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RESEARCH ARTICLE

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Genetic variation among selected pure lines from Turkish barley landrace 'Tokak' in yield-related and malting quality traits

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Abstract

Aim of study: Improvement of barley cultivars for malting traits suffers from narrow genetic pool in barley for these traits. Landraces are resources that could be used for this purpose. The present study was conducted to determine the variation for malting quality traits within a Turkish barley landrace.

Area of study: The study was undertaken in Tokat, a province in Black Sea Region of Turkey.

Material and methods: Twenty-five diverse lines, out of 42 unique genotypes previously identified in 'Tokak' landrace (PI 470281) based on DNA markers, were evaluated for malting quality traits along with the malting barley cv. 'Tokak 157/37' in four field trials. Thousand-seed weight, test weight, grain yield, lodging, malt extract percentage, diastatic power, alpha amylase and malt beta glucanase activities, malt protein and starch contents were determined.

Main results: Principal component analysis of malting quality traits revealed that thousand-seed weight, alpha amylase activity, beta glucanase activity and diastatic power were the most discriminatory traits for the lines. As the average of four trials, 15 of the 25 lines evaluated had higher grain yields and 10 of 25 lines had higher malt extract percentages than the standard cultivar 'Tokak 157/37'. Malt extract was highest in Line 59 in all environments, and this line also had the highest values for beta glucanase activity and starch content. Line 215 had highest values for alpha amylase activity. Lines 59 and 215 clearly had superior malting quality.

Research highlights: These lines could harbor novel alleles for these traits to be used in malting barley improvement.

Additional key words: alpha amylase; diastatic power; diversity; malt beta glucanase; malt extract; principal component analysis

Abbreviations used: 20° DU (dextrinizing units); IRV (increase in reciprocal specific viscosity); PCA (principal component analyses); SSR (simple sequence repeat); °WK (Windish Kolbach)

Authors' contributions: NK and AY conceived and designed the experiments, and obtained funding. IS and OAS performed the experiments. IS carried out statistical analyses. IS and NK wrote the manuscript.

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Introduction

Plant breeding is based on genetic variations. However, due to intense use of some genotypes, limited number of elite genotypes are used in breeding programs today (von Korff *et al.*, 2008). Thus, plants become vulnerable to biotic and abiotic stresses (Elakhdar *et al.*, 2018), which poses a threat for the sustainability of plant breeding in long term. Therefore, there is a need to widen the genetic base of plant breeding programs (Langridge & Waugh, 2019).

Landraces and wild relatives are the sources that could be harnessed to widen the genetic base of crops. Introgressed chromosomal segments of wild relatives have poor recombination with their homeologous counterparts in the developed cultivar receiving those segments. Landraces, on the other hand, do not have the problem of chromosomal recombination when crossed to cultivars. Besides, they do not have deleterious genes impacting yield and quality that could be introgressed into the developed cultivar because of linkage drag. Thus, use of landraces in

crop improvement programs is more advantageous (Mikić *et al.*, 2016).

Landraces are morphologically similar, but genetically diverse populations (Hagenblad *et al.*, 2019). Landraces of self-pollinating crops such as barley are made of many different homozygous genotypes. Therefore, landraces have considerable amount of genetic variation. It was mentioned that about 50-60% of the genetic variations in gene banks are within landraces (Parzies *et al.*, 2000; Jaradat *et al.*, 2004). For an efficient use in plant breeding, this variation needs to be characterized. High levels of variations were reported at DNA level within (Alemayehu & Parlevliet, 1997) and among barley landraces (Hamza *et al.*, 2004; Pandey *et al.*, 2006; Hua *et al.*, 2015; Shakhatreh *et al.*, 2016) based on DNA marker data.

Despite extensive work in genetic diversity of landraces in barley at DNA level, our knowledge for quantitative traits is relatively scarce, and is limited to the studies with purelines selected from the landraces to develop new cultivars. Cultivar candidates with good yield potentials were identified among the genotypes of some barley landraces (Lakew *et al.*, 1997; Akinci & Yildirim, 2009; Akgun *et al.*, 2012). Similarly, genotypes with superior quality traits were found among the genotypes of barley landraces studied (Akgun *et al.*, 2012; Yahiaoui *et al.*, 2014). However, most of these studies examined lines from a multitude of landraces, and variation for quality traits in individual landraces remain to be elucidated.

Investigations with quantitative traits pose a unique challenge because they are heavily influenced by the environment. Besides, high number of lines cannot be evaluated in powerful experimental designs with plots of enough size and replications. Since the available data mostly come from the pureline isolation studies where the major focus is on selecting superior lines, only brief information about the variation level for quantitative traits in landraces is available. Therefore, a systematic decrease of line numbers through eliminating the identical or similar lines based on the DNA marker data could allow a more efficient evaluation of the potential of landraces.

Turkey includes some parts of "Fertile Crescent", important center of origin for barley (Kilian *et al.*, 2006). Barley landraces from Turkey could have exotic variations that could be used in barley breeding. Although measuring the genetic variation levels in landraces using DNA markers are subject of much research, studies into the variation levels in quantitative traits, especially for quality related traits, are rare (Fernie *et al.*, 2006). The aim of this study was to determine whether the high variation determined in a Turkish barley landrace at DNA level is present in yield-related and malting quality traits.

Material and methods

Plant material

Turkish barley landrace 'Tokak' (PI 470281), maintained in Germplasm Resources Information Network of the USA, was subjected to detailed DNA and agronomic evaluations. A total of 52 single plants from PI 470281 were analyzed using 30 single copy SSR markers and 46 different lines were found (Kandemir *et al.*, 2010). Twenty-five lines out of 46 were sampled mostly based on their genetic diversities, and were evaluated in multilocational field trials along with a commercial barley cultivar ('Tokak 157/37') which has a considerable acreage in Turkey.

Field trials

Field trials were conducted in Kazova region of Tokat Province of Turkey in 2009, 2010 and 2011, and in Artova region of Tokat Province in 2010. Kazova experiments were conducted in Middle Black Sea Transitional Zone Agricultural Research Institute in 2009, and in experimental areas of Tokat Gaziosmanpaşa University in 2010 and 2011, while Artova trial was conducted in a farmer's field. Some soil characteristics of the experimental areas are given in Table 1.

Table 1. Soil properties of experimental areas

Location	Elev.	Lat.	Long.	Planting date	Soil texture	Total salt (%)	pH	$CaCO_3$ (%)	P_2O_5 (t/ha)	K_2O (t/ha)	Organic matter (%)
2009 Tokat	574	40.3265	36.4475	6 March	Clayed-loam	0.023	7.88	13.6	75.6	14.6	1.82
2010 Tokat	592	40.3325	36.4686	21 February	Clayed	0.014	8.25	3.9	73.4	29.9	1.19
2010 Artova	1106	40.0542	36.2954	12 March	Clayed	0.012	8.33	3.5	69.1	76.9	0.65
2011 Tokat	589	40.3332	36.4773	19 February	Clayed	0.013	8.17	4.5	124.2	44.1	1.38

Average long term (35 years) daily temperature was 14.1 °C in Kazova and 8.1 °C in Artova. Average daily temperature in Kazova was 13.0 °C in 2009, 14.9 °C in 2010 and 12.0 °C in 2011, while average daily temperature in Artova was 11.9 °C in 2010. Long-term yearly total amount of precipitation was 557.7 mm in Kazova and 464.1 mm in Artova. Precipitation was 582.6, 518.2 and 472.2 mm in Kazova in 2009, 2010 and 2011 years, and 665.0 mm in Artova in 2010. Monthly distributions of precipitation and temperature are given in Fig. 1.

Field trials were conducted in randomized complete blocks design with three replications in Kazova 2009 and Kazova 2011 locations, and with four replications in Kazova 2010 and Artova 2010 trials. Each plot had five 3-m long rows. Row spacing was 30 cm. Thus, area of each plot was 4.5 m^2. A short stature wheat cultivar was sown in the two rows between each plot and at the ends of the blocks to eliminate the effect of lodging in one plot on another. Seeding rate was 200 kg/ha, and trials were planted manually. Phosphorus and nitrogen fertilizers were applied as 75 kg/ha P_2O_5 and 100 kg/ha N, respectively. All of the phosphorus fertilizer and half of the nitrogen fertilizer were applied during the planting while the remaining half of the nitrogen fertilizer was applied before the stem elongation.

Measured traits

Thousand-seed weight (g), test weight (kg) and grain yield (t/ha) were determined according to Kandemir *et al.* (2000). Lodging percentage was determined visually as the percentage of lodged plants in a plot (Kandemir *et al.*, 2000). Malt production was performed as described by von Korff *et al.* (2008) with some modifications. Forty grams of grain with a known moisture content was immersed in two cycles of nine hours of soaking in water and 16 hours of draining. Germination was realized at 14 °C for five days. Moisture level was kept at an equivalent of 45% of the initial grain weight via spraying water over the grains. Germination was terminated when the plumula reached to 75% of the grain length. Kilning was carried out in a gradual way with steps of eight hours at 60 ºC, six hours at 70 ºC and five hours at 80 ºC. The rootlets were mechanically removed. Malt extract percentage was determined using the method described by Fox & Henry (1993) on 3 g of ground malt passing through a 0.5 mm screen. Diastatic power was determined based on the method described by Fox *et al.* (1999). Alpha amylase and malt beta glucanase activities were analyzed using K-CERA and K-MBGL commercial kits (Megazyme Int. Ireland Ltd), respectively, based on manufacturer's instructions. Malt protein content was determined according to IACC (1960), and EBC method (EBC, 1987) was used to analyze starch content.

Statistical analyses

Data from agronomic and quality traits were analyzed using ANOVA. Data from locations were analyzed separately since the Bartlett's homogeneity test showed that variances were not homogeneous for most traits across the environments. Lodging values were subjected to Arc-Sin transformation. Lines were grouped using Duncan's multiple range test. All statistical analyses were performed using MSTAT-C software (Freed & Eisensmith, 1986). Minitab V17 was used to perform multivariate analyses such as principal component analyses (PCA) and UPGMA clustering on means of agronomic and quality traits in locations based on normalized Euclidean distance matrices. Correlation (n=(n*n+1)/2=325) between Nei's genetic distance (Nei, 1972) and the Euclidean distance were analyzed to determine relationship between the phenotypic and genetic diversities (Fufa *et al.*, 2005).

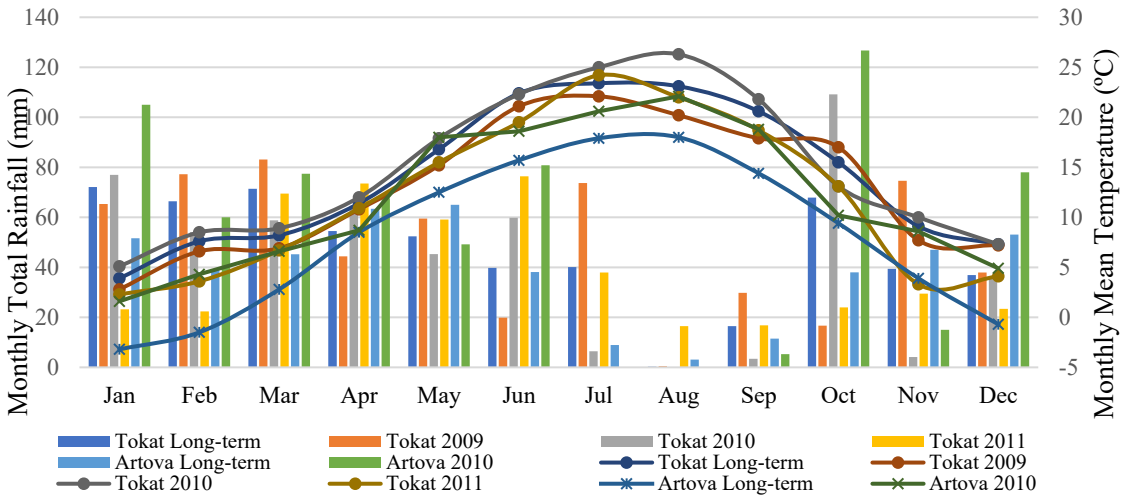

Figure 1. Climatic data of experimental years

Results

In a previous work (Kandemir *et al.*, 2010), a total of 46 different genotypes had been found among 52 plants examined using SSR markers from PI 470281, a barley landrace of Turkey. Besides the DNA markers, morphological characteristics such as rachilla length, rachilla pubescence, size of sterile florets, awn roughness and leaf anthocyanin pigments were also examined. However, no polymorphism was found for any of these characteristics. Of these 46 genotypes, 25 lines were selected based on their genetic distance in order to be used in field trials to reveal the variations among them for the major agronomic and malting quality traits. Results from four field trials using these 25 lines, along with 'Tokat 157/37' as a reference cultivar, are given in Tables 2 and 3.

Yield and related traits

Thousand-seed weight had significant variations in all environments. As the average of four locations, the lowest thousand-seed weight was obtained from Line 46 (43.3 g) and the highest from Line 217 (51.0 g) (Table 2). 'Tokak 157/37' had higher thousand-seed weight than all lines (55.4 g). There were significant differences for test weight among the lines in all locations. As the average of the locations, test weight of the lines varied from 64.7 (Line 208) to 67.6 kg (Line 221). 'Tokak 157/37', on the other hand, had a lower test weight (64.7 kg) than all lines.

Grain yields of lines showed significant differences in three out of four environments. Grain yields of lines across all environments varied from 2.50 (Line 215) to 3.36 t/ha (Line 44). Grain yield of 'Tokak 157/37' was 2.85 t/ha. As the average of four locations, 15 of the 25 lines evaluated had higher grain yields than 'Tokak 157/37'.

Lines showed significant differences for lodging observed at the harvest. Lodging was common among the lines in 2009 and 2011 Tokat locations. In general, all lines had a high level of lodging. All lines lodged considerably in 2009 Tokat trial. 'Tokak 157/37' had a 50% lodging in this trial. Considering the average of all trials, Line 217 had the lowest level of lodging (46.3%). The highest level of lodging, on the other hand, was observed in Line 62 (79.4%). 'Tokak 157/37' had a relatively low level of lodging (52.3%).

Quality traits

Malt extract trait was analyzed in three of four environments, and significant differences were observed among the lines in all three environments. As the average of three locations, Line 213 had the lowest malt extract percentage (76.4%) while Line 59 (80.4%) had the highest level (Table 3). Malt extract of 'Tokak 157/37' was moderate (77.6%). Malt extract of Line 59 was highest in all three environments. In three out of four environments, 10 of 25 lines had higher malt extract percentages than 'Tokak 157/37'.

Lines showed significant differences for alpha amylase activity measured as dextrinizing unit at 20 °C (20° DU), which represents the amount of alpha amylase that will dextrinize one g of soluble starch in an hour at 20 °C in the presence of excess beta amylase (Institute of Brewing, 1969). The lowest activity was observed in Line 217 (9.8 20° DU) and the highest in Line 215 (48.7 20° DU). Alpha amylase activity of 'Tokak 157/37' was 15.0 20° DU. Alpha amylase activities of Lines 44, 46, 212 and 215 were statistically higher than 'Tokak 157/37'.

Beta glucanase activity of the lines varied between 223 (Line 217) to 645 IRV unit/kg (Line 59). IRV is the unit of increase in reciprocal specific viscosity method used by the Institute of Brewing (Buckee & Baker, 1988). Beta glucanase activity of 'Tokak 157/37' (228 IRV unit/kg) was quite low. All lines except for 217 had significantly higher beta glucanase activity than 'Tokak 157/37'.

Significant differences were also observed among the lines for diastatic power, which varied from 320 (Line 67) to 634 ºWK (Line 53). ºWK is the amount of maltose formed from a solution of a standardized soluble starch by an enzyme extract from 100 g of malt (Miller, 2019). Diastatic power of 'Tokak 157/37' (310 ºWK) was lower than all lines. All lines except for 67 and 217 had significantly higher diastatic power than 'Tokak 157/37'.

Starch content in the grain varied between 59.7 (Line 201) and 61.7% (Line 59) and these differences were significant. 'Tokak 157/37' had an average starch content of 60.4%. All lines except for 64, 201, 212 and 217 had higher starch content than 'Tokak 157/37'.

Malt protein content of the lines varied between 11.3 (Line 213) and 14.0% (Line 215). Malt protein content of 'Tokak 157/37' was 13.6%. All lines except for Line 213 had statistically similar malt protein content to that of 'Tokak 157/37'.

Principal component analysis (PCA)

PCA was performed to show the variations for malt quality traits among the 25 lines evaluated in the present study and 'Tokak 157/37', and to determine the relative contribution of each character within the total variance. The first three PCs with eigen value greater than one accounted for 67.7% of the total variability. The PC1 explained 30.6% of the total variance. Thousand-seed weight, alpha amylase activity, beta glucanase activity and diastatic power contributed to the first PC1. On PC2, which constituted 25.1% of entire variation, the most predominant characters were malt protein, starch content and

Table 2. Yield and related traits of the lines selected from PI 470281

Lines	2009 Tokat**	2010 Tokat**	2010 Artova**	2011 Tokat**	Avg	2009 Tokat**	2010 Tokat**	2010 Artova**	2011 Tokat**	Avg
	Thousand-seed weight (g)					Test weight (kg)				
40	50.8 bch	47.5 c-g	45.2 d-i	48.5 b-e	48.0	61.4 abc	68.4 bcd	68.2 abc	66.7 abc	66.2
44	48.6 b-e	43.9 hi	44.2 f-i	49.3 a-d	46.5	62.4 ab	68.3 bcd	68.0 abc	66.4 abc	66.3
46	43.1 e-f	42.7 i	42.6 i	44.9 d-g	43.3	62.6 ab	66.8 de	67.0 abc	65.8 abc	65.6
50	47.1 b-f	45.2 f-i	43.6 ghi	46.5 b-f	45.6	62.6 ab	69.3 abc	67.9 abc	67.3 abc	66.8
51	46.0 b-f	45.3 f-i	44.9 e-i	44.5 d-g	45.2	60.7 abc	66.8 de	66.4 a-d	65.5 bc	64.9
53	46.8 b-f	42.9 i	43.2 ghi	43.7 efg	44.1	62.3 ab	67.5 cde	66.6 abc	64.8 bc	65.3
56	46.9 b-f	47.7 c-g	43.8 ghi	48.3 b-e	46.7	58.7 c	68.5 bcd	66.3 bcd	67.4 abc	65.2
59	48.4 b-e	45.7 e-i	43.5 ghi	42.4 fg	45.0	63.2 a	69.3 abc	66.3 cd	67.5 abc	66.6
61	47.1 b-f	47.8 c-f	46.9 b-f	49.0 a-d	47.7	62.5 ab	68.5 bcd	67.5 abc	68.0 abc	66.6
62	44.0 ef	45.9 e-i	42.6 i	45.2 c-f	44.4	61.5 abc	70.3 ab	68.0 abc	67.0 abc	66.7
64	44.7 def	45.7 e-i	43.9 f-i	44.2 d-g	44.6	61.1 abc	68.8 a-d	67.5 abc	65.5 bc	65.7
67	46.1 b-f	48.9 b-e	47.6 b-e	46.3 b-f	47.2	60.9 abc	69.3 abc	67.3 abc	66.7 abc	66.0
201	46.5 b-f	45.0 f-i	44.6 f-i	46.6 b-f	45.7	61.8 abc	69.0 a-d	67.8 abc	66.5 abc	66.3
206	46.8 b-f	47.8 c-f	47.9 bcd	47.6 b-e	47.5	61.5 abc	67.7 cde	68.0 abc	67.6 abc	66.2
207	47.8 b-e	50.3 abc	48.5 bc	50.6 ab	49.3	60.7 abc	67.5 cde	67.5 abc	66.1 abc	65.5
208	46.4 b-f	49.4 bcd	48.2 bc	50.1 abc	48.5	59.3 bc	68.0 cde	67.3 abc	64.4 c	64.7
210	41.6 f	43.3 hi	43.1 hi	46.7 b-f	43.7	60.6 abc	69.7 abc	68.3 abc	68.3 ab	66.7
212	45.0 c-f	47.4 c-g	44.3 f-i	44.2 d-g	45.2	61.7 abc	69.0 a-d	67.7 abc	67.3 abc	66.4
213	48.8 b-e	51.6 ab	46.0 b-h	48.9 a-d	48.8	59.5 bc	69.3 abc	66.7 abc	65.2 bc	65.2
215	43.2 ef	45.2 f-i	45.6 c-i	40.1 g	43.5	61.0 abc	67.8 cde	68.6 a	67.3 abc	66.2
217	50.9 bc	53.0 a	48.6 b	51.3 ab	51.0	62.3 ab	68.4 bcd	68.0 abc	65.3 bc	66.0
221	45.3 b-f	45.2 g-j	43.0 hi	48.0 b-e	45.4	62.4 ab	70.8 a	68.1 abc	69.3 a	67.6
224	51.1 b	46.5 d-h	43.9 ghi	50.1 ab	47.9	61.6 abc	68.9 a-d	67.3 abc	67.8 abc	66.4
227	50.3 bcd	44.4 ghı	46.2 b-g	46.5 b-f	46.9	62.4 ab	68.2 bcd	68.1 abc	67.0 abc	66.4
228	50.4 bcd	49.2 bcd	49.0 bcd	47.8 b-e	49.1	61.7 abc	68.6 a-d	68.5 ab	67.3 abc	66.5
Tokak 157/37	58.6 a	51.9 ab	57.3 a	53.7 a	55.4	63.6 a	66.0 e	64.6 d	64.6 bc	64.7
	Grain yield (t/ha)					Lodging (%)				
	2009 Tokat*	2010 Tokat*	2010 Artova*	2011 Tokat^{NS}	Avg	2009 Tokat**	2010 Tokat**	2010 Artova**	2011 Tokat**	Avg
40	1.76 b-e	2.66 abc	3.08 a-d	3.66	2.79	100 a	20.0 bcd	2.5 hi	86.7	52.3
44	2.22 ab	2.96 abc	3.28 abc	5.01	3.36	100 a	37.5 abc	42.5 a-f	100.0	70.0
46	1.85 a-e	2.89 abc	3.31 a-b	3.49	2.88	100 a	30.0 abc	15.0 d-i	86.7	57.9
50	2.00 a-d	3.12 ab	3.31 ab	3.84	3.07	100 a	27.5 abc	15.0 d-i	100.0	60.6
51	1.62 cde	3.08 ab	3.11 a-d	3.43	2.80	100 a	31.3 abc	15.0 d-i	100.0	61.6
53	1.90 a-e	2.96 abc	3.39 ab	4.02	3.07	100 a	31.3 abc	21.3 b-i	86.7	59.8
56	1.58 cde	2.64 abc	3.19 a-d	3.61	2.75	100 a	11.3 bcd	10.0 f-i	80.0	50.3
59	1.78 b-e	2.90 abc	3.30 ab	4.04	3.00	100 a	15.0 bcd	0.0 i	86.7	50.4
61	1.44 de	2.91 abc	3.33 ab	4.89	3.14	100 a	47.5 ab	48.8 a-d	100.0	74.1
62	1.68 cde	2.69 abc	2.81 cd	4.08	2.81	100 a	47.5 ab	70.0 a	100.0	79.4
64	1.62 cde	2.58 bc	3.05 a-d	3.30	2.64	100 a	18.8 bcd	12.5 d-i	90.0	55.3
67	1.95 a-e	3.12 ab	3.13 a-d	4.20	3.10	100 a	17.5 bcd	52.5 abc	100.0	67.5
201	1.83 a-e	3.06 ab	3.00 a-d	3.08	2.74	100 a	40.0 abc	7.5 e-i	80.0	56.9
206	1.78 b-e	2.84 abc	3.24 abc	3.40	2.82	100 a	18.8 bcd	5.0 ghi	83.3	51.8

Table 2. Continued.

	Grain yield (t/ha)					Lodging (%)				
	2009 Tokat*	2010 Tokat*	2010 Artova*	2011 Tokat^{NS}	Avg	2009 Tokat**	2010 Tokat**	2010 Artova**	2011 Tokat**	Avg
207	2.38 a	3.03 ab	3.42 ab	4.32	3.29	100 a	20.0 bcd	22.5 b-h	83.3	56.5
208	1.50 de	3.15 a	3.37 ab	4.42	3.11	100 a	37.5 abc	32.5 a-g	100.0	67.5
210	1.83 a-e	2.78 abc	3.12 a-d	3.28	2.75	100 a	67.5 a	46.3 a-d	98.3	78.0
212	1.64 cde	2.40 c	3.47 a	4.49	3.00	100 a	10.0 bcd	21.3 b-i	81.7	53.3
213	1.94 a-e	2.68 abc	3.33 ab	3.74	2.92	100 a	32.5 abc	12.5 d-i	100.0	61.3
215	1.38 e	2.63 abc	2.72 d	3.27	2.50	100 a	7.5 cd	2.5 hi	86.7	49.2
217	1.74 b-e	2.43 c	2.97 bcd	3.43	2.64	100 a	0.0 d	5.0 ghi	80.0	46.3
221	2.12 abc	2.69 abc	2.99 bcd	4.08	2.97	100 a	42.5 abc	57.5 ab	100.0	75.0
224	1.76 b-e	2.99 ab	3.13 a-d	5.02	3.22	100 a	45.0 ab	35.0 a-g	100.0	70.0
227	1.70 b-e	2.75 abc	3.29 ab	3.69	2.86	100 a	21.3 bcd	18.8 c-i	86.7	56.7
228	1.64 cde	3.11 ab	2.94 bcd	3.92	2.90	100 a	25.0 bc	42.5 a-e	100.0	66.9
Tokak 157/37	2.13 abc	2.82 abc	3.16 a-d	3.29	2.85	50 b	25.0 bc	37.5 a-f	96.7	52.3

NS: not-significant. *, ** means with the same letter are not different at 5 and 1% significance level, respectively.

lodging (Fig. 2a). The third PC explained 12.0% of overall variation for which malt extract contributed heavily.

In order to show the genotype responses, score plot was prepared (Fig. 2b). The most responsive genotypes could be determined in the score plot through drawing a polygon whose corners were constituted by the genotypes as the extremes. These genotypes could be the ones with the best or worst performance in some or all environments in which they are evaluated (Yuksel & Akcura, 2012). 'Tokak 157/37' and Lines 61, 221, 59 and 215 appeared to be the most responsive genotypes.

Five groups were established based on the dendrogram prepared with the measured traits (Fig. 3). Dendrogram was first divided into two based on beta glucanase activity and thousand-seed weight. Lines 207 and 217 with the lowest levels of beta glucanase activity, and the highest thousand-seed weight along with the standard cultivar 'Tokak 157/37' constituted the first group (shown in black color). Line 207 which had high alpha amylase and beta glucanase activities, diastatic power and grain yield appeared to have a subgrouping within them. The second group formed as a result of 'lines with the highest' diastatic power (showed in green color). Within this group, lines were further divided into two since Lines 64 and 215 had lower beta glucanase activity, grain yield, lodging and higher malt protein content than Lines 51 and 53. Lines 56, 61, 67, 208, 210, 212 and 213 which had low levels of diastatic power and beta glucanase activity formed the third group (shown in purple color). Line 61 grouped separately because it had lower beta glucanase activity and alpha amylase activity, highest grain yield and lodging in the group. Lines 208 and 212 grouped separately as they had high beta glucanase activity, and Lines 67 and 210 had high malt extract. The fourth group was constituted by the lines with higher diastatic power among the remaining lines (shown in crimson color). Since Lines 44 and 221 had lower diastatic power and malt protein content than Lines 62 and 201, group 4 was further divided into two. The fifth group consisted of lines with higher beta glucanase activity. Since the Lines 46 and 59 had higher beta glucanase activity than all other lines, they grouped separately. Lines 40 and 227 were separated from other lines since they had relatively low levels of diastatic power. In this group, Lines 50 and 206 were similar, while Line 228 was different for malt extract and alpha amylase activities, and Line 224 was different in terms of grain yield and lodging.

No significant correlation was found between the Nei's genetic distance values determined among 25 lines using SSR markers examined and the distance matrix obtained from the agronomic and quality trait data ($r = 0.105$, $p > 0.05$). Thus, no relationship was found between diversity estimates based on SSR data and agronomic and quality trait data.

Discussion

Genetic variations in crop plants have been significantly narrowed during the development of modern cultivars with plant breeding. Landraces are of great importance for expanding these shrinking genetic variation (Yadav *et al.*, 2018). We previously characterized genetic variation of Turkish barley landrace 'Tokak' (PI 470281) at DNA level (Kandemir *et al.*, 2010) and found a high level of genetic variation. However, variation for traits that could be easily observed such as thousand-seed weight, test weight and grain yield was

Table 3. Malting quality traits of the lines selected from PI 470281

Lines	Malt extract (%)				Alpha amylase activity** (20º DU)γ	Beta glucanase activity** (IRV unit/kg)γ	Diastatic power** (ºWK)γ	Starch content** (%)γ	Malt protein content** (%)γ
	2010 Tokat**	2010 Artova**	2011 Tokat**	Avg.					
40	78.1 def	78.8 abc	74.3 def	77.1	26.6 b-f	575 bc	332 jk	61.5 a	12.2 a-d
44	78.7 b-f	78.6 a-d	72.6 f	76.6	39.9 abc	464 g-j	448 f	61.0 a-d	11.9 cd
46	79.4 a-e	79.4 ab	74.9 cde	77.9	41.3 ab	636 a	487 e	61.1 a-d	12.9 a-d
50	79.2 a-f	78.6 a-d	75.2 cde	77.7	34.2 a-e	506 e-h	426 f	61.0 a-d	12.4 a-d
51	80.1 abc	80.3 ab	74.4 def	78.3	29.1 b-f	599 ab	561 c	61.2 abc	11.9 cd
53	79.1 a-f	77.8 bcd	74.9 cde	77.3	33.5 a-e	605 ab	634 a	60.6 a-d	12.9 a-d
56	79.0 a-f	78.1 bcd	75.2 cde	77.5	26.6 b-f	463 hij	372 hi	60.6 a-d	12.9 a-d
59	80.8 a	81.0 a	79.2 a	80.4	30.1 a-e	645 a	417 fg	61.7 a	12.4 a-d
61	77.4 ef	79.0 ab	75.9 bcd	77.4	17.2 def	396 k	392 gh	61.4 ab	11.8 cd
62	78.5 b-f	78.2 bcd	75.4 b-e	77.4	28.2 b-f	471 f-j	522 d	60.9 a-d	13.0 a-d
64	79.4 a-e	79.2 ab	74.3 def	77.6	35.1 a-e	517 def	591 bc	59.8 cd	13.2 abc
67	79.8 a-d	78.7 a-d	76.2 bcd	78.2	19.7 def	421 jk	320 k	60.9 a-d	13.1 a-d
201	77.4 ef	76.3 cd	76.9 bc	76.9	35.8 a-e	512 d-h	522 d	59.7 d	13.3 abc
206	77.7 d-f	78.7 a-d	75.0 cde	77.2	29.8 a-e	516 d-g	437 f	60.9 a-d	13.2 abc
207	80.3 ab	78.1 bcd	74.8 cde	77.7	27.5 b-f	309 l	378 h	60.8 a-d	12.4 a-d
208	78.3 b-f	80.3 ab	75.3 cde	77.9	27.1 b-f	482 f-i	378 h	60.3 a-d	12.6 a-d
210	78.6 b-f	79.7 ab	75.7 b-e	78.0	28.4 b-f	437 ijk	340 ijk	61.6 a	12.3 a-d
212	77.2 f	77.9 bcd	75.9 bcd	77.0	36.7 a-d	509 d-h	390 gh	59.8 cd	13.8 ab
213	77.5 ef	76.2 d	75.5 b-e	76.4	29.6 a-e	471 f-j	341 ijk	61.6 a	11.3 d
215	79.1 a-f	79.3 ab	76.9 bc	78.5	48.7 a	514 d-h	613 ab	60.7 a-d	14.0 a
217	77.4 ef	79.0 ab	73.6 ef	76.7	9.8 f	223 m	327 k	60.0 bcd	12.9 a-d
221	78.6 b-f	78.6 a-d	75.0 cde	77.4	31.0 a-e	450 ij	442 f	61.5 a	11.8 cd
224	77.9 def	78.2 bcd	76.4 bcd	77.5	28.0 b-f	560 bcd	430 f	60.6 a-d	13.0 a-d
227	78.4 b-f	79.0 ab	74.8 cde	77.4	21.1 c-f	578 bc	365 hij	60.9 a-d	12.2 a-d
228	78.1 c-f	78.5 a-d	77.5 ab	78.0	18.8 d-f	535 cde	423 fg	60.8 a-d	12.0 bcd
Tokak 157/37	78.8 b-f	78.5 a-d	75.4 b-e	77.6	15.0 ef	228 m	310 k	60.4 a-d	13.6 abc

**Means with the same letter are not different at 1% significance level. γ: data is from 2010 Artova location only. 20° DU is the amount of alpha amylase that will dextrinize one g of soluble starch in an hour at 20 °C in the presence of excess beta amylase. IRV is the unit of increase in reciprocal specific viscosity method. ºWK is the amount of maltose formed from a solution of a standardized soluble starch by an enzyme extract from 100 g of malt.

not very high in the present study. On the other hand, considerable variation was found for malt quality traits. The lower variation in visible agronomic characters compared to the variation for invisible malting quality traits or variation at DNA level could indicate that ancient farmers made conscious selections for the visible traits. Indeed, no significant correlation was found between the diversity estimates based on SSR data and the diversity estimates based on agronomic and quality trait data. Therefore, assessment of variation at landraces beyond the DNA level is necessary to elucidate the usefulness of variation in landraces (Varshney *et al.*, 2010; Chalak *et al.*, 2015).

Most of the lines evaluated had better performance than 'Tokak 157/37' for all traits except for thousand-seed weight. Striking thousand-seed weight characteristics of 'Tokak 157/37' were previously reported (Kandemir, 2004). Furthermore, 'Tokak 157/37' was reported to have better malt extract values than most other barley cultivars (Sipahi *et al.*, 2009). The lines evaluated in the present study had superior malting quality traits compared to 'Tokak 157/37'. Thus, it could be stated that the lines evaluated have good malting quality character.

Variations in landraces could be due to ecological factors and producers' preferences. A major portion of the variations in gene banks were reported to be within

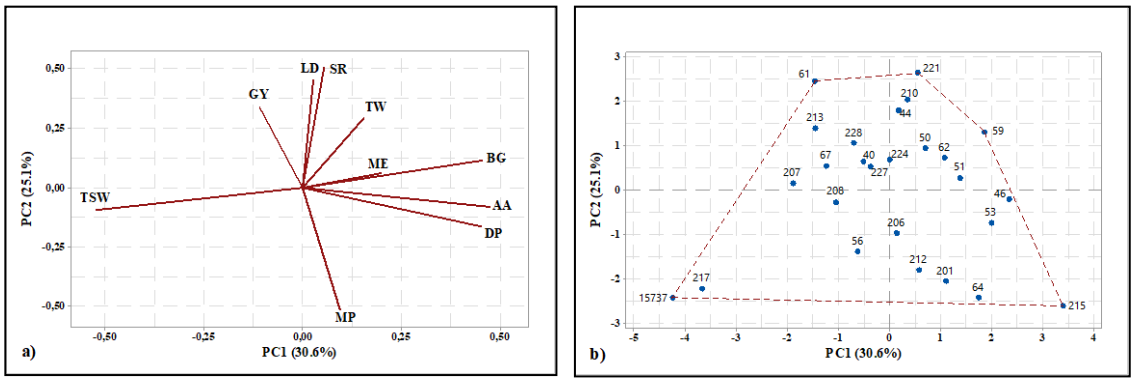

Figure 2. Loading (a) and score (b) plot of first two principal components. TSW: thousand-seed weight. GY: grain yield. LD: lodging. SR: starch content. TW: test weight. ME: malt extract. BG: beta glucanase activity. AA: alpha amylase activity. DP: diastatic power. MP: malt protein content.

landraces (Parzies *et al.*, 2000). Amezrou *et al.* (2018) evaluated a barley population using PCA and found that 66.4% of the variation was explained by the first three PCs. Studying Ethiopian barley landraces, Abebe *et al.* (2010) reported that 73% of the variation was attributed to the first three PCs, and speculated that elevation and ecological factors affect the level of variation. Considering the fact that landraces are relatively homogeneous for visible characters (Hagenblad *et al.*, 2019) but possess genetic variation at DNA level, it could be stated that the ancient farmers might have carried out some visual selections based on their preferences. On the other hand, visually undetectable traits such as biochemical and malting quality characters could have sustained their variability in the hands of ancient farmers since these farmers harvested their seeds as bulks rather than growing progeny from single plant selections. Similar conclusions were drawn from variations detected in various barley populations by PCA in other studies (Demissie & Bjornstad, 1996; Yadav *et al.*, 2018).

Of the lines in the corners of the score plot analysis which allows a better evaluation of the general performance of the lines, Line 59 had the highest values for malt extract, beta glucanase activity and starch content, while Line 215 had highest values for alpha amylase activity and malt protein content (Table 3). 'Tokak 157/37' had lower values than the average for most malting quality traits. Similar to 'Tokak 157/37', Lines 61 and 221 had values lower than the average for most malting quality traits. Lines 59 and 215 had clearly superior malting quality, which might indicate that these lines harbor superior alleles of the genes associated with malting quality traits.

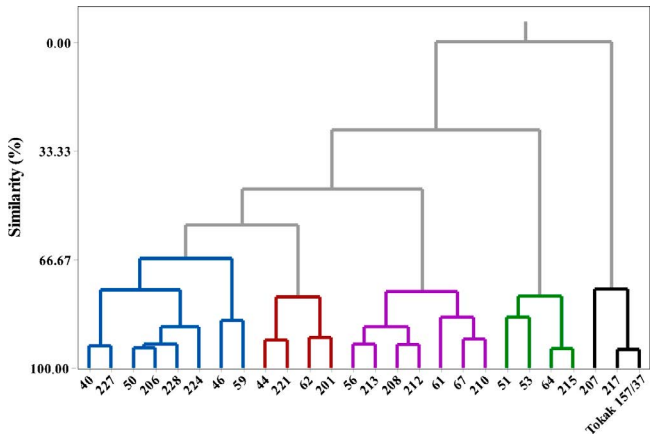

Figure 3. Cluster based on agronomic traits using average linkage and Euclidean distance.

Cluster analysis is a useful tool that allows detailed evaluation of lines for all traits studied using visual images produced. A dendrogram based on all the traits measured classified the lines in five groups differing in thousand-seed weight, beta glucanase activity and diastatic power. Beta glucanase, alpha amylase activities and diastatic power were the traits with the highest positive loadings in the first PC. PC analysis confirmed the groupings of the lines to a large extent. The finding that the trait with the highest positive loading was more effective for the grouping in cluster analysis was also found by Manjunatha *et al.* (2007) and Amezrou *et al.* (2018).

Development of cultivars for quality traits through plant breeding are usually implemented using gene pools with narrow genetic backgrounds (Muñoz-Amatriaín *et al.*, 2010; Augustinos *et al.*, 2016) and there is a need for novel alleles. Due to the deleterious alleles of the genes affecting grain yield and quality characteristics, wild species are not the preferred source of variation to this aim. However, landraces could provide these alleles. For this purpose, it is necessary to evaluate landraces to determine their quality characteristics and identify novel alleles before an efficient use of these novel alleles in breeding programs. 'Tokak' (PI 470281) is a barley landrace with high level of genetic variation and environmental adaptability. With the present study, we evaluated the malting quality characteristics of the selected lines of 'Tokak' landrace from the Fertile Crescent and identified some lines with superior malting quality traits which could carry potential novel alleles for these traits.

Direct use of our selected lines as cultivars do not seem plausible due to the high level of susceptibility for fungal diseases (powdery mildew, leaf stripe) observed during the seed multiplication. Besides, the lines had high levels of lodging which could be a major threat for yields in areas with high yielding potential especially in fall plantings. Thus, the selected lines could better be used as parents in plant breeding programs especially for their superior malting quality gene alleles. Detailed evaluation of alleles of already known gene/QTL regions of our selected lines on the same genetic background could better reveal the value of these novel genes for plant breeding programs.

References

Abebe TD, Bauer AM, Léon J, 2010. Morphological diversity of Ethiopian barleys (*Hordeum vulgare* L.) in relation to geographic regions and altitudes. Hereditas 147: 154-164. https://doi.org/10.1111/j.1601-5223.2010.02173.x

Akgun N, Topal A, Akcura M, 2012. Evaluation of central Anatolian barley landraces for crop improvement. Rom Agric Res 29: 87-93.

Akinci C, Yildirim M, 2009. Screening of barley landraces by direct selection for crop improvement. Acta Agr Scand B-Soil Plant Sci 59: 33-41. https://doi.org/10.1080/09064710701827782

Alemayehu F, Parlevliet JE, 1997. Variation between and within Ethiopian barley landraces. Euphytica 94: 183-189. https://doi.org/10.1023/A:1002946004268

Amezrou R, Gyawali S, Belqadi L, Chao S, Arbaoui M, Mamidi S, *et al.*, 2018. Molecular and phenotypic diversity of a worldwide ICARDA spring barley collection. Genet Resour Crop Evol 65: 255-269. https://doi.org/10.1007/s10722-017-0527-z

Augustinos AA, Petropoulos C, Karasoulou V, Bletsos F, Papasotiropoulos V, 2016. Assessing diversity among traditional Greek and foreign eggplant cultivars using molecular markers and morphometrical descriptors. Span J Agric Res 14: e0710. https://doi.org/10.5424/sjar/2016144-9020

Buckee GK, Baker CD, 1988. Collaborative trial on the determination of beta-glucanase in malt by viscometric and dye labelled method. J Inst Brew 96: 387-390. https://doi.org/10.1002/j.2050-0416.1988.tb04599.x

Chalak L, Mzid R, Rizk W, Hmedeh H, Kabalan R, Breidy J, *et al.*, 2015. Performance of 50 Lebanese barley landraces (*Hordeum vulgare* L. subsp. vulgare) in two locations under rainfed conditions. Ann Agri Sci 60: 325-334. https://doi.org/10.1016/j.aoas.2015.11.005

Demissie A, Bjornstad A, 1996. Phenotypic diversity of Ethiopian barleys in relation to geographical regions, altitudinal range and agro-ecological zones: as an aid to germplasm collection and conservation strategy. Hereditas 124: 17-29. https://doi.org/10.1111/j.1601-5223.1996.00017.x

EBC, 1987. Analysis by the European Brewery Convention. Brauerei-und Getränke-rundschau, CH-8047 Zurich Switzerland.

Elakhdar A, Kumamaru T, Qualset CO, 2018. Assessment of genetic diversity in Egyptian barley (*Hordeum vulgare* L.) genotypes using SSR and SNP markers. Genet Resour Crop Evol 65: 1937-1951. https://doi.org/10.1007/s10722-018-0666-x

Fernie AR, Tadmor Y, Zamir D, 2006. Natural genetic variation for improving crop quality. Curr Opin Plant Biol 9: 196-202. https://doi.org/10.1016/j.pbi.2006.01.010

Fox GP, Henry RJ, 1993. A rapid small-scale method for the determination of malt extract. J Ind Brew 99: 73-75. https://doi.org/10.1002/j.2050-0416.1993.tb01150.x

Fox GP, Logue SJ, Harasymow S, Ratcliffe M, Taylor H, Tansing P, *et al.*, 1999. Standardization of diastatic power method for barley breeding programs. Proc 9th Australia Barley Technical Symp, Melbourne, 12-16 Sept. pp: 2351-2355.

Freed R, Eisensmith SP, 1986. MSTAT - Statistical Software for Agronomists. Agronomy Abstract.

Fufa H, Baenziger PS, Beecher BS, Dweikat I, Graybosch RA, Eskridge KM, 2005. Comparison of phenotypic and molecular marker-based classifications of hard red winter wheat cultivars. Euphytica 145: 133-146. https://doi.org/10.1007/s10681-005-0626-3

Hagenblad J, Leino MW, Afonso GH, Morales DA, 2019. Morphological and genetic characterization of barley (*Hordeum vulgare* L.) landraces in the Canary Islands. Genet Resour Crop Evol 66: 465-480. https://doi.org/10.1007/s10722-018-0726-2

Hamza S, Hamida BW, Rebai A, Harrabi M, 2004. SSR-based genetic diversity assessment among Tunisian winter barley and relationship with morphological traits. Euphytica 135: 107-118. https://doi.org/10.1023/B:EUPH.0000009547.65808.bf

Hua W, Zhang X, Zhu J, Shang Y, Wang J, Jia Q, *et al.*, 2015. A study of genetic diversity of colored barley (*Hordeum vulgare* L.) using SSR markers. Genet Resour Crop Evol 62: 395-406. https://doi.org/10.1007/s10722-014-0165-7

IACC, 1960. ICC Standard No: 105. International Association for Cereal Chemistry.

Institute of Brewing Analysis Committee, 1969. Method for determination of alpha-amylase activity. J Inst Brew 75: 430-432. https://doi.org/10.1002/j.2050-0416.1969.tb06376.x

Jaradat AA, Shahid M, Al-Maskri A. 2004. Genetic diversity in the Batini barley landrace from Oman: I. Spike and seed quantitative and qualitative traits.

Crop Sci 44: 304-315. https://doi.org/10.2135/cropsci2004.3040

Kandemir N, 2004. Determination of malting barley cultivars suitable for Tokat-Kazova conditions. J Agric Fac Gaziosmanpasa Univ 21: 94-100.

Kandemir N, Jones BL, Wesenberg DM, Ullrich SE, Kleinhofs A, 2000. Marker assisted analysis of three grain yield QTL in barley (*Hordeum vulgare* L.) using near isogenic lines. Mol Breed 6: 157-167. https://doi.org/10.1023/A:1009602514106

Kandemir N, Yildirim A, Gunduz R, 2010. Determining the levels of genetic variation using SSR markers in three Turkish barley materials known as Tokak. Turk J Agric For 34: 17-23.

Kilian B, Ozkan H, Kohl J, von Haeseler A, Barale F, Deusch O, *et al.*, 2006. Haplotype structure at seven barley genes: relevance to gene pool bottlenecks, phylogeny of ear type and site of barley domestication. Mol Genet Genomics 276: 230-241. https://doi.org/10.1007/s00438-006-0136-6

Lakew B, Semeane Y, Alemayehu F, Gebre H, Grando S, 1997. Exploiting the diversity of barley landraces in Ethiopia. Genet Resour Crop Evol 44: 109-116. https://doi.org/10.1023/A:1008644901982

Langridge P, Waugh R, 2019. Harnessing the potential of germplasm collections. Nat Genet 51: 200-201. https://doi.org/10.1038/s41588-018-0340-4

Manjunatha T, Bisht IS, Bhat KV, Singh BP, 2007. Genetic diversity in barley (*Hordeum vulgare* L. ssp. vulgare) landraces from Uttaranchal Himalaya of India. Genet Resour Crop Evol 54: 5-65. https://doi.org/10.1007/s10722-005-1884-6

Mikić S, Zorić M, Stanisavljević D, Kondić-Špika A, Brbaklić L, Kobiljski B, *et al.*, 2016. Agronomic and molecular evaluation of maize inbred lines for drought tolerance. Span J Agric Res 14: e0711. https://doi.org/10.5424/sjar/2016144-9116

Miller GH, 2019. Whisky science: A condensed distillation. Springer, Berlin/Heidelberg, Germany. 530 pp. https://doi.org/10.1007/978-3-030-13732-8

Muñoz-Amatriaín M, Xiong Y, Schmitt MR, Bilgic H, Budde AD, Chao S, Smith KP, Muehlbauer GJ, 2010. Transcriptome analysis of a barley breeding program examines gene expression diversity and reveals target genes for malting quality improvement. BMC Genom 11: 653. https://doi.org/10.1186/1471-2164-11-653

Nei M, 1972. Genetic distance between populations. Am Nat 106: 283-292. https://doi.org/10.1086/282771

Pandey M, Wagner C, Friedt W, Ordon F, 2006. Genetic relatedness and population differentiation of Himalayan hulles barley (*Hordeum Vulgare* L.) landraces inferred with SSR. Theor Appl Genet 113: 715-729. https://doi.org/10.1007/s00122-006-0340-0

Parzies HK, Spoor W, Ennos RA, 2000. Genetic diversity of barley landrace accessions (*Hordeum vulgare* ssp. vulgare) conserved for different lengths of time in ex situ gene banks. Heredity 84: 476-486. https://doi.org/10.1046/j.1365-2540.2000.00705.x

Shakhatreh Y, Baum M, Haddad N, Alrababah M, Ceccarelli S, 2016. Assessment of genetic diversity among Jordanian wild barley (Hordeum spontaneum) genotypes revealed by SSR markers. Genet Resour Crop Evol 63: 813-822. https://doi.org/10.1007/s10722-015-0285-8

Sipahi H, Akar T, Ozkara R, Yildiz MA, Baspinar E, Sayim I, 2009. Determination of relationship between seed storage protein electrophrograms and malting quality in barley (*Hordeum vulgare* L.). Biotech Stud 18: 1-10.

Varshney RK, Baum M, Guo P, Grando S, Ceccarelli S, Graner A, 2010. Features of SNP and SSR diversity in a set of ICARDA barley germplasm collection. Mol Breed 26: 229-242. https://doi.org/10.1007/s11032-009-9373-9

von Korff M, Wang H, Léon J, Pillen K, 2008. AB-QTL analysis in spring barley: III. Identification of exotic alleles for the improvement of malting quality in spring barley (*H. vulgare ssp. spontaneum*). Mol Breed 21: 81-93. https://doi.org/10.1007/s11032-007-9110-1

Yadav RK, Gautam S, Palikhey E, Joshi BK, Ghimire KH, Gurung R, *et al.*, 2018. Agro-morphological diversity of Nepalese naked barley landraces. Agr Food Secur 7: 86. https://doi.org/10.1186/s40066-018-0238-5

Yahiaoui S, Cuesta-Marcos A, Gracia MP, Medina B, Lasa JM, Casas AM, *et al.*, 2014. Spanish barley landraces outperform modern cultivars at low-productivity sites. Plant Breeding 133: 218-226. https://doi.org/10.1111/pbr.12148

Yuksel S, Akcura M, 2012. Pattern analysis of multi-environment yield trials in barley (*Hordeum vulgare* L.). Turk J Agric For 36: 285-295.