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Association Analysis for morphological traits in *Triticum urartu* and *Triticum boeoticum* populations using IRAP and REMAP markers

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Abstract

The two einkorn wheats, *Triticum boeoticum* and *Triticum urartu,* that known as 'A' genome donor to cultivated wheat are the main elements of many wheat breeding programs. In order to analyze association of IRAP and REMAP markers with some morphological traits, 44 genotypes of *T. urartu* and 76 genotypes of *T. boeoticum* from west and north-west of Iran were used. Stepwise regression analysis between molecular data as predictors and each morphological traits data as dependent variables was performed to identify informative markers associated with the studied traits. The associated IRAP and REMAP markers explained %16-85 and %24-89 of the variation of each individual trait, respectively. The most explained variation belongs to traits days to heading and harvest index with association of 11 REMAP loci. Totally, 15out of 26 scorable IRAP combinations showed significant regression with 12 morphological traits. While, 28 out of 41 REMAP scorable combinations had significant regression with all 15 morphological traits. Totally, insertional genomic region of *NIKITA* and *LTR7286* retrotransposons indicated the highest association with most of the studied traits. Therefore, it's possible to use these retrotransposons along with morphological traits in wheat breeding programs to identify suitable parents to produce mapping populations and also Marker-assisted selection programs, after necessary validation.

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Introduction

Three species including *T. monococcum*, *T. boeoticum* and *T. urartu* belongs to the einkorn wheat group (Mizumoto *et al.*, 2002). Einkorn wheats are diploid and self-pollinated species (2n=2X=14), known as closely wild relatives of bread and durum wheats (Kilian *et al.*, 2007).*T. boeoticum* and especially *T. Urartu* are known as two main candidate species for "A" genome donor to bread and durum wheats (Eslami Farouji *et al.*, 2015).

West and north-west of Iran includes a large area of Fertile Crescent and is one of the important centers for wild wheat distribution (Kimber and Feldman, 1987). So, this area is potentially ideal for exploring suitable genes to further introgression into cultivated wheat (Van Slagren, 1994). Marker assisted selection is one of the main applications of molecular markers. Selection of plants with superior morphological traits is mainly objected in plant breeding programs. So, any technique helping the early selection of the favorite attributes can make the breeding period shorter and increase the efficiency of the selection (Gupta *et al.*, 1999). This point will be highlighted about some wild species that are trouble in field phenotyping such as *T. boeoticum* and *T. urartu*.

retrotransposon sequences are easily accessible from any plant genome, widely dispersed throughout the length of all chromosomes, commonly found in the euchromatic region and as molecular markers are highly informative (Kumar and Hirichika, 2001).

IRAP and REMAP are two retrotransposon based marker systems that used in a variety of applications, including measurement of genetic diversity and population structure, chromatin modification and epigenetic reprogramming, similarity and cladistic relationships, determination of essential derivation and marker-assisted selection (Kalendar*et al.*, 2011). Using linkage-based association analysis in wheat, a large number of genes for various traits have already been tagged (Gupta *et al.*, 1999). However, previous linkage-based studies usually allowed identification of genes at distance 10-30 CM from the closest markers which is hardly suitable either for marker-assisted breeding or for identification of functional genes. Furthermore, in linkage-based analysis, only few genotypes that are used as parents of mapping populations could be screened for marker trait associations, placing another limitation of linkage based analysis. In the recent past, association studies have been conducted which not only allow mapping of genes with higher level of confidence but also allow detection of genes, which would otherwise escape detection on linkage-based studies (Neale and Savolainen, 2004).

Multiple regression analysis based on association of a marker with the phenotype gives estimates and test of significance of the parameters of multiple linear regression equations. It also provides the coefficient to determination (R^2) which indicates the proportion of variability of a dependent variable that can be explained by a linear function of independent variables (Gomez and Gomez, 1984). Roy *et al.* (2006) reported association of the total 131 SSR, 43 SAMPL and 166 AFLP markers with at least one of the 14 agronomical studied traits in bread wheat via multiple regression analysis Associated SSR markers with flowering time were identified under different growing regimes in barley by means of regression approach (Ivandic *et al.*, 2002). Khaled *et al.* (2015) used regression analysis to identify linked ISSR and RAPD markers with yield traits in bread wheat under normal and drought conditions and showed that, *UBC-881* and *OPF-10* markers significantly regressed with traits. In the study of ISSR Marker-trait association analysis using regression in bread wheat highly significant associations were reported with the studied traits (Motawea *et al.*, 2015). Marsafari *et al.* (2014) used multiple regressions to identify RAPD, ISSR informative markers for traits in Iranian date palm and reported high association with morphological traits. Basaki *et al.* (2011) analyzed stepwise regression of morphological traits in pomegranate with microsatellite markers. Each of 14 traits showed significant association on with 14 of the

23 polymorphic SSR bands. Khaled and Hamam, (2015) reported significant regression for days to heading and spike length using 61 ISSR and 40 SRAP markers in bread wheat. The aim of this study was to identify informative IRAPs and REMAPs associated with morphological traits in *T. boeoticum* and *T. urartu* populations using multiple regression.

Materials and methods

Plant materials and field experiment

Plant materials of current study consisted of 14 populations (44 genotypes of five *T. urartu* populations and 76 genotypes of nine *T. boeoticum* populations) collected from west and north-west of Iran (Table1). In order to vernalize the materials, seeds were germinated during 4 days in 20 °C, then planted in plastic pots and kept in growing chamber for 4 weeks under moisture of %70 and 16 hours lighting condition. Genotypes were assessed during growing season of 2014 in a randomized complete block design with two replications at the research station of Faculty of Agriculture, University of Tabriz. The following traits were measured during growing season: flag leaf length, flag leaf width, plant height, main spike length, main spike weight, number of spikelet, number of seeds, seeds weight, total number of tiller, number of fertile tiller, total spikes weight, biomass weight, days to heading, seed yield and harvest index.

DNA extraction and PCR reaction

Genomic DNA was extracted from leaf samples using Lodhi *et al.* (1994) method. The quality and concentration of DNA were measured using spectrophotometry and %0.8 agarose gel electrophoresis. Eight single IRAP primer, 28 IRAP primer combinations and 88 REMAP combinations (combined from eight IRAP and 11 ISSR primers) were employed to study association between markers and traits (Table 2). PCR Amplification was performed in a total volume of 10 µl containing 1.65 µl of dH_2O, 4 µl of *Taq*-polymerase master mix, 1 µl of each primer and 0.35 µl $MgCl_2$. The amplification condition was conducted at 94°C for 4 min for initial denaturation step; followed by 38 cycles of three steps: denaturation for 1 min at 94 °C, annealing for 55s at respective temperature and extension for 2 min at 72 °C with a final extension for 7 min at 72 °C.PCR products were separated by %1.8 agarose gel electrophoresisin 1X TBE buffer with constant voltage of 130V for 2 hours. The amplified DNA fragments were visualized under UV light and photographed using a gel documentation system.

Statistical analysis

Clear and distinguishable amplified fragments were scored in binary system whereas 1 for presence and 0 for absence of bands. The Number of polymorphic loci, Polymorphic Information Content (PIC), expected heterozygosity (H_e), number of effective allels (N_e) and shannon's information index (I) were calculated using GenAlEx 6.4 (peakall and Smouse, 2006).Stepwise regression analysis between morphological traits as dependent and molecular markers as independent variables was performed using SPSS (version 16).

Results

Variation of morphological traits

Analysis of variance revealed significant differences among the accessions of *T. boeoticum* at %1 level of probability in all 15 traits, indicating the existence of high diversity in these *boeoticum* populations. While, ANOVA showed significant differences among genotypes in terms of all traits except days to heading in *T. urartu* accessions (Table 3).

Markers efficiency and polymorphism

26 out of 36 IRAP and 41 out of 88 REMAP combinations amplified polymorphic and scorable banding patterns. High percentages of polymorphism were detected by IRAP (%84.62) and REMAP (%75.53) markers. Analysis of molecular variance results showed significant phi for IRAP and REMAPs. Estimation of variance within population was higher than among populations (Table 4). IRAP combinations amplified a total of 170 loci in range of150-2500 bp.

Table 1. The geopositionof studied*T. urartu* and *T.boeoticum* populations.

Species	Code	Collectingregion	Longitude	Latitude
T.boeoticum	114	ghazvin	50-40-32	34-23-27
T.boeoticum	12	kermanshah	47-32-32	34-19-33
T.boeoticum	126	hamedan	48-8-38	33-11-27
T.boeoticum	10	kordestan	46-28-27	36-19-31
T.boeoticum	163	Chaharmahalbakhtiari	50-51-40	35-17-26
T.boeoticum	171	lorestan	48-13-32	30-44-25
T.boeoticum	257	Kermanshah	47-33-21	33-24-25
T.boeoticum	19	Kermanshah	46-57-26	33-32-19
T.boeoticum	3	West azerbaijan	45-45-28	36-39-20
T.urartu	154	Kohgiluyehboyerahmad	51-11-34	34-47-32
T.urartu	15	Kordestan	46-16-18	34-27-22
T.urartu	165	Kermanshah	47-22-25	33-31-28
T.urartu	162	Chaharmahalbakhtiari	50-51-29	33-53-30
T.urartu	486	kordestan	42-19-31	35-37-34

Table 2. Name and sequence of primers.

Code	Primer	Sequence	Primer	Sequence5'→3'
825	*ISSR1*	$(AC)_8T$	*Sukkula*	GATAGGGTCGCATCTTGGGCGTGAC
826	*ISSR2*	$(AC)_8C$	*Nikita*	CGCATTTGTTCAAGCCTAAACC
827	*ISSR3*	$(AC)_8G$	*LTR6149*	CTCGCTCGCCCACTACATCAACCGCGTTTATT
830	*ISSR4*	$(TG)_8G$	*3'LTR*	TGTTTCCCATGCGACGTTCCCCAACA
834	*ISSR5*	$(AG)_8yT$	*LTR6150*	CTGGTTCGGCCCATGTCTATGTATCCACACATGTA
835	*ISSR6*	$(AG)_8yC$	*5'LTR1*	TTGCCTCTAGGGCATATTTCCAACA
840	*ISSR7*	$(GA)_8yT$	*5'LTR2*	ATCATTCCCTCTAGGGCATAATTC
841	*ISSR8*	$(GA)_8yC$	*LTR7286*	GGAATTCATAGCATGGATAATAAACGATTATC
845	*ISSR9*	$(CT)_8rG$		
847	*ISSR10*	$(CA)_8rC$		
848	*ISSR11*	$(CA)_8rG$		

The combinations resulted from seven IRAP primers produced on average 6.53 bands per individual. Maximum and minimum PIC values about IRAP loci resulted from *5'LTR1-LTR6150* (0.481) and *5'LTR2-LTR6149* (0.298) IRAP combinations, respectively (Table 5). In REMAP analysis, 41 combinations amplified 214 loci in range of 150-2500 bp. *ISSR827-5'LTR1* (0.495) and *ISSR825-LTR7286* (0.317) showed the maximum and minimum PIC values, respectively among REMAP loci. Also, the highest and lowest values for H_e was detected respectively for *sukkula-3'LTR*and*5'LTR2-LTR6149*IRAP, *ISSR848-5'LTR2* and *ISSR827-LTR7286*REMAP combinations (Table 5).

Table 3. Analysis of variance for morphological traits.

	SV	Number of spikelet	Number of seeds	Seeds weight	Number of fertile tiller	Days to heading	Harvest index
T.boeoticum	Repeat	9.416^{ns}	97.815^{ns}	0.006^{ns}	29.384^{ns}	1.089^{ns}	35.526^{*}
	Genotype	23.919^{**}	139.458^{**}	0.036^{**}	87.523^{**}	76.773^{**}	19.345^{**}
	Between	70.061^{**}	128.523^{ns}	0.0363^{ns}	215.903^{**}	253.859^{**}	46.384^{**}
	Within	17.412	140.99	0.0366	69.417	51.799	15.532
	Error	6.249	62.983	0.014	27.238	13.943	5.253
T.urartu	Repeat	28.125	236.531	0.012	0.001	0.003	0.005
	Genotype	36.436^{**}	171.96^{**}	0.051^{**}	1.136^{**}	0.227^{ns}	0.019^{*}
	Between	148.530^{**}	175.835	0.050	1.321	0.273	0.037^{*}
	Within	22.424	171.475	0.051	1.112	0.221	0.017
	Error	9.871	62.404	0.018	0.607	0.278	0.013

*, ** and ns Significant at %5 and %1 probability levels and not significant respectively.

Table 3. Continued.

	SV	Flag leaf width	Flag leaf length	Plant height	Yield	Main spike length	Biomass	Total spikes weight	Total number of tiller	Main spike weight
T.boeoticum	Repeat	2.317*	17.175**	90.325ns	2.635ns	2.016ns	18.237ns	25.105ns	46.283ns	0.049ns
	Genotype	2.723**	16.196**	327.842**	7.310**	8.030**	284.296**	69.630**	119.578**	0.230**
	Between	10.675**	89.804**	384.984*	15.826**	29.683**	716.741**	150.759**	278.926**	0.449**
	Within	1.602	5.815	319.783	6.108	4.976	223.309	58.189	97.106	0.198
	Error	0.564	2.146	102.192	2.010	1.646	69.760	19.149	35.865	0.063
T.urartu	Repeat	0.001	4.368	66.427	0.004	1.234	12.734	0.139	9.031	1.017*
	Genotype	3.489**	13.769**	349.581**	3.154**	7.052**	383.708**	81.3**	102.742*	0.345**
	Between	22.055**	67.715**	1486.044**	624.867	30.346**	3.59*	101.089	97.968	0.348
	Within	1.168	7.026	207.523	3.056	4.140	353.563	78.826	103.338	0.345
	Error	0.97	3.587	139.442	1.312	3.19	172.418	35.576	57.095	0.175

* , ** and ns Significant at %5 and %1 probability levels and not significant respectively.

Association between IRAP markers and morphological traits

Totally, 25 out of all 170 polymorphic IRAP loci were associated with all 15 morphological traits (table 6). Biomass showed significant regression with only one marker, days to heading with eight markers, harvest index with five markers, each of the traits flag leaf length, seeds weight, total spikes weight and yield with two markers, traits number of fertile tiller, plant height, main spike length, flag leaf width and main spikes weight with four markers.

Table 5. The polymorphism information content (PIC) value and expected heterozygosity (H_e) of IRAPs and REMAPs.

Primer combination	H_e	PIC	Primer	H_e	PIC
3'LTR	0.298	0.381	*ISSR826-5'LTR2*	0.259	0.478
LTR 6150	0.407	0.444	*ISSR826-LTR6149*	0.288	0.428
SUKKULA	0.342	0.379	*ISSR826-NIKITA*	0.272	0.365
NIKITA	0.364	0.419	*ISSR826-LTR6150*	0.353	0.463
5'LTR1	0.362	0.449	*ISSR826-3'LTR*	0.344	0.447
LTR 6149	0.312	0.379	*ISSR841-3'LTR*	0.299	0.456
5'LTR2	0.331	0.393	*ISSR847-3'LTR*	0.242	0.438
3'LTR - LTR 6149	0.392	0.476	*ISSR826-LTR7286*	0.313	0.439
3'LTR- NIKITA	0.341	0.431	*ISSR835-SUKKULA*	0.282	0.416
SUKKULA - LTR6150	0.299	0.370	*ISSR835-NIKITA*	0.245	0.410
SUKKULA-3'LTR	0.430	0.452	*ISSR847-SUKKULA*	0.323	0.444
SUKKULA-5'LTR2	0.312	0.354	*ISSR840-SUKKULA*	0.347	0.492
5'LTR2-LTR6149	0.209	0.298	*ISSR840-LTR6149*	0.212	0.458
5'LTR2-NIKITA	0.315	0.408	*ISSR840-NIKITA*	0.229	0.426
5'LTR2-3'LTR	0.265	0.420	*ISSR840-3'LTR*	0.308	0.493
5'LTR2-LTR6150	0.316	0.431	*ISSR848-SUKKULA*	0.346	0.489
5'LTR1- LTR6149	0.379	0.464	*ISSR848-5'LTR2*	0.378	0.431
5'LTR1-NIKITA	0.320	0.467	*ISSR848-5'LTR1*	0.358	0.491
5'LTR1-SUKKULA	0.352	0.480	*ISSR834-NIKITA*	0.278	0.400
5'LTR1-LTR6150	0.341	0.481	*ISSR827-SUKKULA*	0.305	0.401
5'LTR1-3'LTR	0.244	0.362	*ISSR827-NIKITA*	0.361	0.474
LTR 6150- NIKITA	0.356	0.439	*ISSR827- 5'LTR1*	0.339	0.495
LTR 6150- LTR6149	0.382	0.454	*ISSR840-5'LTR1*	0.374	0.481
NIKITA-SUKKULA	0.245	0.449	*ISSR841-SUKKULA*	0.341	0.446
SUKKULA-LTR6149	0.351	0.443	*ISSR841-LTR7286*	0.278	0.456
3'LTR- LTR6150	0.367	0.470	*ISSR847-LTR7286*	0.337	0.470
REMAPs			*ISSR835-LTR7286*	0.263	0.428
ISSR845-5'LTR1	0.282	0.465	*ISSR835-3'LTR*	0.315	0.443
ISSR835-5'LTR2	0.309	0.448	*ISSR834-SUKKULA*	0.311	0.417
ISSR825-SUKKULA	0.286	0.446	*ISSR834-LTR7286*	0.307	0.470
ISSR825-LTR6149	0.317	0.450	*ISSR827-LTR7286*	0.175	0.395
ISSR825-3'LTR	0.242	0.425	*ISSR845-3'LTR*	0.349	0.487
ISSR825-LTR7286	0.249	0.317	*ISSR848-LTR6150*	0.263	0.425
ISSR847-5'LTR1	0.335	0.435			
ISSR826-SUKKULA	0.225	0.402			

Traits number of spikelet and total number of tiller didn't show any association with IRAP markers. The highest number of informative bands was identified for days to heading with 8 IRAP markers (adjusted R^2=0.855) and the lowest IRAP associated marker was *3'LTR (e)* with biomass (adjusted R^2= 0.163).

Association between REMAP markers and morphological traits

Totally, 60 out of all 214 polymorphic REMAP loci were associated with all 15 morphological traits (Table 7).

Table 6. Results of Stepwise regression of IRAP markers onmorphological traits.

Trait	Informative marker	Adjusted R^2	Standardized regression coefficient
Biomass	*3'LTR (e)*	0.163	0.426
Days to heading	*5'LTR2-3'LTR(g)*	0.855	-0.478
	5'LTR2-LTR6149(e)		-0.251
	5'LTR1-LTR6150(a)		0.484
	5'LTR1(a)		-0.242
	5'LTR2-LTR6149(c)		-0.345
	5'LTR1-SUKKULA(b)		-0.169
	5'LTR2-3'LTR(b)		-0.196
	5'LTR2-LTR6149(b)		-0.172
Harvest index	*3'LTR- NIKITA(c)*	0.686	0.349
	5'LTR1-NIKITA(i)		0.342
	5'LTR1-SUKKULA(b)		-0.305
	5'LTR2-LTR6149(c)		-0.280
	SUKKULA(g)		-0.252
Flag leaf length	*LTR 6150- NIKITA(e)*	0.335	-0.441
	5'LTR2-3'LTR(e)		0.386
Flag leaf width	*NIKITA-SUKKULA(e)*	0.583	-0.411
	LTR 6150- NIKITA(e)		-0.355
	3' LTR - LTR 6149(c)		-0.319
	5'LTR1(f)		0.302
Main spike weight	*5'LTR1(e)*	0.556	0.378
	5'LTR2-NIKITA(d)		0.384
	5'LTR1-NIKITA(g)		-0.468
	5'LTR1-LTR6150(a)		-0.357
Fertile tiller number	*3'LTR(b)*	0.422	-0.422
	5'LTR1-NIKITA(a)		-0.369
	5'LTR2-3'LTR(g)		-0.312
Plant height	*NIKITA-SUKKULA(e)*	0.428	-0.544
	5'LTR2-NIKITA(h)		0.422
	5'LTR1-LTR6150(a)		-0.373
Main spike length	*5'LTR2-NIKITA(d)*	0.467	0.264
	NIKITA-SUKKULA(e)		-0.499
	5'LTR1-NIKITA(h)		0.434
Seeds weight	*5'LTR2-NIKITA(d)*	0.276	0.427
	5'LTR2-LTR6150(d)		0.363
Total spikes weight	*SUKKULA(g)*	0.318	0.459
	5' LTR2(c)		0.379
Yield	*SUKKULA(g)*	0.264	0.420
	5' LTR2(c)		0.354

The associated markers each explained %24.2 (number of spikelet) to %89.7 (days to heading) of the total available variation for each morphological traits. The variation of days to heading (%89.7) was determined by *ISSR835-5'LTR2(b), ISSR835-5'LTR2(c), ISSR835-NIKITA(e), ISSR825-3'LTR(a), ISSR826-NIKITA(c), ISSR840-LTR6149(a), ISSR826-5'LTR2(a), ISSR825-LTR7286(i), ISSR841-LTR7286(g), ISSR840-NIKITA(c)* and *ISSR845-5'LTR1(e)*.The locus *ISSR826-SUKKULA(c)* was linked with most traits such as: biomass, number of fertile tiller, total number of tiller, total spikes weight and yield. Some other REMAP markers such as, *ISSR827-NIKITA(b), ISSR834-SUKKULA(a)* and *ISSR826-SUKKULA(c)* were jointly associated with traits biomass, total spikes weight and yield. *ISSR825-3'LTR(a), ISSR840-LTR6149(a)* and *ISSR845-5'LTR1(e)* were jointly associated with days

to heading and harvest index and*ISSR847-3'LTR(b)* plus *ISSR825-3'LTR(c)* with main spikes weight and seeds weight. The reason for existing same associating markers for different traits could bepleiotropy effect or linkage of controller loci of these traits. The number of REMAP markers associated with each morphological trait was ranged in 2-11 markers (Table 7).

Table 7. Results of Stepwise regression of REMAP markers on morphological traits.

Trait	Informative marker	Adjusted R^2	Standardized regression coefficient
Biomass	*ISSR827-NIKITA(b)*	0.461	-0.431
	ISSR841-3'LTR(a)		0.303
	ISSR834-SUKKULA(a)		-0.307
	ISSR847-5'LTR1(a)		0.240
	ISSR826-SUKKULA(c)		0.220
Days to heading	*ISSR835-5'LTR2(b)*	0.897	-0.186
	ISSR835-5'LTR2(c)		-0.232
	ISSR835-NIKITA(e)		0.244
	ISSR825-3'LTR(a)		0.208
	ISSR826-NIKITA(c)		-0.195
	ISSR840-LTR6149(a)		0.194
	ISSR826-5'LTR2(a)		-0.270
	ISSR825-LTR7286(i)		-0.221
	ISSR841-LTR7286(g)		-0.140
	ISSR840-NIKITA(c)		-0.143
	ISSR845-5'LTR1(e)		-0.116
Harvest index	*ISSR845-5'LTR1(c)*	0.858	-0.222
	ISSR845-5'LTR1(e)		-0.227
	ISSR825-3'LTR(a)		0.119
	ISSR847-5LTR1(c)		-0.168
	ISSR847-3'LTR(e)		-0.230
	ISSR840-LTR6149(a)		0.229
	ISSR826-SUKKULA(b)		0.276
	ISSR834-NIKITA(b)		-0.171
	ISSR840-LTR6149(b)		-0.203
	ISSR826-NIKITA(e)		-0.134
	ISSR835-5'LTR2(a)		-0.136
Flag leaf length	*ISSR847-5'LTR1(e)*	0.562	-0.299
	ISSR847-5'LTR1(b)		0.319
	ISSR827-LTR7286(b)		-0.217
	ISSR825-LTR7286(h)		0.284
	ISSR827-SUKKULA(a)		-0.245
	ISSR825-LTR7286(f)		-0.226
Flag leaf width	*ISSR835-LTR7286(h)*	0.477	-0.385
	ISSR827-LTR7286(a)		0.393
	ISSR841-LTR7286(a)		0.296
	ISSR840-5'LTR1(c)		0.234
	ISSR826-3'LTR(e)		0.218
Main spike weight	*ISSR847-3'LTR(b)*	0.546	0.414
	ISSR825-3'LTR(c)		-0.423
	ISSR845-5'LTR1(a)		0.343
	ISSR827-LTR7286(c)		0.371
	ISSR840-NIKITA(c)		0.238
	ISSR847-5'LTR1(a)		0.230
Fertile tiller number	*ISSR835-5'LTR2(a)*	0.606	-0.389
	ISSR841-LTR7286(e)		0.198
	ISSR834-SUKKULA(c)		-0.270
	ISSR834-NIKITA(b)		-0.348
	ISSR845-5'LTR1(e)		-0.238
	ISSR826-SUKKULA(c)		0.216
	ISSR826-LTR6150(b)		-0.189
Plant height	*ISSR826-LTR7286(f)*	0.335	0.425
	ISSR825-LTR6149(e)		0.387
	ISSR826-LTR7286(e)		-0.416
	ISSR826-LTR6150(d)		-0.265

Table 7. Continued.

Trait	Informative marker	Adjusted R^2	Standardized regression coefficient
Seeds number	*ISSR834-LTR7286(f)*	0.299	0.407
	ISSR848-LTR6150(c)		0.344
	ISSR835-LTR7286(h)		-0.333
	ISSR848-LTR6150(a)		-0.270
Main spike length	*ISSR848-LTR6150(c)*	0.317	0.462
	ISSR826-NIKITA(d)		0.326
	ISSR827-SUKKULA(d)		-0.314
Number of spikelet	*ISSR826-LTR7286(f)*	0.242	0.428
	ISSR834-LTR7286(f)		0.296
Seeds weight	*ISSR847-3'LTR(b)*	0.255	0.315
	ISSR825-3'LTR(c)		-0.363
	ISSR834-SUKKULA(a)		-0.277
Total tiller number	*ISSR834-NIKITA(b)*	0.329	-0.472
	ISSR847-5'LTR1(a)		0.228
	ISSR826-SUKKULA(c)		0.300
	ISSR835-5'LTR2(b)		0.261
Total spikes weight	*ISSR841-3'LTR(a)*	0.652	0.291
	ISSR827-NIKITA(b)		-0.332
	ISSR834-SUKKULA(a)		-0.251
	ISSR825-LTR6149(c)		0.279
	ISSR826-SUKKULA(c)		0.411
	ISSR847-SUKKULA(a)		-0.325
	ISSR847-LTR7286(f)		0.228
	ISSR841-3'LTR(c)		0.233
	ISSR826-NIKITA(c)		0.239
	ISSR826-LTR6150(e)		-0.205
Yield	*ISSR834-SUKKULA(a)*	0.496	-0.294
	ISSR827-NIKITA(b)		-0.340
	ISSR847-SUKKULA(a)		-0.392
	ISSR826-SUKKULA(c)		0.321
	ISSR826-SUKKULA(b)		-0.276
	ISSR826-5'LTR2(b)		-0.239

Among the studied 15 traits, days to heading and harvest index showed significant regression with 11 marker while, number of spikelet regressed with 2 markers.

Discussion

High percentages of polymorphism detected by IRAPs and REMAPs indicated the activity of these retrotransposon elements in *T. urartu* and *T. boeoticum* speicies. Also high level of diversity observed for morphological traits in both *T.urartu* and *T. boeoticum* populations. High PIC values were observed in this research, mean of PIC for IRAP and REMAPs were 0.38 and 0.40 respectively. This outcome confirmed a high efficiency of these primer combinations. So, they can be used to distinguish closely related species and genetic diversity. Fathi *et al.* (2014) reported the good PIC values from 0.32 to 0.44 for IRAPs in *Aegilops triuncialis*. IRAP primer combinations amplified 170 loci at total and produced on average 6.53 bands for each individual. This value was reported approximately 8 in *Aegilops tauschi* using barley retrotransposons (Saedi *et al.*, 2008). According to analysis of molecular variance (AMOVA), within population variance was higher than among population, in both IRAP and REMAP analysis. It was because of high variation within the populations of these wild species that reported in some other related researches, too. In the study of gene diversity in *T. urartu* and *T.boeoticum* of Iran by means of IRAP markers, %84 of total variation attributed to within population and %16 was ascribed to the among population differences (Eslami Farouji *et al.*, 2015).

Totally,15 out of 26 scorable IRAP combinations showed significant regression with 12 morphological traits. While, 28 out of 41 REMAP scorable combinations had significant regression with all 15 morphological traits. Among the 170 IRAP and 214 REMAP polymorphic loci, respectively 25 and 60 loci showed association with morphological traits. Based on multivariate regression analysis, IRAP and REMAP markers respectively explained %16-85 and %24-89 of the total variation of each individual morphologic trait. These percent of explanation seems reliable comparing with other similar researches. Khaled *et al.* (2015) showed that ISSR and RAPD markers explained a maximum regression (%18.29- 34.95) of the total available variation for individual associated traits. The present results showed that the IRAP combinations *5'LTR1-LTR6150(a), SUKKULA* and *NIKITA-SUKKULA(e)* associated with the most number of traits. Therefore, these retrotransposon combinations had correlation with most of the studied traits. These combinations had also the highest number of polymorphic bands respectively (6, 9 and 9).Two IRAP combinations *5'LTR2-LTR6149(c)* and *5'LTR1-SUKKULA(b)* had significant association with both traits days to heading and harvest index. This could be because of pleiotropy effects or linkage in this marker loci.

Totally *NIKITA* retrotransposon in IRAP and *LTR7286* in REMAPs had the highest associations with most of the traits in this study. Also *NIKITA* produces the highest polymorphic bands (15), and it shows high copy number and important role in evolution of wheat genome. *LTR7286* amplified high number of bands in REMAPs with *ISSR826*, *ISSR841*, *ISSR 847*, *ISSR 835*, *ISSR834* and *ISSR 827*.This may indicates the close insertion of this element near some microsatellite sequences.

According to obtained results of IRAP and REMAP markers, retrotransposons *NIKITA*, *5'LTR1* and *5'LTR2* in IRAP *and LTR7286, SUKKULA, 3'LTR* and *NIKITA* in REMAPs, showed significant associations with more traits; therefore it is possible to validate these markers and use along in future breeding and MAS programs in wheat.

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