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Genomic, Agro-morphological and Chemical Divergence of Wild Barley Populations Differentially Adapted to Microenvironments

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Abstract

Genetic diversity is one of the most important indicator for germplasm assessment, molecular evaluation as well as speciation studies. In this study, ISJ molecular markers together with agro-morphological traits and near-infrared spectroscopy were used to investigate the diversity and divergence of two wild barley populations from Mt Gilboa, Israel, adapted to two microenvironments. High level of polymorphism was observed with ISJ markers and the significant differences were found in agro-morphological traits and near-infrared spectroscopy analysis.

The genetic variation (50.5% polymorphism) was recognized between the populations. Cluster analysis grouped genotypes into two clear groups suggesting that they have adapted to two different microenvironments. Some agro-morphological traits such as plant height, number of tillers, days to flowering, flag leaf length and number of seeds per spike exhibited significant different at $p \leq 0.05$ and $p \leq 0.001$ probability levels and several chemical compounds such as fiber and crude protein content also exhibited significant different at $p \leq 0.05$ and $p \leq 0.001$ probability levels between microenvironments. Hence, our results showed the genomic divergence and adaptation of wild barley to microenvironments. Furthermore, the results demonstrated the effectiveness of ISJ markers, agro-morphological and chemical traits in detecting variation exerted by adaptation to microenvironments.

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Introduction

Barley (*Hordeum vulgare* L.) is one of the oldest cultivated cereal crop and the fourth largest cereal crop produced worldwide, quantity wise and it is utilized in the food industry, feed industry and malt production [1]. Also, being the most extensively adapted cereal grain species can be cultivated in fertile lands as well as desert lands [2]. Wild barley (*Hordeum spontaneum*) is the progenitor of cultivated barley varieties which offers considerable potential as a genetic resource for barley improvement. Naturally occurring wild barley populations are distributed primarily across the Fertile Crescent, Central Asia and Tibet [3-6]. The study of the origin, genetic variation and evolutionary relationship in barley is important for the conservation and restoration of biodiversity of wild germplasm [7]. By studying microenvironmental adaptation, we can have fair idea about the genomic divergence of wild barley adapted to different microenvironments.

Molecular markers are vital and valuable tools in the representation and evaluation of genetic diversity within and between species and populations [8]. The concept of polymorphism is used to define genetic variation in a population, which has been extensively studied in recent years by several established scientific disciplines [9]. Molecular markers are robust tools for the detection of genetic variation within and among populations [10]. The use of intron-exon splice junction (ISJ) markers is an effective method for analysis of genetic diversities, cultivar identification, construction of genetic maps and molecular marker assisted breeding [11,12].

The ISJ markers had been used to identify genetic variation and mapping polymorphisms in several crop species such as, wheat, barley, faba, triticale, tritipyrum [12], cotton, mosses and *Orthotrichum speciosum* [13]. ISJ markers are profitable in term of cost, while showing higher polymorphism and good stability with reproducible bands [13]. Therefore, in this study we used ISJ markers to detect polymorphism in wild barley populations.

Several analytical techniques have been used to analyze different chemicals in barley. These techniques include capillary electrophoresis (CE) [14], nuclear magnetic resonance (NMR) [15], mass spectrometry (MS) [16] and ultraviolet and visible (UV-VIS) spectroscopy [17]. Though these techniques are accurate, most of them are destructive, costly and time-consuming. Furthermore, most of these techniques, such as NMR, required skilled labor, which limit their applications in the laboratory. The near-infrared (NIR) spectroscopy is recorded as a simple, fast, nondestructive and chemical free analytical tool, compared to other analytical techniques [18]. Hence, in this study near-infrared spectroscopy was used to analyze chemical composition of wild barley samples.

Primarily, response to physical characteristics of the environment can be reflected as plant stress. The changes in abiotic factors such as temperature, climatic factors and chemicals create extrinsic stress which is considered the most vital stress agent [19-20]. In addition to that, competition, predation and parasitism, are considered as biotic stress factors, which may also cause for the development of stress [21]. Even though abiotic and biotic stresses may act as independent units, these two regularly act synergistically. For example, the organism that has suboptimal fitness due to abiotic stress, often suffers more from parasites and predators.

Environmental stress could only be measured or valued in connection to the organism which is going through stress. These genetic changes in organism or population give rise by inbreeding or other changes in the genetic architecture of organisms or population can change the perception of a different unchanged environment [22]. In this study, we selected wild barley samples to investigate microenvironmental adaptation from Mt. Gilboa, Israel, which belongs to Fertile Crescent, where wild barley originated.

The present study was conducted to evaluate the genetic diversity and genomic divergence of wild barley populations, differentially adapted to microenvironments in Mount Gilboa, Israel by using molecular markers, agro-morphological traits and NIR spectroscopy analysis.

Material and Methods

Plant materials

In this study, we used 27 wild barley samples which were collected from Mount Gilboa mountain, Israel. Samples were collected from the top and bottom of the rock situated in Mount Gilboa, which had two different microenvironments where top of the rock faced harsh stress conditions due to high sun exposure, heat, wind and low amount of water compared to bottom of the rock which received low sun exposure, low heat, low wind and water stress. Among them, sixteen samples were collected from the bottom of the rock and eleven were from the top of the rock. Samples were arbitrarily named using the codes of B1 and B2 (Bottom population) for the samples obtained from the bottom of the rock (Low Elevation) and T1 and T2 (Top population) for the samples obtained from top of the rock (High Elevation).

DNA extraction

DNA was extracted from fresh young leaves of above 27 wild barley accessions using the modified DS buffer method [23]. In brief, leaf tissues were grounded to a powder in 2 ml Eppendorf tubes in liquid nitrogen. The powder was then mixed with 0.6 ml of DS buffer (4% Sarkosyl, 0.1 M Tris-HC1, 10 mM EDTA, pH 8.0) and subsequently added 0.6 ml phenol/ chloroform/ isoamylalcohol (25:24:1). The whole mixture was shaken well for 30 seconds, left it on ice for 20 minutes and centrifuged at 10,000 rpm for 10 minutes. The aqueous phase was recovered and transferred to fresh tubes. Then, 0.6 ml of chloroform was added to the obtained solution and subsequently shaken well before centrifugation for 10 minutes.

The upper phase was collected and 0.5 ml of isoproponol and 50 µl of 3 M NaAc were added to the tube. Then the tube was inverted gently for few times to precipitate the DNA and centrifuged at 10,000 rpm for 5 minutes. After discarding the supernatant, the pellet was washed two times with 70 % ethanol. Pellet was air dried at room temperature for twelve hours and then 50 µl of double distilled H_2O was added. DNA concentration was checked by Thermo Scientific NanoDrop 2000 spectrophotometer and quality was checked using 1% agarose gel electrophoresis.

Molecular markers analyses

Seven ISJ primers were used in this study (Table 1). PCR amplification was carried out in a total volume of 20 µL reaction mixture containing 1.0 µL of template DNA, 2.0 µL of 10 x buffer, 1.6 µL dNTPs (2.5 mmol/L), 1.6 µL $MgCl_2$ (25 mmol/L), 1 µmol/L primer, 0.2 µL of Taq polymerase (Takara) and 12.6 µL of

double-distilled H_2O. The amplification reaction was performed using Bio-Rad C1000 thermal cycler according to the following cycling program: Initial denaturation for 5 minutes at 94 ^{0}C, followed by 9 thermal cycles of 1 minutes at 94 ^{0}C, 108 seconds at 48 ^{0}C, 2 minutes at 72 ^{0}C, 20 thermal cycles of 1 minutes at 94 ^{0}C, 90 seconds at 55 ^{0}C, 2 minutes at 72 ^{0}C and a final extension at 72 ^{0}C for 10 minutes.

Finally, the PCR amplified products were separated by gel-electrophoresis in 2 % agarose gels with 1xTAE buffer. Gels were stained with ethidium bromide and imaged in Biometra (UV-solo model) gel documentation system. Each PCR reaction was carried out twice and only reproducible bands were considered for analysis.

Molecular data analysis

Polymorphic Information Content (PIC) values for each ISJ primer were calculated according to the formula;

$PIC=1-\Sigma(Pij)^2$

where Pij is the frequency of the i^{th} pattern revealed by the j^{th} primer summed across all patterns revealed by the primers [24].

Marker index (MI) was obtained by the formula;

MI = PIC × number of polymorphic bands as proposed by Powell et al [25] and used by Milbourne et al [26]. Effective multiplex ratio (EMR) is defined as the product of the fraction of polymorphic loci and the number of polymorphic loci. The ability of the primers to distinguish genotypes was assessed by calculating their resolving power (Rp) as where Ib is band informativeness, Ib=1–[2× (0.5–pi)] and pi is the proportion of genotypes containing band I [27].

$Rp=\Sigma Ib$

Pair-wise genetic similarity (GS) between individuals for each marker system was estimated using the Jaccard coefficient [28]. All the GS matrices were subjected to appropriate clustering methods using NTSYSpc 2.02 software.

Agro-morphological characterization

The experiment was carried out during two crop seasons of 2015-2016 and 2016-2017 at the experimental field of Northwest Agriculture and Forest University, Shaanxi, China (N 34°10, E 108°10) under rainfed condition. The altitude of the area is 525 m and the climate is semi-humid prone to semi-arid with an average annual temperature of 13 ^{0}C and average annual rain fall of 600 mm.

The rainfall and average temperature in the two crop seasons (October 2015 to June 2016 and October 2016 to June 2017) were 214.8 mm and 389.6 mm and 13.6 ^{0}C and 12.4 ^{0}C respectively. Weeds were controlled manually. Pests and diseases were controlled by conventional methods.

Twenty-seven wild barley accessions collected from the bottom and top of the rock at Mt Gilboa, Israel were used for the experiment. Those all accessions were planted in two locations in the experimental field where the first location was at a lower elevation and the second location was located at comparatively higher elevation representing two microenvironments in Mt Gilboa. Line evaluation was carried out in 5 m rows with 30 cm x 45 cm spacing for within rows and between rows. Five plants per each row were randomly selected representing one plant per one meter and agro-morphological data were recorded for each plant.

Ten agro-morphological traits including plant height (PH), total number of tillers (NT), days to flowering (DF), flag leaf length (FLL), flag leaf width (FLW), peduncle length (PL), spike length (SL), awn length (AL), number of seeds per spike (NSS) and 1000 seeds weight (TSW) were recorded according to the descriptors of barley published by International Plant Genetic Resources Institute (IPGRI), Rome, Italy. To identify the significant differences, variation and correlation within/between the populations and elevations, ANOVA and correlation analysis were performed using IBM SPSS 23.0 software.

NIR spectroscopy analysis

The NIR analysis of wild barley seeds was carried out using Perten Diode array DA7250 NIR analysis system, Perten Instruments, Sweden, according to the protocol described by the manufacturer. The contents of Protein (Wet base), Oil (Dry base), Fiber (Fixed value=10), Crude protein (Dry basis), Starch (Wet base) and Amylose (Wet base) were measured by using NIR spectroscopy. Each sample was analyzed in duplicate as separately prepared replicates in a rotating sample cup. For each sample, four scans were performed and the results were averaged.

Results and Discussion

The potential use of wild barley germplasm has to be exploited largely especially where the wild barley originated. Fertile Crescent is recognized as the originated center of wild and cultivated barley where high genetic diversity was reported[4]. Therefore, to study microenvironmental adaptation and genetic divergence of wild barley, we have selected wild barley germplasms from Israel, as it represents the Fertile Crescent. Wild barley grown in the majority of Israeli climatic, topographic and edaphic habitats experiences many extremely unfavorable conditions [29]. Wild barley populations sampled in this area offered the unique advantage of allowing a comparison between genetic and ecogeographic diversity and finding correlations between genetic parameters and environmental parameters.

There are different criteria for the estimation of genetic diversity such as pedigree analysis[32], agro-morphological traits [30-31], biochemical markers [32-33] and molecular markers [34-35]. In this study, we used molecular markers, agro-morphological traits and biochemical markers to evaluate genetic diversity.

Polymorphism and genetic variation analyzed by ISJ markers

Seven ISJ primers were used in this study and primer sequences and their properties are summarized in table 1. Those ISJ primers produced 85 bands with an average of 6.14 bands per marker and 43 out of 85 bands (50.5%) were polymorphic. All primers except primer R1 detected polymorphism where primer R2 showed the highest polymorphic percentage (75%) while primer R1 showed the lowest polymorphic percentage (0 %). The number of fragments amplified by each reaction ranged from zero (Primer R1) to twelve (Primer R2) with a mean of 6.14. The band fragment size varied from 100 bp to 2000 bp and PIC values were ranged from 0.0 to 0.94 with an average of 0.76, thus indicating sufficient variability in wild barley populations. The Ib, Rp, EMR and MI values were ranged from 0 to 0.13, 0 to 1.19, 0 to 9.00 and 0 to 8.50 respectively. Primer R3 showed

the highest Rp value (1.19), while primer R2 showed the highest EMR (9.00), PIC (0.94) and MI (8.50) values. Primers E4 and R3 showed the highest Ib value (0.13) (Table 1). Moreover, primers E1, E2, R2, R3, R4 and R5 which recorded higher values of PIC, MI, EMR and Rp were identified as more informative in distinguishing wild barley genotypes. Primer R1 has not shown any polymorphic bands and due to that, it cannot use to distinguish wild barley genotypes.

PIC values greater than 0.50 indicate that, those markers enable sufficient level of polymorphism [36]. PCR amplification profile obtained with primer R4 is shown in figure 1. Number of studies have been conducted to evaluate the genetic relationships among different barley genotypes using molecular markers such as RADP [37], SSR[38] and ISJ[11,39,40]. Our results are comparable with the results reported by previous authors. Genetic similarities (GS) among barley genotypes were calculated using Jaccard's similarity coefficient and used to construct a dendrogram using NTSYSpc 2.02 software. Two groups were recognized in the dendrogram (Figure 2) with UPGMA algorithm for constructing cluster.

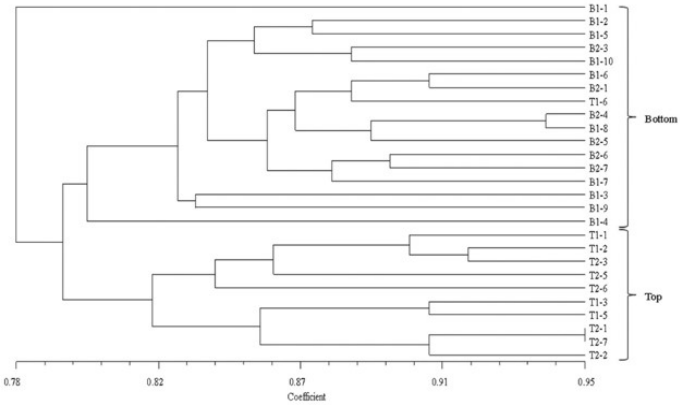

Figure 1

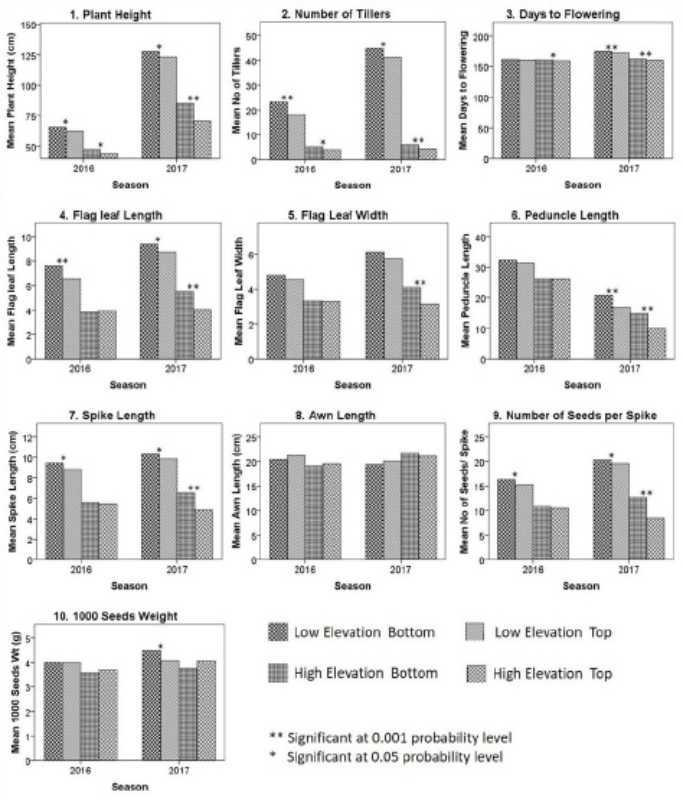

Figure 2

Cluster analysis showed a clear separation between two groups as Top population (T1-1 to T2-7) and Bottom population (B1-1 to B2-7). Accession T1-6 as an outlier, was grouped together in the bottom cluster, since it showed more similarity to the bottom cluster. Similar kind of cluster analysis have been conducted to identify genotype groups in previous studies [39, 41,42].

Diversity of agro-morphological traits

In both growing seasons (2015-2016 and 2016-2017), mean, standard deviation, maximum, minimum and coefficient of variation (CV %) for the traits under both conditions (Low Elevation and High Elevation) in both populations (Top and Bottom populations) are indicated in table 2. Analysis of variance (ANOVA) within the microenvironments (between populations) is shown in table 3 and ANOVA between microenvironments (between elevations) is shown in table 4. Figure 3 demonstrates a graphical illustration of variation of agro-morphological traits.

Plant height, number of tillers, flag leaf length, flag leaf width, peduncle length, spike length, number of seeds per spike and thousand seed weight were comparatively higher in the samples tested from bottom of the rock (LE) than top of the rock (HE) in both seasons. This is due to the adaptation to the shade effect, comparatively higher fertility and water availability in the bottom of the rock. Furthermore, all above parameters were low in top of the rock due to the harsh climatic and edaphic conditions they faced. Statistical analysis clearly indicates these microenvironmental adaptations (Table 2).

Average values of agro-morphological traits including plant height, number of tillers, days to flowering, flag leaf length, flag leaf width and 1000 seeds weight were higher in 2017 than 2016. Plant growth is comparatively higher in 2017 due to high rainfall during the 2016-2017 growing season. However, average peduncle length is low in 2017 than 2016. When the plant height is high, peduncle length is low and they have negative correlation with each other. This is different with other agro-morphological characters (Figure 3).

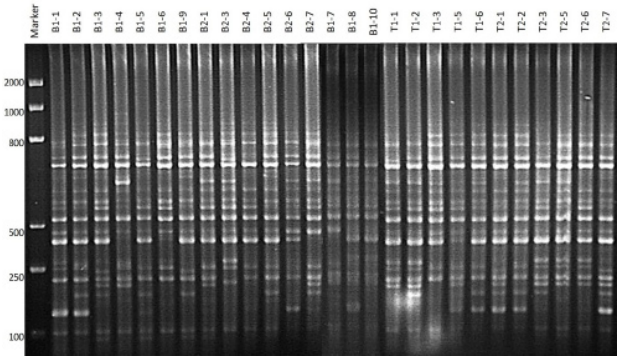

Figure 3

ANOVA between populations

Plant height and number of tillers were significantly different in both 2016 and 2017 seasons between populations. Flag leaf length, spike length and number of seeds per spike were significantly different in both 2016 and 2017 seasons between populations, except between 2016 HE Btm and Top. Days to flowering was significant in both 2016 and 2017 seasons, between populations, except between 2016 LE Btm and Top. Peduncle length was significant only in 2017 in both LE and HE (Table 3).

ANOVA between elevations

All the agro-morphological traits, except days to flowering and 1000 seeds weight, were significant in both 2016 and 2017 seasons between elevations. Days to flowering was significant only in 2017 between elevations whereas 1000 seeds weight was significant only in 2017 between LE and HE Btm (Table 4).

Agro-morphological characters are useful tools frequently use to evaluate the diversification and to establish the description of a genotype in crops including barley[8]. Agro-morphological characterization is a first step towards conservation and utilization of plant genetic resources. When assessing genetic diversity, the use of agro-morphological variation provides greater complementary information to molecular markers characterization [43].

Along with the results described above, CV % is higher in all agro-morphological traits in high elevation, except, days to flowering and 1000 seeds weight. Except leaf width, number of effective tillers and awn length, all higher CV % values were recorded in Top populations (HE Top). Lower CV % values were observed for agro-morphological traits in low elevation, except for the peduncle length (Table 2). As a whole it implies higher CV % obtained from populations of high elevation and lower CV % values obtained from populations of low elevation. It emphasizes that except for the days to flowering, growth rate of high elevation population is higher than low elevation population. These CV % values differentiate the variation of both wild barley populations.

Significant variations were observed for all agro-morphological traits indicating sufficient genetic variation and diversity in two microenvironments (Table 3 and 4). Similar type of results were also observed in several previous studies [44-46]. These agro-morphological traits analysis confirmed the microenvironmental adaptation of both Top and Bottom wild barley populations obtained from two different microenvironments in Mt. Gilboa, Israel.

NIR spectroscopy analysis

The mean, standard deviation, minimum, maximum and coefficient of variation values of NIR spectroscopy analysis of protein (wet base), oil (dry base), fiber (fixed value=10), crude protein (dry basis), starch (wet base) and amylose (wet base) in 2015-2016 and 2016-2017 growing seasons in both Bottom and Top populations are shown in table 5.

Analysis of variance of the chemical composition of NIR spectroscopy analysis in 2015-2016 seasons between the top and bottom populations showed a significant difference in protein, oil, fiber, crude protein and starch composition at $P \leq 0.05$ probability level. Fiber and crude protein contents were shown significant difference at $P \leq 0.05$ probability level between top and bottom populations in 2016-2017 while oil and amylose were shown significant different at $P \leq 0.001$ probability level.

ANOVA of chemical composition of NIR spectroscopy analysis in both seasons, between Top and Btm populations, oil, fiber and crude protein showed a significant difference at $P \leq 0.05$ probability level (Table 6). Many studies have been conducted to investigate effect of environmental effect on the chemical composition of barley [47-50] and it is a good indicator for the analysis of genetic divergence. This NIR spectroscopy analysis confirmed the microenvironmental adaptation in relation to the chemical composition of both Top and Bottom wild barley populations studied from two different microenvironments in Mt. Gilboa, Israel.

Characterization of differently adapted wild barley populations is an important aspect for the evaluation and preservation of wild germplasm. Such germplasms have undergone local environmental adaptations through natural selection, mutations and genetic drift for a particular geographic region over many generations [51]. Also climatic conditions and epigenetic factors play a major role in the evolution by representing significant levels of variation in response to the selection stress in the environment [41]. Present study depicted the clear variations of genetical, agro-morphological and chemical characteristics in wild barley population differently adapted to microenvironments.

Adaptation in basic terms can be stated as the process of change in an organism to conform successfully with new environmental conditions whereby the organism or group of organisms acquires characteristics involving changes in morphology, physiology or behavior that tend to develop their survival and reproductive success in the particular environment [22]. Those phenotypical changes can occur within a set genotype. As a result, phenotype adaptation which is called “phenotypic plasticity” takes place. This has the potential to change its phenotype according to existing conditions in the environment. Moreover, adaptation can also happen through changes in allele frequencies and it is an outcome of the selection pressure exerted by the environment. This mechanism is known as evolutionary adaptation or genotypic adaptation [52].

The variation in agro-morphological traits is usually determined by both genetic makeup of plant and environmental influences and interactions between them. Breeding programs based on both genomic information and genetic information are quick and accurate than conventional breeding. The characterized barley genotypes were mainly classified according to genetic, morpho-agronomic and chemical characterization which was complex and of multigenic characters. Such characters can be hardly influenced by various environmental conditions and therefore are liable to subjective evolution.

Table 1: List of ISJ primers and its descriptive used in the study.

Marker	Sequence 5'-3'	NPB	PP	PIC	Ib	Rp	EMR	MI
E2	5'-GGAATTCCACGTCCA-3'	8	57.14%	0.93	0.07	1.00	4.57	4.26
E4	5"-GGAATTCCACCTGCA-3'	4	50.00%	0.82	0.13	1.02	2.00	1.65
R1	5'-TCGTGGCTGACTTACCTG-3'	0	0.00%	0.00	0.00	0.00	0.00	0.00
R2	5'-TGCTGGTTTGCAGGT-3'	12	75.00%	0.94	0.07	0.93	9.00	8.50
R3	5'-TGCTGTGTGTGGACG-3'	4	44.44%	0.85	0.13	1.19	1.78	1.51
R4	5'-TCGTGGCTGACTTACCTG-3'	7	41.18%	0.92	0.09	1.04	2.88	2.65

R5	5'-TCGTGGCTGACGTCCATT-3'	8	50.00%	0.90	0.01	0.66	4.00	3.61

NPB: Number of Polymorphic Bands; PP: Polymorphism Percentage; PIC: Polymorphism Information Content; IB: Band Informativeness; Rp: Resolving Power; EMR: Effective Multiplex Ratio; MI: Marker Index.

Table 2: Summary of statistics of agro-morphological traits.

Statistics				PH (cm)	NT	DF	FLL (cm)	FLW (mm)	PL (cm)	SL (cm)	AL (cm)	NSS	TSW (g)
2016	LE	Btm	Mean	65.8	23	162	7.6	4.8	32.3	9.5	20.4	16	40.0
			SD	5.48	8.75	3.48	1.81	0.85	6.62	1.36	2.78	2.34	8.43
			Min	54.0	6	155	4.7	4.0	12.0	7.0	16.0	10	22.6
			Max	79.0	51	170	13.0	7.0	45.0	14.0	32.0	22	69.4
			CV	8.33	37.85	2.15	23.79	17.77	20.52	14.42	13.58	14.35	21.04
2016	LE	Top	Mean	62.7	18	161	605	4.5	31.4	8.8	21.2	15	40.0
			SD	7.48	8.12	4.27	1.76	0.91	6.12	1.17	2.15	1.72	9.34
			Min	43.0	5	154	3.0	3.0	18.0	7.0	17.5	12	24.1
			Max	77.0	41	171	11.0	6.0	46.0	12.0	27.5	20	65.0
			CV	11.92	44.52	2.65	26.93	20.01	19.51	13.27	10.12	11.23	23.37
2016	HE	Btm	Mean	47.5	5	161	3.8	3.3	26.2	5.6	19.1	11	35.8
			SD	6.57	2.84	4.14	0.89	0.70	4.70	1.21	2.26	2.03	4.26
			Min	36.0	1	154	2.0	2.0	14.0	3.0	14.0	8	28.7
			Max	64.0	13	169	6.0	5.0	36.0	9.0	24.0	16	42.0
			CV	13.84	54.46	2.57	23.26	21.10	17.96	21.51	11.79	18.74	11.90
2016	HE	Top	Mean	43.7	4	160	3.9	3.3	26.2	5.5	19.6	10	36.8
			SD	10.89	2.41	3.47	0.82	0.59	7.55	1.60	2.52	2.67	4.21
			Min	23.0	1	153	2.2	2.0	9.0	3.0	15.5	6	29.1
			Max	63.0	12	167	6.0	5.0	43.0	10.0	26.0	16	42.4
			CV	24.93	59.58	2.17	20.96	17.93	28.87	29.25	12.85	25.45	11.44
2017	LE	Btm	Mean	127.6	45	175	9.4	6.1	20.7	10.3	19.4	20	44.7
			SD	8.12	10.18	3.12	1.81	1.15	5.36	1.08	2.43	1.95	5.31
			Min	112.0	27	169	5.6	3.0	9.0	8.5	10.5	18	38.1
			Max	149.0	73	184	13.5	10.0	31.0	14.0	25.5	24	53.9
			CV	6.36	22.74	1.78	19.30	18.80	25.88	10.45	12.50	9.58	11.87
2017	LE	Top	Mean	123.3	41	173	8.7	5.7	16.8	9.8	20.1	20	40.8
			SD	8.34	8.11	4.19	1.74	1.38	5.41	1.12	1.68	1.98	3.80
			Min	104.0	24	164	5.2	3.0	5.0	8.0	15.0	16	35.0
			Max	143.0	55	181	13.2	10.0	29.0	12.5	23.5	24	47.0
			CV	6.76	19.66	2.43	19.86	24.10	32.10	11.37	8.38	10.09	9.32
2017	HE	Btm	Mean	85.2	6	163	5.5	4.1	14.8	6.5	21.7	13	37.5
			SD	15.87	2.55	3.32	1.47	0.95	5.46	1.71	2.61	3.79	4.80
			Min	48.0	2	155	2.0	2.0	4.0	3.5	12.0	6	28.8
			Max	117.0	13	171	9.2	8.0	26.0	11.5	26.0	24	47.5
			CV	18.64	42.82	2.04	26.49	23.15	36.99	26.19	12.03	29.92	12.81
2017	HE	Top	Mean	70.6	4	161	4.1	3.1	10.1	4.9	21.1	8	38.2
			SD	13.57	1.76	3.01	1.68	0.93	4.49	1.52	2.68	2.92	4.92
			Min	40.0	1	155	1.2	2.0	2.5	2.5	15.7	4	31.1
			Max	97.0	9	168	8.2	5.0	20.0	9.5	25.4	18	46.2
			CV	19.20	40.13	1.87	41.28	29.61	44.67	31.22	12.70	34.58	12.89

PH: Plant height; NT: Number of tillers; DF: Days to flowering; FLL: Flag leaf length; FLWL: Flag leaf width; PL: Peduncle length; SL: Spike length; AL: Awn length; NSS: Number of seeds per spike; TSW: 1000 seeds weight; SD: Standard deviation; MAX: Maximum; MIN: Minimum; CV: Coefficient variation (CV).

Table 3: ANOVA of agro-morphological traits within microenvironments (Between populations).

Genotype Class	2016						2017					
	Between LE Btm & Top			Between HE Btm & Top			Between LE Btm & Top			Between HE Btm & Top		
	Mean Square	F Value	P Value	Mean Square	F Value	P Value	Mean Square	F Value	P Value	Mean Square	F Value	P Value
PH (cm)	306.250	7.556	.007 *	430.165	5.877	.017 *	576.864	8.561	.004 *	6862.800	30.583	.000 **
NT	778.923	10.779	.001 **	41.301	5.769	.014 *	398.223	4.513	.029 *	81.434	15.884	.000 **
DF	33.263	2.278	.134	71.765	4.749	.026 *	145.137	11.237	.001 **	131.856	12.896	.000 **
FLL (cm)	37.888	11.798	.001 **	0.147	0.197	.658	12.843	4.052	.046 *	71.446	29.543	.000 **
FLW (mm)	2.106	2.755	.099	0.027	0.062	.803	5.156	3.302	.071	29.697	33.462	.000 **
PL (cm)	25.803	0.626	.430	0.016	0.000	.983	488.050	16.854	.000 **	722.061	27.860	.000 **
SL (cm)	11.988	7.206	.008 *	0.622	0.328	.568	7.113	5.932	.016 *	89.431	33.473	.000 **
AL (cm)	20.394	3.161	.078	5.720	1.025	.313	13.218	2.845	.094	10.763	1.546	.216
NSS	31.273	7.002	.009 *	3.605	0.679	.412	17.131	4.461	0.073 *	577.113	48.107	.000 **
TSW (g)	0.182	0.002	.961	6.573	0.366	.551	102.938	4.535	.043 *	2.841	0.121	.731

**Significant at 0.001 probability level and * Significant at 0.05 probability level

Table 4: ANOVA of agro-morphological traits between microenvironments (Between elevations).

Genotype Class	2016						2017					
	Between LE & HE Btm			Between LE & HE Top			Between LE & HE Btm			Between LE & HE Top		
	MeanSquare	F Value	P Value	MeanSquare	F Value	PValue	MeanSquare	F Value	P Value	Mean Square	F Value	P Value
PH (cm)	12948.730	355.992	.000 **	9465.622	110.432	.000 **	71897.681	452.377	.000 **	76375.475	602.270	.000 **
NT	12419.141	285.848	.000 **	5278.343	141.439	.000 **	60178.806	1093.168	.000 **	37388.945	1085.655	.000 **
DF	17.420	1.197	.276	38.173	2.498	.117	5820.156	561.120	.000 **	3936.036	295.441	.000 **
FLL (cm)	554.271	266.308	.000 **	181.959	93.512	.000 **	588.673	216.949	.000 **	604.658	207.673	.000 **
FLW (mm)	83.506	136.587	.000 **	40.566	67.952	.000 **	164.025	147.292	.000 **	183.309	132.207	.000 **
PL (cm)	1435.276	43.094	.000 **	714.394	15.275	.000 **	1417.886	48.405	.000 **	1268.202	51.296	.000 **
SL (cm)	570.129	342.319	.000 **	298.374	153.710	.000 **	575.322	281.921	.000 **	682.509	383.116	.000 **
AL (cm)	65.713	10.204	.002 *	71.934	13.244	.000 **	204.078	32.138	.000 **	30.161	6.025	.016 *
NSS	1170.258	242.517	.000 **	619.998	125.482	.000 **	2348.556	258.869	.000 **	3416.082	548.331	.000 **
TSW (g)	232.925	3.695	.058	85.176	1.102	.298	417.605	16.287	.000 **	36.920	1.911	.182

**Significant at 0.001 probability level and * Significant at 0.05 probability level

Table 5: Descriptive statistics of NIR spectroscopy analysis.

Statistics			Protein (Wet base)	Oil (Dry base)	Fiber (Fixed value=10)	Crude protein (Dry basis)	Starch (Wet base)	Amylose (Wet base)
2016	Btm	Mean	22.11	2.95	15.98	36.89	45.63	35.25
		SD	1.77	0.21	2.32	2.35	1.16	2.00
		Min	20.08	2.58	9.92	33.33	43.99	30.56
		Max	27.35	3.34	18.95	41.97	48.06	39.67
		CV	8.03	7.25	14.51	6.36	2.53	5.69
2016	Top	Mean	20.86	3.07	17.50	35.38	44.92	35.20
		SD	1.53	0.14	1.70	1.91	0.93	1.20
		Min	18.98	2.89	14.03	32.70	43.69	32.60
		Max	24.34	3.35	19.94	39.01	46.73	36.93

		CV	7.34	4.44	9.70	5.39	2.08	3.42
2017	Btm	Mean	20.11	2.26	17.25	34.50	44.79	38.65
		SD	1.35	0.15	1.14	1.41	0.37	1.26
		Min	16.54	2.00	14.89	31.48	44.09	36.38
		Max	22.00	2.48	19.14	36.45	45.44	41.12
		CV	6.72	6.70	6.58	4.09	0.83	3.26
2017	Top	Mean	20.81	1.86	18.05	35.50	44.67	41.45
		SD	1.37	0.14	0.93	1.75	0.39	0.89
		Min	19.35	1.56	16.47	33.14	44.08	39.45
		Max	24.75	2.08	19.75	39.94	45.31	43.01
		CV	6.60	7.75	5.14	4.94	0.87	2.14

SD: Standard deviation; Min: Minimum value; Max: Maximum; CV: Coefficient of variance

Table 6: ANOVA of chemical properties between microenvironments.

Year	Chemical Type		2016				2017		
		Mean Squar	F Value	P Value		Mean Square	F Value	P Value	
2016	Protein (Wet base)	20.590	7.294	.009	*	6.487	3.506	.067	
	Oil (Dry base)	.210	6.040	.017	*	2.106	95.646	.000	**
	Fiber (Fixed value=10)	30.168	6.904	.011	*	8.338	7.468	.009	*
	Crude protein (Dry basis)	29.872	6.290	.015	*	13.002	5.359	.025	*
	Starch (Wet base)	6.570	5.724	.020	*	.191	1.330	.254	
	Amylose (Wet base)	.028	.010	.923		102.273	80.782	.000	**

**Significant at 0.001 probability level and * Significant at 0.05 probability level

Conclusion

The populations analyzed in the present study have been characterized for genetic diversification in several ways such as genetically using ISJ molecular markers, using agro-morphological traits and biochemical analysis using NIR spectroscopy. In this study, we found that wild barley samples obtained from two different microenvironments, under the main common environment in Mt. Gilboa, Israel, have genomic divergence and differentially adapted to the particular microenvironments. Furthermore, our results demonstrated the effectiveness of ISJ molecular markers, agro-morphological traits and chemical characteristics for detecting variation and thus in monitoring the impact exerted by adaptation to the microenvironment on genetic divergence.

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