Indian J. Genet., (2022); 82(3): 289-298

doi: 10.31742/ISGPB.82.3.3

ISSN: 0975-6906

www.isgpb.org

RESEARCH ARTICLE

Studies on effects of terminal heat stress on yield stability, grain iron and zinc contents in wheat (*Triticum aestivum* L.)

Sourav Panigrahi, Yaswant Kumar Pankaj, Vishnu Kumar, Rajeev Kumar* and Santosh Kumar Singh[1]

Abstract

Considering global warming as a major constraint of yield and quality, the present study accessed the impact of terminal heat stress on wheat yield stability and grain Fe and Zn content in wheat. Twenty-three wheat genotypes of CGIAR Research Program (CRP) and two check varieties, PBW343 and HD2967 were evaluated for grain yield stability and the Fe and Zn content under heat stress conditions at terminal stage. Stability measures indicated CRP7, CRP8, CRP33, CRP46, and CRP48 to be the most stable genotypes. Grain iron (Fe) and zinc (Zn) content showed a high degree of variation. Under normal sown conditions the Fe content varied from 20.47 ppm (HD2967) to 76.07 ppm (CRP7) while the Zn content varied from 25.17 ppm (HD2967) to 65.6 ppm (CRP48). Under the stress, variation in the Fe content was observed from 10.17 ppm (PBW343) to 43.93 ppm (CRP54) whereas the Zn content variation ranged from 20.33 ppm (CRP30) to 55.13 ppm (CRP48). The overall average content of Fe was reduced by 31.98 % and Zn by 5.91% under the heat stress indicating grain Fe content to be highly vulnerable to the terminal heat stress than the Zn content.

Keywords: Wheat, terminal heat stress, yield stability, grain Zn and Fe contents

Introduction

Wheat (Triticum aestivum L.) is the second most-consumed cereal crop worldwide. It is considered as one of the sources of carbohydrates, vitamins and minerals for the human body (Shewry et al. 2015). World wheat production stands at 778.6 million metric tonnes for the year 2020–21 (https://www.statista.com). In the year 2020–2021, India achieved a landmark with an estimated total wheat production of 108.75 mt (https://pib.gov.in, Ministry of Agriculture and Farmers Welfare). The tremendously increasing world population demands a very significant increase in wheat production to feed them. Globally, the rise in temperature during the season of wheat crop is noticed which is likely due to the changes in environmental (climatic factors) conditions that affects the crop growth and productivity. Significant reduction in grain yield was reported due to increase in temperature during anthesis to maturity period (Lobell et al. 2011) however, Liu et al. (2016) estimated the loss in grain production to the tune of about 5%. India experienced an unprecedented rise in temperature during 2021–22 (March-April) wheat season that lowered the production by 5.7% (www://www.thehindu.com) and even more (www://www.timesofindia.indiatimes.com) declining the total wheat production. The productivity in the coming decades is threatened by impending climate change as the global mean temperatures are increasing at the alarming rate (Davis 2021; https.www.technologyreview.ac.uk). High-temperature stress is also one of the major constraints leading to lower productivity in the Eastern Gangetic Plain (EGP) of South Asia, particularly in India, Bangladesh, and Nepal (Joshi et al. 2007).

A stable genotype is one that can express its phenotype in any kind of environment. Therefore, for the selection of

Department of Agricultural Biotechnology and Molecular Biology, College of Basic Sciences and Humanities; Dr. Rajendra Prasad Central Agricultural University, Pusa.

[1]Department of Soil Science, College of Agriculture, Dr. Rajendra Prasad Central Agricultural University, Pusa 848 125, Bihar, India

***Corresponding Author:** Rajeev Kumar, E-Mail: rajiv.gen@gmail.com

How to cite this article: Panigrahi S., Pankaj Y.K., Kumar V., Kumar R. and Singh S.K. 2022. Studies on Effects of Terminal Heat Stress on Yield Stability, Grain Iron and Zinc Contents in Wheat (*Triticum aestivum* L). Indian J. Genet. Plant Breed., **82**(3): 289-298.

Source of support: Nil

Conflict of interest: None.

Received: Dec. 2021 **Revised:** June 2022 **Accepted:** July 2022

a genotype, there is a need to analyze the stability and genotype x environment (G x E) interaction along with average performance (Al-Otayk et al. 2010). For assessing the stability, various analyses are conducted. These analyses are focused on selecting the genotypes having the minimum G x E interaction. The analyses use mainly two types of measurements: parametric and non-parametric (Farshadfar et al. 2012). The parametric measurements make use of regression analysis (Lin et al. 1988) or partitioning of G x E interactions (Eberhart et al. 1966) to predict the stability of a genotype based on the performance of the genotype over different environmental conditions whereas additive main effects and multiplicative interaction model (AMMI) (Romagosa et al. 1993) and the genotype main effect plus G x E interaction (GGE) (Akcura et al. 2008) are the most popular among the non-parametric measurements. Both AMMI and GGE biplot analyses make use of principal component analysis (PCA).

Mineral nutrition is one of the most critical aspects of nutritional security. The deficiency of nutrition commonly leads to malnutrition among children and lactating women. More than 3 billion people worldwide are affected by zinc (Zn) deficiency whereas more than 47% of pre-school children globally show various symptoms related to iron (Fe) deficiency, such as impaired physical growth, mental growth, and learning capabilities (Thomas et al. 2010). The Fe and Zn deficiency result from the low amount of available micronutrients in cereals, which form a part of the staple diet for the people (Welch et al. 2002). Along with starch and proteins, the wheat endosperm is also a major source of micronutrients, particularly zinc and iron (Velu et al. 2015). The high variability in the nutritional content of wheat is due to several genetic as well as environmental factors (Hellemans et al. 2018). The reduction in yield and grain quality due to heat stress is a major problem in the world, most likely due to global warming (Schmidhuber et al. 2007). Therefore, the various agronomic, as well as breeding approaches have been focused on increasing world wheat production and the quality of produced wheat in terms of its nutritional value (Ortiz-Monasterio et al. 2007). Recently, various breeding approaches have been followed for increasing grain micronutrient content, as a result of which several genotypes having 20–40% more (8–14ppm) Zn content (e.g. T.SPELTA PI348449//2*PBW343*2/KUKUNA and CROC_1/AE.SQUARROSA (210)//INQALAB 91*2/KUKUNA/3/PBW343*2/KUKUNA have been introduced to farmers field (Velu et al. 2015). However, most of the varieties lack the ability to consistently produce more and better quality grains under heat stress conditions. The present study was, therefore, undertaken to enhance the understanding of the impact of heat stress on the wheat genotypes with respect to yield stability and dynamism of Fe and Zn content in grains by analysing genetic variation.

Materials and methods

Plant materials and experimentation

Twenty-five wheat genotypes selected from CGIAR Research Program (CRP) and two commercially grown check varieties, PBW343 and HD2967 were considered for the present investigation. The pedigree of the studied genotypes is provided in Supplementary Table S1. The sowing was done in Completely Randomized Block design (CRBD) at two different dates, i.e., normal sown (November) and late sown (December) under field conditions following three replications in rabi during 2015-16 and 2016-17. Row spacing was maintained at 3.5 cm x 20 cm for the experiment. Maximum and minimum temperatures for dates of sowing (DOS) and the range of maximum and minimum temperatures for days to heading, days to anthesis and physiological maturity were noted. The temperature data of rabi season for both the years was obtained from agro-meteorological station at the farm of Dr. Rajendra Prasad Central Agricultural University, Pusa, Bihar India. The grain yield was recorded in each year for both dates of sowing.

Statistical analysis

The data were analyzed by running the three-way analysis of variance (ANOVA) for yield recorded on two dates of sowing in both the years using least significant differences (LSD). The associations between genotypes were computed using linear regression. The genotypes were classified and grouped based on LSD test as given by (Gomez and Gomez 1984). Clustering of genotypes was done using Ward's minimum variance method and dendrograms were obtained for genotypes based on yield by using R software (Ward 1963).

Heat Susceptibility Index

Heat Susceptibility Index (HSI) was used to evaluate the effect of heat stress on different genotypes based on the variation in Fe and Zn content in seeds over two dates of sowing. The formula for calculating HSI was taken from Fisher & Maurer (Fischer and Maurer 1978). The formula is given as,

Heat Susceptible Index (HSI) = (1-Y/YP)/D

Where, D = 1-X/XP,

Y = Mean performance of the genotype under heat stress condition,

YP = Mean performance of the genotype under normal condition,

X = Mean performance of all the genotypes under heat stress,

XP = Mean performance of all the genotypes under normal conditions.

Stability analysis and G x E interaction

The additive main effects and multiplicative interaction model (AMMI) (Romagosa et al. 1993) was performed on grain yield under the four environments (two seasons and two dates of sowing which compose four environments). Then the genotype main effect plus G x E interaction (GGE biplot) (Akcura et al. 2008) was used to visualize the G x E interaction. The stability and G x E analysis was conducted using R (software) package GEA-R (Version 4.1, 2017, CIMMYT, El Batán, Mexico) (Pacheco et al. 2016).

Analysis of Fe and Zn contents in grain

The seeds harvested in the year 2016-17 were crushed. 0.5 g of each sample was digested by the wet digestion method (Nitric acid: Perchloric acid::3:1) and their mineral content in Parts Per Million (PPM) was calculated using Atomic Absorption Spectrophotometer. The data were analyzed by running two-way analysis of variance (ANOVA) for the Fe and Zn content data separately for the two dates of sowing and the means were compared. Then, the HSI was calculated for Fe and Zn content in grain (Fischer and Maurer 1978). Moreover, on the basis of Fe and Zn content the genotypes were represented in a dendrogram using Ward's minimum variance method (Ward 1963) by using R software.

Results

The range of maximum and minimum temperatures during different phenological growth stages of the crop planted on two different dates have indicated that the late sowing led to imposition of the heat stress on the crop (Fig. 1).

Analysis of variance and clustering based on yield

The analysis of variance of yield data recorded in each year separately showed significant difference in yield among the genotypes (Table 1). Significant G x E interaction was also

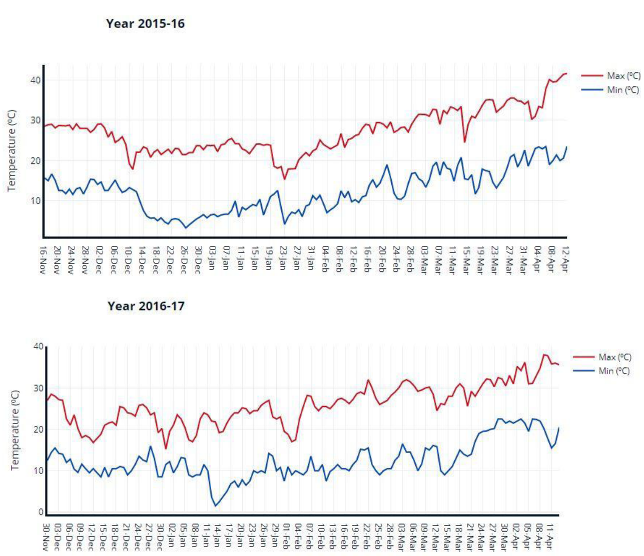

Fig. 1. Maximum and minimum temperatures recorded during the years, 2015-16 (A) and 2016-17 (B)

Table 1. Three factorial Analysis of Variance for yield

	Df	Sum of squares	Mean squares
Genotypes	24	1685613	70234***
Year	1	161379	161379***
DOS	1	22933463	22933463***
Genotypes: Year	24	2493902	103913***
Genotypes: DOS	24	934856	38952***
Year: DOS	1	2416698	2416698***
Genotypes: Year: DOS	24	559394	23308***
Residuals	200	1169627	5848

DOS= Date of sowing

Table 2. LSD t-test for genotypes mean and individual (95%) Confidence Interval

Genotypes	Yield	SD	r	LCL	UCL	Min.	Max.
CRP-15	942.5	416.5	12	899.0	986.0	550	1808
CRP-16	765.8	265.5	12	722.3	809.4	380	1212
CRP-18	1003.0	442.2	12	959.5	1046.5	430	1704
CRP-20	940.0	379.9	12	896.5	983.5	480	1400
CRP-21	819.2	306.9	12	775.6	862.7	544	1416
CRP-27	834.8	374.8	12	791.3	878.4	420	1440
CRP-30	950.0	370.6	12	906.5	993.5	572	1720
CRP-33	939.5	310.8	12	896.0	983.0	488	1350
CRP-34	917.0	263.7	12	873.5	960.5	520	1276
CRP-37	868.8	352.4	12	825.3	912.4	400	1372
CRP-40	887.7	245.3	12	844.1	931.2	520	1200
CRP-42	792.7	319.9	12	749.1	836.2	324	1184
CRP-43	850.5	338.6	12	807.0	894.0	450	1412
CRP-45	827.3	292.2	12	783.8	870.9	440	1250
CRP-46	927.3	312.3	12	883.8	970.9	560	1456
CRP-48	976.8	327.1	12	933.3	1020.4	476	1380
CRP-49	852.7	238.7	12	809.1	896.2	550	1200
CRP-50	1054.2	384.4	12	1010.6	1097.7	636	1616
CRP-51	949.8	394.6	12	906.3	993.4	516	1560
CRP-52	854.2	279.7	12	810.6	897.7	544	1392
CRP-54	822.5	318.1	12	779.0	866.0	428	1200
CRP-7	860.3	272.6	12	816.8	903.9	530	1320
CRP-8	887.7	265.9	12	844.1	931.2	552	1328
HD-2967	1018.2	349.0	12	974.6	1061.7	584	1560
PBW-343	1019.0	410.1	12	975.5	1062.5	576	1596

SD= Standard deviation; r=replicates; LCL= Lower critical limit; UCL= Upper critical limit; Min.=Minimum and Max.=Maxiumum

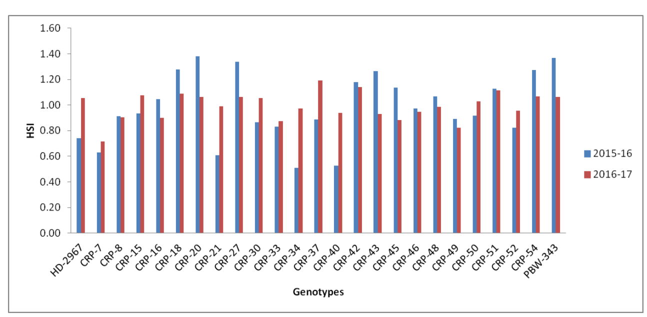

Fig. 2. Graph showing HSI based on yield of 25 genotypes for 2015-16 and 2016-17

observed. The coefficient of variance obtained was 8.474. LSD t test provided following values for the 25 genotypes based on their difference in yield during 2 years for two dates of sowing (Table 2). The critical t-value was determined to be 1.972 at 5% alpha. The least significant difference in yield among genotypes was determined to be 61.563. Taking this into account the 25 genotypes were classified into different yield groups. The different yield groups were represented with different letters, i.e., the genotypes represented with the same letter are not significantly different (Table 3). The reduction in yield was observed for all the 25 genotypes under late sown condition and was quantified into heat susceptibility indices (Table 4). The HSI of all the 25 genotypes gave positive values, revealing that their yield was affected by the heat stress due to late sown conditions. The genotype CRP-34 showed the highest tolerance to heat stress during 2015-16, whereas CRP-7 was observed to be the highly tolerant to heat stress during 2016-17. The HSI of different genotypes for both the years have been graphically represented in Fig. 2. Based on the yield of genotypes under study obtained during two years of study from sowing under normal and late conditions, the genotypes were broadly classified in to 2 clusters. One of the clusters further sub-divided in to 2 sub-clusters. Upon comparison of yield among clusters the major cluster consisting of CRP20, PBW343, CRP15, CRP46, CRP30, CRP51, HD 2967, CRP18 and CRP50 belong to the category of highly susceptible to heat stress as they show significant reduction in yield over both the years consistently having high HSI values. The second major cluster consists of the genotypes that are moderately/highly tolerant to heat stress. The sub cluster consisting of CRP16, CRP48, CRP27, CRP43, CRP7 and

Table 3. Yield groups based on genotypes

Genotypes	Mean yield	Groups*
CRP-50	1054.2	a
PBW-343	1019.0	ab
HD-2967	1018.2	ab
CRP-18	1003.0	abc
CRP-48	976.8	bcd
CRP-30	950.0	cd
CRP-51	949.8	cd
CRP-15	942.5	cde
CRP-20	940.0	de
CRP-33	939.5	de
CRP-46	927.3	def
CRP-34	917.0	defg
CRP-40	887.7	efgh
CRP-8	887.7	efgh
CRP-37	868.8	fghi
CRP-7	860.3	ghi
CRP-52	854.2	hij
CRP-49	852.7	hij
CRP-43	850.5	hij
CRP-27	834.8	hij
CRP-45	827.3	hijk
CRP-54	822.5	ijk
CRP-21	819.2	ijk
CRP-42	792.7	jk
CRP-16	765.8	k

*The genotypes represented with the same letter are not significantly different

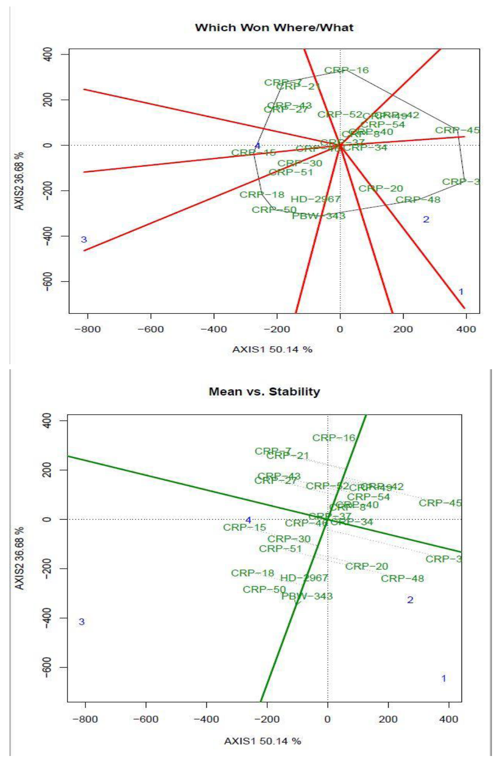

Fig. 3. Vector view of GGE biplot for identification of winning cultivars across environments (A) and their mean vs stability plot (B)

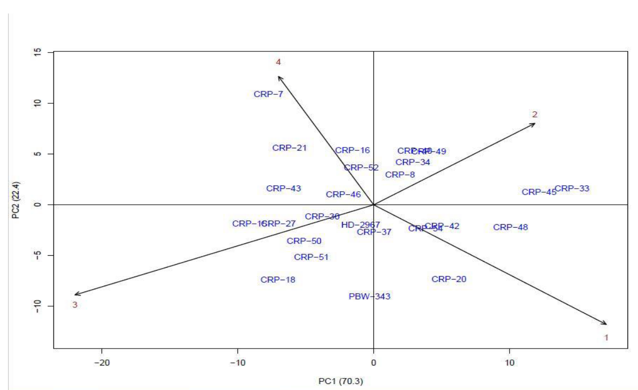

Fig. 4. AMMI model biplot of 1st and 2nd principal components across different environments

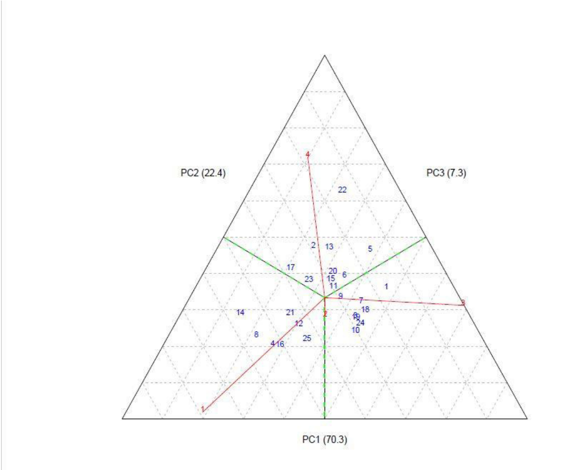

Fig. 5. Triplot of PC1, PC2 and PC3 based on the AMMI model

CRP21 show increased heat tolerance during 2016-17. The remaining genotypes namely, CRP33, CRP45, CRP42, CRP54, CRP37, CRP34, CRP40, CRP52, CRP8 and CRP49 belong to the second sub-cluster and show of very high tolerance towards heat stress. Stability analysis was done by the genotype x environment interaction analysis using additive main effect and multiplicative interaction (AMMI) analysis and genotype main effect plus genotype x environment interaction (GGE). The ANOVA of the AMMI model revealed that the variation in yield due to environmental influences was 98.48% (Table 5). The genotypic variance amounted to 0.81% whereas the variation due to G x E interaction amounted to 0.64%. Based on AMMI analysis, the G x E interaction was divided into 3 principal components. The 1st two principal components were able to explain 92.7% of the variance due to G x E interaction. The biplot and triplot given in Figures 4 and 5 represented the relationships

Table 4. Heat susceptibility index (HSI) based on yield obtained in 2015-16 and 2016-17

		2015-16			2016-17		
Sl no	Genotypes	Normal sown	Late sown	HSI	Normal sown	Late sown	HSI
1	HD-2967	1153.3	850.0	0.74	1476.0	593.3	1.05
2	CRP-7	776.7	603.3	0.63	1292.0	769.3	0.71
3	CRP-8	1053.3	713.3	0.91	1200.0	584.0	0.91
4	CRP-15	956.7	640.0	0.93	1564.0	609.3	1.08
5	CRP-16	856.7	540.0	1.04	1118.7	548.0	0.90
6	CRP-18	1150.0	630.0	1.28	1614.7	617.3	1.09
7	CRP-20	1323.3	676.7	1.38	1258.7	501.3	1.06
8	CRP-21	786.7	616.7	0.61	1301.3	572.0	0.99
9	CRP-27	923.3	486.7	1.34	1381.3	548.0	1.06
10	CRP-30	1043.3	723.3	0.87	1449.3	584.0	1.05
11	CRP-33	1323.3	933.3	0.83	998.7	502.7	0.88
12	CRP-34	1036.7	850.0	0.51	1229.3	552.0	0.97
13	CRP-37	1030.0	706.7	0.89	1312.0	426.7	1.19
14	CRP-40	1003.3	816.7	0.53	1180.0	550.7	0.94
15	CRP-42	1070.0	623.3	1.18	1092.0	385.3	1.14
16	CRP-43	916.7	506.7	1.26	1342.7	636.0	0.93
17	CRP-45	1210.0	723.3	1.14	917.3	458.7	0.88
18	CRP-46	1046.7	686.7	0.97	1350.7	625.3	0.95
19	CRP-48	883.3	550.0	1.07	1173.3	517.3	0.99
20	CRP-49	1033.3	706.7	0.89	1089.3	581.3	0.82
21	CRP-50	1160.0	783.3	0.92	1604.0	669.3	1.03
22	CRP-51	1093.3	656.7	1.13	1497.3	552.0	1.11
23	CRP-52	950.0	673.3	0.82	1230.7	562.7	0.96
24	CRP-54	1100.0	603.3	1.28	1137.3	449.3	1.07
25	PBW-343	1320.0	680.0	1.37	1486.7	589.3	1.06
	Mean	1048	679.2		1291.9	559.4	

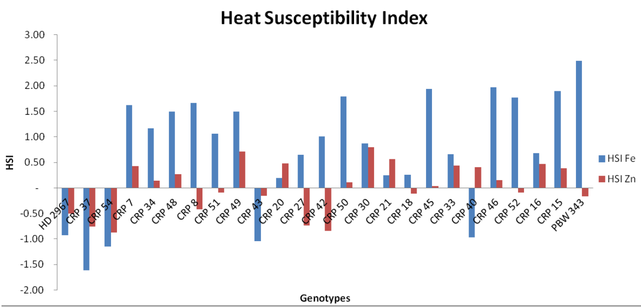

Fig. 6. Bar graph showing HSI of various genotypes on basis of Fe and Zn contents

Table 5. ANOVA and partitioning of G x E interaction by AMMI model for Yield

	Df	Sum of squares	Mean squares	percent
Environment	3	25511541	8503847***	98.48%
Genotype	24	1685613	70234***	0.81%
Environment x Genotype	72	3988152	55391***	0.64%
PC1	26	2802103	107773.2***	70.30%
PC2	24	893788.7	37241.19***	22.40%
PC3	22	292260.4	13284.56***	7.30%
Residuals	192	1016958	5297	

Table 6. Two way mean and HSI of Fe and Zn content in wheat grains

Genotypes	Fe content		Mean Fe	HSI	Zn content		Mean Zn	HSI
	DOS1	DOS2		Fe	DOS1	DOS2		Zn
HD 2967	20.467	26.500	23.483	-0.92	25.167	32.633	28.900	-0.50
CRP 37	25.600	38.800	32.200	-1.61	31.667	45.933	38.800	-0.76
CRP 54	32.167	43.933	38.050	-1.14	30.333	46.000	38.167	-0.87
CRP 7	76.067	36.667	56.367	1.62	34.367	25.767	30.067	0.42
CRP 34	33.467	20.967	27.217	1.17	35.800	32.800	34.300	0.14
CRP 48	56.000	29.233	42.617	1.49	65.600	55.133	60.367	0.27
CRP 8	23.467	11.000	17.233	1.66	27.967	34.833	31.400	-0.42
CRP 51	34.100	22.500	28.300	1.06	43.167	45.600	44.383	-0.10
CRP 49	70.333	36.633	53.483	1.50	48.767	28.133	38.450	0.72
CRP 43	31.100	41.500	36.300	-1.05	35.900	39.267	37.583	-0.16
CRP 20	30.933	28.967	29.950	0.20	42.000	30.200	36.100	0.48
CRP 27	41.200	32.633	36.917	0.65	27.067	38.833	32.950	-0.74
CRP 42	62.700	42.400	52.550	1.01	25.633	38.400	32.017	-0.84
CRP 50	50.233	21.500	35.867	1.79	38.767	36.267	37.517	0.11
CRP 30	31.300	22.633	26.967	0.87	38.267	20.333	29.300	0.79
CRP 21	38.200	35.167	36.683	0.25	44.400	29.567	36.983	0.57
CRP 18	32.900	30.167	31.533	0.26	37.600	40.000	38.800	-0.11
CRP 45	40.867	15.467	28.167	1.94	37.200	36.400	36.800	0.04
CRP 33	31.833	25.167	28.500	0.65	44.533	32.900	38.717	0.44
CRP 40	27.200	35.667	31.433	-0.97	40.933	31.100	36.017	0.41
CRP 46	72.967	26.967	49.967	1.97	41.667	37.967	39.817	0.15
CRP 52	34.633	15.000	24.817	1.77	30.100	31.633	30.867	-0.09
CRP 16	40.167	31.433	35.800	0.68	38.133	27.533	32.833	0.47
CRP 15	32.933	13.000	22.967	1.89	46.267	35.700	40.983	0.39
PBW 343	49.500	10.167	29.833	2.48	28.767	31.567	30.167	-0.16

DOS=Dates of sowing

between the two principal components and yield based on the AMMI model. The biplots showed that the genotypes closer to the centre, viz., CRP46, CRP30, and CRP37 along with the check HD2967 were considered to be highly stable across all the environments and the genotypes presented in the 1st quadrant were the least affected due to G x E interaction. In AMMI triplot, genotypes clustered nearer to the origin against the environment axes showed general adaptation while those located farther showed adaptation to that specific environment. Hence the genotypes nearer to the origin were considered stable over all environments such as CRP27, CRP33 and CRP42.

Analysis of Fe and Zn content in grain

The analysis of variance for Fe and Zn contents were done in the wheat grains harvested in the year 2017. It was found that the variance among the genotypes, between the two DOS and the interaction between them, on the basis of their Fe and Zn content was highly significant. The accumulation of Fe in seeds varied from 17.2–56.4 mg/g in CRP7 with an average of 34.3 mg/g. Accumulation of Zn varied from 28.9–44.4 mg/g of seed except for CRP 48 which showed highest accumulation of Zn (60.4 mg/g) with an average of 36.5 mg/g. HSI was calculated based on the reduction in Fe and Zn content in various genotypes over the two dates of sowing (Table 6). It was observed that the HSI for most of the genotypes were positive, i.e., the Fe and Zn accumulation in grains reduced from the normal sown to late sown conditions. But in few genotypes, such as HD 2967, CRP 37, CRP54 and CRP43 the opposite held true. The genotypes, CRP8, CRP51, CRP27, CRP42, CRP18, CRP52 and PBW 343 showed increase in only their Zn content but not Fe content. Increase in Fe content but reduction in Zn content in grains was observed in CRP 40. Out of 25 genotypes, 5 genotypes showed negative HSI values for Fe accumulation in seeds and 11 genotypes showed negative HSI values based on Zn accumulation. Four of the five genotypes with negative HSI values for Fe content were common with that of genotypes with negative HSI values for Zn accumulation. This showed that these four genotypes, namely HD2967, CRP 37, CRP 54 and CRP 43 were tolerant to heat stress for both Fe and Zn accumulation. The HSI values for Fe and Zn content in wheat grains was plotted for the 25 genotypes (Fig. 6). Three major clusters were observed for the distribution of Fe content data over two dates of sowing. By comparing the Fe content of the genotypes in various clusters, it was seen that the first cluster consisted of genotypes CRP42, CRP50, CRP7, PBW 343, CRP46, CRP49 and CRP48 which were high Fe accumulators. The second cluster consisted of HD 2967, CRP20, CRP18, CRP34, CRP51, CRP30, CRP33, CRP45, CRP52, CRP15 and CRP8 which were low Fe accumulators. The rest of the genotypes fell under 3rd cluster and were average Fe accumulators. Four major clusters were observed for the distribution of Zn content data over two dates of sowing in 25 genotypes. The first cluster consisted of genotypes CRP27, CRP42, CRP52, CRP8, PBW 343 and HD 2967. The second cluster consisted of genotypes CRP37, CRP54, CRP51, CRP43, CRP18, CRP46, CRP50 and CRP45. CRP48 formed a monogenotypic cluster as it had the highest Zn and Fe accumulation in grain. The fourth cluster consisted of the rest of the genotypes.

Discussion

The abiotic stresses, particularly, the heat stress is becoming an alarming situation for crop production. It poses a challenge to the breeders for developing cultivars which can resist high temperature stress. Evaluation of breeding material and the comparative performance of genotypes under unusually warm conditions has been the most common method in selecting heat stress tolerant genotypes (Ehlers and Hall 1998; Annicchiarico et al. 2002). Besides several physiological and statistical parameters, heat stress indices (HSIs) are valuable tools for selection of stable genotypes possessing both heat stress tolerance and high yield (Devi et al. 2021). The present study indicated that reduction in yield was observed for all the 25 genotypes under late sown condition as shown by the heat susceptibility indices (Table 4) and graphically depicted in Fig. 2 for both the years. The HSI of all the 25 genotypes gave positive values, revealing that their yield was affected by the heat stress due to late sown conditions. The genotype CRP-34 showed the highest tolerance to heat stress during 2015-16, whereas CRP-7 was observed to be highly tolerant to heat stress during 2016-17. Furthermore, the genotypes yielding better quality grains rich in minerals such as iron and zinc under challenging climatic scenario are essential for meeting the mineral nutrition of the growing population. Therefore, breeding genotypes on the basis of their natural ability to accumulate more nutrients is highly essential. In this study we were able to identify various high yielding genotypes which were relatively stable under heat stress condition on the basis of their yield as well as Fe and Zn content. The seven genotypes including a check, PBW343, showed increase in only their Zn content but not Fe content. Increase in Fe content but reduction in Zn content in grains was also recorded in CRP 40. Wide variation was observed in accumulation of Fe and Zn among the genotypes due to HSI (Fig. 6). Narendra et al. (2021) also found a significant genetic variation for physiological traits and Fe and Zn contents under the optimum temperature and heat stress conditions, although the negative correlation was observed between grain Zn/Fe and yield under heat stress conditions, but they did not report a single genotype with higher Zn and Fe contents.

The reasons for difference in genotypic yield between the year 2015-16 and 2016-17 may be the different environmental conditions prevalent during each year of the study. Though dates of sowing for the two years are different under both normal as well as late sown conditions, but it was observed that the dates of harvesting almost coincided for both the years for the second date of sowing. It might be due to hastening of maturity due to increase in temperature (Ayeneh et al. 2002) and change in the duration of photo-period during the crop growth. The genotypes differed significantly with respect to yield between the two dates of sowing. The normal sown wheat yielded more than the late sown wheat. This is due to the fact that the length of both vegetative and reproductive phases in late sown wheat was greatly reduced. The variability in yield due to heat stress in the present experiment has been supported by other studies (Fu et al. 2016). The ability to yield higher under heat stress condition is a highly desirable quality for selection of a genotype while breeding for crop improvement (Aziz et al. 2018). Based on the present study the genotypes have been clustered into different yield groups. Each group contains genotypes with different sensitivity to heat stress. The inherent capacity of the genotype to show resistance to different environmental conditions is governed by various genetic components or genes and is a purely quantitative character. Previous studies have shown that the variability in performance of different genotypes under stress conditions is due to differences in combinations of favourable genes (Lopes et al. 2015). Therefore, a lot of effort has been given to identify the genotypes containing the optimum combination of genes governing these characters. Since the molecular mechanisms governing stability of genotypes have not been studied in detail, efforts have been given instead to optimize the performance of the existing genotypes by minimizing the G x E interactions (Philanim et al. 2022). Many statistical methods have been employed to aptly predict the G x E interactions, resulting in various stability coefficients (Philanim et al. 2022; Arya et al. 2022). Often in various studies, a combination of these measures has been used to better explain the G x E interactions (Dwivedi et al. 2020). In this study different methods were used to predict the stable genotypes and each of the measures led to inconsistent results, as supported by previous studies (Elbasyoni 2018). The difference in results of different stability indices may be attributed to the variation in the methods of statistical analysis performed (Witcombe 1988). From the present study GGE biplot has been represented in two forms (Fig. 3). The 'which-won-where' plot presents a polygon whose vertices are made up of the best or least performing genotypes which placed 8 genotypes the farthest from the origin. The biplots showed that the genotypes closer to the centre, *viz.*, CRP46, CRP30, and CRP37 along with the check HD2967 were considered to be highly stable across all the environments and the genotypes presented in the 1st quadrant were the least affected due to G x E interaction. A similar trend has been reported earlier (Kempton 1984).

The lines passing through the polygon's sides divide the polygon into sectors. Since each of the rays depicting environments is present in different sectors, different genotypes performed best in different environments. The 'mean vs stability' model represents the genotypic and G x E interaction along with the average environment coordination (AEC) (Poudel et al. 2020). The genotypes projected closer to the origin on the AEC vertical axis are the most stable while their projected distance on the AEC horizontal axis denoted their mean yield. The most stable genotypes included CRP46, CRP34, CRP37, CRP8 and CRP40 while the genotypes with highest mean yield were observed to be CRP45, CRP48, CRP3 and CRP7. AMMI biplot (Fig. 4) shows that there is a lot of variation among the genotypes due to G x E interaction. AMMI triplot (Fig. 5) shows the interrelationship between the different principal components while all the genotypes and environments are plotted from the centre. The arrows depicting the environments show the effect of each environment. The environment with the shortest arrow has the least interactive effect (Late sown, 2015-16) while the environments with longer arrows exert more interaction effect (Ebdon and Gauch 2002). The mineral content in wheat is an important aspect of grain quality. The increase in wheat yield leads to a reduction in grain mineral content (Murphy et al. 2008). The accumulation of Fe on an average reduced drastically from the first date of sowing to the second date of sowing. The result shows that the heat stress on the plant led to reduced Fe accumulation in the seeds. The average Zn accumulation in the seeds also reduced from 37.6 mg/g on the 1st date of sowing to 35.4 mg/g on the second date of sowing. The variation in grain Fe and Zn content as seen in ANOVA was significant among genotypes, environment as well as due to interaction effect between genotype and environment. The fact that there's varied deposition of Fe and Zn in wheat and that the mineral contents may increase or decrease or remain constant has been supported by previous studies (Moreira-Ascarrunz et al. 2016). Based on the difference in Fe and Zn concentration, the genotypes have been clustered into various groups. The sudden increase in temperature during the reproductive phase in late sown condition leads to lower accumulation of nutrients in grains (Blanco et al. 2012). The genotypes accumulate nutrients at a higher rate during heat stress condition but are ultimately unable to compensate for the reduction in period of maturity, resulting in low yield and less nutrient content, as supported by previous studies (Fu et al. 2016). In the current study, genotype CRP48 showed the highest Zn content and consistently high concentration of Fe in grains. The reason for this lies in its pedigree (WDLL1*2/KURUKU*2/5/REH/HARE//KACHU), which involves desirable parents for improving nutrients. BARI Gom 33 (=Kachu/Solala) released in Bangladesh in the year 2017 showed 7–8 mg/kg Zn advantage (Velu et al. 2019). This study can be useful to the wheat breeders for improving heat tolerance along with high yield and nutrient content of grains.

Supplementary material

Supplementary Table S1 is provided.

Author's contributions

Conceptualization of research (RK); Designing of the experiments (RK and SP); Contribution of experimental materials(RK); Execution of field/lab experiments and data collection (SP, SKS); Analysis of data and interpretation (SP and YKP); Preparation of the manuscript (SP,RK and VK).

Acknowledgments

The authors are thankful to Department of Biotechnology, Government of India for M.Sc. Agricultural Biotechnology Program; CGIAR Research Program for utilizing the research material and Department of Agricultural Biotechnology and Molecular Biology, Rajendra Prasad Central Agricultural University, Pusa, Bihar for providing the research facility.

References

Akcura M. and Kaya Y. 2008. Nonparametric stability methods for interpreting genotype by environment interaction of bread wheat genotypes (Triticum aestivum L.). Genet. Mol. Biol., **31**: 906–913.

Al-Otayk S. M. 2010. Performance of Yield and Stability of Wheat Genotypes under High Stress Environments of the Central Region of Saudi Arabia. Meteorol. Environ. Arid Land Agric. Sci., **21**: 81–92.

Annicchiarico P. 2002. Genotype X Environment Interactions, Challenges and Opportunities for Plant Breeding and Cultivar Recommendations. Food and Agriculture Organization.

Arya, R.K. and Verma, A., 2022. Long-term evaluation of new elite genotypes of Faba bean (Vicia faba L.) by Superiority Index and AMMI analysis. Indian J. Genet. Plant Breed., **82**(2): 232-235. doi: 10.31742/IJGPB.82.2.14

Ayeneh A., Ginkel V., Reynolds M. P. and Ammar K. 2002. Comparison of leaf, spike, peduncle and canopy temperature depression in wheat under heat stress. Field Crops Res., **79**: 173–184.

Aziz A., Mahmood T., Mahmood Z., Shazadi K., Mujeeb-Kazi A., Rasheed A. 2018. Genotypic Variation and Genotype x Environment Interaction for Yield-Related Traits in Synthetic Hexaploid Wheat under a Range of Optimal and Heat-Stressed Environments. Crop Sci., **58**: 295–303.

Blanco A., Mangini G., Giancaspro A., Giove S., Colasuonno P., Simeone R., Signorile A., De Vita P., Mastrangelo A. M. and Cattivelli L. 2012. Relationships between grain protein content and grain yield components through quantitative trait locus analyses in a recombinant inbred line population derived from two elite durum wheat cultivars. Mol. Breed., **30**: 79–92.

Cakmak I., Pfeiffer W. H., McClafferty B. 2010. Biofortification of durum wheat with zinc and iron. Cereal Chem., **87**: 10–20.

Davis J. 2021.The global temperatures are now likely to rise by more than 1,5C. https://www.nhm.ac.uk

Devi K., Chahal S., Singh S., Venkatesh K., Mamrutha H. M., Raghav N., Singh G., Singh G.P. and Tiwari R. 2021. Assessment of wheat genotypes based on various indices under different heat stress conditions. Indian J. Genet. Plant Breed., **81**(3): 376-382. DOI: 10.31742/IJGPB.81.3.4.

Dwivedi A., Basandrai D. and A. K. Sarial A.K. 2020. AMMI biplot analysis for grain yield of basmati lines (*Oryza sativa* L.) in North Western Himalayan Hill regions. Indian J. Genet. Plant Breed., **80**(2): 140-146

Ebdon, J.S. and Gauch Jr, H.G., 2002. Additive main effect and multiplicative interaction analysis of national turfgrass performance trials: II. Cultivar recommendations. Crop Sci., **42**(2): pp.497-506.

Eberhart S. A. and Russell W. A. 1966. Stability Parameters for Comparing Varieties. Crop Sci., **6**: 36–40.

Elbasyoni I. S. 2018. Performance and stability of commercial wheat cultivars under terminal heat stress. Agronomy, **8**: 37.

Ehlers J. D. and Hall A. E. 1998. Heat tolerance of contrasting cowpea lines in short and long days. Field Crops Res., **55**: 11-21.

FAOSTAT, F. Statistical Databases 2018. Food and Agriculture Organization of the United Nations: Rome, Italy.

Farshadfar E., Sabaghpour S. H., Zali H. 2012. Comparison of parametric and non-parametric stability statistics for selecting stable chickpea (*Cicer arietinum* L.) genotypes under diverse environments. Aust. J. Crop Sci., **6**: 514–524.

Fischer R. A. and Maurer R. 1978. Drought resistance in spring wheat cultivars. I. Grain yield responses. Aust. J. Agric. Res., **29**: 897-912.

Fu G., Feng B., Zhang C., Yang Y., Yang X., Chen T., Zhao X., Zhang X., Jin Q., Tao L. 2016. Heat stress is more damaging to superior spikelets than inferiors of rice (*Oryza sativa* L.) due to their different organ temperatures. Front. Plant Sci., **7**: 1637.

Gomez K. A. and Gomez A. A. 1984. Statistical Procedures for Agricultural Research. John Wiley and Sons: New York, NY, USA

Hellemans S., Landschoot K., Dewitte. 2018. Impact of crop husbandry practices and environmental conditions on wheat composition and quality: a review. J. Agric. Food Chem., **11**: 2491–2509.

Joshi A. K., Mishra B., Chatrath R., Ferrara G. O. and Singh R. P. 2007. Wheat improvement in India: present status, emerging challenges and future prospects. Euphytica, **157**: 431–446.

Kempton, R.A., 1984. The use of biplots in interpreting variety by environment interactions. J Agric Sci., **103**(1): 123-135.

Lin C. S. and Binns M. R. 1988. A superiority measure of cultivar performance for cultivar x location data. Can. J. Plant Sci., **68**: 193–198.

Liu B., Asseng S., Müller C., Ewert F., Elliott J., Lobell D. B., Martre P., Ruane A. C., Wallach D. and Jones J. W. 2016. Similar estimates of temperature impacts on global wheat yield by three independent methods. Nat. Clim. Change, **6**: 1130-1136.

Lobell D. B., Schlenker W. and Costa Roberts J. 2011. Climate trends and global crop production since 1980. Science, **333**(6042): 616–620. https://doi.org/10.1126/science.1204531

Lopes M. S., El-Basyoni I., Baenziger P. S., Singh S., Royo C., Ozbek K., Aktas H., Ozer E., Ozdemir F. and Manickavelu A. 2015. Exploiting genetic diversity from landraces in wheat breeding for adaptation to climate change. J. Exp. Bot., **66**: 3477–3486.

Moreira-Ascarrunz S. D., Larsson H., Prieto-Linde M. L. and Johansson, E. 2016. Mineral nutritional yield and nutrient density of locally adapted wheat genotypes under organic production. Foods, **5**(4): 89.

Mulusew F., Bing D. J., Tadele T., Amsalu A. 2014. Comparison of biometrical methods to describe yield stability in field pea (*Pisum sativum* L.) under south eastern Ethiopian conditions. Afr. J. Agric. Res., **9**: 2574–2583.

Murphy K. M., Reeves P. G. and Jones S. S. 2008. Relationship between yield and mineral nutrient concentrations in historical and modern spring wheat cultivars. Euphytica, **163**: 381–390.

Narendra M.C., Roy C., Kumar S., Virk P. and De N. 2021. Effect of terminal heat stress on physiological traits, grain zinc and iron content in wheat (*Triticum aestivum* L.). Czech. J. Genet. Plant Breed., **57**: 43-50. https://doi.org./10.17221/63/2020-CJGPB.

Ortiz-Monasterio J. I., Palacios-Rojas N., Meng E., Pixley K., Trethowan R. and Peña R. J. 2007. Enhancing the mineral and vitamin content of wheat and maize through plant breeding. J. Cereal Sci., **46**: 293-307.

Pacheco A., Vargas M., Alvarado G., Rodríguez F., López M., Crossa J. and Burgueño J. 2016. GEA-R (Genotype x Environment Analysis with R for Windows.) Version 4.1, http://hdl.handle.net/11529/10203. Int. Maize and Wheat Improvement Center.

Philanim W.S., Kumar A., Shittegar N., Sankar S.M., Bharadwaj C., Ngangkham U. and Bhattacharjee B. 2022. Stability analysis of yield and yield related traits in ricebean [*Vigna umbellata* (Thunb.) Ohwi and Ohashi]. Indian J. Genet. Plant Breed., **82**(02): pp.208-216.

Pinthus M. J. 1973. Estimate of genotypic value: A proposed method. Euphytica, **22**: 121–123.

Poudel, M.R., Ghimire, S., Pandey, M.P., Dhakal, K., Thapa, D.B. and Poudel, H.K., 2020. Yield stability analysis of wheat genotypes at irrigated, heat stress and drought condition. J. Biol. Today World, **9**(5): p.220.

Romagosa, I. and P.N. Fox. 1993. Genotype-environment interaction and adaptation. In: Plant Breeding, Principles and Prospects, (Ed. M.D. Hayward, N.O. Bosemark and I. Romagosa), London: Chapman and Hall, pp 373-390.

Schmidhuber J. and Tubiello F. N. 2007. Global food security under climate change. PNAS, **104**: 19703–19708.

Shewry P. R. and Hey S. J. 2015. The contribution of wheat to human diet and health. Food Energy Secur., **4**: 178–202.

Shewry P. R., Hawkesford M. J., Piironen V., Lampi A. M., Gebruers K., Boros D., Andersson A. A., Åman P., Rakszegi M. and Bedo Z. 2013. Natural variation in grain composition of wheat and related cereals. J. Agric. Food Chem., **61**: 8295–8303.

Singh C., Gupta A., Kumar P., Sendhil R., Gopalareddy K., Gupta V., Singh S. K., Sharma A. K., Tyagi B. S., Singh G., Chatrath R. and Singh G. P. 2020. Multi-environment analysis of grain yield in a diverse set of bread wheat genotypes. J. Cereal Res., **12**: 29–39.

Spiegel H., Sager M., Oberforster M., Mechtler K., Stüger H. P. and Baumgarten, A. 2009. Nutritionally relevant elements in staple foods: Influence of arable site versus choice of variety. Environ Geochem Health, **31**: 549-560

Thomas S. M. and Toulmin C. 2010. Food security: The challenge of feeding 9 billion people. Science, **327**: 812–818.

Vasantrao J. M., Pankaj Y. K. and Kumar R. 2019. Characterization of wheat (*Triticum aestivum* L.) genotypes unraveled by molecular markers considering heat stress. Indian J. Biotechnol., **18**: 204-213.

Velu G., Singh R., Arun B., Mishra V., Tiwari C., Joshi A., Virk P., Cherian B. and Pfeiffer W. 2015. Reaching out to farmers with high Zinc wheat varieties through public-private partnerships–An Experience from Eastern-Gangetic Plains of India. Adv. Food Nutr. Res., **1**: 73-75

Velu G., Crespo Herrera L., Guzman C.,Huerta J., Payne T. and Singh RP. (2019) Assessing Genetic Diversity toBreed Competitive Biofortified WheatWith Enhanced Grain Zn and FeConcentrations. Front. Plant Sci., **9**: 1971.doi: 10.3389/fpls.2018.01971

Verma S. K., Chaurasia S. K., Pankaj Y. K. and Kumar R. 2020. Study on the genetic variability and pathogenicity assessment among isolates of spot blotch causing fungi (Bipolaris sorokiniana) in wheat (*Triticum aestivum* L.). Plant Physiol. Rep., **25**: 255–267

Ward J. H. 1963. Hierarchical grouping to optimize an objective function. J Am Stat Assoc., **58**: 236-244.

Welch R. M. 2002. The impact of mineral nutrients in food crops on global human health. Plant Soil., **247**: 83–90.

Witcombe J. R. 1988. Estimates of stability for comparing varieties. Euphytica, **39**: 11–18.

Supplementary Table S1. The passport information/ pedigree of all the 25 genotypes considered in the study

S.No.	Genotype	Pedigree
1	CRP 07	CNDO/R143//ENTE/MEXICO/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER/5/PASTOR
2	CRP 08	MILAN/KAUZ/3/URES/JUN//KAUZ/4/CROC_1/AE.SQUARROSA (224)//OPATA
3	CRP 15	TILHI
4	CRP 16	NL 750
5	CRP 18	W462//VEE/KOEL/3/PEG//MRL/BUC
6	CRP 20	JUPARE C 2001
7	CRP 21	ATTILA/3*BCN//BAV92/3/TILHI
8	CRP 27	VORB/4/D67.2/PARANA66.270//AE.SQUARROSA (320)/3/CUNNINGHAM
9	CRP 30	CROC_1/AE.SQUARROSA(205)//KAUZ/3/SASIA/4/TROST
10	CRP 33	PFAU/MILAN//TROST/3/PBW65/2*S ERI.1B
11	CRP 34	TILHI/PALMERIN F 2004
12	CRP 37	Youngmy-6
13	CRP 40	UP2338*2/KKTS*2//YANAC
14	CRP 42	UP2338*2/KKTS*2//YANAC
15	CRP 43	MURGA/KRONSTAD F 2004
16	CRP 45	BAV92//IRENA/KAUZ/3/HUITES/4/FN/2*PASTOR/5/BAV92//IRENA/KAUZ/3/HUITES
17	CRP 46	BABAX/LR39//BABAX/3/VORB/4/SUNCO/2*PASTOR
18	CRP 48	WDLL1*2/KURUKU*2/5/REH/HARE//KACHU
19	CRP 49	#1/4/CROC_1/AE.SQUARROSA (205)//KAUZ/3/SASIA/5/KACHU
20	CRP 50	SKAUZ*2*FCD'S'//VORB
21	CRP 51	OPATA//SORA/AE.SQUARROSA (323)
22	CRP 52	CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER/5 TICUS/6/2*PBW65/2*PASTOR
23	CRP 54	BABAX/KS94U76//BABAX/3/2*SOKOLL
24	HD 2967	ALD/COC//URES/HD2160M/HD2278
25	PBW 343	ND/VG9144//KAL/BB/3/Y/ACO'S'/4/VEE#5'S'