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The application of zinc fertilizer reduces Fusarium infection and development in wheat

Muhammed Alsamir[1], Esraa Al-Samir[2], T A Kareem[3], Mohammed Abass[4], Richard Trethowan[1]

[1]Plant Breeding Institute, Faculty of Agriculture and Environment, University of Sydney, 107 Cobbitty Road, Cobbitty, NSW 2570, Australia
[2]Biology Department, College of Science, University of Basra, Iraq
[3]Collage of Agriculture Engineering Sciences, University of Baghdad, Iraq
[4]Plant Protection, College of Agriculture, University of Basrah, Iraq

***Corresponding author: alsamir.hameed@sydney.edu.au**

Abstract

Fusarium pseudograminearum and *Fusarium graminearum* commonly cause crown rot (FCR) and head blight (FHB) in wheat, respectively. Disease infection and spread can be reduced by the deployment of resistant cultivars or through management practices that limit inoculum load. Plants deficient in micronutrients, including zinc, tend to be more susceptible to many diseases. On the other hands, and zinc deficiency in cereals is widespread in Australian soils. Zinc deficiency may have particular relevance to crown rot, the most important and damaging Fusarium disease of wheat and barley in Australia. Four wheat genotypes; Batavia, Sunco and two lines from the International Maize and Wheat Improvement Center (CIMMYT) were tested for response to FHB and FCR under differing levels of Zn,1 and 2 g/kg and its correlation with disease severity. Sunco and CIMMYT line 146 were previously rated resistant to crown rot and Zn efficient. Zn application 2 g/kg soil enhanced resistance to FCR of the disease susceptible and Zn in-efficient in Batavia and 48 as its recorded 0.75 and 0.5 respectively compared to Sunco and CIMMYT line 146 as it recorded 0.2 and 0.3 respectively, but did not increase resistance to FHB. However, Zn application did enhance the resistance of Zn efficient genotypes to FHB. Results suggest that higher levels of Zn fertilization could reduce the expression of Fusarium diseases in wheat.

Keywords: Fusarium, crown rot, head blight, wheat, Zn efficiency.
Abbreviations: FCR_Fusarium crown rot, FHB_Fusarium head blight, Zn_zinc.

Introduction

Several fungal soil-borne plant pathogens belong to the diverse genus Fusarium; with high ability to cause serious diseases on important plant families. These diseases damage small-grain cereals by rotting seed, causing seedling death, infecting crowns and spikes. Additionally, the head blight, produces a toxin detrimental to humans and animals (Goral et al., 2019). A number of Fusarium species reported to be a true pathogen of crown rot (FCR) and head blight (FHB) such as the species of F. culmorum, F, avenaceum , F, poae and Microdochium nivale and induced a range of symptoms ranging from severe disease symptoms to only mild symptoms (Parry et al.,1995). Two important Fusarium species present in Australia are Fusarium pseudograminearum (Fp), most commonly associated with FCR and Fusarium graminearum (Fg) which causes FHB. FCR in particular, can cause significant yield losses in sub-optimal cereal rotations during dry grain-filling periods in parts of Australia (Alahmad et al., 2018). FCR is also emerging as an important disease internationally (Smiley et al., 2005a, 2005b). It occurs in most cereal-producing regions of the world and has been reported in Australia (Wildermuth et al., 1997; Burgess et al., 2001), Europe (Rossi et al. 1995; Pettitt and Parry 2001), North America (Fernandez and Zentner 2005; Smiley et al. 2005b), South America (Burgess et al. 2001),West Asia and North Africa (Braun et al. 2004; Nicol et al. 2004) and South Africa (Lamprecht et al., 2006, VanWyk et al., 1987). Recently, natural infections of FCR reduced the winter wheat yield by 35% in commercial fields of the Pacific North-West of the USA; field inoculation with F. pseudograminearum in this region demonstrated potential yield losses up to 61% (Smiley et al., 2005b). Annual losses due to FCR in Australia are estimated at $A80 million (Murray & Brennan, 2009). Crown rot was first recorded in Australia in 1951 although the cause was unknown (McKnight and Hart, 1966). During the 1960s, the pathogen associated with crown rot was described as Fusarium graminearum Schwabe (McKnight and Hart, 1966; Purss, 1966) and later as Fusarium pseudograminearum (Aoki and O'Donnell, 1999). FHB is significant in vast wheat areas of the world with recent epidemics reported in Canada, China, Europe, South America and the USA (McMullen et al., 1997; Goswami and Kistler 2004), prompting the establishment of

coordinated research and development initiatives in most affected areas. FHB is sporadic in Australia and restricted geographically to areas in northern New South Wales and Queensland (Obanor et al., 2013) during wetter than average seasons. Crop losses in Australia from FHB are difficult to estimate due to its sporadic occurrence, but reliable data are available elsewhere. During 1998–2000, FHB inflicted an estimated US$2.7 billion loss attributed to reduced yield and price discounts from lower grain quality in the northern Great Plains and central USA (Goswami and Kistler, 2004).

Investigations on isolates of F. graminearum Schwabe established two distinct populations or groups of F. graminearum Schwabe (Francis and Burgess, 1977): Group 1 caused crown rot in wheat and Group 2 stalk rot of maize and head blight of wheat. The pathogen associated with crown rot was then identified as F. graminearum Group 1 (Fg G1). The existence of these specific sub-groups was further confirmed by genotyping the pathogen using restriction fragment length polymorphism (RFLP) (Benyon et al., 1995). Further morphological and DNA studies of Group 1 and Group 2 strains from different geographical regions showed that the Group 1 isolates were a distinct species; now designated F. pseudograminearum sp nov (Fp) (Aoki and O'Donnell, 1999).

Fp has been reported in Australia in New South Wales and Queensland, the USA, Argentina and South Africa (Carranza, 1961; Cook, 1968; Van Wyk et al., 1988). Damage caused by Fp in spring and winter cereals is most apparent when whiteheads appear shortly before maturity leading to a high frequency of shriveled grains after harvest. Damage can be highly variable within fields and can reduce yield by 35%. Both FHB and FCR result in the accumulation of mycotoxins in the grain (Desjardins, 2006; Mudge et al., 2006). Increased levels of the trichothecenes nivalenol (NIV) and deoxynivalenol (DON) and other mycotoxins from FHB infection can render the grain and grain products unsafe for human and animal consumption. Zinc deficiency in cereals is widespread and poses a threat to sustainable production (Graham et al., 1992; Takkar and Walker, 1993). Dang et al., (2010) found that the concentration and accumulation of Zn in all wheat tissues was high during early and middle growing periods, while accumulation of Zn in grains during late growth primarily depended on redistribution from other organs. Accordingly, it was concluded that Zn should be applied as a seed dressing or basal fertilizer to accelerate early growth and Zn absorption in wheat. This paper examines wheat genotypic responses to FCR and FHB diseases and the impact of Zn fertilizer on disease expression.. To the best of our knowledge, no previous experiments of zinc applications and their interaction with wheat Fusarium pathogens have been conducted, so the present study was designed to assess the Zinc effects on the progress of Fusarium crown rot and Fusarium head blight depending on disease severity as well as protein profile analysis of several wheat cultivars (resistance and susceptible cultivars).

Results

The response of wheat genotypes to inoculation with FCR under different levels of zinc

All four wheat genotypes showed significantly different crown rot disease responses at different zinc treatments (Figure 1). The wheat variety Sunco and CIMMYT 146 were rated more tolerant to disease than Batavia and CIMMYT 48. The addition of zinc significantly increased the tolerance of the crown rot susceptible genotypes Batavia and CIMMYT 48 to crown rot.

The response of wheat genotypes to inoculation with FHB under different levels of zinc

A differential response of the four wheat genotypes to inoculation with FHB under three zinc levels was observed (Figure 2). A significant increase in the FHB tolerance of Sunco and CIMMYT 146 was evident; however, no change in the responses of Batavia and CIMMYT 48 was observed.

Protein profiling of wheat genotypes inoculated with FCR and FHB at different levels of zinc

An equal quantity of protein was extracted from all grain samples and subsequently evaluated using SDS-PAGE analysis. Several proteins were expressed differentially, in particular in the molecular mass range of 30-45 kDa, under high Zn that were either not present or less well expressed under low Zn (Figure 3). The expression level of these proteins was different among the genotypes and treatments evaluated. Batavia and CIMMYT 146 showed maximum up-regulation of the aforementioned proteins at high Zn (2g/kg soil), irrespective of disease inoculation with FHB. However, these specific proteins were upregulated in Sunco following FHB inoculation at intermediate and high levels of Zn (1g and 2g/kg soil). CIMMYT 48 showed maximum up-regulation of these proteins at high Zn (2g/kg soil) without FHB inoculation.

Discussion

Zinc deficiency in cereals is widespread and a threat to sustainable production (Graham et al., 1992; Takkar and Walker, 1993). Unlike micronutrients, Zn deficiency is a common feature in both cold and warm climates irrespective of soil pH and texture (Graham et al., 1992). Crown rot disease in wheat is a widespread problem in the chronically Zn-deficient soils of Australia. A link between FCR and Zn deficiency was established by Sparrow and Graham, (1988) and Zn-efficient wheat cultivars that scavenge Zn from Zn-deficient soils tend to express better resistance to pathogens (Grewal et al., 1996). Zinc inefficient cultivars tend to require extra Zn fertilization to maintain performance comparable to more Zn efficient materials.

In the current study, Sunco and CIMMYT 146 are Zn efficient and better able to maintain resistance to both Fusarium diseases in the absence of Zn fertilization. However, the FCR susceptible genotypes Batavia and CIMMYT 48 required Zn fertilization to express even a low level of resistance to both pathogens. The current study suggests that Zn-efficient cultivars of wheat, combined with crown rot resistance, will enhance wheat performance in Zn-deficient soils prone to FCR. The judicious use of Zn fertilizer may also reduce the severity of the disease regardless of the resistance of the wheat cultivar. .

The four wheat genotypes also exhibited a differential response to inoculation with FHB. However, the application of Zn enhanced the tolerance of Sunco and CIMMYT 146 to the

Table 1. The scores of disease severity.

FCR disease severity	No Zinc	1g/kg soil	2 g/kg soil	FHB disease severity	No Zinc	1g/kg soil	2 g/kg soil
Batavia	3.65	1.2	0.75	Batavia	4.0	4.0	4.0
Sunco	1.3	0.55	0.2	Sunco	3.7	2.7	2.0
146	0.9	0.5	0.3	146	3.6	3.6	2.5
48	2.7	1.55	0.5	48	4.0	4.0	3.8

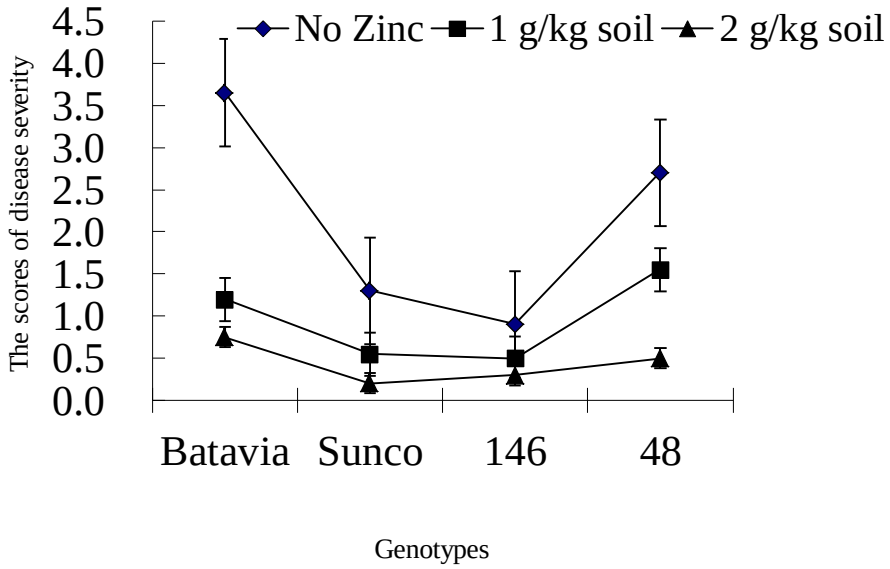

Fig 1. The change in resistance of four wheat genotypes to crown rot disease under zinc levels of 0, 1 and 2 g per kg of soil.

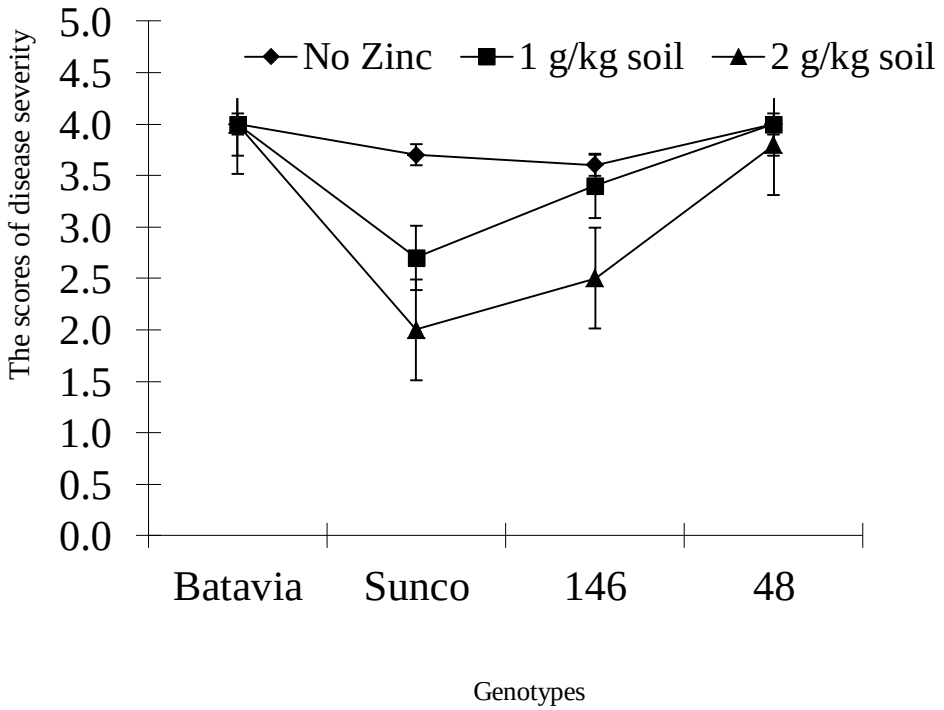

Fig 2. The change in resistance of four wheat genotypes to *Fusarium* head blight under zinc levels of 0, 1 and 2 g per kg of soil.

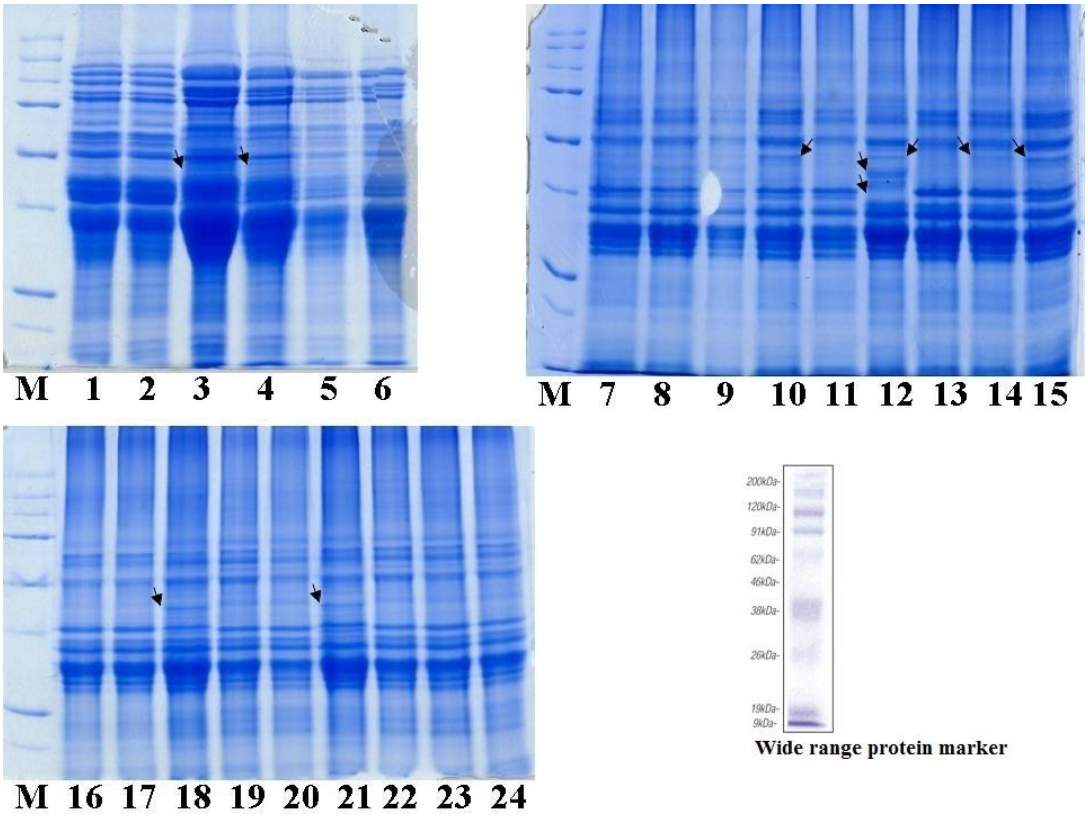

Fig 3. Protein profile of different wheat genotypes. Batavia (B)+no disease inoculation (NDI)+Zinc 1 g/kg of soil (Zn1); 2, B+NDI+No Zinc (Zn0); 3, B+NDI+Zinc 2 g/kg of soil (Zn2); 4, B+disease inoculation (DI)+Zn 2; 5, B+DI+ Zn0; 6, B+DI+Zn1; 7, Sunco (S)+NDI+Zn0; 8, S+NDI+Zn1; 9, S+NDI+Zn2; 10, S+DI+Zn1; 11, S+DI+Zn0; 12, S+DI+Zn2; 13, 146+NDI+Zn0; 14, 146+NDI+Zn1; 15, 146+NDI+Zn2; 16, 146+DI+Zn0; 17, 146+DI+Zn1; 18, 146+DI+Zn2; 19, 48+NDI+Zn0; 20, 48+NDI+Zn1; 21, 48+NDI+Zn2; 22, 48+DI+Zn2; 23, 48+DI+Zn1; and 24, 48+DI+Zn0.

disease, while Batavia and CIMMYT 48, both susceptible to FHB, did not express improved disease resistance to Zn inoculation. These later two genotypes did show a response to Zn under FCR but this was not evident for FHB. It may be that the level of susceptibility to FHB in Batavia and CIMMYT 48 was so high that minor impacts attributable to Zn nutrition simply disappeared.

Zinc is an essential micronutrient for many vital metabolic processes in plants and an important cofactor for several enzymes (Aravind and Prasad, 2003). As Zn application increased the FHB resistance of Zn efficient wheat genotypes, it may also require a much higher level of Zn fertilization to elicit a positive response in less Zn efficient genotypes.

Zinc is known to have a stabilizing and protective effect on bio-membranes against oxidative and peroxidative damage, loss of plasma membrane integrity and alteration of the permeability of the membrane. This effect may also reduce the impact of Fusarium toxins on plant cells and the effect of fusaric acid on plasma membrane integrity (Brion et al, 1997).

Protein profiling revealed several differentially expressed proteins in the 30-45 kDa range under Zn application (Figure 3). Proteins of this molecular size are wall-associated kinase 4-like proteins, isoflavone reductase , oxygenase activase 2, chloroplastic and chloride carrier (Shin et al., 2011). These proteins play an important role in plant responses to disease and mineral imbalances (Shin et al., 2011). Sunco showed an additional band with an approximate molecular weight of 35 kDa at high Zn levels (2g/kg soil) following disease inoculation. This band may correspond to a defense protein; cytochrome c oxidase (Shin et al., 2011).

The disease reducing effects of Zn application has been reported for a range of fungal pathogens in many crops including cotton, rice and banana (Agrios, 1988; Marino et al., 2003). However, our results are showed the suppression of FCR and FHB in wheat. If genetic resistance is combined with genetic Zn-efficiency or Zn application through management, then the expression and impact of these important Fusarium diseases is likely to reduce.

Materials and Methods

Plant material and growth conditions

Four wheat genotypes; Batavia, Sunco, CIMMYT line 146 and CIMMYT line with three replication for each treatment, were evaluated for response to FHB and crown rot under different levels of Zn application (Table 1). Wheat genotypes were grown in 15cm plastic pots filled with potting mixture comprising 8 parts composted pine bark and 2 parts coarse sand. Three levels of Zn treatment were imposed: 0, 1 & 2 g/kg of soil applied as pellets one week after planting. The pots were then placed in a greenhouse set at 18 to 20°C.

Pathogenicity test

One monosporic isolate of Fusarium pseudograminearum was tested for pathogenicity after one week of germination. Seedling inoculation was conducted using an agar plug of 5 mm diameter infected with actively growing Fp mycelium placed as close as possible to the root system. The soil surface of the pot was then covered with a thin layer of wheat bran to promote infection. Control plants were treated similarly but without fungal inoculation. Crown rot symptoms were evaluated and recorded 90 days post-planting. The disease severity was recorded according to the following scale:

1 = slight brown discoloration of the upper root system
2= moderate brown discoloration of two-thirds or less of the upper
3 = extreme brown discoloration of the upper root system and numerous necrotic lesions extending up the crown and stem
4 = Plant death
A score of 1 < 3 was considered resistant to moderately resistant and 3 – 4 susceptible.
Symptoms for FHB were scored at maturity using a scale 0-4 where 0 = no disease expression and 4 highly susceptible.

Protein profiling of wheat different cultivars in response to Zn application and Fusarium infection

Total protein was extracted from SDS-PAGE analysis according to the TCA-acetone method (Granier, 1988). Protein concentration was determined using the Coomassie method (Bradford 1976) and total protein was separated by SDS-PAGE based on the method described by Laemmli, (1910). The amount of protein loaded for each sample was 50 pg and gels were repeated at least three times for each sample to confirm reproducibility. Gels were stained with colloidal Coomassie blue stain and examined for differences in protein expression among the different treatments for each genotype. Gels were scanned using a densitometer (Bio-Rad GS-800) and bands of interest were identified by estimating size/mass relative to the 2kD wide range protein marker.

Statistical analysis

The complete randomized design was used with four replicates for each treatment. The obtained data was analysed according to one way analysis of variance (ANOVA), the mean treatments were compared with Least Significant Difference (LSD) test at the probability level of 0.01, statistical analysis was done by using the SPSS-22 statistical software (SPSS In., Chicago, IL., USA) version 22.

Conclusion

Our results highlighted the efficiency of Zinc application at different concentrations (1 and 2 mg/ Kg soil) on Wheat responses to Fusarium head blight and Fusarium crown rot diseases in four wheat genotypes which were Batavia, Sunco, CIMMYT line 146 and CIMMYT line 48. Results revealed that Zn application led to a significant enhancement of Zn- efficient genotypes Batavia and CIMMYT line 48 to Fusarium crown rot disease compared to Sunco and CIMMYT line 146; different trend of responses were observed in Fusarium head blight disease with significant increase in the FHB tolerance of Sunco and CIMMYT 146. Protein profile analysis of different wheat genotypes to the infection with F. pseudograminearum and F. graminearum pathogens showed different patterns of protein expressions, in particular in the molecular mass range of 30-45 kDa as a response to Zn treatment at high concentration (2 mg/ Kg soil) compared with low concentration. Future research could be carried out to examine another concentrations of Zn on different wheat genotypes as well as examining the interaction between macro and micronutrients status in plant on Zn efficiency.

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