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Study of genetic diversity in bread wheat germplasm using nitrogen uptake and nitrogen use efficiency characteristics

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Abstract

In order to evaluate the traits related to nitrogen utilization and to identify the superior genotypes, 33 bread wheat landraces along three check cultivars of Chamran and Koohdasht (from Iran) and Gobustan (from the Republic of Azerbaijan) were studied in simple lattice statistical design with two replications under two treatments of non-usage and application of 200 kg/ha ammonium nitrate fertilizer in research field of Seed and Plant Improvement Institute located in Karaj, Iran. The results indicated that the diversity of nitrogen uptake was higher than that of nitrogen use efficiency based on coefficient of variation. Genotype 4 (from Iran) and Chamran showed the highest value of nitrogen uptake efficiency. The highest amount of nitrogen use efficiency belonged to the genotypes 4 and 7 (from Iran). In the biplot of nitrogen uptake and use efficiency, check cultivar Chamran along with genotypes 4, 19, 21 and 23 (from Iran) and 2, 3 and 9 (from the Republic of Azerbaijan), which were superior for both traits were located in region A of Fernandez grouping. Generally, based on the results , a high varation in nitrogen components was revealed in the studied germplasem, particullary in Iranian landraces which could be used as gene pool for breeding programs.

Key words: Gene pool, Landrace, Nitrogen yield of grain, Nitrogen biological efficiency.

INTRODUCTION

Nitrogen is one of the main inputs in the agricultural production which inevitably leads to soil and groundwater contamination. Providing sufficient nitrogen and water supply, using high yielding varieties and controlling diseases, pests and weeds are important factors in achieving high yield. Due to the increasing costs of chemical fertilizers and their destructive environmental effects, breeding wheat cultivars with high nitrogen uptake and nitrogen use efficiency would reduce the input costs and as a result, would increase farmers' profits and income, as well as preventing soil and water contamination. Today, scientists are seeking to develop varieties which reduce the risk of contamination of the ecosystem and reduce fertilizer use (Le Gouis *et al*., 2000). One third of the world's fertilizer use is devoted to wheat (Guttieri *et al.,* 2017). Identification of wheat genotypes that efficiently absorb soil nitrogen and transform it into protein can play a key role in meeting the global need for grain and grain protein (Guttieri *et al.,* 2017). The efficiency of nitrogen uptake in wheat plants is important in two aspects of absorption and transfer to grains and other organs of the plant.

Genetic diversity is the basis for breeding crops which allows the selection for desirable traits and their transfer to target plants. Wheat genotypes are different in the efficiency of nitrogen uptake and use so that some of the genotypes have high nitrogen uptake efficiency under low input conditions (Hasanzadeh Gorttapeh *et al*., 2008). Under such conditions, it is possible to select superior genotypes in terms of uptake and use of nitrogen in breeding programs (Hasanzadeh Gorttapeh *et al*. 2008; Ortiz-Monasterio *et al*., 1997; Dhugga and Waines, 1989; Van Sanford and MacKown, 1986).

Table 1. Name and origin of 36 bread wheat genotypes used in this study.

No.	Name/ Accession code	Origin	No.	Name/ Accession code	Origin	No.	Name/ Accession code	Origin
1	Gobustan	Azerbaijan	13	Bc14	Azerbaijan	25	Kc-1691	Iran
2	Girmizigul	Azerbaijan	14	Bc17	Azerbaijan	26	Kc-2474	Iran
3	Azamatli	Azerbaijan	15	Kc-219	Iran (Orumieh)	27	Kc-2682	Iran (Marivan)
4	Arta	Iran	16	Kc-264	Iran (Maraghe)	28	Kc-3155	Iran (Mashhad)
5	Chamran	Iran	17	Kc-677	Iran (Khorram_Abad)	29	Kc-4713	Japan
6	Koohdasht	Iran	18	Kc-1196	Iran	30	Kc-5032	France
7	Bc-1	Azerbaijan	19	Kc-1200	Iran	31	Kc-5801	Unknown
8	Bc-3	Azerbaijan	20	Kc-5514	France	32	Kc-6127	France
9	Bc-5	Azerbaijan	21	Kc-868	Iran (Gorgan)	33	Kc-6360	China
10	Bc-6	Azerbaijan	22	Kc-857	Iran (Gorgan)	34	Kc-388	Iran
11	Bc-7	Azerbaijan	23	Kc-987	Iran	35	Kc-3366	Iran (Mashhad)
12	Bc-10	Azerbaijan	24	Kc-1656	Iran	36	Kc-6143	Russia

Bayat *et al.* (2011) studied the genotypic variation and relationship of grain yield and protein with nitrogen remobilization in 12 Iranian wheat cultivars. The results showed that remobilization of nitrogen was different among cultivars so that the highest and lowest nitrogen remobilization belonged to Karkheh and Azar2, respectivley. Khalilzadeh *et al.* (2011) reported genetic diversity of nitrogen uptake and use in 20 bread wheat genotypes and the results indicated a high level of variation. Hosseini *et al.* (2013) concluded that the contribution of nitrogen productivity efficiency was higher than nitrogen uptake efficiency for all the studied cultivars.

Feizi Asl *et al.* (2014) showed that by optimizing the management of nitrogen application, the effect of drought stress on yield of dryland wheat could be reduced. Yosefi *et al.* (2014) concluded that to achieve optimal wheat yield, 78 kg/ha of pure nitrogen (170 kg/ha of urea fertilizer) should be used in splitting manner. Shahrasbi *et al.* (2016) showed that agronomic nitrogen use efficiency, water productivity, grain yield and yield components were reduced and protein content of grain increased under rainfed conditions. Omidi-Nasab *et al.* (2016) showed that increase in nitrogen fertilizer application up to 150 kg/ha significantly increased grain yield and then no significant changes were observed.

Most of studies in the field of nitrogen utilization focus on optimizing amounts or stages of fertilizer application to achieve the maximum productivity. However since nitrogen metabolism is a complex process consisting of several influencing factors, it is unlikely to identify genotypes with superiority in all nitrogen components. Therefore, a genetic diversity is expected to exist for different aspects of nitrogen metabolism. Wheat landraces are considered as valuable genetic resources for valuable traits. Local germplasms have already proved to be suitable plant material sources relating to nitrogen utilization (Khalilzadeh *et al*., 2011; Hasanzadeh Gorttapeh *et al*., 2008). The purpose of this study was to investigate the genetic diversity of nitrogen indices in wheat germplasm and to identify accessions with high efficiency of nitrogen uptake and nitrogen use.

MATERIALS AND METHODS

Thirty-six bread wheat genotypes (Table 1), including 33 collection accessions along with three check cultivars (Chamran, Koohdasht from Iran and Gobustan from the Republic of Azerbaijan) were evaluated in research field of Seed and Plant Improvement Institute located in Karaj during 2009-2010 crop season. Soil samples were taken from depth of 0 to 30 centimeters in three different locations of the experiments to determine soil physical and chemiacal characteristics before planting and land preparation and after harvest (Table 2). After analysis and determination of the main elements in the soil, the experiments were carried out in two separate levels of without fertilizer application (T_0) and applying 200 kg of ammonium nitrate fertilizer per hectare (T_1) in a simple lattice statistical design with two replications. Each plot consisted of four rows of 2.5 meter long and 30 cm distance. The cultivation density was 350 seeds per square meter. Surface fertilization was perfomed three times with a ratio of 1/3, 1/3 and 1/3 of the total fertilizer so that one third of ammonium nitrate fertilizer was applied simultaneously with planting as base fertilizer and the remainder in two stages, at the beginning of the stem elongation and at the beginning of the spike emergence. Irrigation was done according to the routines of the region. Grain yield, biological yield, nitrogen rate of grain yield, nitrogen rate of straw (root, stem and leaf), nitrogen yield of grain, nitrogen yield of straw, nitrogen portioning index, nitrogen biological efficiency, nitrogen uptake efficiency and nitrogen use efficiency were evaluated.

To measure grain yield and biological yield, grain weight and total weight of each plant were measured and recorded in each plot including four rows of 2 meters (with removal of 25 cm from each side of the rows as margins).The Kjeldahl method (Humphries, 1956) was used to measure the nitrogen rate in grain and straw.

Nitrogen exploitation and storage indices were calculated using the following formulas (May *et al*., 1991; Ortiz-Monasterio *et al*.,1997):

Nitrogen yield of grain=(Grain yield)×(Nitrogen rate in grain)

Nitrogen yield of straw=(Straw yield)×(Nitrogen rate in straw)

Nitrogen portioning index=(Nitrogen yield of grain)÷(Nitrogen yield of straw)

Nitrogen uptake efficiency=(Nitrogen yield of grain+ Nitrogen yield of straw)÷(The amount of nitrogen used)

Nitrogen use efficiency= Grain yield÷(Nitrogen rate in grain+ Nitrogen rate in straw)

Analysis of variance was perfomed based on lattice statistical design and the data was then subjected to a combined ANOVA considering levels of nitrogen fertilizer as different locations with fixed (non-random) effect. The interaction of genotype × nitrogen fertilizer was more accurately evaluated with the calculation of the wi^2 equivalence parameter (Wricke, 1962).

The genetic distances between the studied genotypes were obtained by calculating the Euclidean distances separately in each of the T0 and T_1 conditions, as well as considering both conditions. The correlation between matrices of genetic distances was tested using Mantel's method. For this purpose, using the Monte Carlo simulation based on 999 replications, a reference distribution was obtained and the correlation between matrices of genetic distances was tested with respect to its probability of occurrence in the reference distribution.The studied genotypes were grouped by using cluster analysis based on Ward method. Statistical analysis and drawing of the graphs were carried out by coding in R software.

Table 2. Physico-chemical features of soil in the experiment site before planting and after harvest.

Soil parameters	Before planting	After harvest
Clay (%)	25	23
Silt (%)	51	52
Sand (%)	24	25
P (mg.kg^{-1})	6.23	5.46
Organic Carbon (%)	0.53	0.55
Total Nitrogen (%)	0.044	0.046
N (kg/ha)	1518	1587
pH	7.42	7.88
Electrical Conductivity (dS.m^{-1})	1.91	1.27
Soil Saturation (%)	46	52

RESULTS

The results showed no significant difference between the replications for the studied traits. Therefore, the replications of the lattice design were considered as blocks in the frame of a Randomized Complete Block Design. Nitrogen fertilizer treatments of T_0 and T_1 were also included in the combined ANOVA as different environments. The results of combined ANOVA showed that the difference in the means of genotypes, environments (T_0 and T_1 treatments related to different levels of nitrogen fertilizer application), as well as genotype and nitrogen fertilizer interaction, were significant for all studied traits at 1% probability level (results are not presented). Mean comparison of genotypes showed that the highest grain yield in T_0 and T_1 treatments was related to genotype 10 (4112 kg/ha) from the Republic of Azerbaijan and Chemran (5171 kg/ha), respectively.

The highest biological yield belonged to genotype 17 from Khorram Aabad (6274 and 1465 kg/ha, in T_0 and T_1 treatments, respectively). Biological yield of 14 and 13 genotypes were higher than check cultivars in T_0 and T_1 treatments, respectively. The highest nitrogen rate in T_0 treatment was allocated to two genotypes from the Republic of Azerbaijan including genotype 10 (2.20%) and genotype 2 (2.32%). In T_1 treatment, the nitrogen rate of 13 genotypes was higher than that in Gobustan.The highest rate of nitrogen in straw in T_0 treatment belonged to genotype 17 (0.485%) from Khorram Abad and genotype 13 (0.735%) from the Republic of Azerbaijan. Under T_1 treatment, nitrogen rates of straw in genotype 28 (0.680%) from Mahshad and genotype 13 (0.735%) form the Republic of Azerbaijan were higher than in Koohdasht.The highest nitrogen uptake efficiency (0.257) related to genotype 4 (Arta cultivar from Iran). Genotype 4 also had the

Table 3. Values of descriptive statistics for the studied traits in bread wheat germplasm under condition of non-usage of ammonium nitrate fertilizer.

Trait	Mean	Standard deviation	Minimmum	Maximum	Coefficient of variation (%)
Grain yield (kg/ha)	2721.00	90.73	1416.50	4189.60	28.29
Biological yield (kg/ha)	9499.00	167.68	6176.00	14845.20	14.98
Nitrogen rate of grain yield (%)	2.10	0.00	2.02	2.21	1.71
Nitrogen rate of straw (%)	0.28	0.01	0.14	0.50	31.96
Nitrogen yield of grain (kg/ha)	57.18	1.94	29.10	91.90	28.84
Nitrogen yield of straw (kg/ha)	18.69	0.90	9.20	54.30	40.95
Nitrogen portioning index	3.51	0.20	1.26	8.60	49.10
Nitrogen biological efficiency	1146.70	329.41	570.83	1716.49	28.73

Table 4. Values of descriptive statistics for the studied traits in bread wheat germplasm under application of ammonium nitrate fertilizer (200 kg/ha).

Trait	Mean	Standard deviation	Minimmum	Maximum	Coefficient of variation (%)
Grain yield (kg/ha)	3301.00	104.73	1741.50	5206.10	26.92
Biological yield (kg/ha)	11250.00	178.67	7482.60	15531.80	13.48
Nitrogen rate of grain yield (%)	2.19	0.01	2.05	2.33	2.78
Nitrogen rate of straw (%)	0.37	0.01	0.23	0.75	27.71
Nitrogen yield of grain (kg/ha)	72.32	2.31	40.30	111.40	27.14
Nitrogen yield of straw (kg/ha)	29.63	1.14	15.30	61.80	32.61
Nitrogen portioning index	2.69	0.14	1.11	5.95	43.44
Nitrogen biological efficiency	1289.90	359.25	661.47	2132.29	27.85
Nitrogen use efficiency	2.90	0.215	0.88	9.02	62.78
Nitrogen uptake efficiency	0.13	0.007	0.02	0.26	48.46

highest nitrogen use efficiency (8.94).

Yousefi *et al.* (2014) showed that the effects of rate and time of nitrogen application on wheat bilogical yield, grain yield and leaf area index at anthesis was significant. In this study, the maximum yield was 5530 kg/ha, which was obtained at 78 kg/ha pure nitrogen. Shahrasbi *et al.* (2016) reported that highest grain yield and biological yield were obtained in treatments of 150 and 225 kg/ha of urea fertilizer in non-stress condition, respectively. Omidi-Nasab et al. (2016) concluded that on wheat yield and yield components in response to reduced nitrogen fertilizer application, Chamran cultivar with a mean grain yield of 4131 kg/ha was significantly superior to Behrang cultivar.

Descriptive statistics for the studied traits in the absence of nitrogen fertilizer (T_0) and with application of nitrogen fertilizer (T_1) are presented in Tables 3 and 4, respectively. Based on the minimum and maximum values, the range of all traits except biological yield and nitrogen portioning index increased under nitrogen fertilizer conditions (T_1) compared to the non-nitrogen fertilizer conditions (T_0). Nitrogen portioning index and nitrogen yield of straw in T_0 treatment, and nitrogen uptake and use efficiency in T_1 treatment had the highest coefficient of variation. The correlation coefficients between the traits (Tables 5 and 6) showed that in both conditions, T_0 and T_1, nitrogen yield of grain was highly correlated with grain yield. Nitrogen yield of straw in T_0 condition was not significantly correlated with nitrogen rate of straw. However, in T_1 condition, there was a significant correlation between these two traits. Nitrogen use efficiency was significantly correlated with grain yield, nitrogen yield of grain and nitrogen portioning index. Nitrogen uptake efficiency was significantly correlated with grain yield, biological yield, nitrogen rate in straw, nitrogen yield of grain and nitrogen yield of straw. Nitrogen uptake efficiency and nitrogen uptake efficiency also showed a significant correlation.

In order to study the effect of nitrogen fertilizer on the studied traits, distribution pattern of genotypes under T_0 and T_1 treatments was investigated (Figure 1). As shown in Figure 1, the slope of the regression line for nitrogen yield of straw had the highest deviation from the reference line (b = 0), which means that this trait was more affected by application of nitrogen fertilizer than nitrogen yield of grain, nitrogen portioning index, and nitrogen biological efficiency. In the biplot of nitrogen yield of grain (Figure 1A), all genotypes were located on top of the reference line, indicating the positive effect of nitrogen fertilization on increasing this trait in all studied genotypes. Genotypes 4 and 23 (from Iran) and Chamran (check cultivar) had the highest deviation from the reference line and the highest amount of Wricke's equivalence (w_i^2) for this trait (Table 7),

Table 5. Coefficient of correlation among the studied traits in bread wheat germplasm under condition of non-usage of ammonium nitrate fertilizer.

Trait	Biological yield	Nitrogen rate of grain	Nitrogen rate of straw	Nitrogen yield of grain	Nitrogen yield of straw	Nitrogen portioning index	Nitrogen biological efficiency
Grain yield	0.48**	0.37*	-0.21	0.998**	-0.10	0.76**	0.99**
Biological yield		0.34*	-0.18	0.49**	0.40*	0.12	0.47**
Nitrogen rate of grain			-0.18	0.43**	-0.06	0.30	0.33*
Nitrogen rate of straw				-0.21	0.79**	-0.64**	-0.32*
Nitrogen yield of grain					-0.10	0.75**	0.99**
Nitrogen yield of straw						-0.64**	-0.21
Nitrogen portioning index							0.82**

Table 6. Coefficient of correlation among the studied traits in bread wheat germplasm under application of ammonium nitrate fertilizer (200 kg/ha).

Trait	Biological yield	Nitrogen rate of grain	Nitrogen rate of straw	Nitrogen yield of grain	Nitrogen yield of straw	Nitrogen portioning index	Nitrogen use efficiency	Nitrogen uptake efficiency	Nitrogen biological efficiency
Grain yield	0.39*	0.004	-0.02	0.99**	-0.12	0.76**	0.51**	0.33*	0.99**
Biological yield		-0.03	-0.01	0.39*	0.43**	-0.06	0.15	0.32	0.39*
Nitrogen rate of grain			0.12	0.11	0.11	0.01	-0.10	0.11	-0.10
Nitrogen rate of straw				0.00	0.84**	-0.46**	-0.17	0.52**	-0.15
Nitrogen yield of grain					-0.10	0.76**	0.49**	0.35*	0.97**
Nitrogen yield of straw						-0.66**	-0.22	0.51**	-0.23
Nitrogen portioning index							0.46**	-0.03	0.82**
Nitrogen use efficiency								0.49**	0.53**
Nitrogen uptake efficiency									0.26

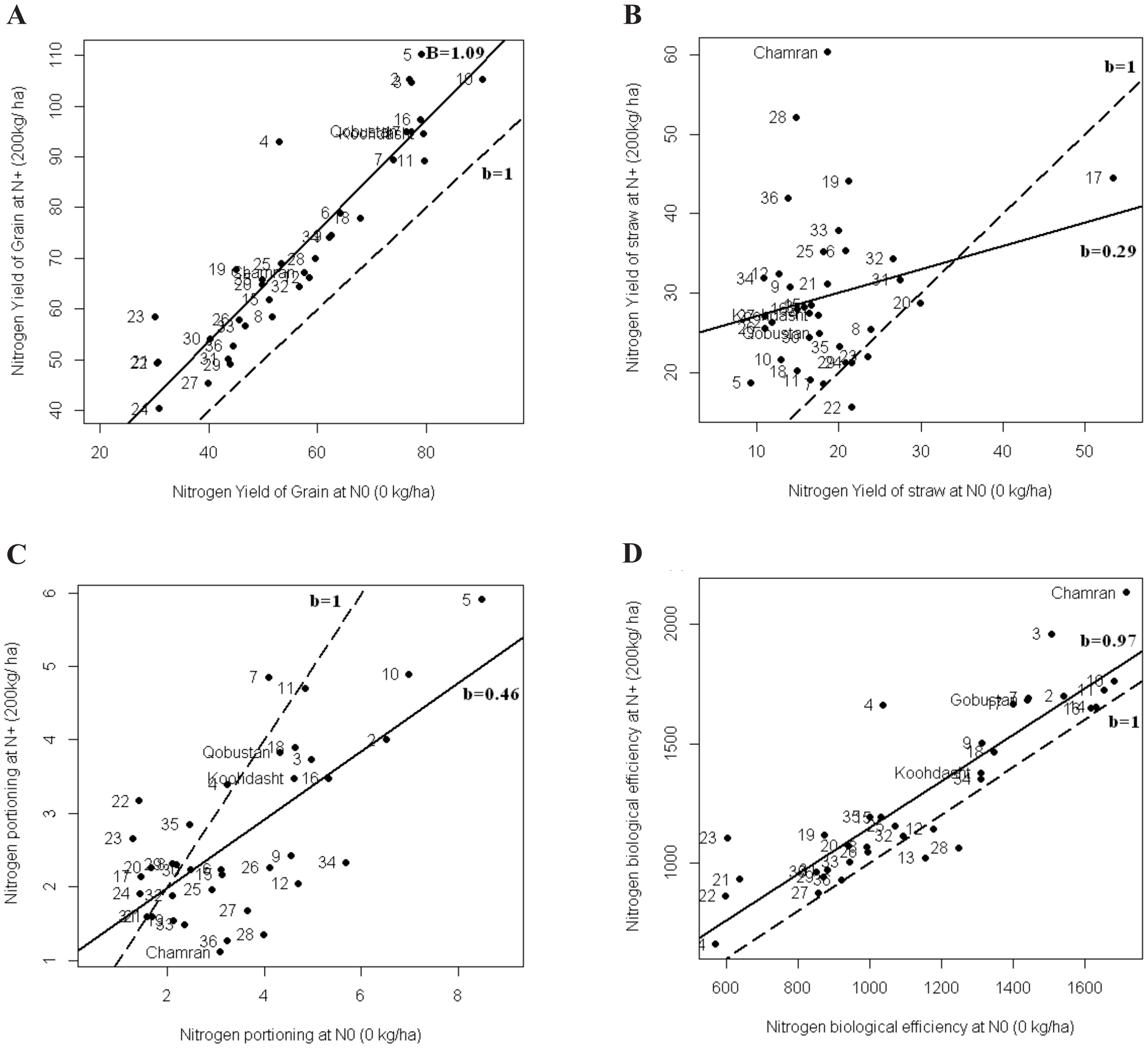

Figure 1. Graphical presentaion of distribution of bread wheat genotypes in biplot based on two treatments of non-usage and application of 200 kg/ha ammonium nitrate fertilizer for the traits **A:** nitrogen yield of grain, **B:** nitrogen yield of straw, **C:** nitrogen proportioning index and **D:** nitrogen biological efficiency.

indicating the highest impact rate of nitrogen fertilizer on these genotypes for nitrogen yield in the grain. In the biplot of the nitrogen yield of straw (Figure 1B), except for the accessions 17, 22, 23, 24 (from Iran) and 20 (from France), other genotypes were located above the reference line, indicating increased nitrogen yield of straw by application of nitrogen fertilizer. Accession 13 (from the Republic of Azerbaijan) and genotypes 17 and 28 (from Iran) had the highest amount of Wricke's equivalence (w_i^2) (Table 7), which indicates the highest nitrogen yield of straw in these samples due to nitrogen fertilizer application. In the graph of nitrogen portioning index (Figure 1C), genotypes 4, 17, 22, 23, 24 and 35 (from Iran), 7 and 8 (from the Republic of Azerbaijan), 20 (from France), 29 (from Japan) and 31 (from unknown origin) were placed on top of the reference line, indicating the allocation of more nitrogen to seeds in these genotypes by using nitrogen fertilizer. All three check figures were situated below the reference line in this biplot. Genotypes 22 and 23 had the highest amount of Wricke's equivalence (w_i^2) (Table 7), which indicated that compared to the non-application of nitrogen fertilizer, more nitrogen is allocated to grains than straw in these genotypes by the application of nitrogene fertilizer. In the biplot of the nitrogen biological efficiency (Figure 1D), all genotypes except genotypes 12 and 13 (from the Republic of Azerbaijan) and 28 (from Iran) were placed above the reference line, indicating an increase in the biological efficiency of nitrogen in these genotypes by using nitrogen fertilizer. Genotypes 4, 23 and 28 (from Iran) also had the highest amounts of Wricke's equivalence (w_i^2) (Table 7).

In order to distinguish the genotypes with higher

Table 7. Values of Wricke's equivalence (*wi*^{2}) for the traits nitrogen yield of grain, nitrogen yield of straw, nitrogen portioning index and nitrogen biological efficiency in evaluation of bread wheat genotypes under two treatments of non-usage and application of 200 kg/ha ammonium nitrate fertilizer.

Genotype	Nitrogen yield of grain	Nitrogen yield of straw	Nitrogen portioning index	Nitrogen biological efficiency	Genotype	Nitrogen yield of grain	Nitrogen yield of straw	Nitrogen portioning index	Nitrogen biological efficiency
1	5.90	6.75	0.05	4730.75	19	29.53	71.36	71.36	5117.95
2	87.33	6.58	1.43	85.49	20	0.00	73.73	73.73	72.95
3	75.22	1.17	0.09	47659.66	21	7.09	1.29	1.29	11614.86
4	309.65	0.03	0.47	115233.93	22	6.94	143.53	143.53	7637.93
5	126.02	1.21	1.53	37156.72	23	87.33	77.17	77.17	64972.26
6	0.08	6.40	0.00	2730.74	24	15.93	64.45	64.45	1381.02
7	0.07	55.05	1.26	5440.49	25	0.12	18.83	18.83	1699.72
8	34.90	43.55	0.44	2324.86	26	4.28	6.61	6.61	3922.19
9	5.30	17.21	0.85	1112.82	27	47.28	13.19	13.19	7727.05
10	0.04	2.72	0.81	2023.54	28	11.59	348.14	348.14	53063.75
11	15.65	35.39	0.23	2691.09	29	50.75	55.05	55.05	2624.42
12	27.49	39.22	1.68	15439.38	30	0.76	4.39	4.39	495.74
13	15.70	474.84	0.67	38396.49	31	35.40	22.73	22.73	1323.68
14	0.00	0.75	0.05	7330.99	32	26.10	5.23	5.23	7610.45
15	9.14	0.43	0.01	133.53	33	13.65	24.62	24.62	3262.08
16	5.07	2.26	0.53	6366.37	34	5.01	50.17	50.17	5310.87
17	3.06	197.07	1.14	7546.25	35	0.49	30.13	30.13	1245.66
18	12.62	16.09	0.00	338.21	36	23.35	147.35	147.35	8975.37

efficiency in nitrogen uptake and use, the distribution pattern of genotypes was plotted based on these two traits (Figure 2). Chamran cultivar and genotypes 2, 3 and 9 (from the Republic of Azerbaijan), and 4, 19, 21 and 23 (from Iran) were located in area A, which is devoted to samples with higher efficiency of nitrogen uptake and use. Koohdasht cultivar and genotypes 12 and 13 (from the Republic of Azerbaijan), 16, 25, 26, 28 and 34 (from Iran), 33 (from China) and 36 (from Russia) with higher nitrogen uptake efficiency and lower nitrogen use efficiency, were situated in B region. Gobustan cultivar, genotype 7 (fromthe Republic of Azerbaijan), and genotypes 17, 22 and 35 (from Iran), with higher nitrogen use efficiency and lower nitrogen uptake efficiency, were placed in region C. D region is devoted to accessions with lower nitrogen uptake and use efficiency and genotypes 15, 18, 24 and 27 (from Iran) 8, 10, 11 and 14 (from the Republic of Azerbaijan), 20, 30 and 32 (from France), 29 from Japan and 31 (from an unknown region) were loated in this region.

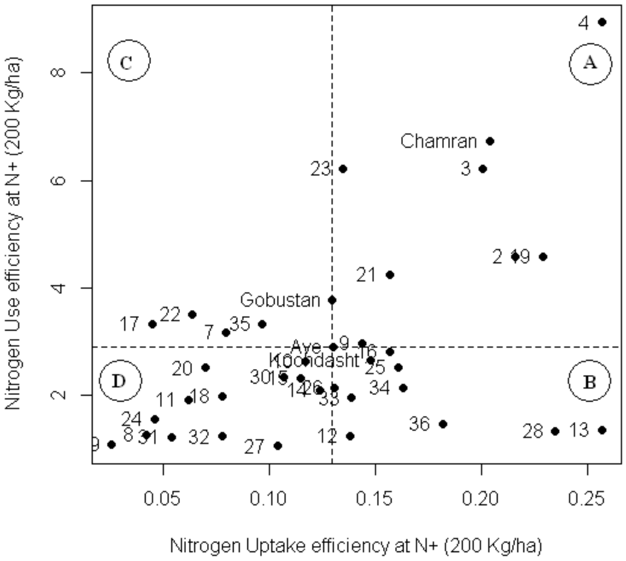

Figure 2. Graphical presentaion of distribution of bread wheat genotypes in biplot based on nitrogen uptake and use efficiency.

The results of analysis of genetic distances showed that in each of T_0 and T_1 treatments, as well as in the total conditions of T_0 and T_1, Chamran cultivar had the lowest genetic distance from genotypes 14 and 10 (from the Republic of Azerbaijan) and then from Gobustan cultivar. In both T_0 and T_1 treatments, the Koohdasht cultivar had the lowest genetic distance with genotypes 34 (from Iran) and 3 and 2 (from the Republic of Azerbaijan). The cultivar Gobustan had the lowest genetic distance with Genotype 2 and 7 (from the Republic of Azerbaijan) and Chamran cultivar. All three check cultivars in all conditions had the highest genetic distance from genotypes 17, 24 and 22 (from Iran) (Figures 3A, 3B and 3C). Mantel test results showed significant correlations between the genetic distances (Figures 4A, 4B and 4C).

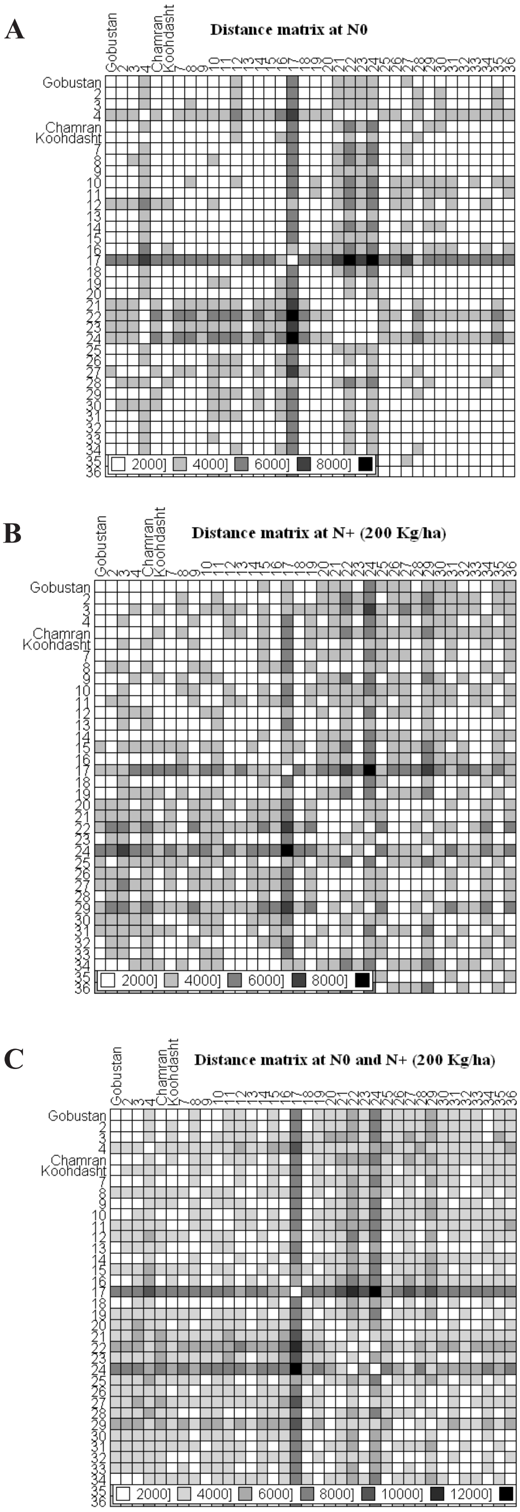

Figure 3. Presentation of genetic distance between bread wheat genotypes in condition of **A:** non-usage nitrogen fertilizer, **B:** under treatment of application of 200 kg/ha ammonium nitrate fertilizer, and **C:** in both treatments.

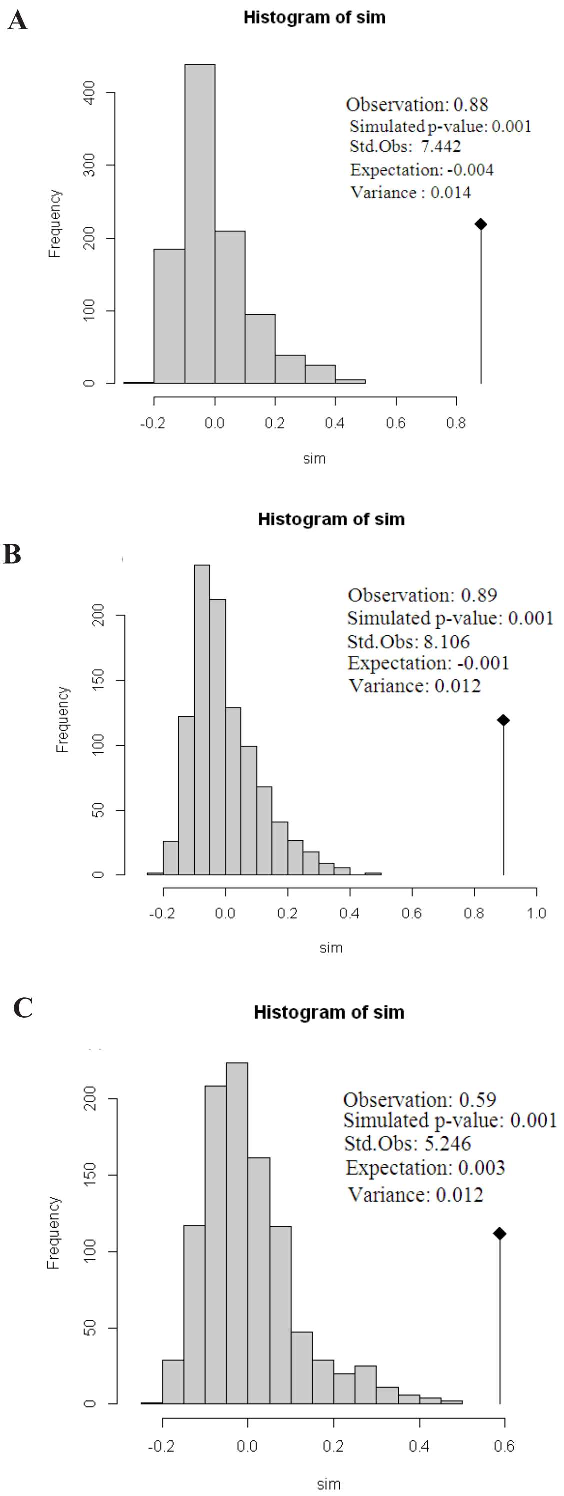

Figure 4. Reference distribution developed through Monte-Carlo approach for Mantel test related to distance matrix of bread wheat genotypes at N0 and N0&N200 (a), at N200 and N0&N200 and at N0 and N200 (c).
N0, N200 and N0&N200 are treatments of **A:** non-usage of nitrogen fertilizer, **B:** application of 200 kg/ha ammonium nitrate fertilizer, and **C:** considering both treatments, respectively.

The dendrogram of cluster analysis under T_0 treatment divided the genotypes in four groups (Figure 5A). Genotypes 34, 2, 3, 11, 16, 10, 14 and 18, along with check cultivars, were located in one group. The studied genotypes were clustered under T_1 treatment in six groups (Figure 5B). Genotypes 3, 2, 16, 7 and 14, along with Chamran and Gobustan were placed in the same group and genotypes 18, 11, 4 and 10, along with Koohdasht were placed in a separate group. The dendrogram divided the genotypes into five groups based on total conditions of T0 and T1 treatments (Figure 5C). Genotypes 9, 34, 2, 3, 11, 10, 16, 14 and 7 were located with Chamran and Gobustan in one group and genotypes 32, 20, 26, 13, 18, 12, 30, 33, 35, 8 and 28, along with Shahid Kuhdasht cultivar, were situated in a separate group.

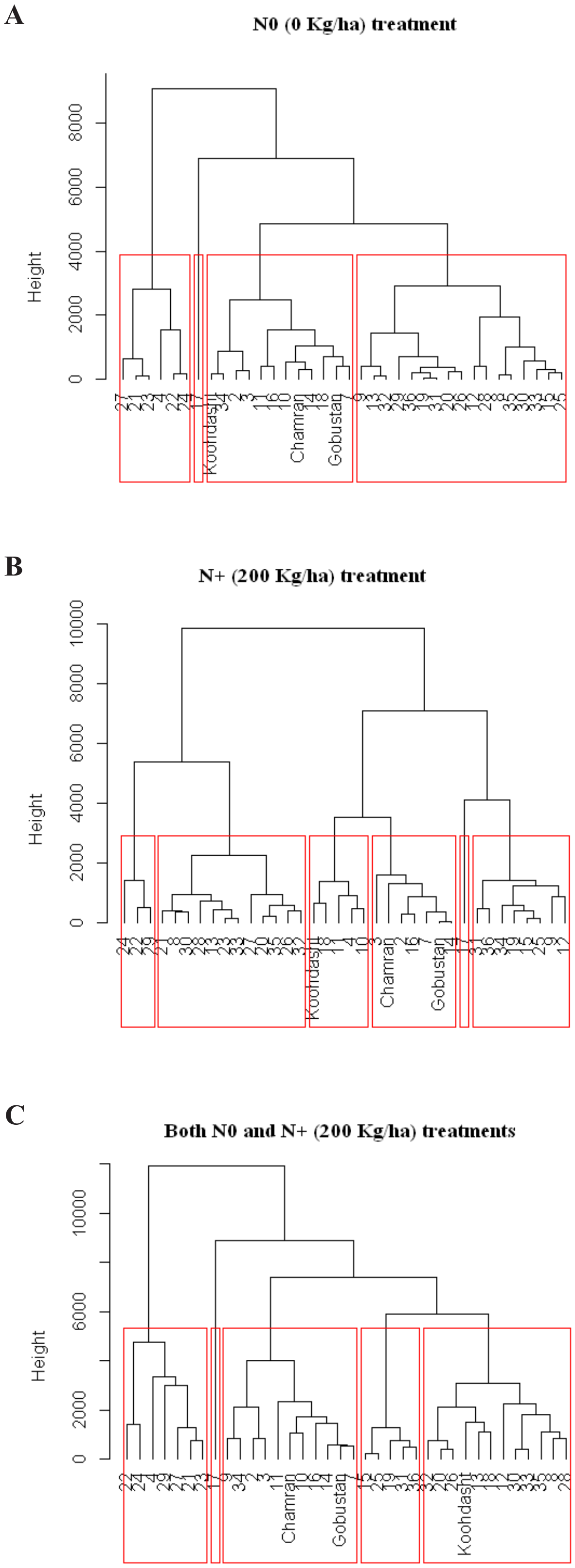

Figure 5. Dendrogram of cluster analysis related to bread wheat genotypes under condition of **A:** non-usage of nitrogen fertilizer, **B:** application of 200 kg/ha ammonium nitrate fertilizer, and **C:** in both treatments.

DISCUSSION

Four basic components involved in nitrogen absorption and conversion are grain yield, nitrogen rate in grain, straw yield and nitrogen rate in straw (Guttieri *et al.,* 2017). In the present study, these four components were considered as the basis for evaluating the genetic diversity of nitrogen utilization components. The combination of two components of grain yield and grain nitrogen are expressed in terms of nitrogen yield of grain. Obviously, increase in this index is influenced by changes in both of its components, and hence, the genotypes that can be successful in increasing both components are more desirable in terms of this index. In the present study, it can be seen that the two components of grain yield and nitrogen rate in grain played different roles in the nitrogen yield of grain since the genotypes with highest amount of grain yield in T_1 condition (Chamran and genotype 3) were not the same as the ones with the highest nitrogen rate in grain. On the other hand, the genotypes with the highest increase in grain yield under nitrogen fertilizer (genotypes 23 and 4) were different with the ones having the highest increase in nitrogen rate of grain (genotypes 22 and 2). Therefore, the superiority of genotypes in nitrogen yield of grain may result from their superiority in one of the two components or in both components. For this reason, it is necessary to investigate which of these two factors contributed to higher performance of superrior genotypes in nitrogen yield of grain. In this regard, it seems that high nitrogen yield of grain in Chamran cultivar is most likely due to higher grain yield rather than higher nitrogen rate in grain since it was situated in the 25th place in terms of nitrogen rate in grain. The performance of genotype 10 can also be mentioned as another evidence for this finding since it did not show a high increase in yield and nitrogen in grain under application of nitrogen fertilizer, while it was ranked among superior genotypes for high nitrogen yield of grain which could be attributed to its initial high grain yield and nitrogen rate in grain in T_0 condition. The same was true for the nitrogen yield of straw. Genotypes 13 and 28 had the highest nitrogen yield of straw in T_1 condition, while they were not among the top genotypes in terms of straw yield in T_1. In contrast, genotype 17 had a high straw yield and also a relatively high straw nitrogen rate in straw content in T_1 condition. Therefore, it ranked third after genotypes 13 and 28 based on nitrogen yield of straw. On the other hand, nitrogen portioning between grain and straw is also very important. In this regard, it is worth mentioning that nitrogen in the grain is supplied form two sources including nitrogen remobilization from vegetative tissues and asymiliated nitrogen after anthesis.Studies show that more than 70 percent of nitrogen in the grain results from nitrogen remobilization prior to anthesis (Kichey *et al.,* 2007; Bogard*et al.,* 2010). However, low genetic variation in the amount of grain nitrogen from nitrogen remobilization from vegetative tissues has been reported (Bogard*et al.,* 2010). The results of nitrogen portioning index showed that there is a variation between genotypes, but according to regression coefficient (Figure 1), this index is also influenced by nitrogen fertilizer application, and therefore, some genotypes are expected to be more different in terms of nitrogen portioning under application of nitrogen fertilizer. However, this interaction seems to be less important in genotypes that have a higher degree of nitrogen portioning index, such that Chamran and Genotype 10 were superior to other genotypes based on this index. However, genotype 7 which was the third highest in terms of nitrogen portioning index under T_1 condition, was ranked 14th for this index unnder T_0 treatment. Also, despite the fact that Chamran cultivar showed superiority for this index, another check cultivar (Koohdasht) did not appear to be suitable as it was ranked 20 and 21 for this index, respectively under T_0 and T_1 treatments.

Significance of the correlation between genetic distances in T_0 and T_1 conditions, which was determined by Mantel test, means that nitrogen storage situation of the genotypes in nitrogen fertilizer application could be predicted to some extent based on the reaction of genotypes under non-nitrogen fertilization. However the precision of this prediction depends on the amount of genotype × nitrogen effect so that at higher levels of this interaction, the correlation between two conditions is reduced. Therefore, examining the response of genotypes at different levels of nitrogen fertilizer application is required to identify the best genotypes in terms of nitrogen uptake and use. Reports indicate that efficiency of nitrogen use is generally low in cereals (Ladha *et al.,* 2005; Raun and Johnson, 1999). Therefore, it is important to search for genetic diversity in nitrogen components so that genotypes with high efficiency in nitrogen absorption or nitrogen consumption are identified. In the present study, biplot of nitrogen uptake and use efficiencies were used to distinguish genotypes that are superior in one or both of these traits. In this biplot, three check cultivars were distinguished in three groups, which showed the effectiveness of this method for differentiating genotypes in terms of nitrogen uptake and use efficiency. However, in cluster analysis under T_0 condition, all three check cultivars were located in the same group, and under T_1 condition, Chamran and Gobustan were situated in a group and Koohdasht was placed in a separate group. These results indicate that the analysis based on the biplot was more successful than cluster dendrograms in making differentiotaion among the check cultivars. Chamran were located in region A of biplot and proved to be superior to two other check cultivars in terms of nitrogen utilization. Koohdasht and Gobustan were situated in B and C regions, respectively which dedicate to genotypes with higher nitrogen uptake and nitrogen use efficiencies, respectively. These results show that the check cultivars responded differently to nitrogen application, therefore, they could be utilized in environments with different status of nitrogen supply dependening on their superiority in each regarding index of nitrogen utilization.

The total results of genotypes differentiation in this biplot indicated that there was a genetic variation in nitrogen absorption and storage components.

CONCLUSION

The results of this research led to the identification of high-performance genotypes in terms of nitrogen absorption and storage.The genotypes belonging to group A of biplot are recommended for cultivation in high yielding areas with favorable climatic conditions and unrestricted application of inputs since they had high efficiency in nitrogen uptake and use. Group B genotypes have higher uptake efficiency and lower use efficiency. In other words, despite the high efficiencyof nitrogen uptake from soil, these genotypes do not succeed in its transfer from vegetative tissues to seeds. In this case, the main part of nitrogen remains in straw.

For this reason, it was also observed that this group had a higher nitrogen yield of straw and a lower nitrogen portioning index than other groups under nitrogen fertilizer application. Based on these characteristics, it is recommended that these genotypes to be used as forage as a result of the possibility of having a higher protein content in straw. Group C genotypes have lower uptake efficiency and higher use efficiency. In other words, these genotypes are able to optimally utilize the limited amount of absorbed nitrogen. Therefore, Group C genotypes are considered as low-demanding and high-yielding genotypes in terms of soil nitrogen productivity. Therefore, their cultivation is recommended in areas exposed to environmental contamination due to the excessive use of nitrogen fertilizers. Group D had lower efficiency in nitrogen uptake and use than other groups and none of the check cultivars were included in this group. Therefore, these genotypes are suitable in terms of nitrogen productivity. Of the plant materials used in this research, only four genotypes (20%) were included in group D, while four genotypes from the total of 11 genotypes and cultivars with the origin of the Republic of Azerbaijan (about 36%), the three genotypes with French origin, and the only genotype of the Japanese origin used in this research were located in this group. Due to the lower frequency of Iranian genotypes (compared to the total number) in Group D, it seems that local bread wheat germplasms have high capacity for nitrogen uptake and use, and it is recommended that the research continues for screening and selecting for higher efficiency in exploitation of the soil nitrogen in the bread wheat genetic reseources.

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