



Evaluation of Bread Wheat Varieties in Terms of Grain Yield and Zn and Fe Accumulation in Grain using Stress Tolerance Index.

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ABSTRACT: Global population is increasing significantly and supplying nutrition for these people requires access to new cultivars with high quality and quantity. Among the factors, enhancing the quality of agricultural products, especially grains, is the presence of micronutrients like zinc and iron. To assess and determine superior wheat genotypes in terms of performance as well as zinc and iron micronutrients in the grain, 37 bread wheat genotypes with two types of facultative and winter growth were studied in two separate trials (with/without zinc foliar application) in a randomized complete block design with three replications at Khalatpoushan Research Station of Agriculture Department, Tabriz University. Then, the combined analysis of variance was performed on it. Zinc foliar (0.68 kg/ ha) was applied in an experiment equipped with foliar application. The agronomic trait of grain yield was measured per plant. Moreover, the two content traits of zinc and iron in whole grains were assessed using DTZ and PPB methods and scans obtained by Photoshop software. The analysis results of variance showed presence of high genetic diversity in terms of the traits considered in this study. Based on correlation analysis of the STI calculated indices, the results indicated a significant negative correlation between the STI index of zinc content and the STI index of iron content and STI index of grain content. On the other hand, there was a positive and significant correlation between STI index of zinc yield and STI index of grain yield. Based on the analysis of three-dimensional charts obtained from STI indicators, the cultivars of back cross roshan, tous,omid, sardari and Gaspard in terms of the combination of iron and yield and Navid cultivars were among the best genotypes in this study.

Key words: Wheat (*Triticum aestivum* L.), Micronutrients, Stress Tolerance Index.

INTRODUCTION

Malnutrition is a major and growing problem in the developing world. Nowadays, more than three billion people are suffering from malnutrition caused by micronutrients (Welch and Graham, 2004). In Iran, due to several reasons such as lime, bicarbonation of irrigation water, drought, and low organic matter, farming soil has faced micronutrients' deficient, particularly in zinc and iron (Malakouti and Tehrani, 2005). Although micronutrients are used in small quantities in plants, they leave significant effects. In case of their deficiency, these elements sometimes act as restrictors of absorption of other nutrients and growth, so it is required to reconsider their applications more cautiously (Malakouti, 2000). Two- year experiments conducted on 700 farms showed that 37% and 40% of soils in Iran suffer iron and zinc deficiencies, respectively (Balali *et al.*, 2000). Iron is an essential element in plant nutrition and any disruption in its ability would reduce growth.

Iron deficiency in plants leads to yellowing of leaves and reduction of photosynthesis and chlorophyll

concentrations (Mahmoodi *et al.*, 2005), shoot and root dry weight (Tabatabai *et al.*, 2009), changes of iron concentration and content (Tabatabai *et al.*, 2009) and other metal elements in plant tissues (Chen *et al.*, 2004), i.e., those characteristics which are closely related to crop yield. Yellowing caused by iron deficiency is one of the factors limiting plant growth in calcareous and alkaline soils. Moreover, Iron chlorosis is related with soil compaction, poor drainage after irrigation, and wet weather conditions. Iron is a very important micronutrient in human's diet. Around the world, especially in developing countries, millions of people have faced anemia caused by its deficiency (Welch *et al.*, 1991). Significance of iron as the central atom of hemoglobin and anemia resulting from its deficiency are well known (Tuman and Doisy, 1978). As a component of myoglobin (Zhang *et al.*, 2004), one of iron's functions is to accumulate oxygen in muscle tissues and cytochrome system (Tuman and Doisy, 1978). Iron, zinc, and selenium play a significant role in immunity system of body (Lyons *et al.*, 2004).

In addition, iron is involved in supplying energy from cellular respiration and it contributes with electron transfer and construction of iron- dependant enzymes to produce energy (King *et al.*, 1999).

In addition, iron is required for metabolism of Group B also Vitamins. It can also promote growth, prevent fatigue, increase resistance to diseases, and treat anemia induced by iron deficiency (Malakouti and Tehrani, 2005). Anemia caused by iron deficiency is one of the most prevalent nutritional problems in the world (Ninh *et al.*, 2002). In addition to anemia, iron deficiency can result in behavioral and physical disorders among under two- year- old children, limit the ability of physical activity, impair immune system and the like (Lynch, 2003). The role of iron deficiency in reducing the effect of iodine supplementation is also proven.

Another energy-saving and essential element for plant growth which performs important tasks is zinc which is mostly used in parts of enzymes' structure or act as regulating co- factors in a great number of enzymes (Malakouti and Homai, 2003). Zinc is involved in RNA metabolism and ribosome content of plant cells, stimulates carbohydrates' construction, and helps in formation of proteins and DNA. This element is also essential for building tryptophan, an IAA I precursor which activates the growth- inducing substance (Amberger, 1982). Zinc has three functions: catalytic, co-catalytic, and structural (Valle and Auld, 1990; Vallee and Falchuk, 1993) and during recent decades, its role in protein molecules involved in DNA transcription has been considered (Coleman, 1992). This element also plays an important role in biomass production (Kaya and Hakyz, 2002; Cakmak, 2008). Zinc may be required for production of chlorophyll II and fertility (Kaya and Higgs, 2002; Pandey *et al.*, 2006).

After iron, zinc content is the second factor that has a unique importance in human body without which body cannot survive. It is required for metabolism of carbohydrates and proteins, excretion of carbon dioxide, and optimal use of the vitamins needed by the body (Hotz and Brown, 2004). In other words, zinc is required for almost all aspects of cellular metabolism (Ruz, 2003). Since it is mostly essential in short cellular mechanisms such as adsorption and desorption needed for body, it is clear that lack of sufficient zinc in diet will lead to adverse consequences (Solomons, 2003).

However, shortage of micronutrients can be treated by traditional methods such soil plowing before and after of planting and spraying after germination. yet, due to presence of problems caused by using fertilizers containing zinc and iron, including their high cost in developing countries, the most important way to increase the amount of zinc and iron in grains will be exploitation of naturally- available genetic variation in terms of concentrations of micronutrients in seeds (Cakmak *et al.*, 2004).

Likewise, through identification of the cultivars resistant to iron and zinc deficiencies, new genotypes can be produced which have greater efficiency and ability to absorb such elements. Therefore, researchers can use this option as an alternative way to compensate lack of iron and zinc (Haji Saleoglu *et al.*, 2001). On the other hand, yield is the most vital and economic feature of crop plants is influenced by environmental conditions, genetic structures of plants and their interactions (Naderi *et al.*, 1999). So, finding genotypes suitable for both environments and compatible with lack of micronutrients deficiency seems quite complicated (Biswas *et al.*, 2001). Hence, the researchers have proposed various methods one of which is the use of different measures that determines the reactions of genotypes in different environmental conditions and analyzes their tolerance or sensitivity (Naderi *et al.*, 1999). These indicators are calculated based on grain yield in stress and non-stress environments (Fernandes, 1993). Among indices, determining efficiency in plants is stress tolerance index (STI) which based on Fernandez's classification (1993) identifies type A genotypes, i.e., the superior genotypes in both with and without deficiency cases. based on the significance of identifying cultivars which have a high yield and contain great amounts of zinc and iron, this study was conducted to assess the reactions of brain wheat genotypes under condition with/ without zinc spraying on zinc deficiency and its effect on iron content in the grain. It also uses STI index to screen superior genotypes in terms of both yield and zinc and iron content per grain.

MATERIALS AND METHODS

The experiment (2011-2012) was implemented in Agricultural Research Station, Tabriz University, located in Karkaj lands, 12 km East from Tabriz, with an altitude of 1360 meters elevation above sea level. Its climate is mountainous cold and semi-arid. The soil of station is was sandy- loam with moderate alkaline pH. The plant materials used in this study consisted of 37 wheat cultivars prepared from the Seed and Plant Improvement Institute in Karaj, IR Iran.

The names of the genotypes used in this study are mentioned in Table 1. Agronomic traits measured in this research were grain yield, the amount of zinc, and total iron content of grains. STI index was also calculated for grain yield, zinc content, and iron content of all grains (Equation1).

$$STI = \frac{Y_s Y_p}{(\bar{Y}_p)^2} \quad \dots(1)$$

where Y_s and Y_p are respectively yield in terms of without zinc foliar application and with of foliar application and \bar{Y}_p is the mean yield of all genotypes in conditions of without zinc foliar application (Fernandez, 1993).

Table 1: Genotype studied in the trail.

Cultivar	Growth type	Cultivar	Growth type	Cultivar	Growth type	Cultivar	Growth type
Bam	facultative	Azar	facultative	Pishgam	winter	Sabalan	winter
Navid	facultative	Alvand	facultative	Biston	winter	shahpasand	winter
Qods	facultative	Shahriar	facultative	Zare	winter	Tous	winter
Roshan	facultative	Karaj 2	facultative	MV-17	winter	Alamout	winter
Inia66	facultative	Zareen	facultative	Omid	winter	gaskogen	winter
Azar2	facultative	Rashid	facultative	Gaspard	winter	Shahi	winter
Kaveh	facultative	WS- 982	facultative	Back cross roshan	winter	Azadi	winter
Zagros	facultative	Karaj 3	facultative	Mihan	winter	Bezostaya	winter
Mahdavi	facultative	Syson	facultative	Sardari	winter	Erom	winter
						Noursetar	winter

In order to determine the location and concentration of zinc and iron in grains, DTZ and PPB staining methods were used. DTZ Staining method leads to production of a red- purple complex, zinc, which is used to determine Zn concentration (Mc Nary, 1954). For preparation of this reagent, 500 mg of DTZ powder was dissolved in 1L pure methanol (Ozturket *et al.*, 2006). PPB staining method produces a blue complex in case of iron accumulation, which is used to determine Fe concentration (Zarcinaset *et al.*, 1987).

PPB solution was prepared by dissolving 60 g potassium hexacyanoferrate (II) dihydrate powder in 1L distilled water. HCl solution was obtained by adding 125 ml of a 32% hydrochloric acid to 1L distilled water. Then, for the final usage, equal volumes of PPB and HCl solution were mixed.

In order to determine the overall amount of zinc and iron per grain, 12 grains were randomly selected from the harvested grains of each experimental unit and were weighed to calculate the number of color pixels to the total grain weight¹. Then, four grains were bonded onto a glass slide by epoxy resin adhesive. In each iteration, three glass slides were prepared for each genotype.

Then, the prepared slides were placed for 24 hours at room temperature to get the adhesive completely dry (Choi *et al.*, 2007). In order to determine the location and concentration of zinc and iron found in the seeds on each slide by DTZ and PPB Reagents, the seeds were sanded so that the three seed layers, aileron, endosperm, and embryo appeared (Choi *et al.*, 2007).

Next, the slides were washed twice with distilled water, the relevant samples used to determine zinc and iron concentrations were respectively for 30 and 10 minutes in DTZ and PPB reagents to get optimal staining. After that, the slides were taken out of the reagents and were washed twice- distilled water and were put under the hood to get completely dry. After drying, the slides are placed inside the flatbed scanner machine and the scanned images were analyzed by Photoshop software. The seed section was cropped of the photos in a similar width and length for all samples in Adobe Photoshop software. Based on the degree of fuzziness (1999) for zinc and 46 for iron), the total number of color pixels (TPN) in red and blue stained samples was determined and eventually, it was divided by their grains' weight to calculate the concentration of zinc and iron available in all grains in pixels per gram.

RESULTS AND DISCUSSION

The results of Analysis of aggregate variance of wheat genotypes studied in terms of these traits are shown in Table 2. The effect of the group on the studied traits was significant. Zinc effect was also significant on all studied traits, except iron content per grain. The interactional effect of the group with zinc was insignificant for all traits. Moreover, the differences between the studied genotypes per group were significant in terms of the traits indicating presence of genetic variation among the genotypes.

Table 2: Combined Analysis of variance for traits studied under condition with and without zinc foliar application.

Sources of variation	Degree of freedom	Mean square		
		Grains yield (gr)	Grain's zinc content (pixel/ g)	Grain's iron content (pixel/ g)
Group	1	217.757**	1549.061**	26.809**
Zinc	1	13.556**	1549.377**	0.155
Group* zinc	1	0.943	101.523	0.001
Error 1	8	0.978**	48.508	0.303
in- group cultivar	35	1.332**	490.020**	1.959**
In- group cultivar* zinc	35	0.199	70.081	0.165
Error 2	140	0.188	52.373	0.196

** significant at the probability level of 1%.

In the meantime, the interactional effect of within-group genotype* zinc was insignificant all traits. Comparison of genotypes in terms of grain yield under conditions with zinc foliar application (no deficiency) and without zinc foliar (deficiency) showed that the average grain yields per plant in terms of with and without deficiency were 3.46 and 2.98 g respectively. Likewise, the average zinc contents per grain in terms of conditions with and without zinc foliar were 33.88 and 28.72 pixel per gram. Moreover, in terms of iron content of grains, the average rates in conditions with and without zinc foliar were 4.50 and 4.60 pixel per gram. Therefore, after zinc foliar, the grain yield and the zinc content increased by 16 and 18%, respectively, whereas iron content of the grain reduced by 0.02%. It should be noted after foliar, the highest rates of increase in terms of zinc yield and content belonged to Navid

(1.1 g per mono- plant unit) and Azar cultivars (23.02 pixel/ g); whereas the highest rate of reduction in iron content of each grain was observed in Omid cultivar (- 4.51 pixel / g).

Based on the results of Table 3, there was a significant difference between the genotypes in terms of STI indices. Moreover, STI indicators for the three traits- yield, zinc content, and iron content of grains, the cultivars of Shahi, Biston, and a back cross roshan were respectively the best, whereas the cultivars of Zagros, Omid, and Shahriar were the weakest genotypes (Table 4). According to the results presented in Table 5, STI indices resulting from each feature and its reaction to the attribute had a positive and significant relationship with both conditions with and without zinc foliar application.

Table 3: Analysis of variance of STI indices for traits of zinc content and iron content in the investigated genotypes.

Sources of variation	Degree of freedom	Mean square								
		Yield in without zinc foliar application	Yield in with zinc foliar application	Grain's zinc content in without zinc foliar application	Grain's zinc content in with zinc foliar application	Grain's iron content without zinc foliar application	Grain's iron content in with zinc foliar application	STI index of grain yield	STI index of grain's zinc content	STI index of grain's iron content
Replication	2	0.421	0.066	28.542	8.686	8.463	5.456	0.138	0.088	1.026
Genotype	36	4.143**	3.421**	3.421**	463.273**	415.967**	20.714**	25.104**	1.052**	7.89**
error	72	0.179**	0.282	0.282	49.47	57.134	3.143	3.442	0.049	0.733

According to the results of this table, STI of performance had a negative and positive relationship with the reactions of iron and zinc attributes in both cases with and without zinc foliar. STI solidarity of zinc attribute had a negative and significant relationship with the reaction of zinc and iron attributes in both conditions, but STI solidarity of iron attribute had a negative and positive relationship with them, respectively. Moreover, based on the results of correlation analysis between STI indicators, there was a negative correlation between STI of zinc content and STI of iron content with STI of zinc content per grain. STI of iron attribute had also a positive relationship with the grain yield (Table 5).

The group effect in case of three attributes was significant indicating that among these three attributes, the cultivars with winter growth type had a different facultative growth type. At the same time, it is possible that there is a somewhat slight difference in environmental conditions. Effects of zinc were not significant on iron content of grains. Therefore, zinc foliar had no significant effect on the iron content of grains. Using cultivars with high performance and high efficiency in absorption of zinc and iron is also one of

the effective solutions to meet and deal with zinc and iron deficiency and its resulting malnutrition problems. Hence, taking advantage of proper indicators, which can be used to select high performance cultivars in terms of zinc and iron under deficiency conditions, is very important (Fernandes, 1993; Reynolds *et al.*, 2001).

Analysis of STI indices showed that there is a significant difference between genotypes in terms of STI indicators for all three attributes- yield, zinc content, and iron content of grains. Also, some studies have indicated that the indices caused by each attribute which have high correlation with it in both cases (with and without deficiency) are known as the best indices, since these indices are able to identify superior genotypes in both conditions, so they can be used for estimating stability of that trait or attribute (Fernandes, 1993). The results of correlation analysis between STI indicators gained from three studied traits and the way each of them responds to such traits under conditions with and without zinc showed that STI indices had a high correlation with the way they respond under both conditions of zinc deficiency and lack of deficiency.

Table 4: The mean of wheat genotypes studied under conditions with and without zinc foliar application.

Row		Yield in without zinc foliar application	Yield in with zinc foliar application	Grain's zinc content in without zinc foliar application	Grain's zinc content in with zinc foliar application	Grain's iron content without zinc foliar application	Grain's iron content in with zinc foliar application	STI index of grain's iron content	STI index of grain's zinc content	STI index of grain yield
1	Sayson	3.99	3.80	47.76	39.99	5.23	4.37	1.27	1.66	1.31
2	Pishgam	3.88	4.62	27.92	31.73	5.81	3.75	1.47	0.82	1.07
3	Biston	4.03	4.36	52.03	50.93	3.36	3.15	1.39	2.36	0.49
4	Zare	3.45	4.54	46.74	43.87	4.89	3.17	1.53	1.79	0.85
5	Karaj-3	3.45	4.22	40.13	27.86	4.11	4.72	1.22	0.95	0.95
6	MV-17	4.14	4.11	23.58	36.08	2.79	3.44	1.18	0.82	0.60
7	Omid	3.05	5.00	8.51	15.92	11.50	6.99	1.75	0.12	4.13
8	Gaspard	4.45	3.72	24.13	34.09	9.23	10.47	0.95	0.74	4.87
9	Back cross Roshan	4.67	5.44	9.27	18.81	10.26	11.91	2.01	0.16	6.11
10	Mihan	3.66	4.84	22.39	30.81	5.01	4.01	1.90	0.60	1.02
11	Sardari	4.05	3.64	17.84	27.10	5.70	8.43	1.12	0.42	2.43
12	Sabalan	4.63	4.29	15.34	18.15	3.44	1.83	1.45	0.24	0.30
13	Shahpasand	3.82	5.05	11.44	14.81	4.45	5.09	1.96	0.15	1.27
14	Tous	3.79	4.59	17.03	26.33	9.63	11.05	1.47	0.39	5.57
15	Alamout	4.02	4.03	13.66	27.46	3.16	1.98	1.27	0.32	0.23
16	Gascogen	5.30	3.62	20.90	27.64	3.14	3.67	1.22	0.49	0.73
17	Shahi	3.78	4.75	13.73	15.46	5.90	7.71	2.08	0.17	2.26
18	Azadi	3.69	4.62	22.51	14.83	7.09	6.98	1.44	0.30	2.62
19	Bezomtaya	3.43	3.69	17.50	26.70	5.19	4.37	1.14	0.41	1.02
20	Orom	2.91	3.74	13.71	15.60	5.30	6.26	1.08	0.19	1.78
21	Noursetar	2.17	3.03	15.03	20.34	4.80	5.19	0.74	0.27	1.28
22	Bam	1.97	2.68	40.96	47.79	3.13	2.02	0.49	1.73	0.28
23	Mahdavi	1.17	2.71	44.40	44.06	1.11	0.92	0.44	1.72	0.05
24	Ws- 82-9	1.29	1.97	36.38	39.40	4.28	5.04	0.19	1.24	1.07
25	Navid	2.08	2.39	32.41	50.27	6.24	5.36	0.26	1.40	1.55
26	Azar	1.64	2.77	22.11	48.13	0.66	0.95	0.48	1.06	0.03
27	Qods	2.06	2.36	41.99	49.05	2.96	1.42	0.33	1.78	0.21
28	Alvand	2.06	2.32	40.22	52.19	6.20	4.95	0.41	1.83	1.52
29	Roushan	2.33	3.01	27.18	34.51	0.95	0.83	0.61	0.81	0.04
30	shahriar	2.37	2.52	40.40	43.95	0.36	0.28	0.51	1.55	0.01
31	Inia66	1.44	1.87	32.37	42.70	1.88	2.86	0.22	1.20	0.20
32	Karaj-2	1.96	2.92	32.20	40.43	4.66	6.99	0.51	1.14	1.69
33	Azar2	1.70	2.40	40.58	40.05	4.54	2.81	0.35	1.41	0.64
34	Zareen	1.91	2.68	31.68	36.51	3.62	1.24	0.43	1.00	0.15
35	Kave	1.66	2.25	46.46	49.50	1.22	3.19	0.32	2.01	0.17
36	Rashid	1.65	2.00	37.15	37.80	6.41	5.83	0.29	1.23	1.84
37	Zagros	0.98	1.61	32.15	32.54	1.86	1.93	0.14	0.91	0.18
	LSD(0.05)	0.69	0.87	11.40	12.30	2.89	3.02	0.36	0.55	1.39

Therefore, based on the analysis results of variances, the correlation and due to classification of type A cultivar in Fernandez's classification (1993) by the STI Index, including genotypes which are superior in both

conditions of deficiency and lack of deficiency, the most appropriate and effective index used to identify genotypes efficient in terms of absorbing iron and zinc is STI index which has high performance.

Table 5: Correlation between STI indices of the traits: yield, zinc content, and iron content per seed and relevant responses of each trait under cases of zinc deficiency and lack of deficiency.

	1	2	3	4	5	6	7	8	9
1. Yield in withzinc foliarapplication	1								
2. Yield in without zinc foliar application	0.885**	1							
3. Grain's zinc content in with zinc foliar application	-0.548**	-0.564**	1						
4. Grain's zinc content in without zinc foliar application	-0.454**	-0.450**	0.700**	1					
5. Grain's iron content in with zinc foliar application	0.362**	0.338**	-0.369**	-0.361**	1				
6. Grain's iron content in without zinc foliar application	0.400**	0.348**	-0.319**	-0.358**	0.692**	1			
7. STI index of grain's iron content	0.957**	0.960**	-0.584**	-0.473**	0.364**	0.391**	1		
8. STI index of grain's zinc content	-0.481**	-0.484**	0.88**	0.923**	-0.365**	-0.328**	-0.505**	1	
9. STI index of grain yield	0.402**	0.346**	-0.350**	-0.39**	0.895**	0.837**	0.389**	-0.370*	1

* and ** represent significance at The 5% and 1% levels, respectively.

Based on the results of three-dimensional graphs and STI indices (Fig. 1,2,3) between genotypes in terms of grain yield, the cultivars of back cross roshan, shahi, shahpasand, mihan, omid, zaree, karaj3, bisoton and alamout, of grain zinc content bisoton, kave, alvand, Qods, bam and mahdavi, grain iron content back cross roshan, tous, Gaspard, omid and sardari were among the best genotypes in this study and placed in group A. Based on the results of three-dimensional graphs, there was no cultivar which can be placed in group A in terms of zinc and iron content. It can be due to the

negative correlation between zinc and iron contents per grain. A negative relationship is also reported by Alloway (2008) between grain's zinc and iron contents. Based on the significant negative relationship between STI yield, STI index of iron content, and STI index of zinc content, it is impossible to rely just on one of them for selecting efficient zinc genotypes equipped with high yield and iron content; therefore, this selection should be performed independently and in accordance of all three indices.

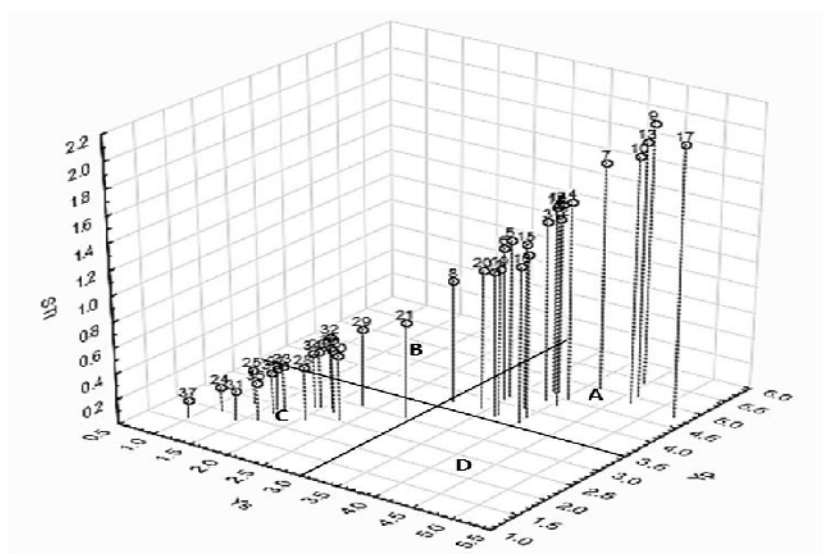


Fig. 1. Grouping of bread wheat varieties of grain yield based on Fernandez model.

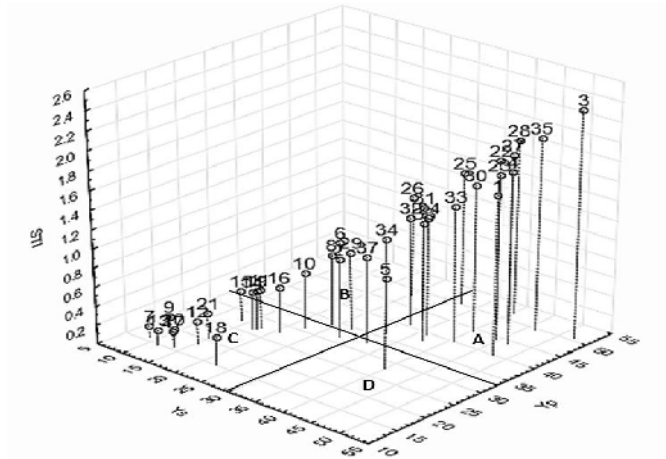


Fig. 2. Grouping of bread wheat varieties of grain zinc content based on Fernandez model.

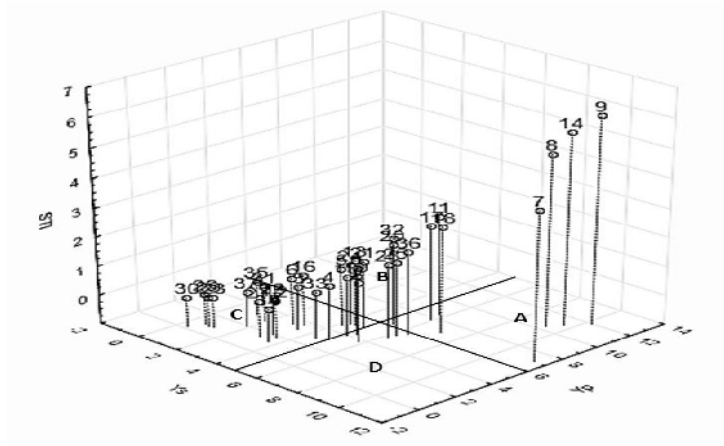


Fig. 3. Grouping of bread wheat varieties of grain Iron content based on Fernandez model.

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