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Effect of terminal heat stress on physiological traits, grain zinc and iron content in wheat (*Triticum aestivum* L.)

Narendra M.C.[1], *Chandan Roy*[1]*, *Sudhir Kumar*[1], *Parminder Virk*[2], *Nitish De*[1]

[1]*Department of Plant Breeding and Genetics, Bihar Agricultural University, Sabour, Bhagalpur, Bihar, India*

[2]*Harvest Plus, C/O International Crops Research Institute for Semi-Arid Tropics (ICRISAT), Patancheru, Telengana, India*

**Corresponding author: chandan.roy43@gmail.com*

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Abstract: Heat stress is one of the major wheat (*Triticum aestivum*) production constraints in South Asia (SA), particularly in the Eastern Gangetic Plains (EGP) of India and Bangladesh. Malnutrition is also a severe problem among children and women in SA. Wheat varieties with high grain Zn/Fe are a sustainable, cost-effective solution in the fight against hidden hunger. Thirty wheat genotypes were characterised under the optimum temperature and heat stress conditions in 2016–2017 and 2017–2018 to study the response of the stress on the yield, physiological traits and grain Zn/Fe content. A significant genetic variation was observed for all the traits under the optimum temperature and stress conditions. The yield was reduced by an average of 59.5% under heat stress compared to that of the optimum temperature. A strong positive association of the canopy temperature depression (CTD) with the grain yield (GY) was observed under the heat stress. A negative correlation of the grain Zn/Fe with the yield was observed under the optimum temperature and heat stress conditions, while the association between the grain Zn and Fe was positive. The genotypes BRW 3723, BRW 3759, BRW 3797, BRW 160, HD 2967, HD 2640 were found to be heat-tolerant in both years. Among the tolerant genotypes, BRW 934, BRW 3807 and BRW 3804 showed a high zinc content and BRW 934, BRW 3797, BRW 3788 and BRW 3807 showed a high iron content, respectively. These genotypes can be explored in future breeding programmes to address the problem of nutritional deficiency.

Keywords: abiotic stress; biofortification; canopy temperature depression; peroxidase activity

Wheat (*Triticum aestivum* L.) is one of the most cultivated cereal crops with an acreage of 220 million ha and a total production of 734.0 million t worldwide (FAO 2018). India reached a landmark achievement in the year 2018–2019 with a total wheat production of 101.2 million t out of a cultivated area of 29.5 million ha (Singh et al. 2020). However, despite the highly fertile Gangetic plain, productivity in the North Eastern Plain Zone of India is lower than the North Western Plain Zone. High temperature stress is one of the associated constraints for the lower productivity in the Eastern Gangetic Plain (EGP) of South Asia (SA), particularly of India, Bangladesh and Nepal (Joshi et al. 2007). The optimum temperatures during the anthesis and grain filling were determined to be 23 °C and 21.3 °C, respectively (Farooq et al. 2011); crop exposure to temperatures above the optimum causes a yield reduction. A high temperature, particularly during grain filling, causes premature ripening of the crop, thus, drastically reducing the grain yield (Sharma & Duveiller 2004).

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High temperatures cause a negative impact on the plant physiology and growth leading to poor seed setting and grain development (Farooq et al. 2011). Rapid chlorophyll loss under heat stress impairs the photosynthetic activity in plants. Chlorophyll stability is an important parameter to be considered while screening wheat genotypes for heat tolerance (Mohammad et al. 2008). The canopy temperature depression (CTD), as a measure of difference between temperature of plant canopy and ambient air, has been successfully used in wheat breeding (Reynolds et al. 1994, 1998). CTD has been shown to be a significant variable among the tested genotypes and positively associated with the yield under heat stress (Kumari et al. 2013; Mondal et al. 2013). Heat stress produces reactive oxygen species (ROS) and a high accumulation of ROS causes cell damage. Antioxidants play an important role in reducing the ROS developed under heat stress. An increased activity of peroxidase and catalase has been recorded under high temperature stress (Almeselmani et al. 2009; Gupta et al. 2013).

Furthermore, the wheat endosperm is a primary source of starch and proteins and a major source of micronutrients, particularly zinc and iron (Velu et al. 2015). Malnutrition is a serious problem among children and lactating women due to micronutrient deficiencies. A zinc deficiency affects human health since it causes mental retardation, poor growth and immunity (Prasad 2013). Approximately, 165 million children under the age of five years suffer from a zinc deficiency, most of them live in Asia and Africa (Velu et al. 2015). Increasing the bioavailability of Zn/Fe in the food grain would maximise the daily intake. It has been estimated that wheat can serve as 50% of the daily requirements of Zn (Kumssa et al. 2015). The development of biofortified wheat varieties can be a cost-effective and sustainable strategy to minimise nutrient deficiency.

Breeding for a high grain Zn/Fe is challenging due to the complex nature of the trait and high G × E interaction. Another limitation for the breeders is the assessment of grain nutrients for a large number of samples which requires expensive instruments, extensive sample preparations and highly trained analysts. Energy dispersive X-ray fluorescence spectrometry (ED-XRF) has been standardised as highly efficient and having a high throughput non-destructive technique and can be effectively used for the estimation of the grain Zn and Fe in wheat (Paltridge et al. 2012). With the increasing problem of adverse climatic conditions, the response of the genotype for the grain zinc and iron content under stress is not well understood. Velu et al. (2016) reported higher protein and zinc concentrations under water and heat stress environments, however, they found a higher zinc and iron yield per hectare in the non-stress environment. A detailed study on the response of non-biofortified varieties for the grain Zn/Fe accumulation under heat stress would help the breeders to develop biofortified varieties using elite genotypes. This study was conducted to establish the relationship of the grain yield with important physiological traits and the grain Zn/Fe content under heat stress and non-stress environments.

MATERIAL AND METHODS

Plant materials and experimental layout. Thirty diverse wheat genotypes comprised of eleven released varieties, seventeen advanced breeding lines and two genotypes, Chirya 3 and BAZ, from the International Maize and Wheat Improvement Center (CIMMYT), Mexico (Table S1 in the Electronic Supplementary Material (ESM)) were evaluated under the optimum temperature (sown in the 3rd week of November) and terminal heat stress condition (sown in the last week of December to expose the wheat to higher temperatures than the optimum at the post-anthesis stage). The experiments were conducted in the year 2016–2017 and 2017–2018 in a randomised complete block design with three replications at Bihar Agricultural University, Sabour, India. Each genotype was sown in a six-row plot of 4.0 m in length with a row to row spacing of 20 cm. To avoid drought stress, five flood irrigations were scheduled at the crown root initiation (21 days after sowing), maximum tillering (Zadoks' growth stage, GS 32), booting (GS 45), milk development (GS 73), and dough stage (GS 85). Besides, there were five rainy days (0.7 to 12.4 mm) in 2016–2017 and two rainy days (6.2 to 24.2 mm) in 2017–2018 (Table S2 in the ESM). The soil moisture was estimated in percentage as the difference between the fresh weight and the oven-dry weight of the soil sample taken at a 5 cm depth from the top in each block of the experiment (Table S3 in the ESM). Proper practices for weed, disease and pest management were followed to raise a healthy crop. A fertiliser dose of 150 : 60 : 40 (N : P : K; nitrogen, phosphorus and potassium) kg/ha in the optimum and 120 : 60 : 40 kg/ha in the heat stressed experiments were applied. The crop was harvested at complete maturity, the seeds were properly dried and stored at a 10–12% moisture content.

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Observations recorded. The days to heading (DH), days to maturity (DM), thousand kernel weight (TKW), soil plant analysis development (SPAD) value, CTD, peroxidase and catalase enzyme activity, grain yield per plant and grain Zn/Fe content were recorded. The SPAD value was estimated using a SPAD 502 (Minolta, Japan) at anthesis on average of three flag leaf traits and each leaf with three readings at the top, middle and bottom. The canopy temperature was measured by an infrared thermometer (FLUKE 62 Mini IR thermometer, FLUKE, China) at anthesis on full sunny days after 12.00 noon. The CTD was measured as the difference between the measured canopy temperature and the maximum air temperature of that day. The peroxidase and catalase enzyme activity in the flag leaf was estimated at anthesis following standard procedures described by Kar and Mishra (1976) and Sinha (1972), respectively.

Estimation of the Zn and Fe concentration in grain. A neatly clean sample of 10 g seeds from each variety was analysed using ED-XRF for the estimation of the Zn and Fe in ppm. The grain zinc and iron content were analysed using energy dispersive X-ray fluorescence spectrometry (ED-XRF, X-Supreme 8000, ThermoFisher Scientific, Oxford, UK) in the laboratory of Pearl Millet Breeding Program at the International Crop Research Institute for Semi-Arid Tropics (ICRISAT), Hyderabad, following the procedure described by Paltridge et al. (2012).

Statistical analysis. The analysis of variance (ANOVA) was calculated to estimate the variance components and the least significance difference (LSD) was used to test the level of significance. The statistical analyses were carried out using the software SPSS (Ver. 16.0). Pearson's correlation coefficient was determined to test the relationship among the traits. The heritability (H^2) was estimated following the formula:

$$H^2 = \sigma^2_g/(\sigma^2_g + \sigma^2_{gy}/y + \sigma^2_e/ry)$$

where:
σ^2_g – genetic variance;
σ^2_{gy} – genotype-by-year interaction;
σ^2_e – error variance;
y – the number of years;
r – the number of replications (Velu et al. 2016).

The stress tolerance index was calculated as per Fisher and Maurer (1978) as follows:

$$\text{HSI} = (1 - Y_s/Y_p)/(1 - \frac{\bar{Y}_s}{\bar{Y}_p})$$

where:
HSI – heat susceptibility index;
Y_s, Y_p – the yield of the genotype evaluated under the heat stress and non-stress conditions;
$\bar{Y}_s$, $\bar{Y}_p$ – the mean overall yield of the genotype evaluated under the heat stress and non-stress conditions, respectively.

RESULTS AND DISCUSSION

Significant genotype and environmental variations ($P < 0.05$ or 0.01) were recorded for all the traits indicating the presence of genetic differences among the genotypes and environmental effects (Table 1). A significant variation in the genotype × environment (G × E) for the DH, peroxidase and grain Zn suggesting the varying environmental conditions influence the expression of these traits; however, the variability attributed by the interaction was much smaller than the genotype and the environment. A significant variation in the G × E interaction for the grain zinc content in the optimum temperature and stress environment was supported by the findings of Velu et al. (2016). Almost all the traits were affected under stress; the maximum reduction was observed for the GY followed by the TKW (Table 1). The heading and maturity duration were reduced under stress with an average of 14 and 23 days, respectively. An average of 10 g TKW was reduced under stress compared to the optimum temperature. A negative effect of heat stress on the DH and TKW was reported in previous studies (Joshi et al 2007; Mondal et al. 2013). Most of the traits exhibited moderate to high heritability under optimum temperatures. High heritability under stress conditions was estimated for the TKW, DH and GY, hence, the effective selection of the genotypes can be made for these traits. A high heritability for the heading date, grain yield and TKW under heat stress environments was reported in an earlier study by Mondal et al. (2013).

Effect of heat stress on yield and physiological traits. In the optimum environment, during anthesis and the grain filling stage, the temperature was within the optimum temperature limit (Figure 1). However, in the stress environment, the crop experienced heat stress at anthesis to post-anthesis depending upon the maturity date of the varieties. The SPAD reading was higher in the optimum conditions than under the heat stress conditions. A significant positive correlation was found between the SPAD and the grain yield in both conditions (Table 2). A posi-

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Table 1. Variance components, pooled mean of the traits, percentage of the reduction and heritability under the optimum temperature and heat stress conditions of the wheat

Source	DF	DH	DM	TKW (g)	SPAD value	CTD (°C)	Catalase	Peroxidase	Zn (ppm)	Fe (ppm)	GY (g)
Replication (environment)	8	1.57	4.39	2.29	1.32	0.38	3.45	12.53	1.84	1.23	0.55
Varieties	29	48.42**	38.2**	24.54	8.73**	2.8*	431.62**	5 858.56**	16.02**	7.8**	6.28**
Environment	3	1 852.66**	5 461.33**	1 652.74**	193.16**	381.18**	7 544.82	205 872.57**	14.03**	23.7**	485.91
Interaction (G × E)	87	16.46*	10.60	12.38	3.62	0.73	199.98	2 499.07**	17.60**	3.77	1.78
Pooled error	232	0.99	3.26	2.46	2.14	0.43	4.90	14.84	7.67	2.17	0.42
Trait in optimum temperature		80.28**	116.49**	34.50**	56.06	4.13**	94.57**	98.06**	27.79	33.98	10.97**
Trait under heat stress		66.66**	93.38	24.19**	55.06**	4.75*	96.99**	174.13**	31.57	34.06	4.44**
Percentage change		−16.96	−19.84	−29.88	−1.77	15.01	2.26	77.57	13.62	0.24	−59.53
Heritability (optimum)		91.14	46.81	38.74	33.12	26.35	89.81	94.33	87.23	58.98	57.74
Heritability (stress)		62.52	47.66	60.47	37.17	42.53	95.6	98.59	85.92	69.507	59.25

*, ***P*-value < 0.05, < 0.01 LSD; DH – days to heading; DM – days to maturity; TKW – thousand kernel weight; SPAD – soil and plant analysis development; CTD – canopy temperature depression; GY – grain yield

tive association of the chlorophyll content with the grain yield under heat stress had been reported in earlier studies (Reynolds et al. 1994, 1998). Though a significant difference existed among the genotypes for the SPAD and CTD in both conditions, no such difference was observed for these traits between the optimum temperature and stress condition. An average of 0.62 °C higher CTD was recorded under stress than the optimum conditions. A significant positive association of the CTD with the GY was observed in both conditions. The association under stress was found to be closer than the optimum temperature as exhibited by the strong positive association in both the years under the stress conditions. A strong association of the CTD with the yield under the heat stress was reported in previous studies (Reynolds et al. 1994; Kumari et al. 2013). Reynolds et al. (1998) proposed the CTD as the selection criteria for heat tolerance genotypes. The genotypes that maintained a cooler canopy under heat stress were PBW 343, HD 2967, SW 129 and SW 139 (Table S4 and S5 in the ESM).

A significant correlation of the catalase and peroxidase enzyme activity with the grain yield was recorded under the stress conditions. The peroxidase activity was increased under the heat stress, which indicated an increased level of thermo-tolerance. The high temperature stress under a controlled environment stimulated the catalase and peroxidase activity in wheat (Almeselmani et al. 2009; Gupta et al. 2013). The genotypic response for the expression of the catalase and peroxidase activity under stress and non-stress environments varied. A significant genetic variability for antioxidants among the tested genotypes was reported by Gupta et al. (2013). The

Table 2. Pearson's correlation coefficient of the physiological, biochemical and nutritional traits with the wheat grain yield

Traits	Optimum temperature		Heat stress	
	2016–2017	2017–2018	2016–2017	2017–2018
SPAD value	0.255*	0.268*	0.235*	0.468**
CTD (°C)	0.349*	0.116	0.791**	0.626**
Catalase	0.240*	−0.248*	0.237*	0.034
Peroxidase	0.279**	−0.230*	0.297**	0.258*
Zn (ppm)	0.205	0.044	−0.28**	−0.203
Fe (ppm)	0.361**	−0.231*	−0.399**	−0.075

*, ***P*-value < 0.05, < 0.01 LSD; SPAD – soil plant analysis development; CTD – canopy temperature depression

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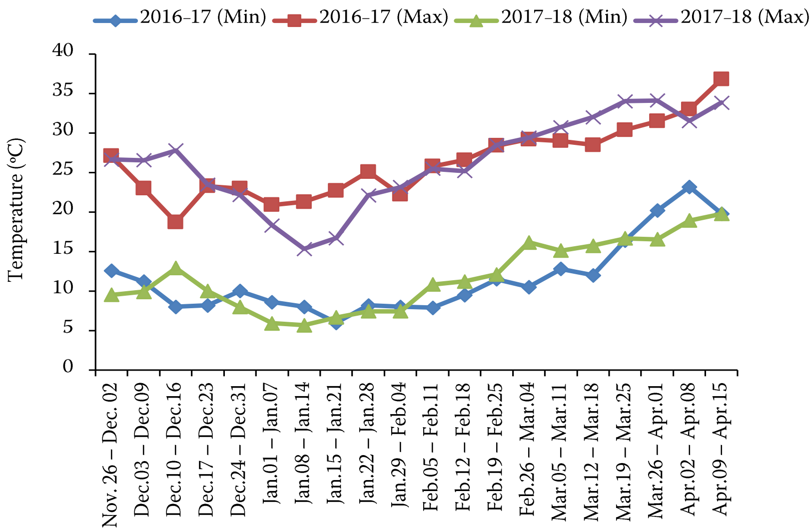

Figure 1. Weekly minimum and maximum temperature (°C) in the growing season of 2016–2017 and 2017–2018

genotypes which showed enhanced catalase activity under heat stress were HD 2643, PBW 343, HI 1563 and SW 129. The genotypes with enhanced peroxidase activity under heat stress were SW 161, SW 139, BRW 3723, HI 1563 and BRW 3807.

An average of a 59.3% grain yield reduction was detected under heat stress. The high heritability for the yield indicated the potential variation among the tested genotypes. The GY reduction under a post-anthesis heat stress was reported in earlier studies (Pradhan et al. 2012; Mondal et al. 2013). Joshi et al. (2007) reported a 45.05% yield reduction under late sown wheat varieties. Late sowing in February compared to November, at Obregon, Mexico resulted in a 3.0 t/ha yield loss (Mondal et al. 2013). The high yielding genotypes under heat stress in 2016–2017 were BRW 3800, BRW 3797, BRW 3790, HD 2643 and, in 2017–2018, they were SW 129, HD 2967, SW 103, BRW 3759, DBW 14 (Table S6 in the ESM). However, variable performance of the genotypes PBW 343, HD 2643, SW 103 under the optimum temperature and BRW 759, HD 2967, BRW 934 under the heat stress environments was observed. A strong significant G × E interaction for the yield under heat stress has been documented earlier (Joshi et al. 2007; Mondal et al. 2013). The heat susceptibility index (HSI) revealed that the genotypes BRW 934, BAZ, Chirya 3, HUW 234, BRW 3807 were heat tolerant in 2016–2017, while SW 103, SW 129, BRW 3797, HD 2967 and BRW 3723 were heat tolerant in 2017–2018 (Table 3). Previous findings also reported the genotypes DBW 14, BAZ, HUW 468 and HUW 234 as tolerant to heat stress (Joshi et al. 2007; Nagar et al. 2015). The genotypes BRW 3723, BRW 3759, BRW 3797, BRW 160, HD 2967, HD 2640 appeared to be heat tolerant in both years and these might be useful as parents for the development of new varieties.

Table 3. A comparison of the genotypes for the heat stress index (HSI) under the optimum temperature and stress conditions of the wheat

HSI range	2016–2017	2017–2018
0–0.5	–	–
0.5–0.75	SW 103, SW 129, SW 161, BRW 3788, BRW 3794, BRW 3762, HD 2733, RAJ 3765	BRW 3807, BRW 3790, BRW 3794, BRW 3762, BRW 3800, BRW 3768, BRW 967, HD 2733, HI 1563, CHIRYA 3, HUW 234, DBW 14, RAJ 3765, PBW 343, HUW 468, BAZ, BRW 934
0.75–1.0	BRW 3807, BRW 3797, SW 160, SW 139, SW 108, BRW 3790, BRW 3759, BRW 3800, BRW 3768, BRW 3723, HD 2967, HI 1563, CHIRYA 3, HUW 234, DBW 14, HD 2643, PBW 343, HUW 468, BAZ, BRW 934	BRW 3797, SW 103, SW 129, SW 161, SW 160, SW 139, SW 108, BRW 3788, BRW 3759, BRW 3804, BRW 3723, HD 2967, HD 2643
> 1.0	BRW 3804, BRW 967	–

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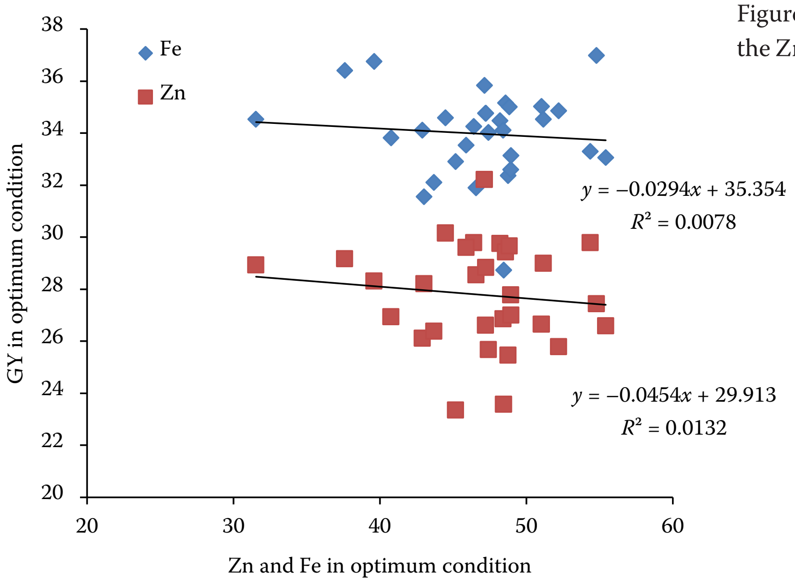

Figure 2. Relationship of the grain yield (GY) with the Zn and Fe content in the optimum conditions

Effect of heat stress on grain Zn and Fe content. The concentration of the grain Zn and Fe varied under the optimum temperature and stress environments. In the optimum condition, the grain zinc content varied from 25.00 ppm (BRW 3804) to 37.33 ppm (BRW 934) in 2016–2017 and 17.15 ppm (PBW 343) to 29.70 ppm (BRW 3768) in 2017–2018. While the iron content varied from 29.17 ppm (PBW 343) to 36.67 ppm (BRW 3797) in 2016–2017 and 25.65 ppm (PBW 343) to 39.80 ppm (BRW 3768) in 2017–2018. When the genotypes were grown under stress conditions, the zinc estimation varied from 28.33 ppm (SW 108) to 41.00 ppm (BRW 3804) in 2016–2017 and 24.05 ppm (SW 103) to 34.95 ppm (BRW 3790) in 2017–2018, while the iron content varied from 29.83 ppm (Chirya 3) to 36.67 ppm (BRW 3807 and BRW 934) in 2016–2017 and from 31.90 ppm (SW 103) to 38.70 ppm (BRW 3797) in 2017–2018. The zinc concentration was higher under the heat stress when compared to the optimum temperature (Table S7 in the ESM). The increased Zn concentration under stress may be due to the reduction in the grain size, which changed the aleurone to endosperm ratio. However, the iron content in the grain did not change much under the heat stress when compared to the optimum conditions. Velu et al. (2016) reported a higher grain Zn concentration under heat and drought stress when compared to

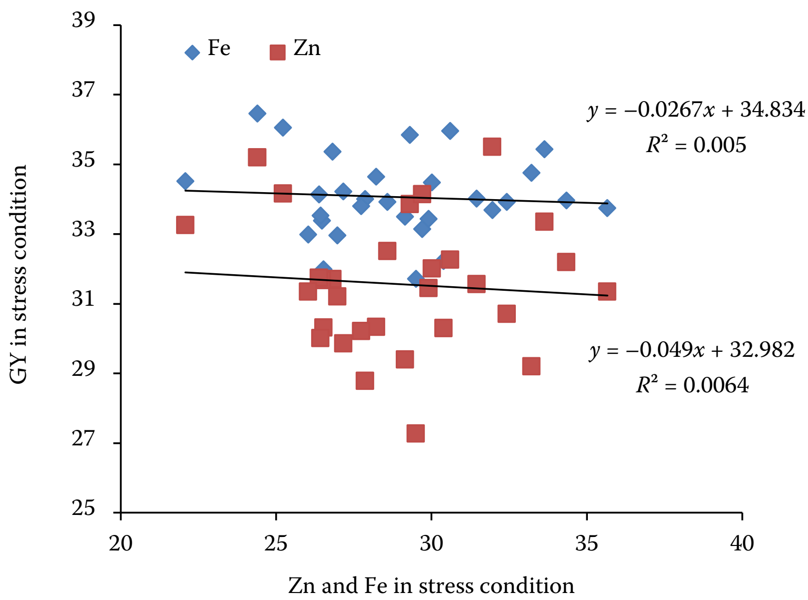

Figure 3. Relationship of the grain yield (GY) with the Zn and Fe content in the stress conditions

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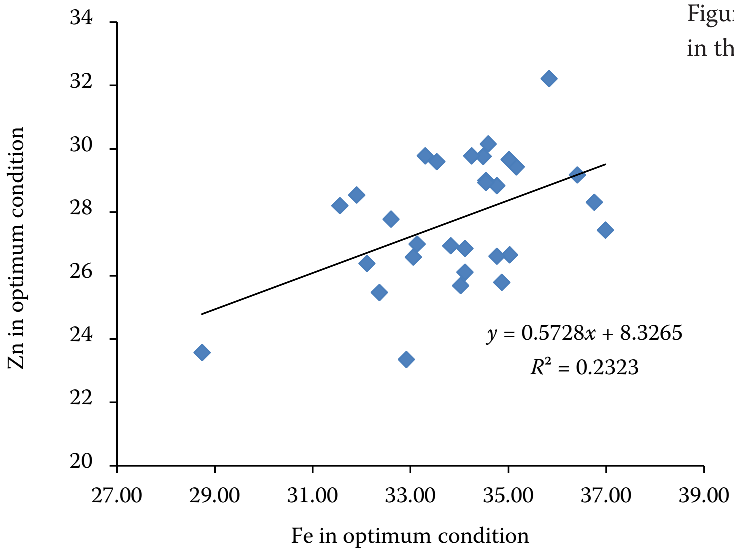

Figure 4. Relationship of the grain Zn and the Fe content in the optimum conditions

the optimum conditions, despite this fact, some of the genotypes, namely BRW 3788, BRW 3807 and BRW 934, showed comparatively high concentrations of zinc and iron under stress and non-stress environments. A negative, but relatively weak, association of the grain zinc and iron content with the yield was recorded in both the conditions (Figure 2 and 3). In an earlier study, Velu et al. (2016) explained the negative correlation for the grain Zn with the yield by the increased grain size and number in the high yielding varieties. Crosses of locally adapted high yielding varieties with a Zn donor would be helpful for high yielding and Zn rich variety development. A positive association was observed between the grain zinc and iron content in both conditions (Figure 4 and 5). The genotypes BRW 934, HI 1563, BR 3804, BRW 3788, BRW 967 with a high concentration of Zn and BRW 3788, SW129, SW 161, BRW 934 with a high concentration of Fe would be useful for further improvement through breeding programmes.

In conclusion, the present study indicated the substantial genetic variability among the tested genotypes for the GY, SPAD reading and peroxidase enzyme activity under the heat stress. A strong association of the CTD with the GY under the heat stress suggested the use of the trait in the selection of heat tolerant genotypes. Most importantly, a positive association between the grain Zn and the Fe content and a significant variability for the grain Zn/Fe content among the genotypes can be further explored for the development of nutrient rich wheat varieties.

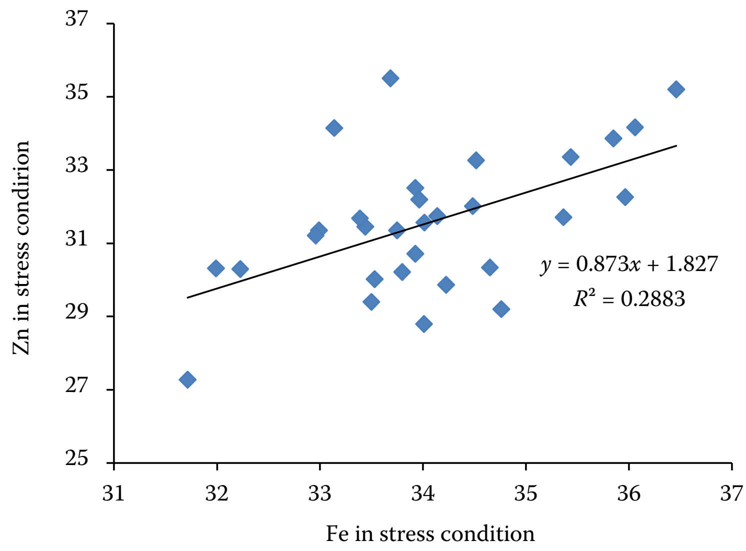

Figure 5. Relationship of the grain Zn and the Fe content in the heat stress conditions

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