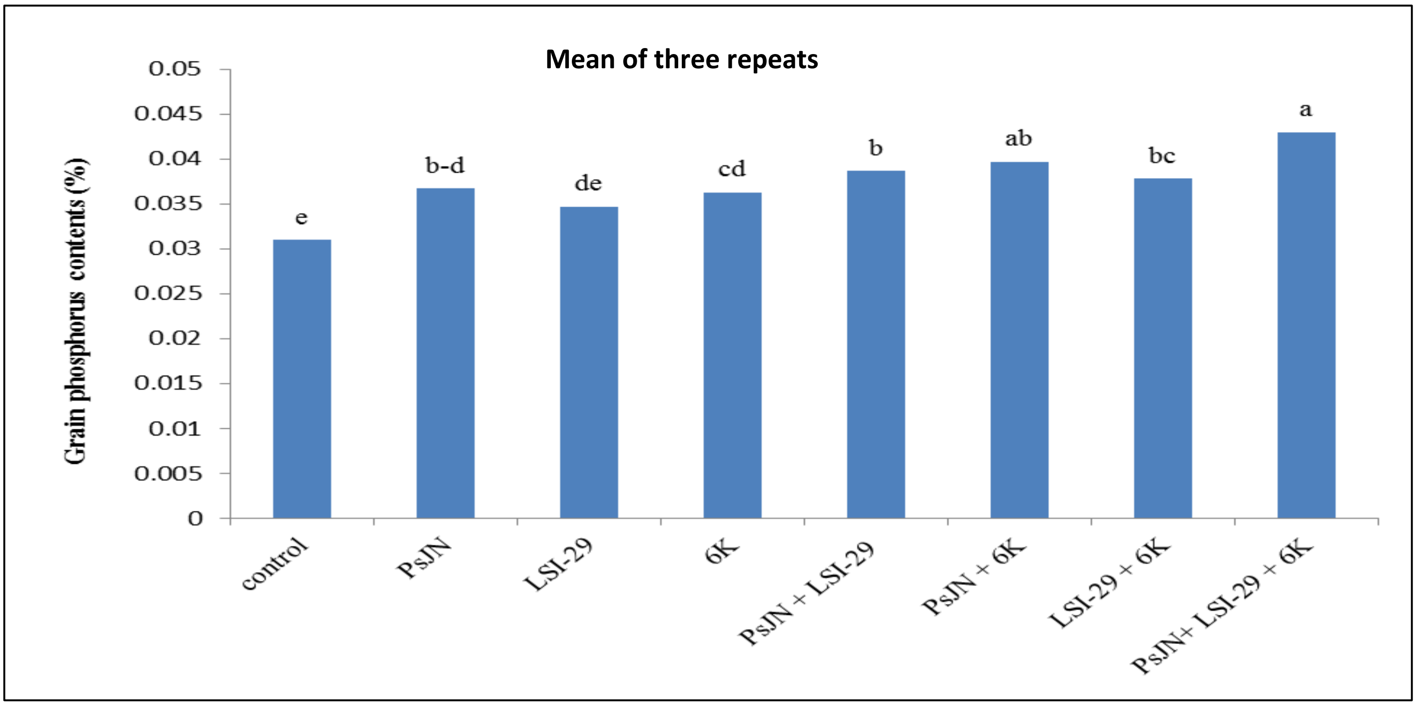

Figure 3. The effect of multi-strain inoculation on grain phosphorus contents of rice crop under field conditions.

Grain potassium contents (%)

Multi-strain inoculation of LSI-29, 6K and PsJN significantly increased the grain potassium contents over all the co-inoculation treatments (i.e., LSI-29 + 6K, PsJN + 6K and PsJN + LSI-29), all the sole inoculation treatments (i.e., LSI-29, 6K and PsJN) and un-inoculated control under field conditions of rice. Multi-strain inoculation of 6K, LSI-29 and PsJN caused maximum increase in grain potassium contents (29%), followed by co-inoculation of PsJN and 6K (24%), co-inoculation of LSI-29 and PsJN (22%), co-inoculation of LSI-29 and 6K (19%), sole inoculation of PsJN (14%), sole inoculation of 6K (10%), and sole inoculation of LSI-29 (8%) over un-inoculated control. Co-inoculation of PsJN and 6K significantly increased the grain potassium contents over sole inoculation of LSI-29 and control while it showed non-significant increase in the parameter over co-inoculation of LSI-29 and PsJN, co-inoculation of LSI-29, sole inoculation of PsJN and sole inoculation of 6K. Similarly, co-inoculation of LSI-29 and PsJN also increased the parameter significantly over sole inoculation of LSI-29 and control. It also increased potassium contents over co-inoculation of LSI-29 and 6K, sole inoculation of PsJN and sole inoculation of 6K but the change was not significant statistically. Co-inoculation of LSI-

29 and 6K prominently increased grain potassium contents over control which was statistically similar to sole inoculation of PsJN, sole inoculation of 6K and sole inoculation of LSI-29. Likewise, all the sole inoculation treatments i.e., LSI-29, 6K and PsJN significantly increased grain potassium contents over un-inoculated control and were statistically at par with each other.

Mean of three repeats

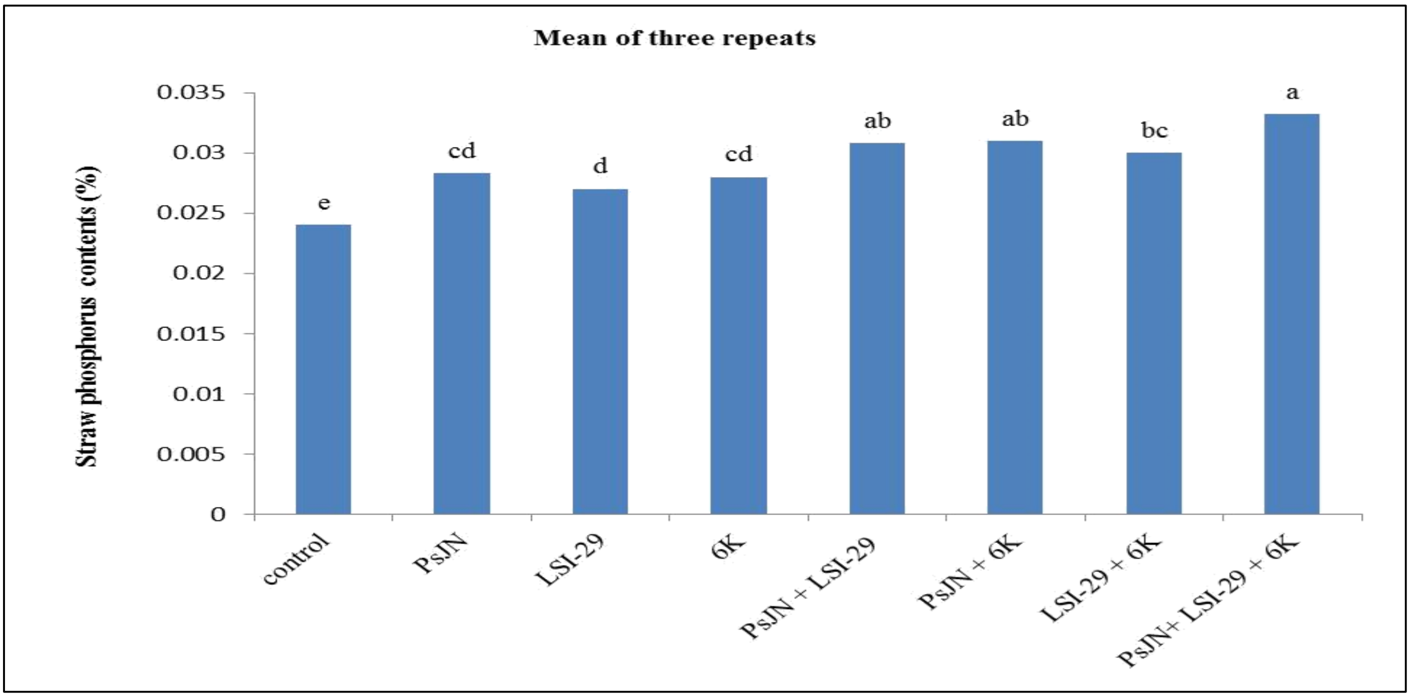

Figure 4. The effect of multi-strain inoculation on grain potassium contents of rice crop under field conditions.

Straw nitrogen contents (%)

Data regarding straw nitrogen contents was analyzed statistically which showed promising results about the potential of multi-strain inoculation. Multi-strain inoculation of LSI-29, 6K and PsJN significantly increased straw nitrogen contents over all the co-inoculation treatments (i.e., LSI-29 + 6K, PsJN + 6K and PsJN + LSI-29), all the single strain inoculation treatments (i.e., LSI-29, 6K and PsJN) and un-inoculated control under field conditions. Multi-strain inoculation of 6K, LSI-29 and PsJN caused maximum increase in straw nitrogen contents (26%), followed by co-inoculation of PsJN and 6K (21%), co-inoculation of LSI-29 and PsJN (19%), co-inoculation of LSI-29 and 6K (16%), sole inoculation of PsJN (15%), sole inoculation of 6K (14%), and sole inoculation of LSI-29 (10%) over un-inoculated control. Co-inoculation of PsJN and 6K significantly increased the straw nitrogen contents over sole inoculation of PsJN, sole inoculation of 6K, sole inoculation of LSI-29 and un-inoculated control which was statistically similar to other co-inoculation treatments i.e., LSI-29 + PsJN and LSI-29 + 6K. Similarly, co-inoculation of LSI-29 and PsJN significantly increased straw nitrogen contents over sole inoculation of PsJN, sole inoculation of LSI-29, sole inoculation of 6K and un-inoculated control. It also increased the parameter over co-inoculation of LSI-29 and 6K but non-significant statistically. Co-inoculation of LSI-29 and 6K prominently enhanced the straw nitrogen contents over sole inoculation of 6K, sole inoculation of LSI-29 and control. It also increased the parameter over sole inoculation of PsJN but non-significant statistically. All the sole inoculation treatments i.e., LSI-29, 6K and PsJN were statistically at par with each other and they all significantly increased the straw nitrogen contents over un-inoculated control.

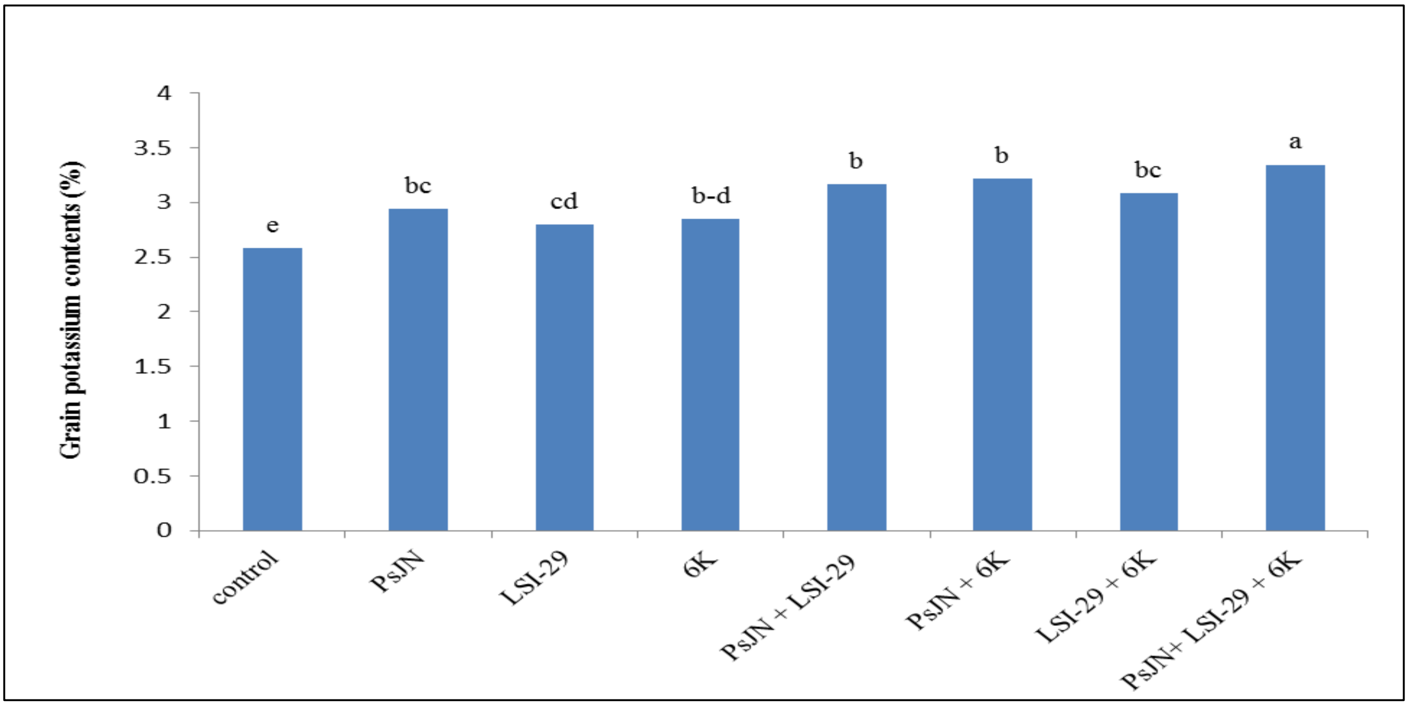

Figure 5. The effect of multi-strain inoculation on straw nitrogen contents of rice crop under field conditions.

Straw phosphorus contents (%)

Multi-strain inoculation of rice with LSI-29, 6K and PsJN significantly increased the straw phosphorus contents over co-inoculation of LSI-29 and 6K, all the sole inoculation treatments and un-inoculated control. The multi-strain combination was statistically at par with co-inoculation of PsJN and 6K, and co-inoculation of PsJN and LSI-29. Co-inoculation of PsJN and 6K significantly increased the parameter over all the sole inoculation treatments and control while it was statistically indifferent with other two co-inoculation treatments i.e., LSI-29 + PsJN and LSI-29 + 6K. Similarly, co-inoculation of LSI-29 and PsJN also significantly improved the parameter over all the sole inoculation treatments and control being non-significant with other co-inoculation treatments. Co-inoculation of LSI-29 and 6K also prominently increased straw phosphorus contents over sole inoculation of LSI-29 and control being statistically at par with sole inoculation of PsJN and sole inoculation of 6K. Likewise, all the sole inoculation treatments significantly increased the parameter over control. The maximum increase in straw phosphorus contents was observed in multi-strain inoculation of LSI-29, 6K and PsJN (38%), followed by co-inoculation of PsJN and 6K (29%), co-inoculation of LSI-29 and PsJN (28%), co-inoculation of LSI-29 and 6K (25%), sole inoculation of PsJN (18%), sole inoculation of 6K (17%), and sole inoculation of LSI-29 (13%) over un-inoculated control under field conditions.

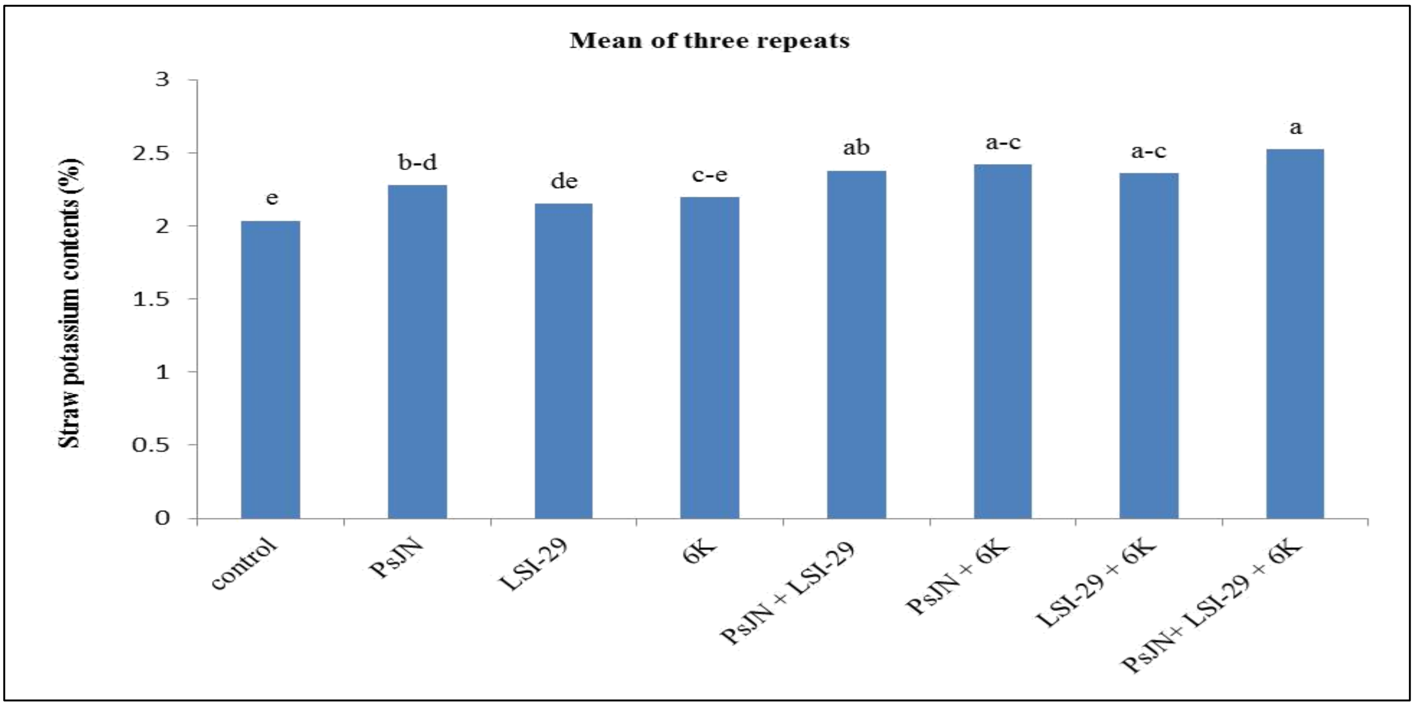

Figure 6. The effect of multi-strain inoculation on straw phosphorus contents of rice crop under field conditions.

Straw potassium contents (%)

Multi-strain inoculation significantly increased straw potassium contents over all the sole inoculation treatments i.e., LSI-29, 6K and PsJN. It also increased the parameter over all the co-inoculation treatments (i.e., LSI-29 + 6K, PsJN + 6K and PsJN + LSI-29) but this increase was non-significant statistically. Co-inoculation of PsJN and LSI-29 significantly increased straw potassium contents over sole inoculation of 6K, sole inoculation of LSI-29 and control being statistically indifferent with other co-inoculation treatments (LSI-29 + PsJN and LSI-29 + 6K) and sole inoculation of PsJN. Co-inoculation of 6K and PsJN significantly increased the parameter over sole inoculation of LSI-29 and control being statistically at par with co-inoculation of LSI-29 and 6K, sole inoculation of PsJN and sole inoculation of 6K. Similarly, co-inoculation of LSI-29 and 6K significantly increased straw potassium contents over sole inoculation of LSI-29 and control being statistically at par with sole inoculation of PsJN and sole inoculation of 6K. Likewise, sole inoculation of PsJN also significantly increased straw potassium contents over control which was statistically similar to sole inoculation of LSI-29 and sole inoculation of 6K. Sole inoculation of 6K and sole inoculation of LSI-29 also increased the parameter over control but this increase was non-significant statistically. The maximum increase in straw potassium contents was produced by multi-strain inoculation of LSI-29, 6K and PsJN (24%), followed by co-inoculation of PsJN and 6K (19%), co-inoculation of LSI-29 and PsJN (17%), co-inoculation of LSI-29 and 6K (16%), sole inoculation of PsJN (12%), sole inoculation of 6K (8%), and sole inoculation of LSI-29 (6%) over un-inoculated control under field conditions.

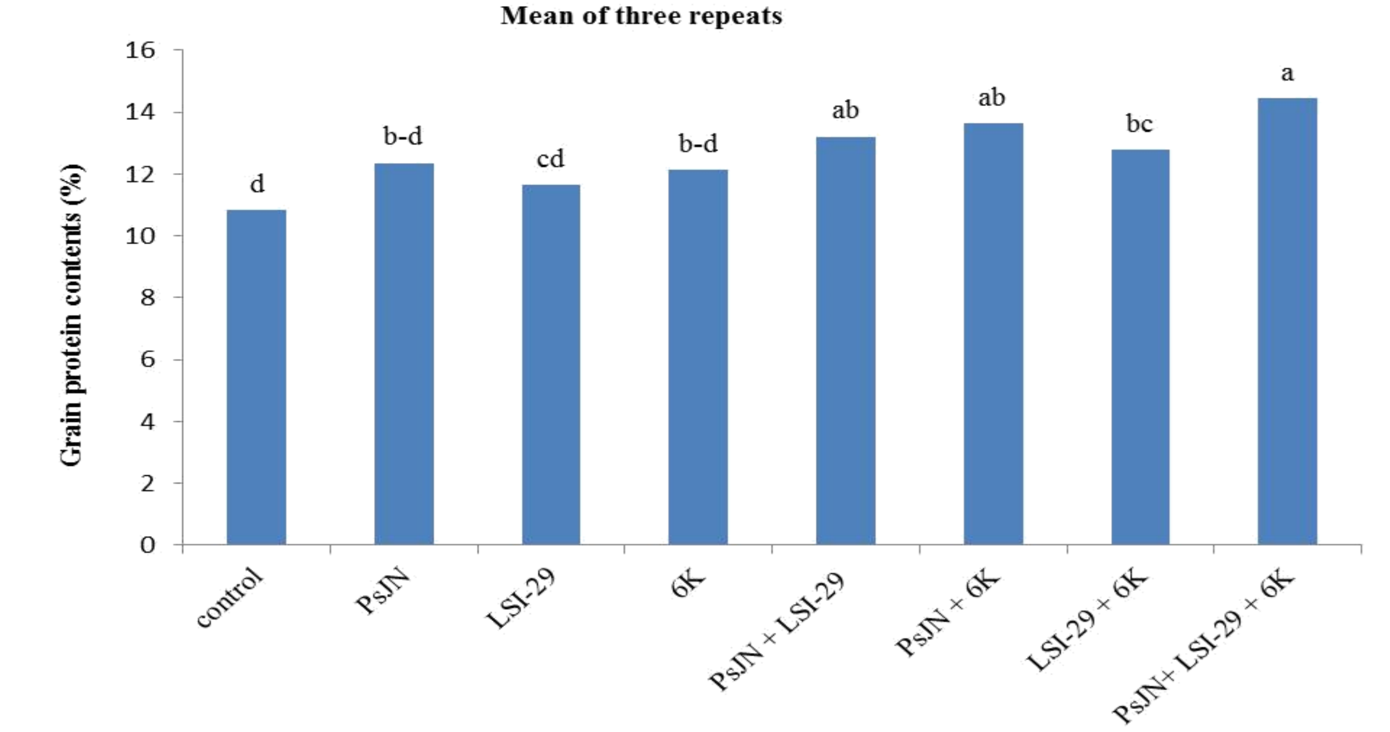

Figure 7. The effect of multi-strain inoculation on straw potassium contents of rice crop under field conditions.

Grain protein contents (%)

Data regarding grain protein contents produced encouraging results about the effectiveness of multi-strain inoculation. Multi-strain combination of LSI-29, 6K and PsJN significantly increased the grain protein contents over co-inoculation of LSI-29 and 6K, all the sole inoculation treatments (i.e., LSI-29, 6K and PsJN), and un-inoculated control. However, multi-strain inoculation was statistically at par with co-inoculation of PsJN and 6K, and co-inoculation of LSI-29 and PsJN. Co-inoculation of PsJN and 6K also significantly increased grain protein contents over sole inoculation of LSI-29 and un-inoculated control being statistically at par with other co-inoculation treatments (i.e., LSI-29 + PsJN and LSI-29 + 6K), sole inoculation of PsJN and sole inoculation of 6K. Similarly, co-inoculation of PsJN and LSI-29 significantly improved the parameter over sole inoculation of LSI-29 and control being statistically similar to co-inoculation of LSI-29 and 6K, sole inoculation of PsJN, and sole inoculation of 6K. Co-inoculation of LSI-29 and 6K significantly increased grain protein contents over control. It also caused a non-significant increase over all the sole inoculation treatments i.e., LSI-29, 6K and PsJN. Sole inoculation of PsJN also increased the parameter over sole inoculation of 6K, sole inoculation of LSI-29 and control. However, this increase was non-significant statistically. Multi-strain inoculation of LSI-

29, 6K and PsJN maximally increased grain protein contents (33%), followed by co-inoculation of PsJN and 6K (26%), co-inoculation of LSI-29 and PsJN (22%), co-inoculation of LSI-29 and 6K (19%), sole inoculation of PsJN (14%), sole inoculation of 6K (12%), and sole inoculation of LSI-29 (7%) over un-inoculated control under field conditions.

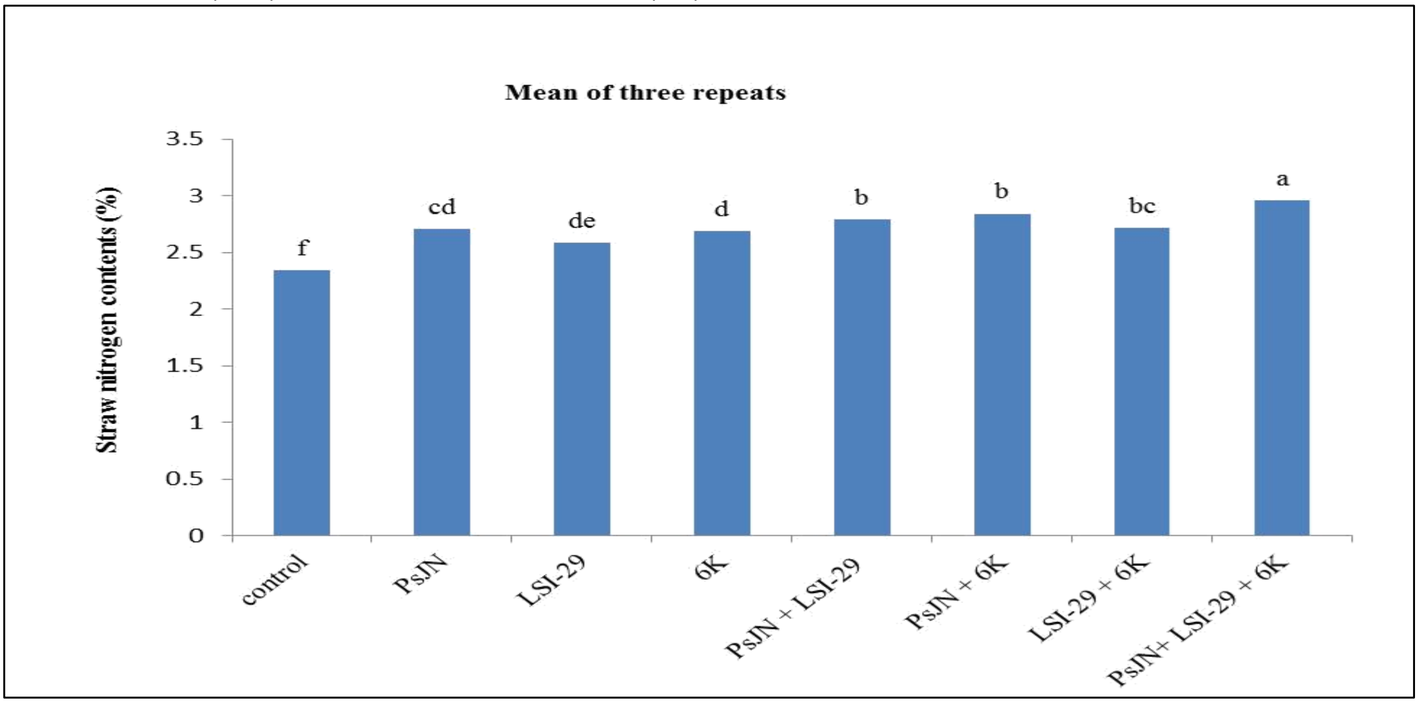

Figure 8. The effect of multi-strain inoculation on grain protein contents of rice crop under field conditions.

Conflict of interest: Nothing to declare

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