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# Transferability of wheat SSR markers for determination of genetic diversity and relationships of barley varieties

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**Abstract**
Simple sequence repeat (SSR) markers are simple PCR-based co-dominant markers, which are highly polymorphic and informative due to the number and frequency of alleles, and thus they are most utilized among the molecular markers. However, the development of SSR markers is costly and time-consuming. Cross-species transferability of SSRs allows the SSRs isolated from one species to apply on a closely related species, which increases the utility of previously isolated SSRs. This study demonstrated the cross-species transferability of 196 SSR primer pairs of wheat in genetic diversity analysis of 40 varieties of barley (*Hordeum vulgare* L.). Of the 196 SSR primer pairs assayed, 59 (30.1%) showed transferability. Of the 59 primer pairs, 21 pairs were polymorphic with the polymorphism information content ranging from 0.19 to 0.70. The number of alleles detected at each locus ranged from one to seven with an average of 3.57. Cluster analysis using the Minimum Evolution algorithm and the coefficient of Number of Differences assigned the genotypes into five groups.

**Keywords**: Barley; SSR; Transferability; Wheat

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## Introduction

Simple sequence repeat (SSR) or microsatellites consist of tandemly-repeated mono, di, tri, tetra, or penta-nucleotide units that exist in the genomes of most eukaryotes with high frequency (Powell *et al*. 1996). SSRs are most preferred because they are co-dominant, detect more alleles per locus and are uniformly dispersed throughout the genome (Sing *et al*. 2011). However, most of the SSRs have been developed for use in only a few agriculturally important crop plants due to high cost and extensive effort required in the microsatellite marker development (Basudeba *et al*. 2013). Sequence data gathered from several crop species show the existence of sufficient homology among genomes in the regions flanking the SSR loci. Therefore, the designed primer pairs based on the sequence from one species may be used to identify SSRs from other related species. The cross-species transferability of SSRs from one species to another has been demonstrated in a lot of species (Kuleung *et al*., 2004) including wheat, barley, maize, sorghum, oat (Zeid *et al*. 2010), vetiver (Singh *et al*. 2014), blueberry (Liu *et al*. 2014), bottle gourd (Yildiz *et al*. 2015), pistachio (Zaloglu *et al*. 2015), sugarcane (Singh *et al*. 2011), rice (Chen *et al*. 2010) and legumes (Erayman *et al*. 2014). Varshney *et al*. (2004) examined 165 EST-SSR markers from a total of 185 assigned to the genetic map of barley for

transferability to wheat, rye and rice. A higher proportion of barley markers were amplified in wheat and rye (78.2% and 75.2%, respectively) followed by rice (42.4%).

This study was aimed verifying the transferability of wheat SSR markers derived from different chromosomal regions into 40 barley cultivars. The effects of different motifs, chromosomes and genomes on transferability were also addressed.

## Material and Methods

### Plant materials and DNA extraction

In this research, 40 barley genotypes (Table 1) were used for the transferability study. Most of these genotypes were selected largely for their economic value. These genotypes were collected from different geographical regions. Young leaves of the plants grown in the greenhouse of the University of Tabriz, Iran, were collected and stored at –80 ºC until the genomic DNA was extracted. The extraction of the genomic DNA from leaf tissues was done by the CTAB method (Saghai-Maroof *et al*. 1984). The DNA solution (5 µl) was loaded on 8% agarose gel for the quality check and DNA was quantified with a spectrophotometer. The working concentration of DNA was adjusted to 25 ng. This DNA was then used for the PCR analysis with wheat SSRs.

### PCR amplification and fragment analysis

In this study, 196 wheat SSR markers were used. The microsatellite primers were taken from the commercially available microsatellite primer kits. A full list of the transferable primers and their annealing temperature is provided in Table 2. PCR amplifications were performed in the 10-µL reaction mixture which consisted of 4 µL Master Mix [including Taq DNA polymerase, dNTP, Tween 0.2%, $MgCl_2$, $SO_4 2(NH_4)$ and Tris-HCl], 2 µL template DNA and 0.5 µL of each primer (forward and reverse primers), and 3 µL water. PCR amplification conditions were as follows: 94 ˚C for 3 min, 30 cycles of 94 ˚C for 1 min, annealing step of 1 min at the optimized annealing temperature for each primer, 72 ˚C for 1.5 min and the final extension at 72 ˚C for 7 min. The amplified products were separated by 4% denaturing polyacrylamide gel electrophoresis. The size of the amplified fragments was estimated by 50 and 100 bp DNA ladder.

### Data analysis

SSRs were scored as dominant markers. The presence of a band was recorded as "1" and the absence as "0." The number of alleles, major allele frequency, gene diversity, observed heterozygosity ($H_o$) and the polymorphic information content (PIC) was calculated by the PowerMarker v3.25 software (Liu and Muse 2005). PIC as a measure of allelic variability and evenness at a particular locus was calculated for each locus using the following formula:

$$PIC=1-\sum_{i=1}^{n} p_i^2 - 2\sum_{i-1}^{n}\sum_{j=i+1}^{n} p_i^2 p_j^2$$

where, n is the number of alleles and $p_i$ and $p_j$ are the sample frequency of the ith and jth alleles, respectively. The effective number of alleles (Ne), Nei's gene diversity (H) or expected heterozygosity (He) and Shannon's information index (I) were determined by the following formulae for each locus using the GenALEx 6.4 software (Peakall and Smouse 2006):

$H_e = 1 - \Sigma p_i^2$ (Nie 1973)

$I = -\Sigma(p_i \ln(p_i))$ (Maguran 2004)

$N_e = 1/(\Sigma p_i^2)$ (Peakall and Smouse 2006)

In the above formulae, $p_i$ and $p_j$ are the frequency of the ith and jth alleles, respectively.

Cluster analysis was performed for 40 barley varieties based on the Minimum Evaluation algorithm and the distance coefficient of Number of Differences using the MEGA program (Tamura *et al*. 2011).

**Results and Discussion**

**Transferability polymorphism**

Of the 196 wheat microsatellite primer sets, 59 (30.1%) yielded band transferability in these varieties. The other primer pairs did not amplify scorable bands. Amplification of PCR products across varieties indicates that primer sites were highly conserved. The annealing temperature is critical for PCR which affects the transferability of SSR markers (Wang *et al*. 2004). Gupta *et al*. (2003) reported that 59 wheat EST-SSR markers were amplified out of 24 fragments in barley, maize, oat, rice and rye. According to Varshney *et al*. (2004), 78.2% of barley EST- SSR markers showed amplification in wheat. Based on Zhang *et al*. (2005), the amount of cross amplification and transferability of SSRs between wheat and four cereals (Agropyron, barley, rice, rye) was 50% on the average. Castilo *et al*. (2008) observed 81% transferability of the barley EST-SSRs markers in the genome of *T. aestivum, T. urartu and T. tauschii*. According to Tang *et al.* (2006), 86.8% of the wheat EST- SSR markers produced amplicons in barley.

SSR markers derived from different chromosomes showed different percentages of transferability in our study. Among the196 SSRs, those located on the 2D, 1B and 6D chromosomes were the most transferable, while those from the 3B and 3D chromosomes exhibited no transferability (Table 3). There are three different motif types for SSRs as follows: pure, compound and interrupted (Peakall *et al.* 1998). In this study, compound motifs showed the highest transferability (40%) and the lowest rate (20%) belonged to interrupted motifs (Table 4). From a total of 196 wheat SSRs, there were 85.16% di-nucleotide repeats, 10.99% tri-nucleotide repeats, 3.3% tetra-nucleotide repeats, 0.0% penta-nucleotide repeat and 0.55% hexa-nucleotide repeats. We compared the transferability of different SSR motifs (Table 5). Tetra-nucleotide motifs had the highest (50%) and hexa-nucleotide motifs had the lowest transferability rate (30.34%). Tetra-nucleotide microsatellites probably represent conserved regions in the genome that can be useful for transferability between species.

**Genetic diversity analysis**

We also used the successfully transferred 21 SSR markers derived from wheat to detect the genetic diversity among barley varieties. From the 59 tested loci, 21 were polymorphic and others had non-polymorphic bands. Thus, polymorphic loci constituted 35.59% of the total loci. The detected polymorphism level depends on the types of primers and the genetic divergence of species (Wang *et al*. 2004).

Table 1. Description of 40 barley varieties under study.

| Genotype | Adaptation area | Growth type | Genotype | Adaptation area | Growth habit |
|---|---|---|---|---|---|
| Gohar | WM | Spring | Dasht | WM | Facultative |
| Zarjo | CM | Facultative | Nimrooz | WM | Facultative |
| Eram | WM | Spring | Nosrat | WM | Facultative |
| Valfajr | CM | Facultative | Abidar | CM | Facultative |
| Aras | CM | Facultative | Torsh | WH | Facultative |
| Reyhan | WM | Spring | Sina | WM | Spring |
| Sahra | WM | Facultative | Shirin | WH | Facultative |
| Afzal | CM | Facultative | Loot | M | Spring |
| Gorgan | WH | Spring | Yuosef | M | Spring |
| Karoon | WH | Facultative | Zahak | WH | Spring |
| Jonoob | WH | Facultative | Behrokh | M | Spring |
| Makoie | MC | Facultative | Produktive | CM | Spring |
| Bahman | MC | Facultative | Abshar | | Winter |
| Bolbol | M | Winter | Clipper | C | Spring |
| Fajr30 | CM | Facultative | Igri | C | Spring |
| Reyhan03 | WM | Spring | Yea/188/ | C | Spring |
| Kavir | WM | Facultative | Sahara | | Winter |
| Seyyedtajoddin | C | Spring | Morex | M | Spring |
| Nik | M | Spring | Steptoe | M | Spring |
| Ghareharpa | MC | Spring | TN-02-216 | | |

Table 2. The characteristics of microsatellite primers used in the polymerase chain reaction.

| Marker | Motif | Chromosome | A. temp | Marker | Motif | chromosome | A. temp |
|---|---|---|---|---|---|---|---|
| BARC164 | (CA)28 | 3B | 58 | BARC128 | (CA)12 | 1B, 2B, 5B, 7D | 55 |
| CFA2173 | (CA)19 | 4A, 4D, 6A | 59 | WMC256 | (CT)16 | 6A | 59 |
| XGWM232 | (CT)(CT)com | 1D | 62 | XGWM325 | (GT)14 | 6D | 57 |
| WMC3 | (GA)14 | 3A | 59 | XGWM642 | (CT)4(GA)10 | 1D | 57 |
| WMC312 | (CA)2GC(GA)3 | 1A | 61 | XGWM271 | (GT)30 | 5D | 57 |
| WMS46 | (GT)6(N)28(GT)16 | 7B | 60 | WMC786 | (CT)46 | 6A, 6B, 6D,7A | 62 |
| WMS129 | (CT)22 | 2B, 5A | 59 | XGWM192 | (CT)9(CA)21 | 4A | 62 |
| XGWM304 | (CA)27 | 2A | 58 | WMC405 | (CT)30 | 1D, 7D, 5B, 7A, 5D | 62 |
| XGWM583 | (CA)27 | 5D | 55 | XGWM257 | (CA)17 | 2B | 56 |
| BARC205 | (CT)10 | 5D | 59 | CFA2234 | (CT)17 | 3A | 59 |
| WMC336 | (GCC)7 | 1D, 1A | 59 | CFA2114 | (CA)32 | 6A | 60 |
| WMC398 | (GT)14 | 6A, 6B | 58 | CFA2185 | (TG)29 | 5D | 59 |
| WMC432 | (GT)14 | 1D | 62 | CFA2240 | (TA)n | 7A | 58 |
| WMC63 | (GA)12 | 2A | 61 | GDM6 | (GT)27 | 2A | 59 |
| WMS295 | (GA)25 | 7D | 60 | WMC640 | | 3A, 5B, 5D | 57 |
| WMS515 | (GT)17(TCAT)(GT)6 | 2A, 2D, 1A | 59 | WMC58 | (GCC)6 | 7A 7B, 7D | 62 |
| XGWM68 | (GA)3(G)3(GA)25 | 7B | 58 | WMS10 | (AT)5(GT)15 | 2A, 2B, 3A, 7A | 62 |
| XGWM146 | (GA)5GG(GA)20 | 7B | 59 | WMS43 | (CA)22 | 4A, 7B | 61 |
| XGWM156 | (GT)14 | 5A | 62 | WMS44 | (GA)28 | 4A, 7D | 56 |
| XGWM311 | (GA)29 | 2D | 61 | WMS46 | GA)2GC(GA)33 | 7B | 59 |
| XGWM374 | (CT)38 | 2B | 56 | XGWM292 | (CT)38 | 5D | 58 |
| XGWM674 | (CT)16CCC(GT)4 | 3A | 59 | XGWM66 | (CA)30(TA)21 | 4B | 59 |
| WMC58 | (GCC)6 | 3A, 7B, 7D | 59 | WMS126 | (CA)15 | 5A | 59 |
| WMC251 | (GT)17 | 4B | 56 | XGWM70 | (GT)7GC(GT)11 | 6B | 55 |
| WMC18 | (CA)(CT) | 2D | 57 | BARC59 | (TAGA)11 | 2D, 5B | 59 |
| WMC139 | (CA)12 | 7A | 62 | BARC3 | CCT)17 | 6A | 59 |
| XGWM129 | (GT)8(N)28(GT)16 | 2B | 62 | BARC100 | (TAA)19 | 5A | 62 |
| XGWM302 | (GA)21 | 7B | 61 | BARC88 | (TGA)9 | 5B | 59 |
| BARC119 | (CT)17 | 1A, 1D | 59 | CFA2043 | (GA)28 | 2A, 2B | 61 |
| XGWM337 | (CT)5(CACT)6(CA)43 | 1D | 60 | | | | |

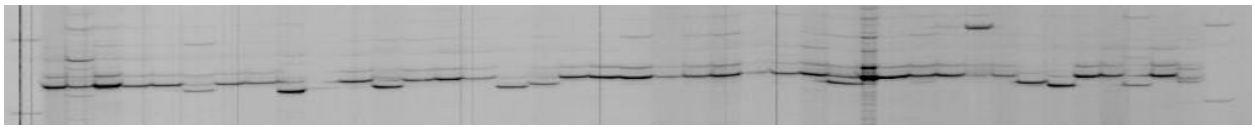

Figure 1. The banding pattern of the microsatellite marker WMS786 for a barley genotype.

Table 3. Transferability of SSR loci according to their assignment to wheat genome and homeologous groups.

| Chromosome | Marker% | Transferability% |
|---|---|---|
| 1A | 3.20 | 0.44 |
| 2A | 7.11 | 0.30 |
| 3A | 7.11 | 0.25 |
| 4A | 3.91 | 0.30 |
| 5A | 7.47 | 0.19 |
| 6A | 4.27 | 0.33 |
| 7A | 6.76 | 0.21 |
| 1B | 3.91 | 0.50 |
| 2B | 7.82 | 0.31 |
| 3B | 6.25 | 0.00 |
| 4B | 3.55 | 0.10 |
| 5B | 7.47 | 0.14 |
| 6B | 4.05 | 0.18 |
| 7B | 6.04 | 0.35 |
| 1D | 5.33 | 0.40 |
| 2D | 2.84 | 0.62 |
| 3D | 2.88 | 0.00 |
| 4D | 2.49 | 0.14 |
| 5D | 6.04 | 0.47 |
| 6D | 0.71 | 0.50 |
| 7D | 3.91 | 0.36 |

Table 4. Transferability of wheat SSR markers into barley genome according to the motif types.

| Motif categories | Primer | Transferability |
|---|---|---|
| Pure | 73% | 38.1% |
| Compound | 17.54% | 40% |
| Interrupted | 8.77% | 20% |

Table 5. Transferability of different motif types of wheat SSR markers into the barley genome.

| Repeated motifs | Di | Tri | Tetra | Penta | Hexa |
|---|---|---|---|---|---|
| Primer (%) | 85.16 | 10.98 | 3.3 | . | 0.55 |
| Transferability (%) | 30.34 | 45 | 50 | . | . |

In the present study, the number of polymorphic bands generated by the 21 wheat microsatellites was 51 with an average of 3.57 bands per primer (Table 6). The highest number of alleles was detected by the microsatellite marker GWM192 with 7 alleles, whereas the Xgwm583, WMS129, WMS46, WMC312, WMC3 and CFA2173 with only 2 alleles, had the lowest allele number. Naceur *et al*. (2012) reported an average of 2.13 alleles by characterizing 31 barley accessions originated from Algeria, Tunisia, Egypt with 11 SSR primer pairs. They indicated that the number

of alleles per primer pair is mainly affected by primer sequences, type of accession and protocol conditions. Observed heterozygosity ($H_o$) ranged from 0.00 to 0.13 with an average of 0.01. PIC values ranged from 0.19 (WMS46) to 0.70 (Xgwm304) with an average of 0.41. The average PIC (0.41) was less than what was obtained by Khodayari *et al*. (2012) (0.651), who analyzed 32 local barley varieties with 17 SSRs. The higher value observed by Khodayari *et al*. (2012) might have resulted from the use of different genotypes, primers and other differences between the two experiments. Mohammadvand Latifi *et al.* (2011) used 14 SSR markers for the identification and differentiation of 34 barley varieties. A total of 77 SSR polymorphic bands were observed overall genotypes. Polymorphic alleles ranged from 3 to 9 among the markers. The estimated highest and lowest PIC scores were 0.84 and 0.39 for Bmac0316 and Bmag0136 markers, respectively.

**Cluster analysis**

Cluster analysis using the Minimum Evolution algorithm and the coefficient of Number of Differences assigned the barley varieties into five groups (Figure 2). Group A comprised of accessions with spring growth type and adapted for the Mediterranean regions. In addition, most of the foreign varieties were included in this group. Group B contained accessions with the facultative growth type and adapted for cold origins. Most of the group C was the spring type and adapted for warm areas. Group D also contained varieties that were grown in the Mediterranean climate. Most of the varieties in group E had the facultative growth habit and are cultivated in warmer areas. However, some varieties with different adaptation areas or growth habits were clustered in the same group, which indicated that these genotypes had a similar genetic basis. Moreover, varieties with the same adaptation area or growth type were included in different groups, because these genotypes had mainly a wide genetic background.

Analysis of molecular variance for the cluster groups indicated that the majority of the total variance belonged to the within-group variance, which accounted for 86% of the total variation. Only 14% of the total variation was attributed to differences among groups (Table 7) indicating a low differentiation between groups.

The effective number of alleles (Ne), number of specific alleles, expected heterozygosity (He) and Shannon's information index (I) for each group are shown in Table 8. The group D (Ne = 3.200, He = 0.688, I = 1.255) showed the highest level of genetic diversity and the group E (Ne = 1.324, He = 0.245, I = 0.410) showed the lowest level of genetic diversity.

**Conclusions**

Our results indicated that barley has close synteny with wheat. SSR markers developed from wheat exhibited good transferability in barley and can be useful in the analysis of the genetic relationship of barley genotypes and also the comparative mapping between wheat and barley. Furthermore, the results show that the SSR loci from wheat can be useful in the genetic studies of other species within poaceae family.

Table 6. The polymorphism information content (PIC), amount of heterozygosity, gene diversity, allele No. and frequency of more common allele for SSR markers concerning barley.

| Marker | Allele No. | Gene diversity | Heterozygosity | PIC | Frequency of more common allele |
|---|---|---|---|---|---|
| BARC205 | 3 | 0.51 | 0.05 | 0.40 | 0.53 |
| BARC128 | 5 | 0.51 | 0.13 | 0.48 | 0.67 |
| Wmc256 | 4 | 0.49 | 0.02 | 0.44 | 0.67 |
| Gwm325 | 3 | 0.50 | 0.02 | 0.40 | 0.59 |
| Gwm642 | 5 | 0.66 | 0.00 | 0.61 | 0.50 |
| Gwm271 | 4 | 0.37 | 0.00 | 0.34 | 0.77 |
| Wmc786 | 3 | 0.35 | 0.00 | 0.32 | 0.78 |
| Gwm192 | 7 | 0.70 | 0.00 | 0.67 | 0.50 |
| Wmc405 | 3 | 0.35 | 0.00 | 0.30 | 0.77 |
| Gwm257 | 3 | 0.32 | 0.00 | 0.28 | 0.80 |
| Cfa2234 | 3 | 0.52 | 0.00 | 0.44 | 0.60 |
| BARC119 | 6 | 0.72 | 0.02 | 0.68 | 0.42 |
| BARC164 | 6 | 0.53 | 0.00 | 0.50 | 0.66 |
| CFA2173 | 2 | 0.50 | 0.00 | 0.37 | 0.50 |
| GWM232 | 3 | 0.32 | 0.00 | 0.28 | 0.80 |
| WMC3 | 2 | 0.47 | 0.00 | 0.36 | 0.61 |
| Wmc312 | 2 | 0.42 | 0.00 | 0.33 | 0.69 |
| WMS46 | 2 | 0.21 | 0.00 | 0.19 | 0.87 |
| WMS129 | 2 | 0.38 | 0.05 | 0.30 | 0.74 |
| Xgwm304 | 5 | 0.74 | 0.05 | 0.70 | 0.36 |
| Xgwm583 | 2 | 0.30 | 0.00 | 0.25 | 0.81 |
| Mean | 3.57 | 0.47 | 0.01 | 0.41 | 0.65 |

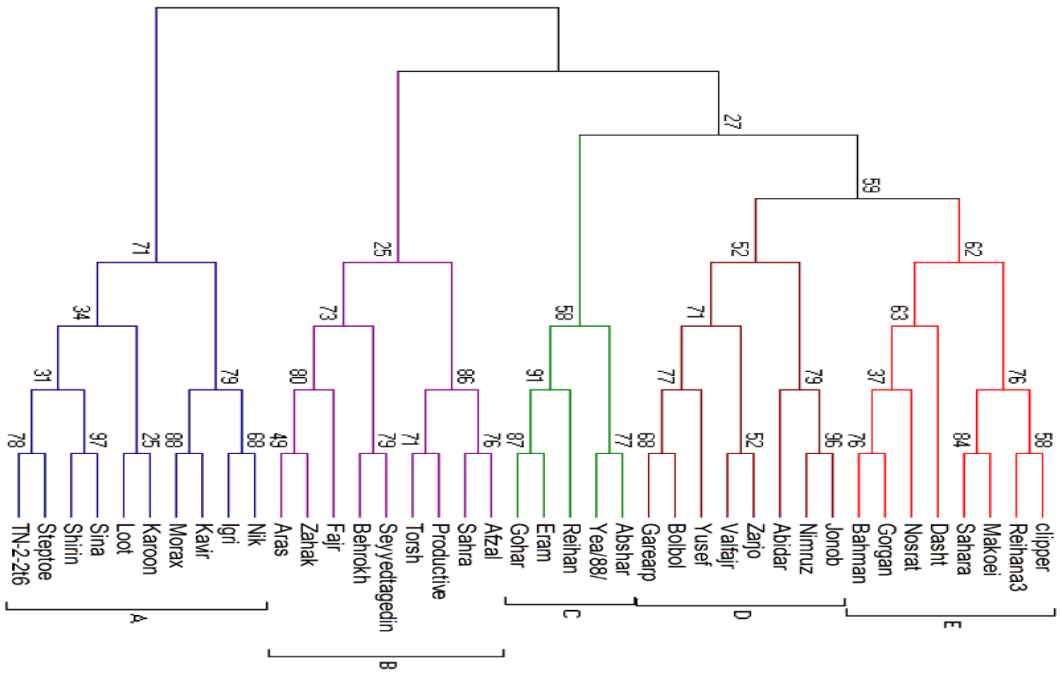


Figure 2. Grouping of barley genotypes based on SSR markers using Minimum Evolution algorithm and Number of Differences distance coefficient.

Table 7. Analysis of molecular variance based on SSR markers for 40 barley varieties from five different groups obtained from the cluster analysis.

| Source of variation | df | Sum of squares deviations | Estimates of variance components | Percentage of variation |
|---|---|---|---|---|
| Among groups | 4 | 69.253 | 17.313 | 14 |
| Within groups | 35 | 363.485 | 4.846 | 86 |

Table 8. The effective number of alleles (Ne), expected heterozygosity (He), Shannon's information index (I) and the number of specific alleles observed among five groups of barley varieties determined by the cluster analysis using SSR markers.

| Group | (Ne) | (He) | (I) | No. of specific alleles |
|---|---|---|---|---|
| A | 1.361 | 0.265 | 0.518 | 1.000 |
| B | 2.909 | 0.656 | 1.213 | 0.000 |
| C | 2.273 | 0.560 | 0.950 | 0.000 |
| D | 3.200 | 0.688 | 1.255 | 0.000 |
| E | 1.324 | 0.245 | 0.410 | 0.000 |

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**Conflict of Interest**

The authors declare that they have no conflict of interest with any people or organization concerning the subject of the manuscript.

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# انتقال پذیری نشانگرهای SSR گندم برای بررسی تنوع و روابط ژنتیکی ارقام جو

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**چکیده**

نشانگرهای SSR نشانگرهای هم بارز مبتنی بر PCR هستند که به دلیل ماهیت چندآللی و فراوانی بالای آللی، دارای چندشکلی بالا و حاوی اطلاعات زیادی می‌باشند و بنابراین کاربرد فروانی در میان نشانگرهای مولکولی دارند. در عین حال، توسعه نشانگرهای SSR بسیار پرهزینه و زمان‌بر می‌باشد. انتقال پذیری بین گونه‌ای نشانگرهای SSR امکان استفاده از نشانگرهای SSR طراحی شده برای یک گونه را در گونه‌های نزدیک فراهم می‌کند و بنابراین فراوانی استفاده از نشانگرهایی را که قبلا طراحی شده‌اند، افزایش می‌دهد. در این مطالعه انتقال‌پذیری و چندشکلی ۱۶۹ نشانگر SSR که برای گندم (*Triticum aestivum* L.) طراحی شده بودند، در ۴۰ ژنوتیپ جو (*Hordeum vulgare* L.) بررسی شد. از ۱۶۹ نشانگر، ۵۹ (۳۰,۱٪) آغازگر انتقال‌پذیری نشان دادند که از این ۵۹ جفت آغازگر، ۲۱ جفت چندشکل بودند (با متوسط ارزش PIC ۰,۱۹ تا ۰,۶۸). تعداد الل مشاهده شده در هر جایگاه بین ۱ تا ۷ الل و با متوسط ۳,۵۷ متغیر بود. تجزیه خوشه‌ای با استفاده از الگوریتم Minimum Evaluation و ضریب فاصله Number of Difference ژنوتیپ‌ها را در ۵ گروه مجزا قرار داد.

**واژه‌های کلیدی:** انتقال‌پذیری؛جو؛ گندم؛ SSR