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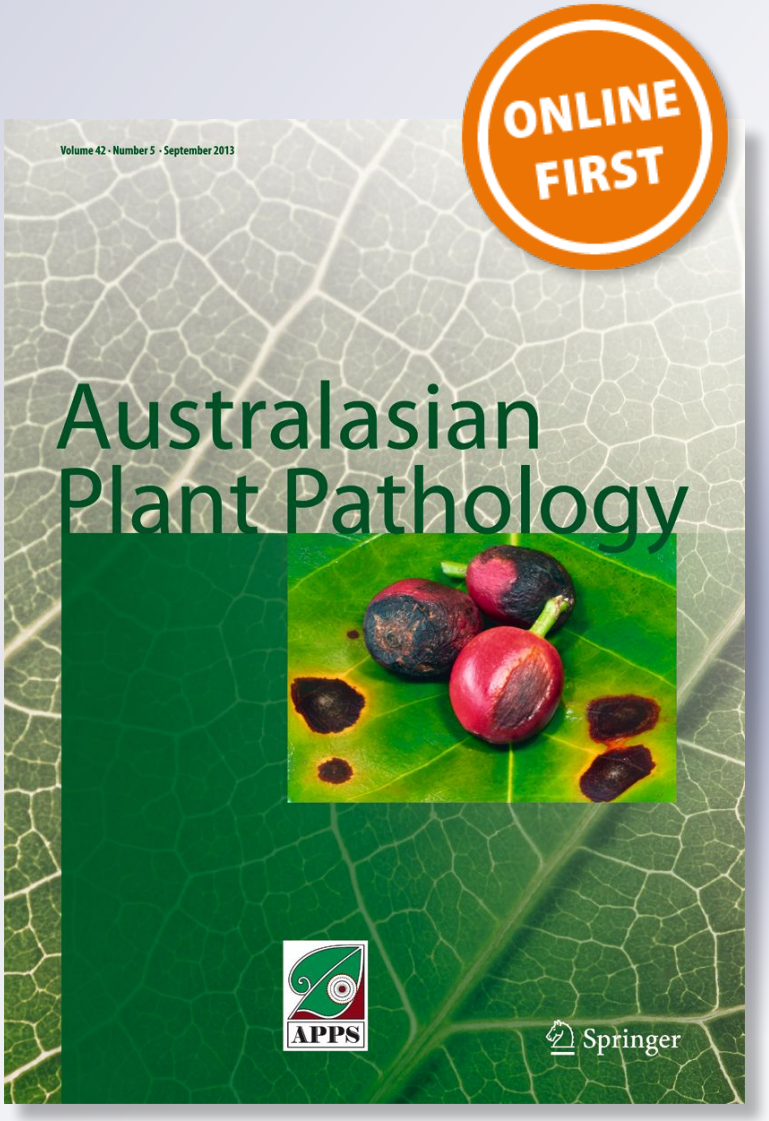

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ORIGINAL PAPER

Retrotransposonable regions of sunflower genome having relevance with resistance to *Sclerotinia* species: *S. sclerotiorum* and *S. minor*

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Abstract

Basal stem rot (BSR), caused by *Sclerotinia sclerotiorum* and *S. minor*, is one of the most important fungal diseases of sunflower (*Helianthus annuus* L.) causing significant yield losses worldwide. Using resistant cultivars is the most effective method to manage BSR in the field. Therefore, identification of resistant genotypes and genomic regions related to the disease resistance is necessary for employment in the field and for development of resistant cultivars. In this study, the reaction of 100 oilseed sunflower lines was investigated against three isolates from each of the *S. sclerotiorum* and *S. minor* species in controlled conditions and association analysis of resistance traits was performed using retrotransposon-based DNA markers. The sunflower germplasm exhibited various reactions against the fungal isolates as the mean necrosis percentage in basal stem of the lines ranged from 30.67 to 100. The genotypes H156A/H543R, 110 and 8A×/LC1064C were resistant to the isolates of both species. Population structure analysis subdivided the genotypes into two subpopulations. Association analysis using general and mixed linear models identified 15 and 14 loci, respectively, which were significantly ($P \leq 0.01$) associated with resistant traits. Phenotypic variance explained by QTLs (R^2) ranged from 1 to 23%. The markers UF1, LTR1064-A13, LTR1061-UBC818 and LTR1064–65 were commonly associated with the traits conferring resistance to more than one fungal isolate. This was the first study on QTL mapping of genomic regions responsible for resistance to *S. sclerotiorum* and *S. minor* using retrotransposon-based DNA markers in and provided an evidence for effectiveness of these markers in association analysis of sunflower. The QTLs and the markers associated with the resistance traits can be useful in marker-aided programs to develop sunflower cultivars with effective resistance to BSR caused by the two *Sclerotinia* species.

Keywords Association analysis · Molecular markers · Quantitative resistance · Sclerotinia · Sunflower

Introduction

Sunflower (*Helianthus annuus* L.) is one of the four major field crops that is cultivated globally for its edible oil and seeds (Paniego et al. 2007; Vollmann and Rajcan 2009). Basal stem rot (BSR), caused by *Sclerotinia sclerotiorum* and *S. minor*, is considered as a major threat for sunflower production in Europe, Argentina, and USA, causing average yield reductions of 10 to 20%, and can even result in loss of the entire harvest (Pereyra and Escande 1994; Gulya et al. 1997; Van Becelaere and Miller 2004; Bolton et al. 2006). The causal agents of disease have strikingly broad host ranges and are distributed globally, although *S. sclerotiorum* is a more common pathogen than *S. minor* in most sunflower-growing regions. Sclerotia of both fungal species can germinate myceliogenically and infect basal stem of the plants but sclerotia of *S. sclerotiorum* are also capable of germinating carpogenically under favorable environmental conditions and infecting upper plant parts in the field (Saharan and Mehta 2008; Valera Rojas 2014; Sharma et al. 2016).

The use of resistant cultivars is considered as the most effective and economic method to manage BSR in sunflower fields (Ebrahimi et al. 2014). However, no complete resistance to the causal agents has been observed in host plants and resistant genotypes of sunflower (Godoy et al. 2005), beans

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(Gilmore et al. 2002), peas (Porter et al. 2009) and soybean (Hartman et al. 2000) exhibit partial resistance to the disease. Several studies have reported sunflower genotypes with various levels of resistance to *S. sclerotiorum* (Castaño et al. 1993; Hahn 2002; Ebrahimi et al. 2014) but a few researches have been conducted on evaluating the reactions of sunflower germplasm to *S. minor* (Sedun and Brown 1989) because the latter pathogen is not a threat to this crop in most regions. Resistance to *S. sclerotiorum* is a complex quantitative trait (Mestries et al. 1998) with polygenic inheritance (Castaño et al. 1993; Gentzbittel et al. 1998; Bert et al. 2002, 2004; Fusari et al. 2012) and resistant sunflower genotypes show different levels of resistance to this disease (Hahn 2002; Rönicke et al. 2004). Therefore, due to the complexity of quantitative trait, little information is available on the number and chromosomal locations of the genes associated with phenotypic expression of resistance to BSR.

Quantitative trait loci (QTL) mapping is a common method to identify molecular markers linked to the loci associated with partial resistance in crop plants (Bernardo 2008; Kole and Henry 2010). These markers can be used in marker-aided selection (MAS) programs to develop new cultivars with effective resistance to plant diseases (Collard et al. 2005). Family-based linkage (FBL) mapping and linkage disequilibrium (LD)-based association mapping (AM) are the two approaches mostly used for QTL mapping in various crop plants (Mackay and Powell 2007; Breseghello and Sorrells 2006). Linkage mapping strategy is very costly, has low resolution, and evaluates few alleles simultaneously in a relatively longer time scale (Flint Garcia et al. 2005; Gupta et al. 2005; Ross-Ibarra et al. 2007) while association mapping provides high mapping resolution due to using all meiosis events accumulated in breeding history of species, richness of allelic polymorphism due to the vast genetic variation represented by diverse genetic background of the genotypes and, availability of populations and phenotypic data collected through multiple environments without any extra cost (Rostoks et al. 2006; Roy et al. 2006; Yu and Buckler 2006; Yu et al. 2006; Andersen et al. 2007; Sorkheh et al. 2008; Hall et al. 2010; Liu et al. 2010; Wang et al. 2012; Zhang et al. 2012). However, population structure, sample size and frequency of specific alleles may affect the results of association mapping leading to detecting false positive associations between markers and traits (Zhang et al. 2012). Therefore, the methods incorporating population structure (Q) and kinship (K) matrix or a combination of Q and K (Q + K) has been proposed to minimize false-positive association (Pritchard et al. 2000; Yu and Buckler 2006; Yu et al. 2006).

BSR is the most important fungal disease of sunflower in Iran and is responsible for significant yield losses to this crop (Ershad 1977). Because of the favorable conditions for disease development and intensive cultivation of susceptible sunflower cultivars, West Azarbaijan province is one of the hotspots for BSR in the country. *Sclerotinia sclerotiorum* has been the main pathogen causing the disease in the province, however, *S. minor* has recently been intensified in some regions so that this pathogen is the dominant species in infected fields. Therefore, identification of resistant genotypes and genomic regions associated with disease resistance is essential to reduce the disease impact by replacing susceptible commercial cultivars with resistant genotypes and developing hybrid cultivars with effective resistance against the disease. The QTLs associated with partial resistance to *S. sclerotiorum* have already been identified in various sunflower populations using a diverse of molecular markers in Iran (Davar et al. 2011; Darvishzadeh 2012; Amoozadeh et al. 2015) and elsewhere in various countries (Mestries et al. 1998; Bert et al. 2002) but no information available on the QTLs associated with *S. minor*. This study was aimed to assess the reaction of 100 oilseed sunflower lines to the isolates of *S. sclerotiorum* and *S. minor* and to detect association between BSR resistance and retrotransposon-based molecular makers in the studied lines.

Materials and methods

Plant material

One-hundred oilseed sunflower inbred lines developed by various research centers (Table 1) were investigated in the present study. Seeds of these lines were provided by Institute National de la Recherche Agronomique (INRA), France. The seeds were planted in rectangular 20 × 60 cm pots filled with peat moss. The pots were kept in a growth chamber at 25 ± 1 °C with 75% relative humidity (RH) and 12 h light photoperiod. The experiment was conducted in a completely randomized design (CRD) with three replications (pots) and six plants of each genotype per replication.

Phenotyping

Reactions of sunflower genotypes were assessed against three isolates from each of the *S. sclerotiorum* and *S. minor* at six- to eight-leaf (V6–V8) growth stage. The fungal isolates which have already been collected from infected sunflower fields in various regions of West Azarbaijan province, Iran, were grown on potato dextrose agar (PDA 39 gL^{-1}, pH 6) medium in dark at room temperature. After 3 days, mycelial disks (3 mm diameter) from growing edges of three-days-old colonies were placed on basal stem of plants and covered by Parafilm for 48 h (Davar et al. 2011). Three days after inoculation, the reaction of genotypes was evaluated for basal stem rot disease visually by measuring percentage of necrosis area in 1 cm above stem base where the mycelial disks were placed (Davar et al. 2011; Amoozadeh et al. 2015).

Table 1 Studied oilseed sunflower lines and their origin

No.	Line	Country	Research center	Q1	Q2	No.	Line	Country	Research center	Q1	Q2
1	DM2	USA	USDA	0.252	0.748	52	H603R	France	INRAMONT	0.712	0.288
2	LC1064C	France	ASGROW	0.304	0.696	53	NSF1 A4 × R5	France	NOVARTIS	0.008	0.992
3	H158A × LC1064C	France	ASGROW	0.398	0.602	54	NSF1 A5 × R5	France	NOVARTIS	0.903	0.097
4	NS-R5	France	NOVARTIS	0.985	0.015	55	H158A/LC1064	France	ASGROW	0.714	0.286
5	HAR4	USA	USDA	0.702	0.298	56	H543R/H543R	France	ASGROW	0.509	0.491
6	SDB1	USA	USDA	0.934	0.066	58	H156A/H543R	France	ASGROW	0.879	0.121
7	AS5305	France	ASGROW	0.972	0.028	59	H543R	France	–	0.026	0.974
8	RHA274	USA	USDA	0.958	0.042	61	H100A/RHA274	France	ASGROW	0.406	0.594
9	SDR18	USA	USDA	0.865	0.135	62	H205A/83HR4	France	ASGROW	0.706	0.294
10	RT931	France	RUSTICA	0.543	0.457	63	H158A/H543R	France	ASGROW	0.194	0.806
11	NS-B5	France	NOVARTIS	0.825	0.175	64	H209A/83HR4	France	ASGROW	0.500	0.500
12	SDB3	USA	USDA	0.713	0.287	65	H157A/LC1064	France	ASGROW	0.882	0.118
13	803–1	Serbia	IFVC	0.854	0.146	66	H100A/LC1064	France	ASGROW	0.774	0.226
15	F1250/03	Hungary	–	0.684	0.316	67	H100A/90R78	France	ASGROW	0.834	0.166
16	HA335B	USA	USDA	0.973	0.027	68	AF1POPA	France	NOVARTIS	0.502	0.498
17	TMB 51	France	INRAMONT	0.974	0.026	69	OES	France	INRAMONT	0.878	0.122
18	LP-CSYB	France	ENSAT	0.980	0.020	70	703-CHLORINA	France	ENSAT	0.695	0.305
19	PM1–3	USA	USDA	0.012	0.988	71	RHA266	USA	USDA	0.532	0.468
20	SDR19	USA	USDA	0.807	0.193	72	PAC2	France	ENSAT	0.708	0.292
21	RHA265	USA	USDA	0.854	0.146	73	AS613	France	ASGROW	0.683	0.317
22	QHP1	–	–	0.705	0.295	79	11 × 12	Iran	SPII	0.034	0.966
23	RT948	France	RUSTICA	0.694	0.306	81	38	Iran	SPII	0.523	0.477
24	ENSAT-283	France	ENSAT	0.966	0.034	82	346	Iran	SPII	0.745	0.255
25	D34	France	INRAMONT	0.687	0.313	85	1059	Iran	SPII	0.832	0.168
26	HA337B	USA	USDA	0.983	0.017	86	36	Iran	SPII	0.757	0.243
27	B454/03	Hungary	–	0.494	0.506	87	4	Iran	SPII	0.390	0.610
28	H100B	France	ASGROW	0.745	0.255	88	30	Iran	SPII	0.223	0.777
29	HA304	USA	USDA	0.873	0.127	89	28	Iran	SPII	0.864	0.136
30	AS5304	France	ASGROW	0.430	0.570	90	110	Iran	SPII	0.075	0.925
31	RHA858	USA	USDA	0.030	0.970	91	SDB2	France	INRAMONT	0.695	0.305
32	AS3211	France	ENSAT	0.600	0.400	92	1,009,370 1(100 K)	France	ENSAT	0.175	0.825
33	AS5306	France	–	0.796	0.204	93	1,009,370 3(100 K)	France	ENSAT	0.500	0.500
34	ENSAT-254	France	ENSAT	0.667	0.333	95	CSWW2X	France	Caussade semences	0.856	0.144
35	ENSAT-270	France	ENSAT	0.653	0.347	96	H100A	France	ASGROW	0.826	0.174
36	1,009,329 2(100 K)	France	ENSAT	0.964	0.036	97	H158A/H543R	France	ASGROW	0.556	0.444
37	1,009,337 (100 K)	France	ENSAT	0.611	0.389	98	H209A/H566R	France	ASGROW	0.455	0.545
38	100,935 0(100 K)	France	ENSAT	0.632	0.368	99	AS6305	France	ENSAT	0.608	0.392
39	5DES20QR	France	BRN	0.770	0.230	100	H100A/83HR4	France	ASGROW	0.235	0.765
40	7CR13 = PRH6	France	C.F	0.652	0.348	101	H205A/H543R	France	ASGROW	0.186	0.814
41	SSD580	France	ASGROW	0.807	0.193	102	H209A/LC1064	France	ASGROW	0.416	0.584
42	SSD581	France	ASGROW	0.032	0.968	103	AS0–1-POP-A	France	ENSAT	0.577	0.423
43	ENSAT-699	France	ENSAT	0.502	0.498	104	BF1POPB	France	NOVARTIS	0.380	0.620
44	9CSA3	France	Caussade semences	0.784	0.216	105	CAY	France	ENSAT	0.702	0.298
45	5AS-F1/A2 × R2	France	ASGROW	0.773	0.227	106	A CONTROL PLASTIPIC	France	ENSAT	0.732	0.268
46	8ASB2	France	ASGROW	0.624	0.376	107	H156A/RHA274	France	ASGROW	–	–
47	12ASB3	France	ASGROW	0.671	0.329	108	sf 076	–	–	–	–
48	AS3232	France	ENSAT	0.986	0.014	109	sf 022	–	–	–	–

Table 1 (continued)

No.	Line	Country	Research center	Q1	Q2	No.	Line	Country	Research center	Q1	Q2
49	H049 + FSB	France	–	0.600	0.400	110	sf-109	–	–	–	–
50	15,038	Hungary	–	0.122	0.878	111	sf-105	–	–	–	–
51	15,031	France	ASGROW	0.942	0.058	112	sf-023	–	–	–	–

Q1 and Q2 present the membership percent of each genotype to each subpopulation

Genotyping

The data from inter retrotransposon amplified polymorphism (IRAP) and retrotransposon microsatellite amplified polymorphism (REMAP) markers that have already been generated for the studied sunflower lines (Basirnia et al. 2016), were used in association analyses. These data consisted of 248 loci amplified by 28 retrotransposon-based primers (Table 2).

Statistical analyses

Descriptive statistics values such as minimum, maximum, mean and standard division of necrosis of genotypes caused by fungal isolates were calculated. Population structure of the lines was analyzed using a model-based Bayesian approach by Structure 2.3.3 software (Pritchard et al. 2000). Five independent runs were performed for K values ranging from 1 to 10 using burn-in period and MCMC (Markov Chain Monte Carlo) each with 100,000 replications. The admixture model and correlated allele frequencies were assumed for the analyses. The most likely K value was determined as described by Evanno et al. (2005). Inferred ancestry estimates of individuals (Q-matrix) were derived for selected subpopulation (Pritchard et al. 2000). Association analysis was performed to infer marker-trait association by incorporating ancestry coefficient (Q values) estimates as covariate in general linear model (GLM), and kinship (K-matrix) and ancestry coefficients (Q values) estimates as covariates in the mixed linear model (MLM) using TASSEL 2.1 software.

Results

Phenotypic evaluation

Descriptive statistics values of fungal infection percentage on the sunflower genotypes are shown in Table 3. The minimum and maximum percentage of necrosis produced by the pathogen isolates was 30.67 and 100, respectively. All six isolates incited the maximum necrosis 100% on highly susceptible genotypes. However, minimum necrosis percentages varied depending on the isolate examined as the lowest and the highest means were produced by 2 *S. sclerotiorum* isolates A37 and J1. None of the genotypes were resistant to J1 (*S. sclerotiorum*), whereas most of genotypes showed resistance to isolate A37 (*S. sclerotiorum*) (Table 3).

Table 2 Names and sequences of retrotransposon-based primers used in the study

Retrotransposon primers	Sequence (5′ → 3′)	ISSR primers	Sequence (5′ → 3′)
1061LTR	AGAGGGGAATGTGGGGGTTTCC	UBC-818	CACACACACACACACAG
1062LTR	TCTCTATTTATAGCCGGAGAGGTG	UBC-826	ACACACACACACACACAC
1063LTR	GATCCGGTTTCACGGGACTTAC	UBC-840	GAGAGAGAGAGAGAGAYT
1064LTR	CGAAGAACAAACCGAATCACC	UBC-857	ACACACACACACACACYG
1065LTR	AGCCTCTGAAAGACTCGTTCG	A13	GTGTGTGTGTGTCC
CF	GGTTTAGGTTCGTAATCCTCCGCG	UBC-812	GAGAGAGAGAGAGAGAA
CR	ACAGACACCAGTGGCACCAAC	UBC-864	ATGATGATGATGATGATG
UF(U81)	TAACGGTGTTCTGTTTTGCAGG	UBC-880	GGAGAGGAGAGGAGA
UR1(U82)	AGAGGGGAATGTGGGGGTTTCC		

Table 3 Descriptive statistics of fungal infection percentage of the studied oilseed sunflower genotypes in responses to *Sclerotinia sclerotiorum* and *S. minor* isolates

Fungal isolate	Min of necrosis (%)	Max of necrosis (%)	Mean of necrosis (%)	STDEV	Mode (%)	Median (%)	Coefficient of variation (%)	Genotype with the lowest necrosis (Resistant genotypes)
S. minor								
A1	69.33	100.00	88.51	8.27	100.00	89.67	9.34	H205A/83HR4, 110
M1	58.33	100.00	90.76	8.85	100.00	92.17	9.75	8A×/LC1064C, ENSAT-699, 1009370 1(100K)
G2	64.00	100.00	94.26	6.66	100.00	96.33	7.06	H156A/H543R
S. sclerotiorum								
J2	30.67	100.00	90.26	10.83	100.00	93.50	12.00	H543R/H543R, 110, NSF1 A4 × R5, 1059
J1	90.00	100.00	97.20	2.01	97.67	97.67	2.07	–
A37	39.33	100.00	82.67	14.71	100.00	84.84	17.79	8A×/LC1064C, HAR4, RHA274, RT931, F125/03, LP-CSYB, HA337B, 5DES20QR, H049 + FSB, ASB28, H543R/H543R, H156A/H543R, H100A/RHA274, H100A/LC1064, AF1POPA, OES, PAC2, AS613, 110, 1059, 1009370 1(100K), H100A/83HR4,

STDEV: standard deviation

Marker-trait associations (MTAs)

Genetic structure of association panel was inferred by 248 loci in Structure software by implementing Bayesian approach. Based on these analyses, K = 2 was found as the optimal number of subpopulations (Fig. 1). This optimized K was considered for estimating the population structure and membership of each individual (Q matrix). Assignment analysis indicated that 63% of lines had a membership of ≥0.7 whilst in 37% of the genotypes membership was <0.7 (Fig. 2). The two subpopulations red and green consisted of 15 and 48% of the genotypes, respectively, whereas 37% had mixed genotypes. Subpopulation red mainly comprised of some French and Iranian lines whilst subpopulation green mostly consisted of some other French and Iranian as well as American genotypes.

To detect the possible association between retrotransposon-based markers and genomic regions responsible for resistance to BSR, association analysis was performed using GLM accounting for population structure (Q model), and MLM accounting for population structure and kinship relatedness (Q + K model) using TASSEL 2.1 software (Supplementary 1 and Table 4). The analyses identified the markers tightly linked to BSR resistance traits. Based on GLM model, 15 markers (loci) showed significant ($P \leq 0.01$) association with resistance to both fungal pathogens. One locus was found to be linked with partial resistance to each of the *S. minor* isolates A1 and M1 and, four loci were associated with G2 resistance. Furthermore, four and five loci showed association with *S. sclerotiorum* isolates A37 and J2 (Supplementary 1). According to these results, phenotypic variance explained by QTLs (R^2) ranged from 1 to 23% (Supplementary 1). In MLM model, 14 loci were identified to be significantly ($P \leq 0.01$) associated with resistance to BSR. This model detected one locus linked with partial resistance to each of the *S. minor* isolates A1 and M1 whereas three loci were associated with the isolate G2. Five and four loci were also found that showed association with resistance to 2 *S. sclerotiorum* isolates A37 and J2, respectively (Table 4).

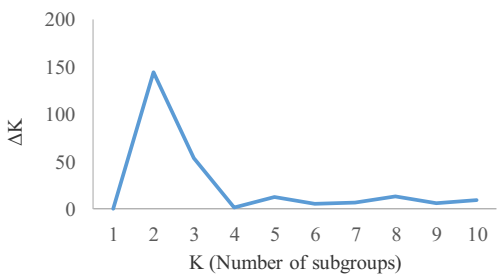

Fig. 1 Estimation of subpopulations number (K = 2) in the studied oilseed sunflower germplasm according to retrotransposon-based loci using Structure software

Discussion

In this study, the reaction of 100 oilseed sunflower lines with diverse genetic backgrounds and origins was evaluated against *S. sclerotiorum* and *S. minor* isolates under controlled conditions and association of IRAP and REMAP markers with partial resistance to the pathogens were analyzed using GLM and MLM procedures. Results indicated that the studied sunflower germplasm had high diversity in terms of fungal infection percentage. The three genotypes H156A/H543R, 110 and 8A×/LC1064C were resistant to both fungal pathogens and thus, had the highest spectrum of resistance to the pathogens. Furthermore, 8A×/LC1064C exhibited good levels of resistance to *S. sclerotiorum* isolate A37 and *S. minor* isolate M1 as less than 60% of necrosis was observed in this

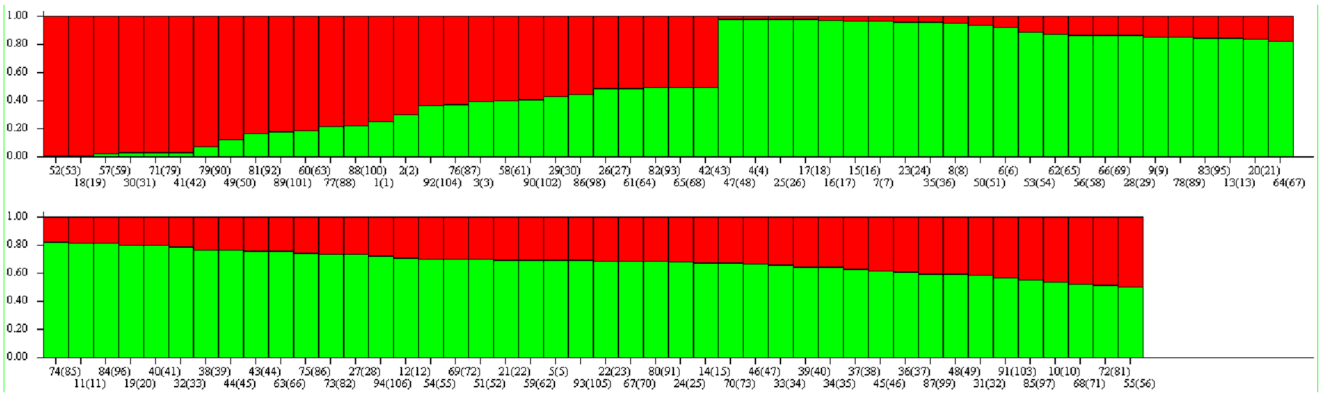

Fig. 2 Bar plot showing the membership of the studied oilseed sunflower germplasm according to retrotransposon-based loci estimated by Bayesian model. Each color shows one subpopulation or cluster. The numbers in the horizontal (Numbers between parenthesis) and vertical axes indicate the individual number (Table 1) and percentage of their membership in the groups, respectively

line. Association analyses identified several DNA markers that were linked with partial resistance to the five isolates of both pathogens.

In genetic studies, population structure is analyzed to provide insight into the genetic structure of populations and evolutionary relationships of individuals in a population (Breseghello and Sorrells 2006). Efficiency of association analysis is significantly influenced by population structure (Sorkheh et al. 2008) and, there should not be structure in the studied association panel (Breseghello and Sorrells 2006). It means that any association analysis without accounting for the effects of population structure may lead to detecting false positive associations between markers and traits (Basirnia et al. 2014). Therefore, knowledge on population structure as a prerequisite for association analysis can be used to avoid such false positive associations (Pritchard and Donnelly 2001). Recent developments in statistical methodologies made it possible to properly infer population structure (Sorkheh et al. 2008). Structure is a widely used software that by implementing Bayesian model divides the total population into subpopulations with different structures so that Hardy Weinberg equilibrium holds within the subpopulations and mixed genotypes can be recognized confidently (Dadras et al. 2014).

Table 4 Retrotransposon-based markers associated with resistance to basal stem rot caused by *Sclerotinia sclerotiorum* and *S. minor* isolates in the studied oilseed sunflower germplasm according to MLM

Fungal isolate	Molecular marker	MLM	
		F	*P*-value
S. minor			
A1	UF1	8.1833	0.0053
M1	CF-CR10	9.4455	0.0031
G2	UR1-UBC8405	8.0446	0.0058
	UF11	7.8311	0.0064
	CR6	7.5797	0.0072
S. sclerotiorum			
J1	LTR1061–658	7.1419	0.0092
J2	LTR1061-UBC8185	10.1696	0.002
	LTR1064–6511	7.7003	0.0069
	LTR1064-A1310	7.0333	0.0096
	LTR1063-UBC8261	7.0216	0.0096
A37	LTR1061-UBC8185	8.8837	0.0038
	LTR1061-UBC8186	9.3413	0.003
	LTR1061-UBC8572	9.6217	0.0026
	CF-UBC8265	7.3988	0.0079

MLM: Mixed linear model. P-value: Probability value

The two methods, GLM and MLM, have been proposed to test association between phenotypic traits and molecular markers although, MLM has generally been used for association analysis of disease resistance (Ghavami et al. 2011), abiotic stress (Saeed et al. 2014) and agro-morphological traits (Yu and Buckler 2006) in plants because this model reduces effectively the false positive associations of markers-traits. In present study, both models were employed to identify association between resistance and retrotransposon-based markers. The markers UF1, CF-CR10, UR1-UBC8405, UF11, CR6, LTR1061-UBC8185, LTR1064–6511, LTR1064-A1310 and LTR1063-UBC8261 showed significant association with resistance to the fungal isolates in both GLM and MLM. Based on GLM, CF-UBC818 with a high phenotypic variance (23%) was associated with resistance to A37 isolate. The markers UF, LTR1064-A13, LTR1061-UBC818 and LTR1064–65 were commonly associated with resistant to two fungal isolates that can result from the linkage or pleiotropic effects (Jun et al. 2008). These common markers can increase the efficiency of marker-aided selection in plant breeding programs via simultaneous selection for several traits (Tuberosa et al. 2002; Hittalmani et al. 2003).

In this study, a number of markers were found that had a significant association with resistance traits in a model but

were non-significant in the other model. These results can result from the difference in model parameters and statistical power of the models. For instance, in MLM both population structure and kinship among individuals is incorporated to identify genetic-phenotype relationship but in GLM, kinship is not considered. Most of the genotypes studied in the present study were from French institutions and to a lesser extent from other countries, and it is likely that at least a number of these lines share similar genetic background and are derived from a limited number of genotypes. Therefore, MLM is considered statistically more powerful and is more effective in adjusting association tests on markers (Pritchard et al. 2000; Yu and Buckler 2006; Yu et al. 2006). Indeed, the results of MLM appear to be more valid and reliable than GLM and the markers identified by MLM can be used more confidently in marker-aided programs.

Several studies have used various molecular markers to identify QTLs for resistance to BSR in biparental populations of sunflower explaining various levels of phenotypic variance (For instance: Micic et al. 2004; Micic et al. 2005a, b; Rönicke et al. 2005; Yue et al. 2008; Amoozadeh et al. 2015). In recent years association mapping have been utilized to dissect disease resistance QTLs and to localize genes involved in resistance to *Sclerotinia* in a diverse of sunflower genotypes (Fusari et al. 2012; Talukder et al. 2014). In all of these genetic researches, QTLs for resistance to *S. sclerotinia* have been studied and no information available on the QTLs conferring resistance to *S. minor* in sunflower. The present study, reports QTLs for resistance to both *Sclerotinia* species and retrotransposon-based markers linked to the traits in different sunflower genotypes. These association panel and molecular markers have already been employed to localize QTLs for agro-morphological traits in sunflower (Jannatdoust et al. 2015; Sahranavard et al. 2015; Darvishzadeh 2016). Therefore, IRAP and PEMAP markers can be effectively used in marker-trait association studies to identify QTLs involved in BSR resistance or other polygenic traits in sunflower.

Conclusion

Results of this study showed that the studied sunflower germplasm had high diversity for resistance to BSR caused by *S. sclerotiurm* and *S. minor*. The minimum and maximum value of necrosis on the lines was 30.67 and 100%, respectively. The genotypes H156A/H543R, 110 and 8A×/LC1064C exhibited resistant to two fungal isolates of *Sclerotinia* spp. The genotype 8A×/LC1064C showed the lowest disease infection percentage in responses to the isolates of both *Sclerotinia* species and thus, is a good candidate for use in BSR breeding programs. Population structure analysis using 248 retrotransposon-based loci subdivided the genotypes into two subpopulations (K = 2). Association analysis using GLM and MLM identified 15 and 14 loci, respectively, that were significantly ($P \leq 0.01$) associated with BSR resistance traits in the sunflower genotypes. The phenotypic variance explained by QTLs (R^2) ranged from 1 to 23%. The results also confirmed the efficiency of MLM model for association mapping. The analyses revealed that the markers UF1, LTR1064-A13, LTR1061-UBC818 and LTR1064–65 were commonly associated with resistance to more than one fungal isolate. Therefore, these common markers can be useful in sunflower breeding programs to select for resistance to BSR and to develop sunflower cultivars with broader spectrum of resistance to the disease. Our study also provided preliminary evidence that retrotransposon-based markers can be effective in association analysis for BSR resistance in sunflower. The identified QTLs and their associated markers have a potential for use in breeding programs although we believe that further studies are required to evaluate the effectiveness of these QTLs against additional fungal isolates and to confirm their performance in the field.

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