RESEARCH ARTICLE

Analyzing Sunn pest resistance in bread wheat genotypes using phenotypic characteristics and molecular markers

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Abstract Sunn pest is one of the most destructive insects in the western and central parts of Asia causing severe reductions of wheat yield and flour quality. Therefore, an effort was undertaken to find effective resistance by analyzing genetic variation among 25 wheat genotypes artificially infested in field-cages as well as using start codon targeted (SCoT) polymorphism and inter- retrotransposon amplified polymorphism (IRAP) markers. High variation was revealed amongst genotypes with Sunn pest resistance characteristics including Bayat, Bezostaya, Sayson, Line93, Line120, Rashagol, Golsepi and AarasGolsoor, which were classified as resistant to moderately resistant. SCoTs and IRAPs were determined as efficient markers for studying genetic diversity. The non-parametric Kruskal–Wallis test was conducted to evaluate the effect of specific SCoT and IRAP amplicons on Sunn pest resistance characteristics for wheat genotypes. The stepwise regression analysis exhibited seven informative SCoTs and IRAPs explaining the highest resistance characteristics variation ranging from 25.7–50.1 to 17.6–40.1, respectively. The relationship between resistance of genotypes and informative SCoT and IRAP amplicons was found based on canonical discriminant analysis showing the capacity of informative markers for functional marker selection method and screening the wheat germplasms for Sunn pest resistance characteristics.

Keywords Bread wheat · IRAP · Informative markers · SCoT · Sunn pest · Stepwise regression

Introduction

Bread wheat (*Triticum aestivum* L.) is the most important and strategic crop in Iran with a total production of 10,600,000 tonnes from approximately 7,300,000 hectares (FAOSTAT 2014). Sunn pest (*Eurygaster integriceps* Puton, 1881) is the most notable pest of wheat and barley in West and Central Asia, Eastern Europe and North Africa (Kinaci and Kinaci 2004; El Bouhssini et al. 2013). Sunn pests consume various structures of the host cereal plants such as leaves, stems and grains creating various degrees of damage (El Bouhssini et al. 2009; Alizadeh et al. 2010) even leading to 100% decreases in yield under severe Sunn pest infestation in some areas (Fatehi et al. 2009; Alizadeh et al. 2010). The pest insect injects digestive enzymes (prolyl endo-protease) into the grains causing degradation of gluten and thus drastically reducing flour baking quality and economic value of harvested wheat grains (Hariri et al. 2000; El Bouhssini et al. 2009). The quality of bread wheat flour decreases with even as little as 2–3% damage to grains (Hariri et al. 2000; El Bouhssini et al. 2009; Duric et al. 2014). The chemical control of Sunn pest is costly, hazardous for humans and the environment and has detrimental side effects for predator insects. The use of Sunn pest resistant wheat genotypes has the potential to reduce crop losses (Krupnov 2012). Therefore, the best sustainable, safe and cost effective method for controlling this insect pest is plant resistance (El Bouhssini et al. 2009; Hossaini et al. 2009; El Bouhssini et al. 2013). Host plant resistance is one of the important components of integrated pest management (IPM) programs for controlling of the

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Sunn pest and has been used by ICARDA (El Bouhssini et al. 2007). Nevertheless, a low number of resistance sources have been identified for Sunn pest in wheat and its wild relatives (El Bouhssini et al. 2013). Identification and deployment of different resistance genes derived from diverse sources can lead to the prevention of the development of new biotypes (Joukhadar et al. 2013) as well as the possible transfer of these genes to agriculturally important wheat cultivars (Myburg et al. 1998).

DNA markers can be used for marker assisting selection (MAS) of pest resistance as well as pyramiding of several resistance genes into a superior cultivar (Myburg et al. 1998; Dewikat et al. 2002). Although the resistance to Sunn pest has been evaluated in wheat genotypes (El Bouhssini et al. 2007; Kinaci and Kinaci 2007; El Bouhssini et al. 2013; Joukhadar et al. 2013), however, very little information (Emebiri et al. 2017) is available regarding genes conferring Sunn pest resistance in wheat. The objective of our study was (a) the evaluation of phenotypic characteristics related to Sunn pest resistance in wheat genotypes (b) assessment of genetic variation of wheat genotypes using start codon targeted (SCoT) and inter-retrotransposon amplified polymorphism (IRAP) markers, and (c) analysis of genetic diversity in association with Sunn pest resistance to obtain informative markers for future studies.

Materials and methods

Plant materials

Genetic diversity of 17 popular bread wheat genotypes in Iran as well as three landraces from Kurdistan and five artificial lines (Table 1) was assessed using start codon targeted (SCoT) polymorphism and inter-retrotransposon amplified polymorphism (IRAP) markers. In addition, their responses to Sunn pest (*E. integriceps*) infestation was evaluated at the research farm of Kurdistan University (Latitude: 35.26N Longitude: 47.03E and Altitude: 1413 m). The experiment was laid out using a randomized complete block design (RCBD) with three replications. Each genotype was planted in separate plots comprised of four rows, spaced 15 cm apart.

Sunn pest resistance evaluation

Wheat genotypes were artificially screened against Sunn pest in 2015 in metal cages covered by a mesh screen (0.3×1 m) established on the each plot. The overwintered pest insects (local widespread biotype) were collected from wheat farms in the Sanandaj County ($14^{\circ}35'N$, $46^{\circ}E$ and the height 1450–1538 m) of Kurdistan Province, Iran. The

overwintered adult male and female insects were weighed stringently before releasing them into the cages. Shortly thereafter, two pairs of female and male insects were released into each cage at the head initiation stage of wheat genotypes around mid-April. Three weeks after infestation, the insects from each plot were collected and weighed again. Based on the following formula, the weight gain of each female (AFW) and male (AMW) insect was calculated:

Weight gain = $(W_2 - W_1)/W_2$ where W_1 and W_2 are weight of each insect before and after feeding in milligram.

After mating and egg laying of adult insects, the leaves containing eggs were collected from each plot and the number of eggs counted on each genotype (EL) per plot. The eggs were reared in a germinator (at $25^{\circ}C$ and 65% humidity) until the appearance of third instars. The third instars were weighed and then each plot was infested with five instars. The nymphs fed on wheat grains for 30 days. After that, weight gain of nymphs (WGN) was measured based on the following formula: Weight gain = $(W_2 - W_1)/W_2$ here W_1 and W_2 are weight of nymphs before and after feeding in milligram.

In addition, the percentage of nymph mortality (PNM) was calculated for each plot as an index of resistance. The spikes were collected and threshed separately at the maturing stage concurrently with the appearance of a new generation of adult insects in each cage. The pierced grains were used for calculating the percentage of damaged seeds (PDS) and damaged grains weight (DGW). Variance components were calculated based on ANOVA results and accordingly, broad sense heritability (H^2) was estimated for studied phenotypic traits using the following formula:

$$H^2 = \frac{\sigma_g^2}{\sigma_p^2} = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_e^2}{R}}$$

were σ_g^2 , σ_e^2 and R respectively representing the variances of genotypes, error and number of replications.

DNA extraction

All wheat genotypes were planted in the greenhouse and young healthy leaves were collected in liquid nitrogen for DNA extraction using the CTAB method (Saghai-Maaroof et al. 1984). The quality and quantity of extracted DNA were verified by an 0.8% agarose gel and a NanoDrop 1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA), respectively. Isolated DNA was diluted to 10 ng/ μ l for SCoT and IRAP markers analysis.

SCoT and IRAP analysis

Seventeen SCoT markers (developed by Collard and Machill 2009 and Luo et al. 2010) and six IRAP markers

Table 1 Studied wheat genotypes, pedigree, origin and their growth habit

Codes	Genotypes	Pedigree	Origin	Growth habit
1	Ghods	Rsh/5/Wu/4/N0r10/K54*2//Fn/3/Ptr/6/Omid//Kal/Bb	Iran (Karaj)	Spring
2	Arvand	Rsh//Mt-Ky/My48	Iran (Ahvaz)	Facultative
3	Zagros	Tan'S'/Vee'S'//Opata85	ICARDA	Spring
4	Roshan	Landrace	Iran (Esfahan)	Spring
5	Line 7	Wheat/Tritipyrum	Iran	–
6	Cross-Shahi	Landrace	Iran	Winter
7	Shoaleh	Landrace	Iraq	Spring
8	Golsepi	Landrace	Iraq (Kurdistan)	–
9	Bezostaya	LUTESCENS17//SKOROSPELKA2	Russia	Winter
10	Line 102	Wheat/Tritipyrum	Iran	–
11	Pishgam	90-Zhong87/Bkt	Iran (Karaj)	Facultative
12	Azar 2	Kvs/ym71/3/May'S'//Bb/Inia/4/Sefid	Iran (Khoy)	Winter
13	Alvand	1-27-6275/CF 1770	Iran (Karaj)	Facultative
14	Line 10	Wheat /Tritipyrum	Iran	–
15	Sardari	Landrace	Iran (Kurdistan)	Winter
16	Aaras Golsoor	Landrace	Iraq (Kurdistan)	–
17	Kave	FTA/PL	CYMMIT	Spring
18	Rashagol	Landrace	Iraq (Kurdistan)	–
19	Bayat	PUNJAB-76/CHENAB-70	Iran (Darab)	Spring
20	Line 120	Wheat/Tritipyrum	Iran	–
21	Alamoot	Pi/Ndr//Hys/3/Inza/5/Kj2/4/Bb/Inia//''3Maya'S/Kvs/Ti	Iran (Karaj)	Winter
22	Line 93	Wheat/Tritipyrum	Iran	–
23	MV17	–	Hungary	Winter
24	Sayson	–	–	–
25	Azadi	Mexp//4820/1-32-15409	Iran (Karaj)	Winter

(Kalendar et al. 1999; Alavi Kia et al. 2008) were used in this study (Table 2). The PCR reaction was performed in a final volume of 10 μ L comprising of 4.7 μ L ddH₂O, 2 μ L of genomic DNA as template, 0.30 μ L dNTPs (10 mM), 0.7 μ L MgCl₂ (50 mM), 1 μ L 10 $\times$ PCR buffer (1 mM Tris-HCl, pH 8.8 at 25°C, 50 mM KCl and 0.1% Triton X-100), 0.1 μ L DNA Taq polymerase (10 U 1 μ L⁻¹), and 1.2 μ L of each primer (5 pmol μ L⁻¹). The PCR reaction was conducted according to the following program: 94°C for 3 min followed by 35 cycles of 94°C for 1 min, annealing temperature (48–59°C) for 1 min and 72°C for 2 min, and final extension at 72°C for 5 min. Fragments were separated using 1.3% agarose gel. The reproducibility of the amplicons was tested in three samples.

Data analysis

For both SCoT and IRAP markers, each sharp and prominent amplified fragment was scored in terms of a binary code as a presence (1) and absence (0). Genetic diversity parameters including Shannon's information

index (I), expected heterozygosity (He), number of amplicons and percentage of polymorphic fragments were estimated using GenAlEx 6.5 (Peakall and Smouse 2012). Genetic similarity matrices based on Dice and Jaccard similarity coefficients were calculated using NTSYS 2.1. Neighbor-joining dendrograms and bootstrap analysis were conducted by DARwin 6 software. Mantel test were performed using XLSTAT. ANOVA and correlation for traits related to resistance to Sunn pest were undertaken using SPSS 18 statistical software. Nonparametric Kruskal–Wallis analysis and stepwise regression were carried out to study the associations amongst SCoT, IRAP markers and resistance parameters. Stepwise regression was used to identify markers explaining the highest phenotypic value. Functional grouping of resistant and susceptible genotypes was tested through canonical discriminant analysis, which was performed based on genetic distance between genotypes from SCoTs (Dice) and IRAPs (Jaccard) linked to the seven resistance characteristics.

Table 2 Primer sequences and PCR condition for the amplification of 25 wheat genotypes

Marker name	Primer sequences (5′–3′)	Ta (°C)	Alleles size range (bp)
<i>SCoT</i> markers			
SCoT ₁	CAACA <u>ATGG</u> GCTACCACCA	48	800–2000
SCoT ₂	CAACA <u>ATGG</u> GCTACCACCC	53	1000–2700
SCoT ₇	CAACA <u>ATGG</u> GCTACCACGC	51	1000–1500
SCoT ₁₂	ACGAC <u>ATGG</u> GCGACCAACG	56	1100–1800
SCoT ₁₇	ACC <u>ATGG</u> GCTACCACCGAC	51	1300–2250
SCoT ₂₂	AACC <u>ATGG</u> GCTACCACCAC	51	300–1700
SCoT ₂₇	ACCAT <u>GGT</u> TACCACCGTG	52	800–2700
SCoT ₂₉	CCAT <u>GGG</u> TACCACCGGCC	56	900–1800
SCoT ₃₀	CCAT <u>GGG</u> TACCACCGGCG	51	900–2000
SCoT ₃₁	CCAT <u>GGG</u> TACCACCGCCT	51	1000–1500
SCoT ₃₃	CCAT <u>GGG</u> TACCACCGCAG	51	100–1000
SCoT ₃₄	ACCAT <u>GGT</u> TACCACCGCA	53	1250–1600
SCoT ₃₅	CAT <u>GGT</u> TACCACCGGCC	58	1150–1650
SCoT ₃₆	GCAACA <u>ATGG</u> GCTACCACC	52	500–1750
SCoT ₅₃	GAAACAT <u>GGG</u> ACCACCGACC	53	950–1550
SCoT ₅₄	ACCAT <u>GCG</u> AACCCGAACC	51	900–1600
SCoT ₇₀	ACCAT <u>GGG</u> TACCAGCGCG	51	150–1350
IRAP markers			
Gaga	GGGAACCAACCGTCACA	49	100–3000
5′LTR	ATGTACACACCTATGTTC TGTACCCGGCTTGCTC	45	100–2300
LTR6150	ATGTACACACCTATGTAT CTGTACCCGGCTTGCTG	59	1000–2000
3′LTR	TGTTTCCCATGCGACGTT CCCCAACA	58	300–1300
Nikita	CGCATTTGTTCAAGCCTA AACC	52	800–1800
Sukkula	GATAGGGTCGCATCTTG GGCGTGAC	54	800–2600

Underlines have determined the ATG codon (start codon of translation) in the primer sequence

Table 3 Analysis of variance based on complete block design for damaged grains weight (DGW), egg laying (EL), weight gain of nymphs (WGN), percentage of damaged grains (PDS), Weight gain of adult female insects (AFW) and adult male insects (AMW) traits

SOV	Df	MS					
		DGW	EL	WGN	PDS	AFW	AMW
Block	2	2.422 ^{ns}	2.333 ^{ns}	158.285*	1.995E–8 ^{ns}	1.213 ^{ns}	18,913.223 ^{ns}
Genotypes	24	2.166*	1013.126***	853.723***	9.932E–8***	1342.778***	2.057E7***
Error	48	1.16	10.222	53.488	3.100E–8	7.533	130,643.631
CV ^a (%)		1.69	5.8	6.37	0.02	1.08	4.25
Broad Sense Heritability (%)		46.45	98.99	93.73	68.79	99.43	99.36

ns,*,***nonsignificant and significant at 0.05 and 0.001 level respectively

^aCoefficient of variation

Results

Evaluation of Sunn pest resistance characteristics

Seven traits including damaged grains weight (DGW), egg laying (EL), weight gain of nymphs (WGN), percentage of damaged grains (PDS), weight gain of adult female insects (AFW), weight gain of adult male insects (AMW) and percentage of nymph mortality (PNM) were used for evaluating Sunn pest resistance in wheat genotypes. Assumptions of ANOVA were not met for studied traits except for PDS, thus boxcox (DGW, EL, WGN, and AMW) and square root (AFW) methods were used to convert the data. After performing all transformation methods, the assumptions were still not met for the percentage of nymph mortality (PNM) character. Therefore, a nonparametric Friedman test was undertaken for PNM that significantly showed great differences ($p < 0.001$) between genotypes. Analysis of variance exhibited high significant differences among genotypes for examined traits (Table 3). The mean of each characteristic was compared using the protected least significant differences (PLSD) test. The mean weights of damaged grains (DGW) were the lowest for Bayat, AarasGolsoor, Bezostaya, Line120, Rashagol, Line93 and Golsepy while higher DGW means were estimated for Line102, Sardari, Shoaleh, Alvand, Azar2 and Arvand. The minimum amount of EL, WGN, AMW, and AFW characteristics as indicators of resistance were obtained for Bayat, Line93, AarasGolsoor, Rashagol, Line120, Sayson and Bezostaya. The percentage of damaged grains (SDP) was less than 2% in Zagros, Bezostaya, AarasGolsoor, Bayat and Line93 genotypes. In addition, the percentage of nymph mortality (PNM) was higher for Rashagol, Golsepi, Cross-Shahi, Sayson, Bezostaya, Line93 and Line120 genotypes (Fig. 1).

High significant positive Pearson correlations were exhibited between damaged grains weight (DGW) and percentage of damaged grains (PDS), weight gain of adult female insects (AFW), weight gain of adult male insects (AMW) and egg laying (EL), and weight gain of adult female insects (AFW) and weight gain of adult male insects (AMW). A significant negative correlation was shown between weight gains of nymphs (WGN) and percentage of nymph mortality (PNM) traits (Table 4).

Cluster analysis was carried out using UPGMA algorithm and Euclidean distance coefficient based on all resistance traits. The genotypes were divided into two main groups and five subgroups. Most of the resistant or semi-resistant genotypes including Bayat, Line120, Bezostaya, Golsepi, Line93 and Rashagol were classified into

Fig. 1 Comparing wheat genotypes based on PNM, EL, WGN, PDS, AFW, AMW and DGW characteristics. The nonsimilar letters indicate significant differences at 0.01 level. The error bars were depicted using standard errors

Fig. 1 continued

subgroups of group I whereas group II comprised of the highest number of susceptible or semi-susceptible genotypes (Fig. 2).

Table 4 Correlation analysis for damaged grains weight (DGW), egg laying (EL), Weight gain of nymphs (WGN), percentage of damaged grains (PDS), Weight gain of adult female insects (AFW), adult male insects (AMW), and percentage of nymph mortality (PNM) traits

Variables	DGW	EL	WGN	PDS	AFW	AMW
DGW						
EL	0.126					
WGN	0.330	0.109				
PDS	0.768***	0.317	0.161			
AFW	0.146	0.964***	0.111	0.342		
AMW	0.107	0.972***	0.096	0.352	0.990***	
PNM	− 0.380	− 0.265	− 0.777***	− 0.316	− 0.249	− 0.240

***Significant at 0.001 level

Genetic variation analysis

Seventeen SCoT markers produced 144 amplicons with an average of 8.47 amplicons per primer for all 25 wheat genotypes. The number of amplicons per primer ranged from 2 to 14. SCoT₇ produced the minimum number of amplicons and the maximum amplicons were obtained using SCoT₃₁. The average Shannon information index was 0.23 ranging from 0.05 for SCoT₂₇ to 0.48 for SCoT₅₄. The genetic diversity as estimated by expected heterozygosity (He) ranged from 0.03 (SCoT₂₇) to 0.35 (SCoT₅₄) with an average overall loci of 0.15 for all wheat genotypes. Of all the obtained amplicons, 47.22 (%) were polymorphic. The percentage of polymorphic amplicons ranged from 8.33 (SCoT₂₇) to 88.89 (SCoT₃₆) with an average of 48.06 (Table 5).

The total 39 amplicons produced using six IRAP markers revealed an average 68.90% polymorphism amongst 25 wheat genotypes. The percentage of polymorphic amplicons ranged from 50 (LTR6150) to 87.5 (Nikita). The number of amplicons per primer ranged from 4 (LTR6150) to 9 (5'LTR) with an average of 6.5. Average of Shannon information index was 0.31, ranging from 0.17 for LTR6150 to 0.42 for Sukkala. The genetic diversity (He) ranged from 0.1 (LTR6150) to 0.29 (Sukkala) with an average overall loci of 0.20 for all wheat genotypes (Table 6).

Analysis of genetic relationships among genotypes

Genetic relationships among genotypes were evaluated for SCoT and IRAP markers separately and then the combination of both markers based on the neighbor-joining method using 1000 bootstrapped data sets. Dice and Jaccard similarity coefficients were used for SCoT and IRAP markers, respectively. Wheat genotypes were classified into five groups based on SCoT markers. Two landraces AarasGolsoor and Sardari from Kurdistan region were grouped together in the same cluster. Cluster 5 was comprised of three subgroups with seven genotypes that consisted of the two landraces Shoaleh and Golsepy originated

Fig. 2 UPGMA dendrogram using Euclidean distance method based on all morphological traits **Table 5** Estimated number of replicated amplicons, percentage of polymorphism, genetic diversity and Shannon information index for SCoT markers

Markers	Number of replicated amplicons	Number of polymorphic amplicons	Polymorphism percentage (%)	Genetic diversity (He) ^a	Shannon information index (I) ^b
SCoT ₁	7	3	42.86	0.13 ± 0.20	0.20 ± 0.28
SCoT ₂	11	3	27.27	0.09 ± 0.15	0.13 ± 0.23
SCoT ₇	2	1	50.00	0.25 ± 0.35	0.34 ± 0.49
SCoT ₁₂	9	2	22.22	0.05 ± 0.14	0.11 ± 0.22
SCoT ₁₇	12	4	33.33	0.06 ± 0.11	0.10 ± 0.17
SCoT ₂₂	9	6	66.67	0.16 ± 0.18	0.26 ± 0.25
SCoT ₂₇	12	1	8.33	0.03 ± 0.12	0.05 ± 0.17
SCoT ₂₉	7	2	28.57	0.12 ± 0.21	0.17 ± 0.29
SCoT ₃₀	8	1	12.5	0.04 ± 0.11	0.06 ± 0.18
SCoT ₃₁	14	8	57.14	0.20 ± 0.22	0.29 ± 0.31
SCoT ₃₃	9	7	77.78	0.12 ± 0.17	0.21 ± 0.23
SCoT ₃₄	7	5	71.42	0.18 ± 0.17	0.28 ± 0.25
SCoT ₃₅	8	4	50.00	0.11 ± 0.19	0.17 ± 0.26
SCoT ₃₆	9	8	88.89	0.31 ± 0.18	0.46 ± 0.23
SCoT ₅₃	6	4	66.67	0.29 ± 0.24	0.41 ± 0.33
SCoT ₅₄	5	4	80.00	0.35 ± 0.20	0.48 ± 0.28
SCoT ₇₀	9	3	33.33	0.07 ± 0.11	0.12 ± 0.18
Total	144	68	—	2.56 ± 3.05	3.84 ± 4.60
Average	8.47	4	48.06	0.15 ± 0.18	0.23 ± 0.27

^aNei's expected heterozygosity: $He = 1 - p^2 - q^2$ ^bShannon's Information Index: $I = -1 \times [p \times \ln(p) + q \times \ln(q)]$

from Iraq. No concordance was observed between data groupings based on SCoTs with the pedigree.

The genotypes were classified into five groups using IRAP markers. Group 1 comprised of six genotypes entailing the two landraces AarasGolsoor and Sardari. Group 2 included four genotypes including two landraces Shoaleh and Golsepi, which derived from Iraq. No

correspondence was revealed between grouping based on IRAP data and pedigree. Mantel test with 10,000 permutations was performed for determining the relationship of two genetic similarity matrices between wheat genotypes for SCoT and IRAP markers. A significant correlation between the matrices were exhibited with $r = 0.315$ ($p \leq 0.01$).

Table 7 Probable related SCoT markers to Sunn pest resistance characteristics based on Kruskal–Wallis test

Linked SCoT markers	Chi-Square	Degree of freedom	Asymptotic significance	Correlations	Amplicons Size (bp)
<i>DGW</i>					
SCoT31-J2	3.932	1	0.047	0.170 ^{ns}	1500
SCoT31-J8	3.932	1	0.047	– 0.316 ^{ns}	900
SCoT33-K6	5.250	1	0.022	0.430*	450
<i>EL</i>					
SCoT1-A6	10.520	4	0.033	0.572**	900
SCoT31-J2	7.944	4	0.019	0.472*	1500
SCoT34-L4	11.316	4	0.023	– 0.460*	1450
SCoT53-O6	12.424	4	0.014	– 0.560**	950
<i>WGN</i>					
SCoT2-B11	6.105	5	0.047	– 0.114 ^{ns}	1000
SCoT31-J6	8.348	5	0.015	0.459*	1200
SCoT17-E1	11.5	5	0.042	– 0.364 ^{ns}	2250
SCoT17-E12	11.5	5	0.042	0.364 ^{ns}	1300
SCoT35-M6	11.25	5	0.047	0.094 ^{ns}	1250
<i>PDS</i>					
SCoT2-B11	4.263	1	0.039	– 0.424*	1000
SCoT33-K3	4.00	1	0.046	– 0.286 ^{ns}	900
SCoT33-K4	4.00	1	0.046	– 0.286 ^{ns}	850
SCoT33-K5	4.00	1	0.046	0.205 ^{ns}	500
SCoT33-K6	4.00	1	0.046	0.569**	450
SCoT35-M8	4.00	1	0.046	– 0.286 ^{ns}	1150
<i>AMW</i>					
SCoT2-B11	14.476	4	0.043	0.433*	1000
SCoT53-O6	14.023	4	0.050	– 0.553**	950
SCoT1-A2	11.407	4	0.022	– 0.468*	1800
SCoT35-M8	11.5	4	0.021	– 0.413*	1150
<i>AFW</i>					
SCoT1-A2	11.054	2	0.004	– 0.511**	1800
SCoT1-A6	6.668	2	0.036	0.559**	900
SCoT53-O6	9.261	2	0.01	0.591**	950
<i>PNM</i>					
SCoT31-J8	9.179	2	0.01	– 0.170	900
SCoT35-M6	6.5	2	0.039	0.099	1250
SCoT36-N3	7.8	2	0.02	0.198	1450
SCoT34-L6	9.179	2	0.010	0.185	1300
SCoT17-E9	6.05	2	0.049	– 0.064 ^{ns}	1500
SCoT70-Q6	9.714	2	0.008	0.432*	750

*, **Significant at 0.05 and 0.01 level respectively

total variations for effective SCoT data on resistance characteristics of DGW, EL, WGN, AFW, AMW and PNM, respectively (Table 9). The genotypes did not discriminate well for effective SCoT markers on the PDS trait. Conversely, for effective IRAP markers on PDS, first discriminant function explained 99.9 of total variance. Canonical correlation and Eigen values were 0.888 and 3.734, respectively. However, the genotypes were not

discriminated based on effective IRAP markers on other traits (Table 9). Stepwise regression analysis was conducted based on effective SCoT and IRAP markers on each DGW, EL, WGN, PDS, AFW, AMW and PNM characteristics. The determination coefficients (R^2) for effective SCoT markers were estimated as 0.321, 0.481, 0.257, 0.501, 0.388, 0.483, and 0.387. Highest variation of DGW, EL, WGN, PDS, AFW, AMW and PNM were explained by

Table 8 Probable related IRAP markers to Sunn pest resistance characteristics based on Kruskal–Wallis test

Linked IRAP markers	Chi-Square	Degree of freedom	Asymptotic significance	Correlations	Amplicons size (bp)
<i>DGW</i>					
5'LTR-B1	3.932	1	0.047	0.183 ^{ns}	2600
5'LTR-B4	3.932	1	0.047	0.466*	1500
<i>EL</i>					
3'LTR-D2	7.632	2	0.022	– 0.494*	1100
Nikita -E4	5.526	2	0.049	– 0.442*	1300
<i>WGN</i>					
5'LTR-B4	6.857	2	0.032	0.419*	1500
<i>PDS</i>					
3'LTR-D3	9.067	2	0.011	0.025	800
Nikita-E5	8.246	2	0.016	– 0.344	1100
3'LTR-D2	10.667	3	0.014	– 0.405*	1100
<i>AFW</i>					
3'LTR-D2	10.147	3	0.017	– 0.463*	1100
Nikita-E3	11.5	3	0.009	– 0.092	1500
Nikita-E7	11.5	3	0.009	– 0.092	900
<i>AMW</i>					
3'LTR-D2	9.859	4	0.043	– 0.529**	1100
Nikita-E4	9.789	4	0.044	– 0.421*	1300
<i>NM</i>					
5'LTR-B4	9.079	3	0.028	– 0.470*	2000
LTR6150-C3	9.333	3	0.025	0.473*	1300
LTR6150-C4	9.333	3	0.025	0.473*	1000
Nikita-E3	11.5	3	0.009	– 0.335 ^{ns}	1500
Nikita-E7	11.5	3	0.009	– 0.335 ^{ns}	900

*Significant at 0.05 level

SCoT₃₃-K₆ (18.5%), SCoT₁-A₆ (32.8%), SCoT₃₁-J₆ (21.1%), SCoT₃₃-K₆ (32.4%), SCoT₅₃-O₆ (34.9%), SCoT₅₃-O₆ (30.6%) and SCoT₃₁-J₈ (48.9%), respectively.

Determination coefficient (R^2) of effective IRAP markers on DGW, EL, WGN, PDS, AFW, AMW and PNM were estimated as 0.305, 0.366, 0.176, 0.250, 0.252, 0.211 and 0.401, respectively. The IRAP markers including 5'LTR-B₄ (30.5%), Nikita-E₄ (23.5%), 5'LTR-B₄ (17.2%), 3'-LTR-D₂ (16.4%), 3'-LTR-D₂ (23.6%), 3'-LTR-D₂ (28.00%) and 5'LTR-B₄ (26.3%) explained the highest variations of DGW, EL, WGN, PDS, AFW, AMW and PNM, respectively.

Discussion

Phenotypic characteristics related to Sunn pest resistance

To enable screening of wheat genotypes for the levels of resistance to Sunn pest, seven characteristics including DGW, EL, WGN, PDS, AFW, AMW and PNM were

calculated as indices of resistance. High variability was observed in wheat genotypes for Sunn pest resistance characteristics. Variation in wheat genotypes to Sunn pest damage allows the selection of potential gene sources for wheat breeding programs utilizing resistance genes against Sunn pest.

Considerable variation for percentage of nymph mortality (PNM) was estimated between the studied wheat genotypes. The genotypes including Rashagol, Golsepi, Cross-Shahi, Sayson, Bezostaya, Line93 and Line120 with high PNM scores were classified as resistant. It seems that variability of PNM between wheat genotypes represented antibiosis resistance mechanism resulting in the death of nymphs in some resistant genotypes. Joukhadar et al. (2013) also reported an antibiosis resistance mechanism in wheat for Hessian fly. The percentages of infested grains were most commonly used to classify the quality of wheat (Duric et al. 2014). Due to the percentage of damaged grains in Bayat, AarasGolsoor, Bezostaya and line93 scoring less than 1%, these genotypes were classified as resistant. Low percentage of damaged grains in wheat genotypes is an important resistance character because

Table 9 Discriminant analysis using associated SCoT and IRAP markers with DGW, EL, WGN, PDS, AFW, AMW and PNM traits

Traits	Function	Eigen value	% of variance	Canonical correlation	Cross-validated groups (%)
<i>SCoT</i>					
DGW	1	4.054	93.8	0.896	84
	2	0.266	6.2	0.459	–
EL	1	7.148	99.5	0.937	48
	2	0.037	0.5	0.189	–
WGN	1	0.793	76.4	0.665	64
	2	0.037	23.6	0.189	–
PDS	1	4.320	100	0.901	–
AMW	1	5.495	74.3	.920	96
	2	1.905	25.7	0.810	–
AFW	1	7.251	96.5	0.937	88
	2	0.265	3.5	0.458	–
PNM	1	11.759	93.6	0.96	96
	2	0.802	6.4	0.667	–
<i>IRAP</i>					
DGW	1	3.480	100	0.880	–
EL	1	0.045	100	0.208	–
<i>WGN</i>					
PDS	1	3.734	99.9	0.888	68
	2	0.033	0.1	0.059	–
AFW	1	0.006	100	0.079	–
AMW	1	0.05	100	0.218	–

according to Arzani et al. (2007) and Mutlu et al. (2014) reports, quantitative losses are generally less important than qualitative losses because infestation might not reduce yield significantly and as little as 2–3% of damaged grains might be deemed unacceptable for baking purposes. When proportions of damaged seeds are less than 3% in wheat genotypes, it should be treated with flour improvers to strengthen bread-making quality (Kinaci and Kinaci 2004). As a result, considerable economic losses will occur for producers and consumers. Broad sense heritability (H^2) of PDS indicating 68.79% of variation between wheat genotypes for this trait is under genetic control. Significant differences for percentage of damaged seeds in infested durum and bread wheat genotypes with Sunn pest was previously reported by Hossaini et al. (2009) and Duric et al. (2014).

Wheat genotypes including Bayat, Line120, Line93, Rashagol, Azadi, Sayson and Bezostaya were determined to be resistant based on minimum estimated values of egg laying (EL), weight gain of nymphs (WGN), and weight gain of adult female and male insects (AFW and AMW). Conversely, genotypes that were more susceptible were Line102, Arvand, Sardari, Roshan and line10. Alamoot, Alvand, Ghods and Shoaleh, which were ranked as semi susceptible genotypes. Other genotypes were grouped as intermediate resistant genotypes. Resistance of wheat cultivars to Sunn pest were evaluated during Sunn pest

outbreaks from 1987 to 1990 in Iran. It was found that Ghods and Karajl cultivars were more susceptible than Bezostaya, Roshan, Omid, Sardari and Tabassi with respect to yield and baking quality (Javahery 1996). Rezabeigi et al. (2007) studied some Iranian bread wheat cultivars and concluded that the cultivar Falat was resistant whereas the Azadi, Golestan, Ghafghaz, and Navid cultivars were grouped as semi-resistant. Other cultivars including the Bezostaya and Sardari were ranked as semi-susceptible to Sunn Pest. Najafian et al. (2008) ranked the Bezostaya, MV17 and Navid as resistant cultivars in their research. The Bezostaya and Navid wheat cultivars were identified as resistant to overwintered Sunn pest in Iran (Najafian et al. 2008; Sanaey and Najafi Mirak 2012). Fatehi et al. (2009) reported that the Sardari and Azadi cultivars were susceptible due to a high percentage of their damaged seeds. It appears that the variation in time of insect infestation could influence resistance and susceptibility of cultivars as previously reported by Fatehi et al. (2009).

In our study, the Bayat, Bezostaya, Line120, Line93, Rashagol, Golsepi and Sayson were ranked as genotypes that are more resistant. Three landraces from the Kurdistan region of Iraq including Rashagol, Golsepi and AarasGol-soor were classified as resistant and semi resistant genotypes category. These landraces were cultivated continuously in the above-mentioned region and constantly exposed to Sunn pest attack; therefore, it is quite possible

that the resistant genes evolved and accumulated in these landraces. These landraces possessed some common morphological features such as long-awns, intense pubescence and glumes enclosing the grains, which might have contributed to antixenosis resistance. Moreover, the gluten percentage of grains were higher relatively in resistant genotypes particularly in the already mentioned landraces (data not shown). High gluten content could also be an indicator of resistance because with Sunn pest infestation the gluten of grains degrades in susceptible genotypes. Two artificial lines, 120 and 93, resulting from Triticum/Tritipyrum hybridization were categorized as the most resistant genotypes indicating the potential of artificial lines for introducing novel superior genes to wheat populations.

Significant positive correlations were obtained between damaged grain weight (DGW) and damaged seeds with Sunn pest (PDS), egg laying (EL) and weight gain of adult females and males (AFW and AMW) meaning that the wheat genotypes with higher PDS, EL, AFW and AMW values are more susceptible. However, high negative correlation was calculated between weight gain of nymphs (WGN) and the percentage of nymph mortality (PNM). The significant positive correlation between DGW and PDS was a contradictory result, which was also previously reported by Hossaini et al. (2009) and could be attributed to some kinds of unknown yield compensate mechanisms in wheat genotypes.

Genetic diversity and relationships

In the present study, two marker techniques SCoT and IRAP were used to assess genetic diversity in 25 bread wheat genotypes and their effect on Sunn pest resistance characteristics.

SCoT and IRAP markers revealed average genetic diversity between bread wheat genotypes detecting the 47.22 and 68.9% of polymorphism, respectively. IRAP markers with higher polymorphism exhibition capacity performed better than SCoT markers. In the previous studies that used SCoT and IRAP markers, wide genetic diversity was reported in durum and bread wheat cultivars (Carvalho et al. 2010; Hamidi et al. 2014; Etminan et al. 2016).

The wheat genotypes were classified in five groups using neighbor-joining (NJ) algorithm based on SCoT, IRAP and combined data. Two landraces from Kurdistan (Sardari and AarasGolsoor) and two landraces from Iraq (Shoaleh and Golsepy) were classified appropriately indicating the ability of these molecular markers to differentiate genotypes based on geographic regions. The clustering of wheat genotypes based on both marker data sets was similar with a few differences represented in significant Mantel correlations between similarity matrices.

Assessment of effective SCoT and IRAP markers on resistance characteristics

Functional analysis means that determining correlation between genetic diversity of DNA markers and resistant traits will lead to the fast and reliable screening methods. The effect of any of the SCoT and IRAP amplicons on the resistant characteristics was estimated by using the Kruskal–Wallis test in which significant effective markers were detected. Effective SCoT markers highly discriminated wheat genotypes based on DGW, EL, WGN, AFW, AMW and PNM characteristics exhibiting high genetic differences for Sunn pest resistance in wheat genotypes. The highest variation of DGW, EL, WGN, PDS, AFW, AMW and PNM was explained by SCoT₃₃-K₆ (18.5%), SCoT₁-A₆ (32.8%), SCoT₃₁-J₆ (21.1%), SCoT₃₃-K₆ (32.4%), SCoT₅₃-O₆ (34.9%), SCoT₅₃-O₆ (30.6%) and SCoT₃₁-J₈ (48.9%), respectively. The highest phenotypic variation for linked IRAP markers were explained by 5′LTR-B₄ (30.5%), Nikita-E₄ (23.5%), 5′LTR-B₄ (17.2%), 3′-LTR-D₂ (16.4%), 3′-LTR-D₂ (23.6%), 3′-LTR-D₂ (28.00%) and 5′LTR-B₄ (26.3%) for DGW, EL, WGN, PDS, AFW, AMW and PNM, respectively. The occurrence of common effective SCoT or IRAP markers on some resistance characteristics indicates common genetic loci. Due to the low number of our genotypes, these effective markers might be considered valuable and informative markers that should be further assessed in larger wheat populations in future studies such as association mapping approach. Therefore, these markers might be of some limited use as functional effective markers for selecting Sunn pest resistant wheat genotypes. The association of protein and DNA markers with Sunn pest resistance characteristics has been reported in a few research studies. According to Fatehi et al. (2009), the 62.1% of the total variation in percentage of damaged seeds are attributed to four glutenin sub-units alleles including 7 + 9 (39.2%), 12 (13.4%), 7 + 8 (5%) and 6 + 8 (4.5%). Joukhadar et al. (2013) reported that seventeen DArT markers associated with visual damage score (shoot/leaf damage by Sunn pest) were localized on 1A, 1B, 3AS, 4BL, 5AL and 6BS chromosomes. Zabet et al. (2008) identified dominant and over dominant QTLs associated with Sunn pest damage located on the large arms of 6B, 3D, 4B and short arm of 5D chromosomes. Emebiri et al. (2017) identified a single major locus (Designated as *Eil*) on chromosome 4BS for Sunn pest resistance in two doubled- haploid wheat populations. Zabet et al. (2008) using diallel analysis showed that both additive and the dominance genetic variances influenced Sunn pest resistance but dominance genetic variance was more important. Conversely, narrow heritability for percentage of Sunn pest damage was estimated as relatively high. Introducing novel resistance genes into locally

adapted elite wheat cultivars and pyramiding of diverse resistance genes could be important in reducing damage to wheat at the vegetative stage (shoot and leaves) consequently reducing Sunn pest adult populations as well as nymphal and new generation adults (El Bouhssini et al. 2009; Joukhadar et al. 2013).

Molecular SCoT and IRAP markers revealed high genetic diversity in bread wheat genotypes and high variation in wheat genotypes for Sunn pest resistance characteristics. Some wheat genotypes including Bayat, Bezostaya, Line120, Line93, Rashagol, Golsepi and Sayson were categorized as resistant which could be valuable gene resources for breeding programs or mapping approaches. In this study, a number of effective SCoT and IRAP markers were identified for Sunn pest resistance characteristics. Informative DNA markers provide simple and reliable selection methods and therefore extension of resistant wheat sources for controlling insect biotypes. Thus, further studies involving larger wheat genotypes including landraces, relatives and cultivars and a large number of DNA markers would be efficient in the study of genetic bases of Sunn pest resistance.

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Compliance with ethical standard

Conflict of interest The authors declare that they have no financial or other conflict of interest.

Ethical standards The authors confirm that ethical standards were considered in this research.

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