Research Article

Bacterial Coated Fertilizer Induced Resistance Against Wheat Stripe Rust

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Abstract: Wheat is the second largest consumed cereal by humans after Rice and its high yield and production is very critical for ever increasing global population. The wheat crop is grown all over Pakistan and threatened by several limiting factors. Stripe rust, caused by *Puccinia striiformis* f. sp. *tritici*, is the most destructive wheat pathogen and can reduce yield up to 70% in Pakistan. The present study aimed at exploring the role of Zabardast urea, a bacterial coated urea with zinc, in inducing resistance against wheat stripe rust. The study involved the collection and maintenance of stripe rust inoculum on Morroco cultivar which later used to inoculate seedlings of Akbar-2019 and Galaxy-2019 resistant and susceptible varieties with three different fertilizer levels viz. specialty fertilizer zabardast urea, plain urea with zinc and plain urea. The results demonstrated the positive role of bacterial coated urea with zinc and reduced the disease severity by 10% and 5% in susceptible and resistant cultivars, respectively, leaving resistant variety asymptomatic. The plain urea with zinc also decreased disease severity in susceptible variety Galaxy-2013 by 6% in comparison with plain urea treatment underlying the role of zinc in combating stripe rust ($p<0.01$). Moreover, the plant height responded very strongly to the fertilizer treatments ($p<0.01$) whereas SPAD chlorophyll value was influenced by varietal type ($p<0.05$) but non-significant behavior was observed in SPAD chlorophyll values with fertilizer treatments. The SOD activity was highly significant in case of fertilizers and varieties respectively ($p<0.01$) while significant SOD levels differences were observed in interaction of fertilizers and varieties ($p<0.05$). The study underlines the importance of specialty fertilizers in inducing resistance against stripe rust in wheat and needs further experimentation exploring the mechanisms involved in disease resistance under field conditions.

Keywords: wheat stripe rust; *Puccinia striiformis*; specialty fertilizer; Zinc; Endophytic bacteria

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1. Introduction

Global wheat consumption has increased due to the change in socio-economic conditions, ever expanding urbanization and people's preference towards wheat as a staple food in daily life. The increasing trend predicted to forecast 60% increase in wheat consumption by year 2050 but it faces numerous challenges which lessen the wheat yield like quality seed availability, decreasing water and arable land, efficient use of fertilizers, pathogens and insect pest pressure [3, 9]. Wheat enjoys prominent status in agriculture of Pakistan with per capita consumption of 124 kg [11] and cultivated by approximately 80% farmers covering almost 9 million hectares of land [4]. One of the major pathogens infecting wheat globally is rust fungi (*Puccinia spp.*) and reported to cause up to 60% (leaf rust), 70% (stripe rust) and 100% (stem rust) yield loss in wheat. Stripe rust is the major challenge to wheat crop in Pakistan because 5.8 million hectares wheat cultivated area is vulnerable to wheat stripe rust [2; 4]. Endophytic bacteria mostly survive inside the roots of the plants in an ecological harmony without harming the host plants and proving beneficial for the plants against the onslaught of pathogens. The endophytic bacteria are being reported to act as biological control agents by suppressing the pathogens in various pathosystems [14] employing either directly regulating the genes [19] or increasing availability of essential nutrients [10]. Moreover, many bacterial species are testified as a potent biological control agent against rust fungi [9]. Zinc is a very vital micronutrient, a proven yield enhancer in wheat [1], which is a cofactor of several enzymes and interacts with structural proteins. It also plays important role in boosting plant immunity and "Zn finger" proteins regulate certain transcription factor which are crucial for plant defense mechanisms [5, 20]. The enzyme superoxide dismutase (SOD) functions as an antioxidant by catalyzing the conversion of superoxide radicals to hydrogen peroxide, which can subsequently be reduced to water by other antioxidant enzyme. They are essential for its defense against the toxicity produced by partially reduced metabolites, generated during the normal biological reduction of molecular oxygen [17]. It was assumed that the presence of consortia of bacteria in Zarbardast Urea may involve the endophytic nature of bacteria which is likely to be responsible for high SOD activity and impart bio-chemical resistance against the stripe rust of wheat, therefore, a study was carried out aimed at exploring the role of bacterial coated zinc over urea in imparting resistance against wheat stripe rust.

2. Materials and Methods

The study was conducted in the Institute of Plant Protection, MNS University of Agriculture Multan.

2.1 Selection of Wheat Varieties

The wheat cultivars Akbar-2019 and Galaxy-2013 were selected for being resistant and susceptible against wheat stripe rust respectively. The Morocco wheat cultivar was used to multiply the *Puccinia striiformis* f. sp. *tritici* inoculum under controlled conditions.

2.2 Maintenance of Stripe rust culture

The stripe rust inoculum was collected from different areas of Multan district and maintained at Plant Disease Diagnostic Lab, MNS University of Agriculture Multan as per protocol demonstrated by Yasmeen et al., [21].

2.3 Fertilizer Application Levels

The three fertilizer treatments were applied in large pots (13 inch diameter) viz. Zabardast Urea (Bacterial coated urea specialty fertilizer with zinc) @ 50kg/1000 tones, Plain urea with zinc sulphate @ 6kg/1000 tones and plain urea @ 75 kg/1000 tones [13].

2.4 Inoculation of stripe rust on wheat varieties

The 20 days old seedlings of wheat varieties Akbar-2019 and Galaxy-2013 were sprayed with stripe rust inoculum with a sprayer having fine nozzle. The inoculation was done with few modification as described by Kiani et al., [9].

2.5 Disease Rating

The disease rating was done according to the modified Cobb's scale described by Peterson et al., [18].

2.6 SOD Activity Essay

The total SOD activity (EC 1.15.1.1) was determined according to the method described by Paoletti et al., [16]. One gram (1g) of plant material was taken from each treatment and was immediately crushed in mortar. To the homogenate obtained, 2ml of potassium phosphate buffer solution containing 100mM NaKP i, pH 7.0; 0.3g polyvinyl-polypyrrolidone (PVPP) and 200ml of fluoride-phenyl-methyl-sulfonyl (PMSF). The mixture was vortexed and kept for 30 min in an ice bath. The solid material was concentrated, centrifuging at 1000g for 10min and the recovered supernatant was centrifuged again at 10,000g for 20min. The entire extraction procedure was carried out at 4°C. All samples were kept at 25°C. To record the enzymatic activity, the change in absorbance at 560nm was measured every 30s for 5min in a spectrophotometer. The method defines a SOD unit as the amount of enzyme that causes 50% of the maximum inhibition of NBT to formazan blue, expressing the activity of the extracts as SOD units per mg of protein. The determination of each extract was carried out in triplicate.

2.6 Experimental Design and Statistical Analysis

The experimental units were laid out in completely randomized design with two-factor factorial. The data was analyzed using ANOVA and means were compared using LSD test in statistix 8.1 software. Performance of fertilizer treatment was assessed by applying Principal Component Analysis (PCA) with the help of GGEBiplot®.

3. Results

The disease resistance recorded from the experimental units showed the performance of zabardast urea (T_1), plain urea + zinc sulphate (T_2) and plain urea (T_3) respectively. In case of T_1, 50 and 0 disease severity (DS) percentage was recorded in Galaxy-2013 and Akbar-2019 with a genotype response of moderately susceptible (MS) to resistant (R). The DS percentage of 54 and 5 with a genotype response of MS and R was observed in T_2 while T_3 resulted in 60 and 5 percent DS with MS to S genotype response in Galaxy-2013 and Akbar-2019 respectively (Table 1; Figure 1).

Figure 1. Impact of fertilizers imparting resistance against wheat stripe rust

Table 1. **Wheat stripe rust disease severity percentage susceptible (Galaxy-2013) and resistant (Akbar-2019) varieties**

	Bacterial Coated Urea (T1)	Plain Urea + Zinc Sulphate (T2)	Plain Urea(T3)
Galaxy-2013	50 (MS) **C**	54 (MS) **D**	60 (S) **E**
Akbar-2019	0 (O) **A**	5 (R) **B**	5 (R) **B**

Value in parenthesis connotes to resistance level
MR= Moderately Resistant; MS= Moderately susceptible; S= Susceptible; O= No disease symptoms; R= Resistant
Letters followed by parenthesis indicate the significant of means differentiated by LSD value 1.7887 ($P < 0.05$)

In terms of control, the zabardast urea (T_1) outperformed other fertilizers and induced 10 percent (50% DS) inhibition as compared to T_2 and T_3 (60% DS) in susceptible Galaxy-2013 cultivar. The T_1 induced 5 percent resistance leading to 0 % DS as compared to other treatments (Table 1; Figure 2)

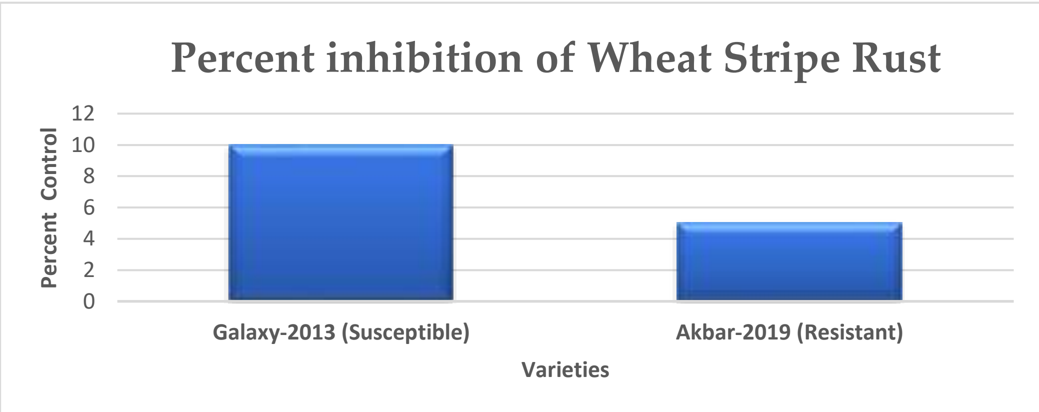

Figure 1. Inhibition of wheat stripe rust by Zarbardast urea

The statistical analysis revealed the high significant P values for fertilizers, varieties and their interactions. The varietal response were different confirming their genetically potential of Akbar-2019 to resist stripe rust and inability of Galaxy-2013 to restrict pathogen's growth. There have been highly significant differences ($p<0.01$) among the fertilizer treatments for plant height, whereas significant differences ($p<0.05$) were observed among the varieties and their interaction with fertilizer treatments for plant height (Table 2). Although there were significant differences among the varieties for SPAD chlorophyll values but the non-significant differences among the fertilizers for SPAD resulted in non-significance of their interaction with the varieties. Highly significant differences for SOD activities among the fertilizers and varieties, and significant differences for their interaction were also observed (Figure 3).

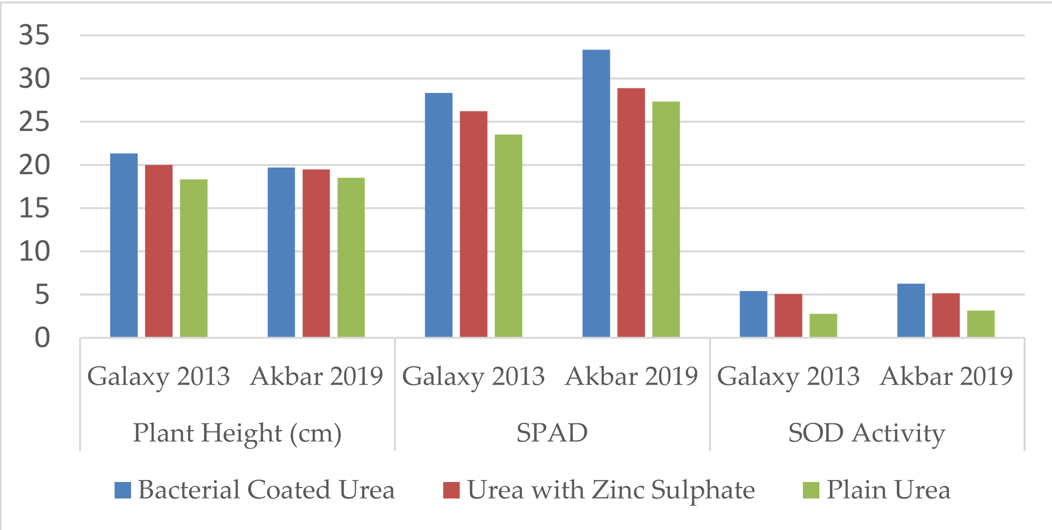

Figure 2. Parametric performance of Bacterial coated Zinc on urea with other fertilizers in wheat varieties

Table 2. P-value obtained from ANOVA

Source	Disease Severity	Plant Height	SPAD	SOD Activity
Fertilizers	0.0000**	0.0001**	0.1068 NS	0.0000**
Varieties	0.0000**	0.0241*	0.02840*	0.0007**
Fertilizers*Varieties	0.0011**	0.0345*	0.33309NS	0.0175*

NS: Non-Significant; * Significant at 5% probability level, **Significant at 1% probability level

Principle Component 1 (PC1) explained 97.6% whereas PC2 explained only 2.4% of the results. It is evident from the results that, for all fertilizers, disease severity (DS) is negatively correlated with SOD activity, plant height, and SPAD value. Higher SOD activity is desirable as observable in figure 4:4A pointing towards top right (positive side) marked with red line, contrary to the disease severity (bottom left) making the bacterial coated zinc on urea a desirable fertilizer. The SOD activity decreased (figure 4:4B) but the disease severity also increased more than that observed in bacterial coated zinc on urea. Disease severity increased and SOD activity decreased in the plants fertilized with the plain urea, marked with red line pointing towards left (figure 4:4C). Plant height (cm), SPAD, and SOD activity were positively related with each other, but the degree of association with plant height and SPAD was more than that of present between SPAD and SOD; and SPAD and plant height. Negative association between disease severity and plant and SPAD was observed (Figure 5).

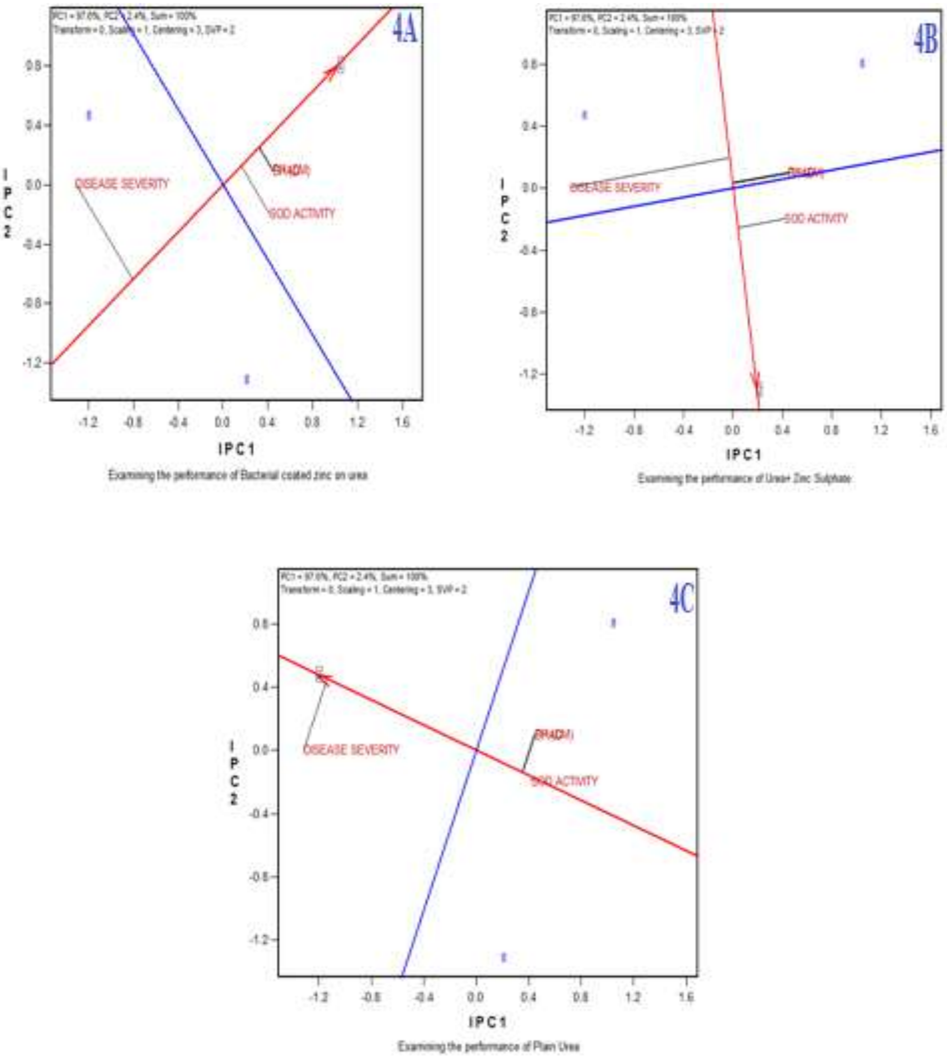

Figure 3. Principal Component Analysis (PCA); 4A: Performance of Bacterial coated zinc on urea; 4B: Performance of plain urea + zinc sulphate; 4C: Performance of plain urea

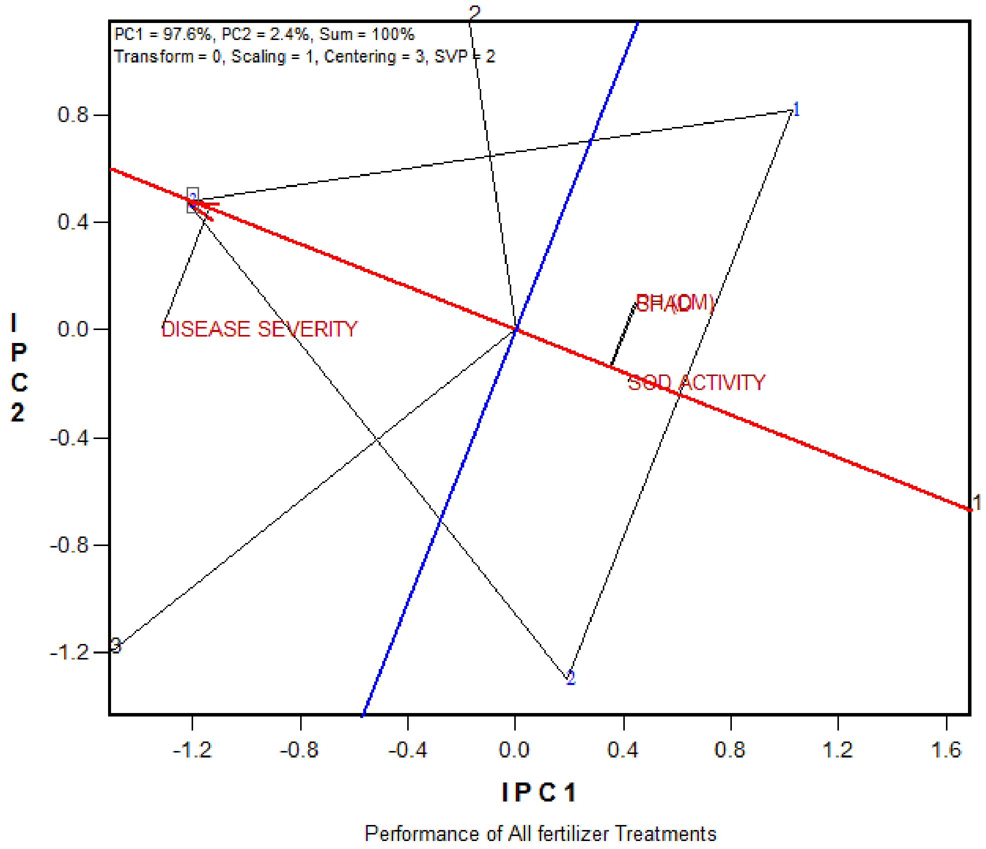

Figure 4. PCA: Performance of all treatments (fertilizers)

4. Discussion

In present study, a specialty fertilizer by Engro fertilizer was tested for incorporating resistance in wheat against stripe rust caused by *Puccinia striiformis* f. sp. *tritici*. The Zabardust urea (bacterial coated with zinc) and plain urea+zinc sulphate induced resistance in susceptible cultivar Galaxy-2013 and reduced disease severity by 10% and 6% respectively when compared with plain urea application. The performance of Zabardast urea also impacted the disease severity in resistant cultivar Akbar-2019 resulting in asymptomatic plants with zero percent disease severity.

Increased level of SOD is the indicators of activated plant defense mechanisms. Although the diseases severity on plant fertilized with zinc sulphate and urea was almost similar with the plants fertilized with plain urea in naturally resistant cultivar Akbar 2019, the high SOD values in zinc fertilized plants indicate the competitive advantage for the forthcoming crop stages. As one of the most recognized functions of Zn is related to its impact on the correct functioning and structural stability of many proteins [5], where about 10% of them need this element (approximately 2,800 proteins) to develop regulatory, catalytic and structural actions. Therefore, the structural and functional integrity of biological membranes depends on a sufficient amount of Zn content. Genetic resistance fully expressed, under provided conditions, with the fertilization of zinc coated with bacteria on urea, hinting towards higher zinc uptake and higher SOD values advocate for the endophytic activity of coated bacteria on zarbardast urea, which activates the enzymes of self-defense against the biotic stresses including the incidence of rust [17].

The SOD activity remained the strongest indicator of efficacy of fertilizer activity as it weighted maximum in explaining principal components. It is observable from figure 5 that performance of all three fertilizer treatments on PCA matrix is expressed as triangular shape with two corners in positive right side and one corner in negative side indicating fertilizer 3 (urea) has maximum disease severity. The remaining two zinc containing fertilizers remain on positive right axis, indicating better performance than that of urea. Although both zinc containing fertilizers performed better than solo urea, the zinc in the form of zinc sulphate had negative value on PC2 whereas the zinc coated with bacteria had both principal components in positive axis, indicating supremacy of bacterial presence over normal zinc sulphate.

The study has three aspects which needs to be considered in assessing the reasons of disease resistance induction in wheat. First one is the association of coated bacteria with wheat roots which could be endophytic or ectophytic in nature as several studies indicated the role of endophytic bacteria in improving plant immunity and inducing rust resistance in wheat and other crops [9, 12, 15, 19]. The second possibility is the role of zinc in systemic induced resistance against biotic stresses particularly fungal pathogens [6, 5, 7, 8, 20, 17]. The third possibility which is quite evident from the results that coated bacteria has a significant role directly or indirectly by providing timely availability of zinc to wheat plants leading to increased resistance as compared to zinc application without bacterial coating. This is the first report of any specialty fertilizer mediated induced resistance against stripe rust of wheat.

5. **Conclusions**

The use of specialty fertilizer manufactured by Engro fertilizers, is a new sphere in crop production sciences and has an enormous potential in precision agriculture driven technologies. The present study has opened a novel domain of disease resistance induction through specialty fertilizer application and warrant the need to explore the mechanism underlying disease resistance through exploring the specialty fertilizer mediated identification and expression of genes through transcription analysis followed by quantitative PCR validation, endophytic nature of coated bacterium aided molecular identification techniques, identification of pathogen's growth patterns by electron microscopy and zinc bio-availability under field conditions.

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