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Identifying Selection Signatures Related to Domestication Process in Barley (*Hordeum vulgare* L.) Landraces of Jordan Using Microsatellite Markers

Nidal A. Odat[1,*], Maen K. Hasan[2], Maher S. Obeidat[1], Mohamad A. Shatnawi[1], Saeid M. Abu-Romman[1], Issam M. Qrunfleh[2], and Muhannad I. Massadeh[3]

[1]*Department of Biotechnology, Al-Balqa' Applied University, Al-Salt, 19117, Jordan;*

[2]*Department of Plant Production and Protection, Faculty of Agricultural Technology, Al-Balqa' Applied University, Al-Salt, 19117, Jordan,*

[3]*Department of Biology and Biotechnology, Hashemite University, Zarqa, 13115.*

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Abstract

Domestication process and the subsequent breeding may result in a genetic mutation and selection pressures that possibly contributed to the emergence of two phenotypes of barley spikelets, namely six-row and two-row barley. The present study used microsatellite genetic markers to investigate the population genetics of a selected set of accessions of domesticated barley representing different populations of Jordan, to identify a signature of selection resulted from domestication process. Genomic and statistical approaches, such as the analysis of molecular variance (AMOVA) and the so-called hierarchical "outlier" tests, were utilized to identify signatures of selection. Generally, there was a high level of polymorphisms in all the studied populations, which ranged from 19.23 for Ramtha to 69.23 (%) for Zarga, with an average of 53.61 (%). The average number of allele per locus for the entire sample set was of 9.346 and it ranged 2 occurring at [(Bmag0136), (EBmac0970_a), (EBmac0970_c) loci], to 27 at (Bmac0040) locus. The results of AMOVA showed that the main portion (~72%) of total diversity was attributed to differentiations within populations. Other portions of diversity (11.040%) were explained by the diversity among populations. As revealed by the hierarchical outlier tests and AMOVA results, 9 microsatellites were identified to be under possible selection pressures, possibly indicates that these loci were important in the past improvement of barley by early cultivators. In conclusion, the present study shows that barley landraces of Jordan possess high levels of genetic diversity and allelic richness that could be utilized in barley improvement and breeding program.

Keywords: Hordeum spp, microsatellite, genetic diversity, population differentiation, domestication, selection signature, Jordan.

1. Introduction

Modern cereals, such as barley (*Hordeum vulgare* L.), have been originated as a result of the domestication process which is believed to be started ~12,000 years before present (yBP) in the Fertile Crescent, a region which encloses the countries of Iraq, Kuwait, Syria, Lebanon, Jordan, Palestine, Cyprus, and Egypt, southeastern border of Turkey, and the western border of Iran (Zohary and Hopf, 2000; Salamini *et al*., 2002). This domestication process and the subsequent selective breeding by early cultivators of cereals involved the selection of individual's plants with certain traits that serve human needs, such as improved seed yield, seed recovery, and other traits (Hammer, 1984; Harlan, 1992). In barley, the selection process by humans during domestication resulted in the emergence of two different phenotypes or morphs, namely six-rowed barley or (*Hordeum vulgare* L. *hexastichum*), and two-rowed barley or (*Hordeum vulgare* L. *distichum*) (Badr *et al*., 2000; Kilian *et al*., 2006). Population genetics studies indicated that during domestication process the six-rowed barley has emerged from the two-rowed wild barley as a result of mutations and selection pressures which lead to the difference in morphology of spikelets (Komatsuda *et al*., 2007).

Studying population genetics of domesticated crops, such as barley, is important not only from an evolutionary stand point, but also from agricultural perspectives (Meyer and Purugganan, 2013; Fuller *et al*., 2011; Kellogg, 2001). For example, an efficient breeding

* Corresponding author. e-mail: nidalodat@gmail.com.

method requires a selection of agriculturally important phenotypes which are often genetically controlled by an array of genotypic spectrum (Fuller *et al*., 2011).

Microsatellites, also called Simple Sequence Repeats (SSRs), are among the advanced genetic markers currently developed (Struss and Plieske, 1998). These genetic markers are considered to be highly informative and reproducible in term of their discrimination among populations and landraces of barley (Ramsay *et al*., 2000)

In the present study, the population genetics of a selected set of accessions of domesticated barley covering ten populations (sites) of Jordan were investigated using microsatellite genetic markers. Specifically, the objectives of this study are to: (1) investigate the level of genetic diversity within and between populations of barley, (2) study the degree of population differentiation between the two cultivated phenotypes (two-rowed and six-rowed barley) and (3) identify microsatellite loci that are under possible influence by selection pressure of domestication.

2. Materials and Methods

2.1. Plant Material and Genomic DNA Isolation

A set of 139 seeds representing accessions of barley landraces (*Hordeum vulgare* L.), originally collected from 10 geographical locations of Jordan, were obtained from the seed bank of the National Center for Agricultural Research and Extension (NCARE, Weltzien, 1988). Of these accessions, 42 were of six-rowed spike barley type and 97 of two-rowed barley spike type. Seeds were grown in greenhouse and genomic DNA was extracted from young leaves of about 4 weeks using the CTAB method (Doyle and Doyle, 1987). An average of ten seeds (individuals) per site were used in the present study. Most seeds present in NCARE are of two-row compared with six-row barley.

2.2. Genotyping of microsatellite markers

Twenty-five microsatellite loci covering the 7 chromosomes of barley were chosen based on the genomic map established by Ramsay *et al*., (2000) (Table 1). Polymerase Chain Reactions (PCR) were carried out in a volume of about 12 µl containing approximately 20 ng of genomic DNA template, 0.5 U Taq polymerase (Qiagen, Hilden, Germany), 1 x PCR reaction buffer (Qiagen), 0.5 pmol each of forward and reverse primers (IRD700- or IRD 800-labelled), and 0.18 mM of each deoxynucleoside triphosphates (dNTPs). Amplifications were performed using a standard amplification cycle in a GeneAmp PCR System 9700 thermal cycler, and SSR polymorphisms were resolved and visualized using a LI-COR GeneReadir 4200 (MWG Biotech, Ebersberg). DNA isolation, PCR conditions, genotyping of microsatellite and gel preparation detailed in (Hasan *et al*., 2006). The laboratories techniques for this were conducted in the laboratories of the Justus Liebig University-Giessen/Germany.

2.3. Analysis of the level of genetic diversity

Summary statistics of genetic diversity, including gene diversity (Nei, 1978), the number polymorphic loci, percentage of polymorphism, and alleles number, were calculated for each microsatellite locus per population using the software Powermaker version 3.25 (Liu and Muse, 2005).

2.4. Identifying selection signature at microsatellite loci in relation to domestication

In order to detect and identify microsatellite loci that were possibly under selection pressure due to domestication, F_{ST}-based outlier test was conducted. This method takes hierarchical population structure into account (Excoffier *et al*., 2009), and is implemented in the software Arlequin. Briefly, the method detects outliers exhibiting significantly high or low F_{ST} values, controlled for heterozygosity at microsatellite loci. Outliers are detected based on differentiation among all populations (F_{ST}) and differentiation among groups of populations (two- rowed barley vs. six-rowed barley) (F_{CT}). The analyses were conducted at: (1) an overall analysis encompassing all analyzed populations (based on F_{ST}) and (2) an analysis at the phenotypic level conducting tests for pair-wise comparisons (F_{CT}). This analysis assumed a simulation model of 25 groups each consisting of 50 populations and based the tests on 50,000 simulated loci for the full data set.

3. Results

The 25 microsatellite loci used in the present study provided a fair coverage of the genome, with a range between 2 and 5 loci for each of the seven *Hordeum* linkage groups (Chromosomes). The final dataset, therefore, was based on results of 229 amplified SSRs with an average of 9.16 alleles per locus in the entire sample. The results show a large variation in the number of alleles among the microsatellite loci, with the lowest of 2 occurring at the (Bmag0136), (EBmac0970_a), and (EBmac0970_c) loci and the highest of 27 at (Bmac0040) locus. Nine of the (25) loci showed more than 10 alleles per locus (Table 1).

To see the level and the extent of genetic diversity within our studied samples of barley, summary statistics of genetic diversity were estimated using several parameters for each populations of barley. The average gene diversity for the entire samples set was found to be 0.468, with a range of 0.096 for Ramtha population to 0.610 for Zarga population (Table 2). Generally, there was a high level of polymorphisms in all the studied populations, which ranged from [19.23 for Ramtha to 69.23 (for Zarga)] with an average of 53.61 %.

To see the level of the population genetic differentiation that might reflect geographical or phenotypical structure between barley landraces, the total genetic diversity was divided within and between-populations components by using the analysis of molecular variances (AMOVA). The results of AMOVA show that the main portion of the total diversity of 71.830% was attributed to differentiations within populations (Table 3). Other portions of diversity (11.040%) were explained by the diversity among populations within phenotype types and (17.130%) percentage of variances was clarified by differentiation between the barley phenotype types, namely two-rowed and six-rowed barley. The high F_{CT} value of 17.13%,

which measures the extent of differences between barley phenotypes, indicated that genetic differentiation between (*Hordeum vulgare* L. *hexastichum*), and two-rowed barley (*Hordeum vulgare* L. *distichum*) is a considerably high. There was no significant correlation between geographic origins and genetic differentiation of barley accessions. As suggested by the hierarchical outlier test, it was found that 8 microsatellite loci to be under selection across all populations (F_{ST}; Figure 1), whereas 9 outliers microsatellite loci were identified to show selection pressures which contributed to the phenotypic characteristics of studied accessions of barley (F_{CT}; Figure 2).

Table 1. Marker name, allele number, chromosome location, primer pairs sequences, and repeat motif for 25 microsatellite markers used in this study.

Marker or Loci	Allele number	Chromosome	Primer sequence (5′–3′)	Repeat motif
Bmag0382	7	1H	5' TGAAACCCATAGAGAGTGAGC 3' 5' TCAAAAGTTTCGTTCCAAATAC 3'	(AG)7AA(AG)7
Bmag0211	10	1H	5' ATTCATCGATCTTGTATTAGTCC 3' 5' ACATCATGTCGATCAAAGC 3'	(CT)16
Bmag0347	14	1H	5' CTGGGATTGGATCACTCTAA 3' 5' AAAACAAGTACTGAAAATAGGAGA 3'	(CT)28
Bmac0093	5	2H	5' CGTTTGGGACGTATCAAT 3' 5' GGGAGTCTTGAGCCTACTG 3'	(AC)24
EBmac0850	5	2H	5' CTCAGATAACACCTTTAAACACA 3' 5' AAGACAGTTGGGTAAGCCT 3'	(AC)13AT(AC)10A-(AC)5
Bmac0134	21	2H	5' CCAACTGAGTCGATCTCG 3' 5' CTTCGTTGCTTCTCTACCTT 3'	(AC)28
Bmag0136	2	3H	5' GTACGCTTTCAAACCTGG 3' 5' GTAGGAGGAAGAATAAGGAGG 3'	(AG)6-(AG)10-(AG)6
HVM60	10	3H	5' CAATGATGCGGTGAACTTTG 3' 5' CCTCGGATCTATGGGTCCTT 3'	(AG)11 and (GA)14
Bmac0181	9	4H	5' ATAGATCACCAAGTGAACCAC 3' 5' GGTTATCACTGAGGCAAATAC 3'	(AC)20
Bmag0353	12	4H	5' ACTAGTACCCACTATGCACGA 3' 5' ACGTTCATTAAAATCACAACTG 3'	(AG)21
HVM67	8	4H	5' GTCGGGCTCCATTGCTCT 3' 5' CCGGTACCCAGTGACGAC 3'	(GA)11
Bmag0384	7	4H	5' TGTGAGTAGTTCACCATAGACC 3' 5' TGCCATTATCATTGTATTGAA 3'	(AG)18
Bmag0337	11	5H	5' ACAAAGAGGGAGTAGTACGC 3' 5' GACCCATGATATATGAAGATCA 3'	(AG)22
Bmac0163	4	5H	5' TTTCCAACAGAGGGTATTTACG 3' 5' GCAAAGCCCATGATACATACA 3'	(AC)6(GC)3(AC)17
EBmac0970_a	2	5H	5' ACATGTGATACCAAGGCAC 3' 5' TGCATAGATGATGTGCTTG 3'	(AC)8
EBmac0970_b	3	5H	5' ACATGTGATACCAAGGCAC 3' 5' TGCATAGATGATGTGCTTG 3'	(AC)8
EBmac0970_c	2	5H	5' ACATGTGATACCAAGGCAC 3' 5' TGCATAGATGATGTGCTTG 3'	(AC)8
Bmac0096	15	5H	5' GCTATGGCGTACTATGTATGGTTG 3' 5' TCACGATGAGGTATGATCAAAGA 3'	(AT)6(AC)16

Bmag0009	7	6H	5' AAGTGAAGCAAGCAAACAAACA 3' 5' ATCCTTCCATATTTTGATTAGGCA 3'	(AG)13
Bmac0040	27	6H	5' ATTATCTCCTGCAACAACCTA 3' 5' CTCCGGAACTACGACAAG 3'	(AC)20
Bmag0321	9	7H	5' ATTATCTCCTGCAACAACCTA 3' 5' CTCCGGAACTACGACAAG 3'	(AG)17(AC)16
Bmac0273_a	9	7H	5' ACAAAGCTCGTGGTACGT 3' 5' AGGGAGTATTTCACCCTTG 3'	(AC)20(AG)20
Bmac0273_b	5	7H	5' ACAAAGCTCGTGGTACGT 3' 5' AGGGAGTATTTCACCCTTG 3'	(AC)20(AG)20
Bmac0031	9	7H	5' AGAGAAAGAGAAATGTCACCA 3' 5' ATACATCCATGTGAGGGC 3'	(AC)28
Bmag0120	16	7H	5' AGAGAAAGAGAAATGTCACCA 3' 5' ATACATCCATGTGAGGGC 3'	(AC)28

Table 2. Summary statistics of genetic variation estimated for the studied populations of Jordanian landraces of barley.

Population/row-type	Sample size	Gene Diversity	No of Polymorphic loci	% of Polymorphic loci
Irbid/Two-rowed	11	0.555	16	61.538
Irbid/Six-rowed	22	0.588	11	42.308
Ramtha/Two-rowed	3	0.096	5	19.231
Jarash/Two-rowed	4	0.366	16	61.538
Mafrag/Two-rowed	8	0.572	12	46.154
Mafrag/Six-rowed	6	0.474	16	61.538
Zarga/Two-rowed	7	0.559	18	69.231
Zarga/Six-rowed	11	0.61	16	61.538
Balga/Two-rowed	2	0.558	14	53.846
Balga/Six-rowed	6	0.383	17	65.385
Madaba/Two-rowed	9	0.475	16	61.538
Madaba/Six-rowed	5	0.491	18	69.231
Karak/Two-rowed	18	0.564	13	50
Karak/Six-rowed	3	0.348	13	50
Tafileh/Two-rowed	16	0.347	14	53.846
Maen/Two-rowed	14	0.498	8	30.769

Table 3. AMOVA of barley populations investigated in this study showing the effect barley inflorescence types (two vs six row) on genetic differentiation.

Source of variation	df	Sum of squares	Variance	% variation	P value
Among genome types	1	99.138	0.777	17.130	P<0.001
Among populations within genotype types	14	150.217	0.501	11.040	P<0.001
Within populations	232	756.693	3.262	71.830	
Total	247	1006.048	4.541	100	

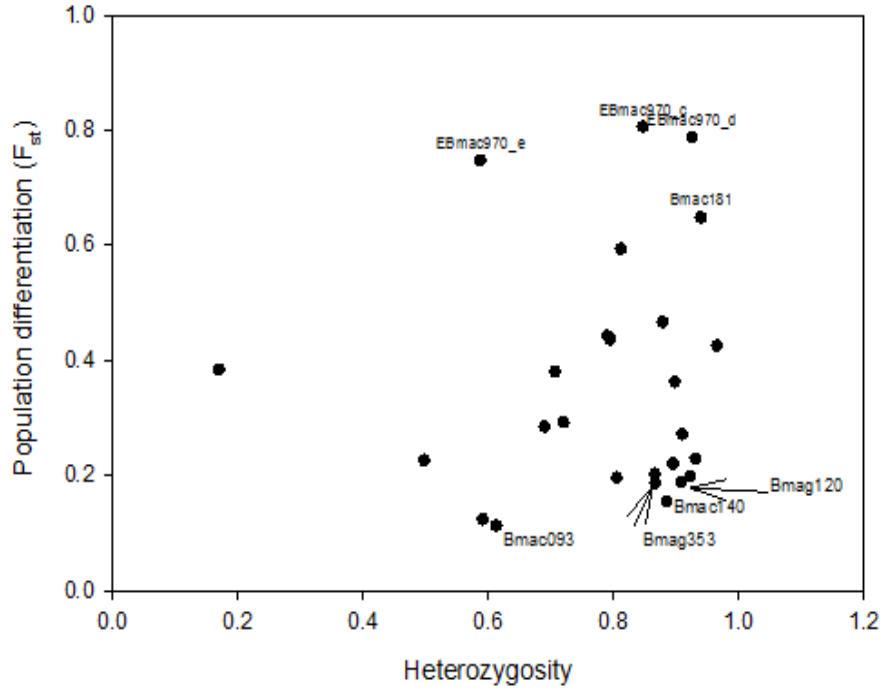

Figure 1. Graphical representation of the relationship between heterozygosity and population differentiation as obtained from hierarchical outlier tests involving the 25 microsatellites in this study. The graph shows the eight loci which are supposed to be under selection based on genetic differentiation between the two barley phenotypes.

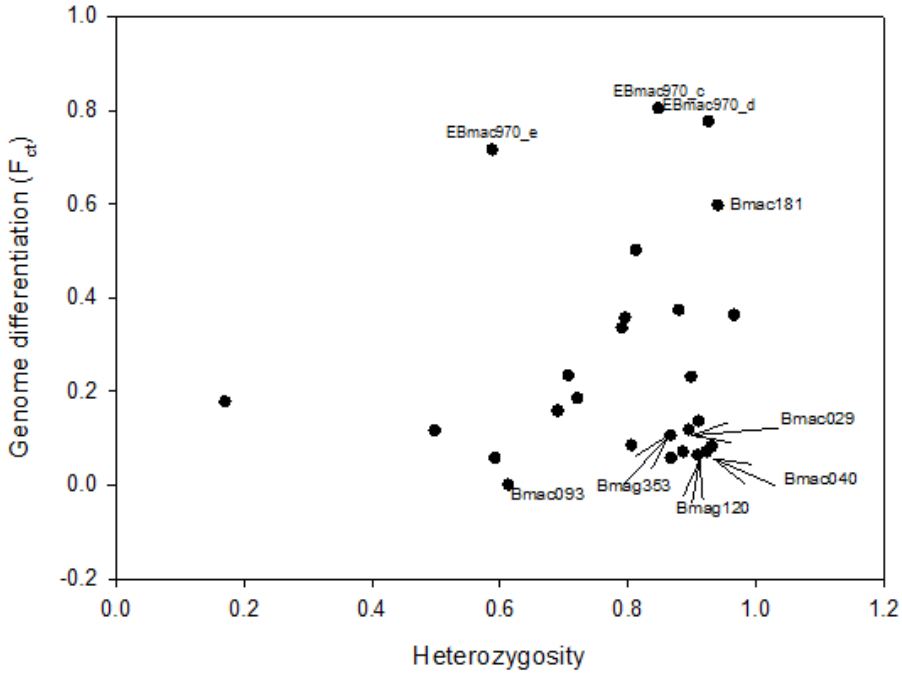

Figure 2. The relationship between heterozygosity and population differentiation as obtained from hierarchical outlier test based on genetic differentiation between the two barley phenotypes indicating the 9 microsatellite loci that show signature of selection pressures of barley domestication.

4. Discussion

With the advances of molecular markers, population genetics of crop plant has become an important tool for conserving and maintaining germplasm collections. Studies of population genetics of crops offer a unique opportunity to identify footprints or signature of selection which can give valuable insight which help to identify new genes of agronomic importance (Vigouroux *et al*., 2002; Burger, 2008; Fuller *et al*., 2011). Similar to other studies, the present study employed microsatellite, as suitable molecular markers to study the population genetic diversity in barley landraces (Ordon *et al*., 1995; Thiel *et al*., 2003; Varshney *et al*., 2006). This study of barley revealed a relatively high overall genetic diversity for all

the populations studied (Table 2). The high level of genetic diversity suggests that gene pool of barley of Jordan possesses a high level of allelic richness and polymorphism that could be exploited for the improvement and management of barley germplasm. The high level of genetic diversity detected in the present study is possibly a consequence of the location of Jordan in Fertile Crescent which characterized by high diversity of crop plants and even wild plants (Badr *et al*., 2000; Zohary and Hopf, 2000; von Bothmer *et al*., 2003; Pourkheirandish and Komatsuda, 2007).

As estimated by summary statistics, most of the genetic diversity found in this study resided within population rather than between the populations of the studied accessions (Table 2). Moreover, AMOVA analysis revealed a low but a significant genetic diversity between populations, and there was no association between genetic diversity and geographical locations of populations. This suggests that the gene flow between barley from different areas in Jordan is relatively high. These results are in agreement with other studies which revealed a differentiation between different barley accessions from different countries (Ordon *et al*., 1995; Hamza *et al*., 2004). According to AMOVA, the barley landraces could be divided into two groups that reflect different barley inflorescence types, namely six-row in one group and two-row in the second group (Table 3). Based on these results, the six-rowed barley populations significantly differed from two-rowed barley likely as a consequence of the gene flow and selective pressures during domestication and selective (Hamza *et al.*, 2004; Malysheva-Otto *et al.*, 2006; Yahiaoui *et al.*, 2008).

The present study also investigated the gene diversity of barley with the intention of identifying microsatellites that show evidence of selection during barley domestication. To achieve this, a population genetic approach was utilized by starting with SSR genetic loci and asking whether these loci were targets of selection (Doebley *et al*., 2006). Of the 25 microsatellites loci studied here, 9 microsatellites were found to be under possible selection pressures between the two barley phenotypes (two-row vs six-rowed) (Figure 2). These loci possibly represent genes that were selected by barley cultivators as they perhaps contributed to agronomic performance, palatability, nutritional quality, or other traits of barley. It could be hypothesized that these loci were important in the past improvement of barley, and this may indicate that these loci are possible candidates for introgressive breeding from wild relatives to increase the pool of diversity with which modern breeders can work (Vigouroux *et al*., 2002). However, the effect of plant breeding on genetic diversity is a controversial issue. For example, a decrease in genetic diversity because of conventional plant breeding has been found in barley (Graner *et al*., 1990; Allard 1996; Ellis *et al*., 1997; Russell *et al*. 1997). On the other hand, Struss and Plieske (1998) found the same level of genetic diversity in wild and in domesticated barley. Our study has a caveat as we used seeds collected in the past (see Material and Methods section). This makes us cautious as the genetic diversity found in the present study may be different if compared with plant materials that represent the current time. Thus, a further study is needed to compare our results with new barley landraces that represent the current time.

In conclusion, the present study reveals that barley landraces, from different areas of Jordan, posses high levels of genetic diversity and allelic richness that could be utilized in barley improvement and breeding program.

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