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Variation in Zinc, Iron and Quality Parameters in Wheat Lines at Different Sowing Locations

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ABSTRACT

Fifty lines of bread wheat representing 5th Harvest Plus Yield Trial were grown at three sites to identify genetically determined differences in micronutrient concentrations. Zn concentration showed positive and significant correlation with Fe concentration. Both Zinc (Zn) and Iron (Fe) concentrations correlated positively and significantly with grain protein, test weight and grain appearance score. Correlation of phenolic reaction score with Fe concentration was strong but with Zn concentration was week. Both Zn and Fe concentrations correlated negatively and significantly with days to heading, thousand grain weight, SDS (Sodium dodecyl sulphate) sedimentation value and grain yield. Both Ze and Fe concentrations showed negative direct effect on grain yield. Analysis of variance showed significant differences between genotypes, locations and interactions between genotypes and locations for grain Fe and Zn. 5th HPYT entry 409 had the highest mean Zn concentration (50.17 ppm), followed by 449(48.63 ppm), 412(48.21 ppm), 443(47.58 ppm) and 404(47.27 ppm). In case of Fe mean concentration, HPYT entry (449) had the highest value (53.81 ppm), followed by 409(51.71 ppm), 412(50.74 ppm), 436(49.25 ppm) and 443(49.25 ppm). Mean data from three locations showed one entry (417) yielded the more than the mean of the check (104%), while entry (410) yielded 98% of the check. Pooled data across locations showed an increment of 20.3% (50.17 ppm) over the check (41.71 ppm) for Zn. Eleven entries showed more than half of the target Zn (4 ppm) and 2 entries with more than full target Zn (8 ppm). The results from this study are useful for developing micronutrient biofortification strategies.

Key words: *Wheat Biofortication, Grain Fe and Zn, Grain Yield, Grain Protein*

INTRODUCTION

More than three billion people are affected by micronutrient malnutrition, a serious health problem worldwide[16,33]. The most widespread deficiencies of micronutrients are Fe, Zn, vitamin A and I, which occur particularly among women and children in the developing countries[35]. About one-third of the world's population is affected with each Fe and Zn deficiencies[15,35]. Number of people and the proportion of global population suffering from micronutrient malnutrition have increased in the last four decades[14,34].

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Two reasons might be caused this distressing trend. First, cultivation of pulse crops in South Asia has decreased compare to high yielding varieties of rice and wheat in the last 2-3 decades. This trend may be a main causative factor to the rise in micronutrient deficiency in South Asia because edible legume seeds are better source of micronutrients than cereals, especially after the cereal grains have been processed before consumption[34]. Second, new plant breeding has been mainly focused to high agronomic yield rather than the nutritional quality[23]. Increased grain yield may have resulted in a low quantity of minerals in grain, though facts for this existing up to now are ambiguous[12,13,20,26]. Biofortification, which aims to improve micronutrient concentrations and bioavailability in plant based foods through genetic enhancement, is a cost effective way of solving the micronutrient deficiency problem[3,24]. Micronutrient fertilizers are required to meet biofortification targets, where micronutrient concentrations in foods are low because of the soil supply[5,14].

For humans, cereals are the main resource of micronutrient minerals. Knowledge of the difference in the trait among the available germplasm is required for breeding of cereal crops with improved micronutrient concentration. Many trials screening wheat germplasm for mineral concentrations have been reported, indicating the existence of considerable variation in micronutrient concentrations in grain[13,17,22,30].

Farmer's acceptable varieties and which are commercially competitive should have acceptable to millers, manufacturers and consumers and acceptable processing quality. For instance, in South Asia where chapatti is the main local unleavened flat bread, biofortified wheat products should have medium-to-hard grain texture, as well as extensible and medium-strength gluten, to produce flat bread with acceptable (long-lasting) textural characteristics. Lines showing superior dough extensibility combined with medium-to high gluten strength can also be used for products such as yeast leavened bread, thereby promoting small-to-intermediate-scale local industry. A strong positive correlation between grain Zn and protein content was shown in several studies[10,22,30] suggesting that breeding for high Zn concentration may not affect processing and end-use quality. Conversely, Morgounov *et al*.[22], reported a significant negative correlation between glutenin content and Zn and Fe concentrations among central Asian wheat varieties; however, more studies are needed to define this relationship wheat germplasm.

The main objective of the present study was to investigate the variation in the micronutrient concentrations in wheat grains among 50 bread wheat of HPYT trial to represent a wide range of diversity in the gene pool available for plant breeders. The second objective was to explore the relationships between micronutrient concentrations and other agronomic or grain quality traits.

MATERIAL AND METHODS

Plant material

Fifty lines of bread wheat (*Triticum aestivum* var. aestivum) including one control cultivar WH 1105 were grown as 5th HPYT at three sites in north west India (Ludhiana, Bathinda, Gurdaspur) during 2014-15 crop season. Each line was sown in two replicate plots of 5 metre long with six rows spaced at a distance of 20 cm. Recommended package of practices to raise a good crop was followed. Observations were recorded on days to heading, 1000 grain weight (gm) and grain yield (kg/plot).

Grain analysis

The concentration of elements Fe and Zn in wheat grains was determined using a bench-top, non-destructive, energy-dispersive X-ray fluorescence spectrometry (EDXRF) instrument (model X-Supreme 8000, Oxford Instruments plc, Abingdon, UK), previously standardized for high throughput screening of Zn and Fe in whole wheat grain[28]. The grain protein content was estimated using the whole grain analyzer, Infratec 1241 supplied by M/S Foss Analytical AB, Sweden. Test weight was determined using the apparatus developed by the Directorate of Wheat Research, Karnal, India, which employs a standard container of 100cc[21]. Grain appearance score was evaluated subjectively out of a maximum score of ten, giving due weightage to the grain size (3), shape (2), colour (2) and luster (3). The phenol reaction score (PRS) was evaluated by treating about 100 grains soaked overnight, with 1% phenol solution for four hours. The grains are evaluated for extent of darkness out of a score of 10, half an hour after draining off the phenol solution. The SDS sedimentation value of wholemeal samples was determined by employing the method given by Axford et al[2].

Data analysis

The data were analyzed for variability, divergence, correlation and path coefficient study. Correlation and regression analysis were performed on the wheat data from three locations. Analysis of variance (ANOVA) was calculated by Factorial Randomized Block Design analysis on multiple environment trials to evaluate the significance of the differences between lines.

RESULTS

Estimate of genetic variability

Genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability (h^2) and genetic advance as percent of mean (GA) are given in Table 1. PCV and the GCV were near to close for days to heading, grain protein, test weight, grain appearance score and phenolic reaction score. High estimates of PCV were observed in 1000 grain weight, Zn and Fe concentrations, SDS sedimentation value and grain yield as compared to GCV. PCV in Fe and Zn concentrations was observed as 11.81 and 8.75 and GCP was observed as 0.42 and 2.66, respectively. High heritability was recorded for grain protein, test weight, grain appearance score and phenolic reaction score. Zn concentration had high heritability (9.27) than Fe concentration (0.12) and these were lowest heritability values among all traits recorded. High GA was recorded for days to heading and test weight.

Genotypic and phenotypic correlation coefficient

Correlation analysis showed that Zn concentration correlated highly significantly ($p < 0.001$) with Fe concentration (Table 2). Both Zn and Fe concentrations correlated positively and significantly with grain protein, test weight and grain appearance score. Correlation of phenolic reaction score with Fe concentration was strong but with Zn concentration was week. Both Zn and Fe concentrations correlated negatively and significantly with days to heading, thousand grain weight, SDS sedimentation value and grain yield. Apart from Fe and Zn concentrations, days to heading exhibited a negative and significant correlation with grain yield. All other traits showed positive correlation with grain yield. 1000 grain weight, grain protein and test weight showed significantly positive correlation with grain yield but correlation of grain yield with GAS, PRS and SDS sedimentation value was weak.

Path coefficient analysis for direct and indirect effects on grain yield

Partitioning of the total correlation coefficient into direct and indirect effects for grain yield per plot showed a positive direct effect of many yield contributing traits like 1000 grain weight, grain protein, test weight, phenol reaction score and SDS sedimentation value (Table 3). Traits like days to heading, Fe and Zn concentrations and GAS showed negative direct effect on grain yield. Traits like days to heading, 1000 grain weight, grain protein, test weight, phenolic reaction score and SDS sedimentation value contributed more positive indirect effects than negative indirect effects. Traits like Zn concentration and GAS showed more negative indirect effects.

Analysis of variance

The concentrations of two minerals (Zn and Fe), grain protein, SDS sedimentation value, phenol reaction score and grain appearance score in grain were determined. Among the 50 bread wheat lines, Fe concentration varied by 1.4-fold, ranging from 38.70 to 53.81 ppm and grain Zn by 1.3-fold, from 37.37 to 50.17 ppm (Table 4). Other traits like days to heading

(96-109), 1000 grain weight (31.0-43.3), grain protein (10.1-14.0), test weight (72.1-79.6), grain appearance score (4.7-6.1), phenolic reaction score (1.1-4.5), SDS sedimentation value (39.8-58.8) and grain yield (42.1-65.0 q/ha) had wide range of mean values. 5th HPYT entry 409 had the highest mean Zn concentration (50.17 ppm), followed by 449(48.63 ppm), 412(48.21 ppm), 443(47.58 ppm) and 404(47.27 ppm). In case of Fe mean concentration, HPYT entry (449) had the highest value (53.81 ppm), followed by 409(51.71 ppm), 412(50.74 ppm), 436(49.25 ppm) and 443(49.25 ppm). Analysis of variance showed a highly significant difference between wheat lines and between locations for traits days to heading, 1000 grain weight, grain Fe and Zn concentrations, grain protein, test weight, grain appearance score, phenol reaction score, SDS sedimentation value and grain yield (Table 5). Interaction between wheat lines and locations were significantly differing for traits days to heading, 1000 grain weight, grain Fe and Zn concentrations. Mean data from three locations showed one entry (417) yielded the more than the mean of the check (104%), while entry (410) yielded 98% of the check. Entry 417 showed an increment of 18.0% (61.7 q/ha) over the check (52.3 q/ha). Pooled data across locations showed an increment of 20.3% (50.17 ppm) over the check (41.71 ppm) for Zn. Eleven entries showed more than half of the target Zn (4 ppm) and 2 entries with more than full target Zn (8 ppm). (Table 4). For Fe, an increment of 30.77% (53.81 ppm) was observed over the check (41.15 ppm).

DISCUSSION

Perusal of data revealed that PCV and the GCV were close for most of the traits except 1000 grain weight, Fe and Zn concentrations, SDS sedimentation value and grain yield, indicating primarily the genetic control for these traits rather the environment effect alone. Also high estimates of GCV and PCV were observed in phenolic reaction score, suggesting that selection based on these characters would facilitate successful isolation of desirable types. GCV in Fe and Zn concentrations were observed as low, suggesting that selection based on these characters would not successful isolation of yield trait. However, the genetic variability together with heritability estimates would give a better idea on the amount of GA expected from selection[4]. Days to heading and test weight had low GCV values indicating little scope of genotypes for improvement in these traits.

Traits having high heritability and high genetic advance are supposed to be under control of additive genes; hence, these can be improved by selection based on phenotypic performance[1,7,11]. Traits like grain protein, grain appearance score and phenolic reaction score had high h^2 but low values of GA suggesting the involvement of non-additive gene action in their inheritance. Traits like test weight showed high heritability coupled with high PCV suggesting greater scope for selection of these traits on analytical basis.

Grain Fe and Zn concentrations are complex characters controlled by several components which reflect positive and negative effects on these traits. Thus, for achieving rational improvement in grain Fe and Zn concentrations and its components, knowledge of mechanism of association, cause and effect relationship provides a basis for formulating suitable selection methods for these components. Results indicate that 1000 grain weight, grain protein and test weight had positive (significant) correlation and GAS, phenol reaction score and SDS sedimentation value had positive (non-significant) correlation at genotypic as well as phenotypic level with grain yield (Table 2) and the selection based on this trait will result in improving the seed yield in wheat.

Both grain Fe and Zn concentrations correlated positively with grain protein content among 50 lines of wheat. This result is similar with other wheat genotypes trials and indicates a feasible link between grain protein and the concentrations of the two trace elements[22,30]. Other studies have shown that the Gpc-B1 (Grain protein content-B1) locus of wild wheat affects both grain protein content and the concentrations of Fe and Zn in grain[8]. This locus encodes an NAC transcription factor (NAM-B1) that accelerates senescence and enhanced remobilization of nutrients (N, Fe and Zn) from leaves to developing grains[31]. The wheat genome contains three NAM genes, but modern wheat varieties carry a non-functional NAM-B1 allele, which causes delayed leaf senescence and low levels of grain protein, Fe and Zn in modern wheat varieties compared with wild emmer wheat. The positive relationship between grain protein, Fe and Zn concentrations may be a pleiotropic effect of NAM genes[5]. The positive correlations between Fe and Zn concentrations and protein content would be helpful information for wheat breeders, and further studies included a larger set of modern varieties with high protein content[36].

Wheat lines producing lower grain yields may contain higher levels of trace elements and this is because of concentration-dilution effect by the dry matter accumulated in grain. It is not helpful in the breeding for biofortification of high-yielding wheat. It is reported in some studies that grain yield and trace element concentrations were correlated negatively[12,19,22,26,36]. It is also found that increasing grain yield by N fertilisation was resulted in high micronutrient concentrations in wheat grain[20]. Zhao *et al*.[36], showed that higher yielding varieties contained significantly lower levels of Zn in grain than old varieties. In present study a significant negative relationship was found between grain yields and both Fe and Zn concentration (Table 2), which is similar with the results reported Zhao *et al*[36]. Garvin *et al.*[12], and Zhao *et al*[36]., also showed that a significant decreasing trend in grain Zn concentration with the release date of the 26 wheat lines tested in the multiple site trials. This means that improved grain yield as a result of genetic enhancement may have resulted in decreasing Zn concentration in grain. The negative effect of yield enhancement was less visible on grain Fe concentration. One other study has shown a decreasing pattern in grain mineral concentrations (including Zn, Cu, Mn, Mg) in the 160-year history of the Broadbalk Experiment in England, with the reduce coinciding with the introduction of the short-straw, high-yielding modern varieties of wheat[9] (Fan et al. 2008). It is significant to break this negative relationship and to breed genotypes that contain high levels of trace elements without any yield penalty, which help in biofortification programes.

Aleurone layer and germ of the wheat grain, which are separated as the bran part during milling contain majority of the trace elements like Fe and Zn[18,25,27,29,36]. Therefore, it was thought that the concentrations of Fe and Zn in the whole grain may associate negatively with grain size, because larger grain would have a relatively smaller bran portion. Though Because of this localization pattern, the correlation among grain size and grain Fe and Zn concentrations was either weak or not significant, indicating that smaller grain does not necessarily lead to smaller trace element concentrations (Table 2). This finding is also consistent with study of McDonald *et al*[19]. It indicates that yield components other than the thousand grain weight must be contributing to the negative relationship between yield and trace element concentration. Calderini and Ortiz-Monasterio[6] reported that the concentrations of Zn and Fe reduced at grain positions more distal from the rachis within the ear. Therefore, a higher number of grains per ear may be associated with low average concentrations of these minerals.

Positive genotypic correlation values of traits like 1000 grain weight (2.38), grain protein (0.18), test weight (0.24), phenolic reaction score (0.04) and SDS sedimentation value (0.12) with grain yield and their high direct positive effect values on grain yield i.e. 0.4586, 0.0054, 0.1417, 0.0070 and 0.0039, respectively, indicated a true picture of association between these traits (Table 2, 3).

It is evident from the range of mean values for different traits among the genotypes being evaluated that these had diverse genetic background (Table 4). The range of Fe and Zn concentration in this trial was similar to that reported by Graham *et al*.[13], Morgounov *et al*.[22], and Zhao *et al*[36]. Analysis of variance showed a highly significant difference between wheat lines and between locations for traits days to heading, 1000 grain weight, grain Fe and Zn concentrations, grain protein, test weight, grain appearance score, phenol reaction score, SDS sedimentation value and grain yield (Table 5). Interaction between wheat lines and locations were significantly differing for traits days to heading, 1000 grain weight, grain Fe and Zn concentrations. Nutritionists have established that in South Asia the target is to increase Zn and Fe levels by 8 and 25 mg/kg, respectively, above baseline mega-varieties such as ‘PBW343’, grown on 8 million ha in India, and Pakistani mega-variety ‘Inqalab 91’, which possess about 25 mg/kg Zn and Fe. This translates into total Zn and Fe levels in the grain of 33 and 50 mg/kg, respectively. The absolute target level for Fe is significantly higher than for Zn, due to lower bioavailability of Fe as compared to Zn[32]. The range of Fe and Zn concentration in this trial was similar to that reported for 27 genotypes tested in the US[30], 132 genotypes tested by CIMMYT[13] and 66 genotypes tested in central Asia[22] and 150 genotypes tested in Hungary[36]. In general, grain Fe concentration showed greater variation across genotypes than Zn. Identifying the best lines with wider adaptation across locations is highly important, but identifying the best site-specific lines (adapted to favorable environment) is also essential for promoting candidate varieties to specific environments[32].

Table 1: Estimates of genetic parameters for different traits in wheat

Characters	h^2 (%)	GA	%GA	PCV	GCV	GM	CV
Days to heading	45.92	3.24	3.12	3.30	2.24	103.81	2.43
Thousand grain weight	13.95	1.16	3.06	10.66	3.98	37.89	9.88
Zn	9.27	0.73	1.67	8.75	2.66	43.86	8.34
Fe	0.12	0.01	0.03	11.81	0.41	45.49	11.81
Grain protein	75.86	1.52	12.68	8.12	7.07	11.96	3.99
Test weight	99.72	3.13	4.14	2.02	2.01	75.65	0.11
Grain appearance score	99.98	0.60	11.34	5.51	5.51	5.33	0.08
Phenolic reaction score	100.00	1.35	46.63	22.64	22.64	2.89	0.04
SDS sedimentation value	24.24	0.17	6.23	12.48	6.14	2.71	10.86
Grain yield	24.24	0.17	6.23	12.48	6.14	2.71	10.86

h^2= heritability (broad sense); GA= Genetic advance; %GA= Genetic advance as percentage of mean; PCV= Phenotypic coefficient of variability; GCV= Genotypic coefficient of variability; GM- Grand mean

Table 2: Genotypic and phenotypic correlation coefficients among various traits of wheat

Characters		Days to heading	Thousand grain weight	Zn	Fe	Grain protein	Test weight	Grain appearance score	Phenolic reaction score	SDS sedimentation value
Thousand grain weight	G	-0.27**								
	P	0.03								
Zn	G	-0.49**	-1.08**							
	P	-0.05	-0.01							
Fe	G	1.74**	-8.32**	35.18**						
	P	-0.17*	0.01	0.22**						
Grain protein	G	-0.33**	0.18*	0.19*	1.21**					
	P	-0.22**	0.02	0.14	-0.10					
Test weight	G	-0.03	0.28**	0.66**	3.94**	-0.09				
	P	-0.02	0.11	0.20*	0.14	-0.07				
Grain appearance score	G	0.15	0.19*	0.27**	3.41**	-0.42**	0.73**			
	P	0.10	0.07	0.08	0.12	-0.37**	0.72**			
Phenolic reaction score	G	-0.37**	0.14	0.15	0.43**	0.02	-0.24**	-0.15		
	P	-0.25**	0.05	0.05	0.02	0.02	-0.24**	-0.15		
SDS sedimentation value	G	0.12	0.20*	-0.38**	-3.88**	0.34**	-0.04	-0.20*	0.05	
	P	-0.08	0.08	-0.12	-0.13	0.30**	-0.04	-0.20*	0.05	
Grain yield	G	-0.17*	2.38**	-0.60**	-6.63**	0.18*	0.24**	0.11	0.04	0.12
	P	-0.05	0.47**	-0.11	0.00	0.02	0.12	0.05	0.02	0.06

Critical value of ‘r’ at 5%=0.16 and that at 1%= 0.21; G= genotypic correlation coefficient; P= phenotypic correlation coefficient

Table 3: Phenotypic path coefficient analysis for direct (bold) and indirect effects on grain yield/plant in wheat

Characters	Days to heading	Thousand grain weight	Zn	Fe	Grain protein	Test weight	Grain appearance score	Phenolic reaction score	SDS sedimentation value
Days to heading	**-0.0640**	0.0151	0.0063	0.0004	-0.0012	-0.0029	-0.0063	-0.0018	0.0003
TGW	-0.0021	**0.4586**	0.0009	0.0000	0.0001	0.0149	-0.0043	0.0004	0.0003
Zn	0.0030	-0.0031	**-0.1325**	-0.0005	0.0008	0.0285	-0.0049	0.0003	-0.0005
Fe	0.0109	0.0062	-0.0290	**-0.0021**	-0.0006	0.0193	-0.0071	0.0001	-0.0005
Grain protein	0.0142	0.0069	-0.0185	0.0002	**0.0054**	-0.0105	0.0222	0.0001	0.0012
Test weight	0.0013	0.0483	-0.0266	-0.0003	-0.0004	**0.1417**	-0.0438	-0.0017	-0.0002
Grain appearance score	-0.0067	0.0330	-0.0108	-0.0002	-0.0020	0.1026	**-0.0604**	-0.0010	-0.0008
Phenolic reaction score	0.0162	0.0244	-0.0062	0.0000	0.0001	-0.0334	0.0090	**0.0070**	0.0002
SDS sedimentation value	-0.0053	0.0344	0.0155	0.0003	0.0016	-0.0059	0.0122	0.0003	**0.0039**

Table 4: Mean of locations for various traits under study of 50 wheat lines

Entry	Days to heading	Thousand grain weight	Zn	Fe	Grain protein	Test weight	Grain appearance score	Phenolic reaction score	SDS sedimentati on value	GY (q/ha)	% local Check
401	105	43.33	41.71	41.15	11.8	76.1	5.8	4.1	54.8	62.43	100
402	104	37.33	40.73	41.48	11.2	77.1	5.6	3.1	47.8	52.68	84
403	103	40	37.37	41.28	12.2	74.6	5.3	3.7	54.8	52.83	85
404	96	37.67	47.27	48.56	14.0	76.6	5.0	3.6	47.8	49.18	79
405	103	41	41.98	42.94	12.0	76.1	5.3	2.9	52.8	55.93	90
406	108	43	43.78	46.46	12.0	75.1	5.2	3.9	46.8	58.99	94
407	101	36.33	42.97	44.46	12.4	76.6	5.5	3.9	51.8	51.59	83
408	102	41	46.17	48.32	12.8	75.1	5.2	3.1	53.8	54.89	88
409	101	38.67	50.17	51.71	13.5	79.1	5.6	2.9	46.8	54.89	88
410	103	42.33	40.29	38.7	13.4	75.1	4.9	3.1	58.8	61.27	98
411	101	38.33	44.21	46.63	13.4	74.6	5.1	2.8	50.8	50.13	80
412	105	34.83	48.21	50.74	13.3	76.1	5.2	3.3	55.8	42.88	69
413	103	36	42.59	43.87	13.3	73.6	4.9	3.2	55.8	44.28	71
414	104	37.33	45.59	48.09	13.3	75.6	5.0	3.7	54.8	48.23	77
415	103	38	40.39	39.83	12.0	74.1	5.1	2.8	46.8	50.72	81
416	101	38.67	42.67	44.15	12.3	76.6	5.5	2.7	48.8	52.31	84
417	99	43	44.17	46.88	11.3	77.1	5.3	3.1	47.8	65	104
418	104	36.67	43.3	45.28	12.8	74.1	5.3	2.9	56.8	52.02	83
419	104	31	43.3	44.31	11.5	73.6	4.9	2.9	49.8	43.28	69
420	104	36.67	44.35	47.29	12.4	72.1	4.7	2.9	50.8	53.44	86
421	103	37.33	42.88	44.26	11.3	75.1	5.3	3.0	46.8	51.14	82
422	109	37	42.08	42.66	12.3	73.6	5.1	3.0	51.8	49.22	79
423	109	39.67	43.93	46.67	10.9	75.6	5.5	2.1	46.8	54.61	87
424	108	39	41.99	43.08	11.4	75.1	5.2	1.3	48.8	54.78	88
425	106	39.17	44.83	47.98	11.9	75.6	5.4	3.3	44.8	55.55	89
426	104	38.83	43.78	45.31	12.2	75.6	5.6	3.3	39.8	54.65	88
427	99	41.33	43.59	46.38	11.1	73.6	5.5	3.2	44.8	57.43	92
428	105	38	41.16	41.65	13.1	74.6	5.6	2.8	49.8	52.41	84
429	105	38.33	44.32	47.06	12.3	75.1	5.5	2.5	48.8	51.67	83
430	103	41	42.26	43.46	12.4	73.6	5.1	2.6	54.8	57.67	92
431	104	38	42.98	44.54	12.2	77.6	5.7	1.7	49.8	52.97	85
432	104	37.33	46.28	48.39	12.2	75.6	5.2	2.8	53.8	51.38	82
433	102	39	43.88	46.48	11.6	76.1	5.4	2.3	50.8	54.13	87
434	104	36.33	43.13	45.12	11.8	75.6	5.4	3.1	58.8	45.53	73
435	105	40	46.68	48.48	11.3	79.6	6.1	2.9	55.8	54.06	87
436	103	36.33	47.25	49.25	11.6	77.6	5.9	3.1	51.8	49.39	79
437	103	36	44.01	46.78	11.0	77.6	5.8	3.1	44.8	48.37	77
438	108	36	42.88	43.42	11.9	77.1	5.6	1.3	50.8	50.24	80
439	108	35	43.41	46.1	11.7	77.1	5.6	1.1	48.8	49.32	79
440	101	35.33	41.43	42.52	11.7	75.1	5.4	2.9	50.8	47.79	77
441	103	34.33	44.33	47.12	10.2	75.6	5.7	3.5	43.8	43.33	69
442	106	38.17	41.58	40.88	10.6	77.1	5.7	3.0	55.8	52.43	84
443	103	36.17	47.58	49.25	10.3	74.6	5.1	3.5	47.8	48.78	78
444	108	40.67	43.37	45.43	10.1	76.1	5.5	3.3	49.8	55.04	88
445	108	37.67	46.83	48.85	11.6	77.1	5.7	2.7	51.8	51.43	82
446	106	36.33	41.84	42.88	11.6	75.6	5.3	2.8	50.8	47.74	76
447	104	33	45.66	48.1	10.9	72.6	5.3	4.5	46.8	42.14	67
448	105	32	46.23	47.6	12.3	75.1	5.2	2.7	46.8	43.23	69
449	101	38.33	48.63	53.81	12.4	77.1	5.8	2.5	46.8	52.05	83
450	101	37.83	43.18	45.26	11.3	77.1	5.6	2.6	46.8	50.72	81
SE±	2.74	2.64	2.41	3.11	0.89	1.53	0.29	0.65	4.09	4.92	
CD	1.31	1.00	3.01	6.70	0.71	1.17	0.68	0.07	1.38	7.83	
Mean	104	37.89	43.86	45.49	11.96	75.65	5.3	2.9	50.3	51.72	
Minimum	96	31	37.37	38.17	10.13	72.08	4.7	1.1	39.8	42.14	
Maximum	109	43.33	50.17	53.81	14.02	79.58	6.1	4.5	58.8	65	

Table 5: Analysis of variance for experimental design for various traits in wheat

Source of variation	d.f.	M S									
		Days to heading	Thousand grain weight	Fe	Zn	Grain protein	Test Weight	Grain appearance score	Phenolic reaction score	SDS sedimentation value	GY
Replications	1	367.31 **	182.51**	48.37	4.94	0.23	352.04**	6.75**	6.75**	1008.32**	411.93**
Locations (A)	2	200.35**	745.02 **	11290.58**	620.50**	7.87**	2.10	10.75**	10.75**	108.32**	13037.88**
Genotypes (B)	49	45.01**	41.71 **	57.87**	34.93**	4.75**	13.93**	0.52**	2.57**	100.22**	94.64**
AB	98	12.69**	28.06 **	57.71**	26.75**	0.46	0.01	0.00	0.00	0.00	48.12
Error	149	1.32	0.76	34.15	6.90	0.39	1.04	0.00	0.00	1.45	46.65

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