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Analyses of genomic regions linked with resistance to basal stem rot in sunflower (*Helianthus annuus* L.) under field conditions

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Abstract

Sunflower (*Helianthus annuus* L.) is one of the four important sources of edible oil in the world. Fungal diseases are considered as major constraints for its seed yield and quality. Basal stem rot resulted by *Sclerotinia sclerotiorum* (Lib.) de Bary fungus, is known as a serious disease on oily sunflower, worldwide. In this project, genomic region linked with partial resistance to basal stem rot disease was identified using a population of recombinant inbred lines (RILs) created from the hybridization between PAC2 (♀) and RHA266 (♂) lines. Nine phenotypic characters related to disease resistance including PN4D, PN8D, PN12D, NCW100S, CW100S, NCPY, CPY, DP100S and DPY were measured under artificial infection in the field conditions. Newly developed genetic linkage map of sunflower was used for detecting and mapping QTLs. The linkage map includes 210 SSR and 11 SNP markers distributed in 17 groups. The analysis was carried out using composite interval mapping (CIM) procedure. High coefficient of variation (CV) was detected for those studied characters that reveal high genetic variability for susceptibility to disease in the studied sunflower RIL population. Totally, 56 putative QTLs were identified for the studied nine quantitative characters. The number of QTLs for each character ranged from 1 to 9, explaining 0.91 to 80.75% of phenotypic variation (R^2). Additive effect sign was positive for 17 QTLs, suggesting that the promising allele has been transmitted from male parent (RHA266). In this project, major QTLs (LOD≥2.5 and R^2≥10%) were identified for all of the studied characters, exceptifor NCW100S and CW100S characters. The major QTLs are important for running marker-aided selection (MAS) in resistant breeding programs.

***Key words*:** Linkage mapping, Oily sunflower, Partial resistance, QTL mapping, *Sclerotinia sclerotiorum*.

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is a diploid plant with 2n=2x=34 chromosomes. It is one of the four important sources of edible oil in the world. The plant was domesticated from wild ancestors about four thousand years ago by early American farmers as a source of consumable oil and for nonfood impetus (e.g., for coloring the textiles) (Soleri *et al.,* 1993). The farmers cultivate the plant either for oilconsumption or confectionery purposes. Although, the main type cultivated is the oil type, the market and production area of confectionery sunflower has augmented in the recent years (Seiler and Jan, 2014).

Fungal diseases are considered as major constraints for seed yield and quality of sunflower. The necrotrophic pathogen *Sclerotinia sclerotiorum* (Lib) de Barry is a fungus with worldwide distribution and wide range of

hosts (>400) mainly broadleaf plant species including sunflower (Filippi *et al.,* 2020), soybean (Mbedzi *et al.,* 2019) and rapeseed (Qasim *et al.,* 2020). Mainly three types of diseases are caused on sunflower by *S. Sclerotiorum* that include basal stem rot, mid-stalk rot, and head rot. Meanwhile, basal stem rot and head rot are more seen on sunflower in humid temperate and tropical and subtropical areas of the glob (Gulya *et al.,* 1997). Depending on environmental states, Sclerotia infects host roots when it germinates myceliogenically or it infects aerial parts of host plants when it germinates carpogenically (Gulya *et al.,* 1997; Bolton *et al.,* 2006). In the last situation, Sclerotia produce apothecia and then ascospores (Gulya *et al.,* 1997; Bolton *et al.,* 2006). The fungus causes average yield reductions of 10 to 20%. However, under severe infection and favorable climatic conditions and without any efficient means of chemical control of Sclerotinia attack, it results in yield loss up to 100% (Pereyra and Escande, 1994; Van-Becelaere and Miller, 2004). Introduction of DNA-based molecular markers and development of linkage map technology have provided appropriate tools for understanding the genetic bases of resistance to diseases. Till today, several genetic maps have been established using various DNA-based markers, including RAPD, RFLP, SSR and SNP in cultivated sunflower (Kane *et al.,* 2011; Talukder *et al.,* 2020). Polygenic inheritance has been found for resistance against *S. sclerotiorum* in various genetic research programs (Micic *et al.,* 2004; Micic *et al.,* 2005a; Micic *et al.,* 2005b; Fusari *et al.,* 2012; Amouzadeh *et al.,* 2013, 2015; Talukder *et al.,* 2014a; Talukder *et al.,* 2016; Filippi *et al.,* 2020). Identification of genetically linked DNA-markers to genomic region associated with resistance is important for pyramiding appropriate alleles into common genetic context by selection via identified markers (MAS) (Laroche *et al.,* 2019), conducting map-based cloning activities (Jaganathan *et al.,* 2020) and elucidating the genetics basis of complex characters (Ibrahim *et al.,* 2020).

Resistance to basal stem rot disease is genetically complex and governed by several small effect genes (Davar *et al.,* 2010; Amouzadeh *et al.,* 2013, 2015; Talukder *et al.,* 2014b; Talukder *et al.,* 2016; Najafzadeh *et al.,* 2018). There are a few reports on the identification of genomic regions involved in resistance to Sclerotinia basal stem rot in oily sunflower under field conditions. Davar *et al.* (2010), detected 7 QTLs for resistance to Sclerotinia disease severity in sunflower on linkage groups (LGs) 1, 2, 4, 6, 8, 14, and 17 by inoculating the recombinant inbred lines (RILs) population using a moderately aggressive isolate of *S. sclerotiorum*, SSU107 in controlled conditions. In another research work, Amouzadeh *et al.* (2013), mapped 5 QTLs for resistance to Sclerotinia disease severity on LGs 1, 3, 8, 10, and 17 by inoculating the same RILs population using other moderately aggressive isolates of fungal agent; SSKH41. The explained phenotypic variations by the identified QTLs in the two above mentioned studies were small and varied between 0.5 to 8 percent. Any QTL with major effect, till now, has not been detected for resistance against *S. sclerotiorum* rot diseases in oil seed sunflower. However, statistically significant variations in susceptibility to basal stem rot have been observed during field screening of diverse germplasm collections (Talukder *et al.,* 2014b; Seiler *et al.,* 2017; Delgado *et al.,* 2020).

In West Azarbaijan province of Iran, the basal stem rot is one of the major threats for the development of sunflower cultivation, due to favorable weather conditions for the disease spread. This region is known as hotspot for basal stem rot epidemy. Using sunflower genotypes with high resistance to disease in corporation with suitable crop operations is an effective procedure to control the threat. So, the main aims of our study were to dissect the genetic control of resistance against *S. sclerotiorum* in oilseed sunflower under field conditions and approximate the number, genomic locations and effects of QTLs implicated in the resistance reaction.

MATERIALS AND METHODS

Plant population and field experiment

A collection of 98 RILs obtained from the hybridization between PAC2 (♀) and RHA266 (♂) oilseed sunflower lines, was kindly supplied by the National Institute of Agronomy Research (INRA) of France. The pedigree of the parental lines was presented in detail by Gentzbittel *et al.* (1995). Both paternal and maternal lines were partially resistant to Sclerotinia rot disease (Poormohammad Kiani *et al.,* 2007). However, the RHA266 was more tolerant to disease than PAC2 (Poormohammad Kiani *et al.,* 2007). Resistance reaction of RILs and two parental lines against basal stem rot was evaluated in 2015 under field condition in Vaghaslo Village located in Urmia in the northwest of Iran. Experiments were carried out in the frame with 10×10 simple lattice layout, with two replicates. Each plot comprised 10 plants on one row with 1.25 m long. The space between rows was considered 0.65 m. For guarantee the infection process, about 5 plants of each genotype were artificially inoculated per

replication, by A107 isolate of *S. sclerotiorum*. The fungal isolate was collected from naturally infected sunflower plants at Vaghaslo Village. The potato dextrose agar (PDA 42 g L^{-1}, pH=6) medium was used for culturing the fungal isolate. The isolate was grown in the dark at room temperature (25±1 °C). Mycelia containing disks with 3 mm diameter from the growing area of the colonies were prepared (two day-old on the medium) and were inoculated on the base of stem of plants. The disks containing mycelia were covered by wet cotton and Parafilm for 48 hours (Davar *et al.,* 2010). Parafilm retains moisture for fungal growth. Four, eight and twelve days post inoculation, the necrotic areas on one cm of the stem base of the inoculated plants were visually evaluated in the scale of zero (no symptoms) to 100 percent (totally necrotic). The sunflower plants were infected in the time of V6-V8 from developmental stages (Schneiter and Miller, 1981). V6-V8 correspond to developmental stages in sunflower plants with at least 6 to 8 leaves. These are determined by counting the number of true leaves at least 4 cm in length. Weeds were mechanically removed during the experiment and 100 kg urea per hectare was applied at the time of V8 from developmental stages and irrigation was done immediately. For preventing the damage from sparrows during the seed filling stage, the sunflower heads were coated by white color paper covers. Nine morphological characters including percentage of necrotic area after 4 days (PN4D), percentage of necrotic area after 8 days (PN8D), percentage of necrotic area after 12 days (PN12D), weight of 100 seeds in non-contaminated plants (NCW100S), weight of 100 seeds in contaminated plants (CW100S), plant yield in non-contaminated plants (NCPY), plant yield (CPY) in contaminated plants, 100 seeds weight loss (DP100S), per plant yield loss (DPY) were measured at the maturity stage.

Field data analysis

Normality distribution of errors was tested by Univariate command in the SAS 9.4 package (SAS Institute Inc., Cary, N.C.). Analysis of variance was carried out with General Linear Model command by SAS.

Genetic linkage map and QTL mapping

Newly developed genetic linkage map of sunflower was used for detecting and mapping QTLs (Haddadi *et al.,* 2012; Amouzadeh *et al.,* 2015). The analysis was carried out using composite interval mapping (CIM) procedure in Version 2.5 of Windows QTL Cartographer package (Wang *et al.,* 2005). LOD score (the ratio of Log10 likelihood; Likelihood that the outcome results via linkage to likelihood that the outcome results via chance) of 2.5 identified by test of permutation (n=1,000) (Doerge *et al.,* 1996) was used. The sunflower genome was checked at 2 cM intervals with a size of window of 15 cM, till 15 background markers as cofactors were incorporated in the CIM. The phenotypic variation (R^2) described by each QTL was approximated at the peak of the LOD graph by Windows QTL Cartographer. The QTL locations on the linkage map were drawn by using the software package MapChart version 2.2 (Voorrips, 2002).

The linkage map includes 210 SSR and 11 SNP markers distributed in 17 groups. Some candidate genes including drought inducible genes, genes related to tocopherol and phytosterol pathways, genes related to enzymatic antioxidants and Arabidopsis Sec14 homologue genes have been previously mapped in novel version of linkage map (Haddadi *et al.,* 2012; Amouzadeh *et al.,* 2015). Linkage groups have been specified with 1 to 17 in agreement with sunflower reference linkage map (Tang *et al.,* 2002). Length of linkage groups ranged from 8.2 to 127.1 cM that cover a total map length of 1653.1 cM with a mean interval distance of 7.44 cM between markers.

RESULTS

Phenotypic analysis

The majority of plants reveal Scleronia rot disease symptoms on the basal stem four days after inoculation. The mean of infection rate was 93%. The fungal growth on RHA266 line was 21.81 percent, 4 days-post inoculation and reached 58.19 percent 12 days-post inoculation; while in PAC2 line, the fungal growth was 41.25 percent, 4 days-post inoculation and reached 100.00 percent, 12 days post inoculation. In the other words, the speed of fungal growth on RHA266 line was slower than PAC2. Considering other morphological characteristics, the RHA266 had a better situation in comparison with PAC2 (Table 1). These results justify its higher tolerance compared to PAC2. High coefficient of variation (CV) revealed a high genetic variability for susceptibility to disease in the studied sunflower RIL population (Table 1). Traits with high coefficients of variation have a better chance for selection. Maximum and minimum values of genetic gain were observed for NCPY and NCV100S (Table 1). Genetic gain is calculated as the difference between 10% of best RILs with mean of the parents. Concerning the necrotic area percentage, the maximum and minimum genetic gains were detected in PN12D (-42.95) and PN4D (-23.26), respectively (Table 1).

Table 1. Statistical parameters of characters related with resistance to *S. sclerotiorum* in sunflower RIL population under field conditions.

Item	Characters (%)								
	PN4D	PN8D	PN12D	NCW100S	CW100S	NCPY	CPY	DP100S	DPY
PAC2 (P1)	41.25	82.50	100.00	3.25	2.35	8.75	5.05	27.46	41.17
RHA266 (P2)	21.81	25.42	58.19	4.47	4.46	28.53	18.39	1.89	31.24
$\bar{X}_P$	31.53	53.96	79.10	3.86	3.40	18.64	11.72	14.67	36.20
$\bar{X}_{RILs}$	22.84	48.27	74.72	5.74	5.02	26.83	20.64	16.40	25.91
$\bar{X}_{RILs}$-$\bar{X}_P$	-8.7	-5.69	-4.38	1.88	1.62	8.19	8.92	1.73	-10.29
$\bar{X}_{10\% \text{ best RILs}}$-$\bar{X}_P$	-23.26	-37.8	-42.95	5.15	4.43	43.65	35.72	-12.24	-33.71
GG10%=$\bar{X}_{10\% \text{ best RILs}}$-$\bar{X}_P$									
Coefficient of variation	43.92	42.73	23.14	34.65	33.24	65.44	61.49	74.71	69.11

$\bar{X}_P$: Mean of parents, $\bar{X}_{RILs}$: Mean of recombinant inbred lines, $\bar{X}_{10\% \text{ best RILs}}$: Mean of the 10% of selected recombinant inbred lines, GG10%: Genetic gain when the mean of 10% of selected recombinant inbred lines are compared with the mean of parents, PN4D: Percentage Necrotic area after 4 days, PN8D: Percentage Necrotic area after 8 days, PN12D: Percentage Necrotic area after 12 days, NCW100S: Non-Contaminated Weight of 100 Seeds, CW100S: Contaminated Weight of 100 Seeds, NCPY: Non-Contaminated Plant Yield, CPY: Contaminated Plant Yield, DP100S: Decreased Performance of 100 Seeds, DPY: Decreased Yield per Plant, CV: Coefficient of variation, RILs: Recombinant inbred lines, P1: Maternal line, P2: Paternal line.

Mapping of QTLs

The map location and characteristics of the identified QTLs for the studied characters are presented in Table 2 and Figure 1. QTL names were coined using the character abbreviations appended with the map and the characteristics of QTLs associated with the studied characters. Totally, using composite interval mapping (CIM), 56 putative QTLs were identified for nine studied characters (PN4D, PN8D, PN12D, NCW100S, CW100S, NCPY, CPY, DP100S and DPY) in the studied sunflower population (Table 2). The QTLs related to various characters mapped throughout the sunflower genome except for LGs 6, 7 and 13 (Figure 1). Among the identified loci, 5 QTLs (8.92%) with favorable effects were related to disease severity associated traits including PN4D, PN8D and PN12D (Table 2). In detail, 2, 1 and 2 QTLs were identified for PN4D, PN8D and PN12D on linkage groups 11, 14 and 15, respectively. QTLs identified for PN4D possessed 14.30% and 1.85% of the phenotypic variation of character R^2. Concerning the QTL identified for PN8D, R^2 was 8.8%. R^2 values of QTLs detected for PN12D were 21.9 (PN12D-15.1) and 37.3 (PN12D-15.2). In the identified QTLs, the positive sign of additive effects shows that promising allele came from RHA266 (paternal line).

QTLs controlling other morphological characters were located on LGs 1, 2, 3, 4, 5, 8, 9, 10, 11, 12, 14, 15, 16 and 17 (Table 2 and Figure 1). Six QTLs were detected for NCPY distributed on LGs 15, 4, 1, 14 and 16. The identified QTLs explained 1.11 to 10.24 percent of the phenotypic variation of character. Based on the sign of the identified QTLs, the promising alleles were transmitted from the maternal line (PAC2) to descendants. Seven QTLs were identified for DPY on LGs 12, 3, 16, 17 and 9, accounting for 1.43 to 11.13 percent of the phenotypic variation of character.Based on the sign of additive effect of identified QTLs, the promising alleles were transmitted from both paternal and maternal lines. Five QTLs were identified for NCW100S on LGs 16, 17, 8, 10 and 14, accounting for 1.66 to 0.91 percent of the phenotypic variation of character. Based on the sign of additive effect of identified QTLs, one promising allele comes from the maternal line (PAC2) and three alleles out of four come from the male parent (RHA266). Five QTLs were found for CW100S, on LGs 14, 16, 17 and 10, that account for 1.04 to9.89 percent of phenotypic variations and the promising allele comes from the female parent (PAC2). Concerning DP100S, the identified QTLs explained 3.42 to 78 percent of the phenotypic variation of the character and the promising alleles were originated from both paternal and maternal lines. Among 56 putative QTLs, 19 QTLs were detected for plant yield in contaminated plants (CPY) and 9 QTLs for 100 seeds weight loss (DP100S). About CPY, the explained phenotypic variation by the detected QTLs varied between 3.25% and 80.75% (CPY-11.3) and the promising alleles were transmitted from both parental lines to descendants.

Table 2. Map location and effect of QTLs detected for studied characteristics in sunflower RILs population.

Traits	QTLs	LG	Position (cM)	LOD	Additive	R^2 (%)	Traits	QTLs	LG	Position (cM)	LOD	Additive	R^2 (%)
PN4D	PN4D-11.1	11	15.01	3.16	-13.98	14.30		DP100S-17.3	17	0.01	6.48	-2.293	13.77
	PN4D-14.1	14	39.01	2.85	0.50	1.850		DP100S-12.2	12	8.01	3.80	-2.738	10.40
PN8D	PN8D-11.2	11	32.01	3.68	-9.26	8.800		DP100S-5.3	5	28.01	3.89	-2.454	4.412
PN12D	PN12D-15.1	15	29.01	4.32	-15.68	21.90		DP100S-8.3	8	31.01	2.50	1.939	3.420
	PN12D-15.2	15	41.01	5.72	-13.67	37.30	DP100S	DP100S-2.3	2	43.01	2.84	11.93	78.00
NCPY	NCPY-15.3	15	1.01	5.46	-4.124	5.636		DP100S-14.6	14	59.01	4.29	-3.538	6.800
	NCPY-4.1	4	11.01	2.76	-2.241	6.482		DP100S-14.7	14	68.01	5.00	-2.415	7.397
	NCPY-15.4	15	12.01	3.19	-2.689	3.634		DP100S-17.4	17	74.01	2.91	0.307	8.739
	NCPY-1.1	1	17.01	3.91	-0.943	1.325		DP100S-15.5	15	86.01	4.77	1.965	4.668
	NCPY-14.4	14	68.01	2.79	-8.382	10.24		CPY-10.4	10	1.01	3.95	-1.774	6.041
	NCPY-16.3	16	69.01	4.86	-0.346	1.116		CPY-16.4	16	6.01	5.05	0.076	4.268
DPY	DPY-12.3	12	10.01	2.84	-4.117	7.719		CPY-5.1	5	8.01	6.31	-1.804	14.31
	DPY-3.1	3	15.01	3.45	-4.354	11.13		CPY-5.2	5	15.01	4.38	-11.71	11.31
	DPY-3.2	3	42.01	2.52	-4.662	5.117		CPY-11.3	11	20.01	2.53	12.02	80.75
	DPY-16.8	16	52.01	2.76	-2.298	1.433		CPY-16.5	16	21.01	3.40	-0.207	3.303
	DPY-17.5	17	59.01	3.11	3.937	3.748		CPY-10.5	10	22.01	6.24	-3.170	20.68
	DPY-12.3	12	61.01	3.31	-2.468	3.747		CPY-10.6	10	28.01	3.53	-2.478	9.237
	DPY-9.1	9	69.01	2.51	3.054	2.236		CPY-10.7	10	34.01	3.39	-1.276	6.498
NCW100S	NCW100S-16.1	16	1.010	6.36	0.235	1.537	CPY	CPY-12.1	12	38.01	4.20	1.663	3.988
	NCW100S-17.1	17	10.01	4.99	-0.080	1.668		CPY-11.4	11	44.01	3.45	2.305	5.629
	NCW100S-8.1	8	35.21	3.33	0.031	0.916		CPY-1.2	1	47.01	3.82	-0.454	4.747
	NCW100S-10.1	10	42.01	5.14	0.896	9.720		CPY-10.8	10	49.01	3.29	-1.325	5.483
	NCW100S-14.2	14	71.01	7.04	0.549	1.099		CPY-8.2	8	57.01	4.15	-1.917	4.889
CW100S	CW100S-14.3	14	0.01	12.56	-0.091	1.619		CPY-14.5	14	69.01	5.06	-1.521	8.792
	CW100S-16.2	16	1.01	12.05	-0.528	9.898		CPY-2.1	2	84.01	4.22	0.267	3.254
	CW100S-17.2	17	10.01	10.01	-0.046	1.046		CPY-2.2	2	94.01	4.39	-0.527	3.267
	CW100S-10.2	10	55.01	12.27	-0.206	1.397		CPY-16.6	16	96.01	3.20	-0.621	4.844
	CW100S-10.3	10	65.01	9.09	-0.060	1.932		CPY-16.7	16	104.01	5.78	0.011	6.044

cM: centi Morgan, LG: Linkage Group, CPY: Contaminated Plant Yield, CW100S: Contaminated Weight of 100 Seeds, DP100S: Decreased Performance of 100 Seeds, DPY, Decreased Yield per Plant, LOD: Log10 likelihood ratio (likelihood that the effect occurs by linkage/likelihood that the effect occurs by chance), NCPY: Non- Contaminated Plant Yield, NCW100S: Non-Contaminated Weight of 100 Seeds, PN4D: Percentage Necrotic area after 4 days, PN8D: Percentage Necrotic area after 8 days, PN12D: Percentage Necrotic area after 12 days, QTL: Quantitative Trait Loci, R^2: Percentage of phenotypic variance explained by the individual QTLs. The positive additive effect shows that PAC2 allele increase the trait and negative value shows that RHA266 allele increases the trait. Ns: non-significant, *, **: significant at 0.05, 0.01 probability level. QTLs with LOD≥2.5 and R^2≥10% are considered major QTLs.

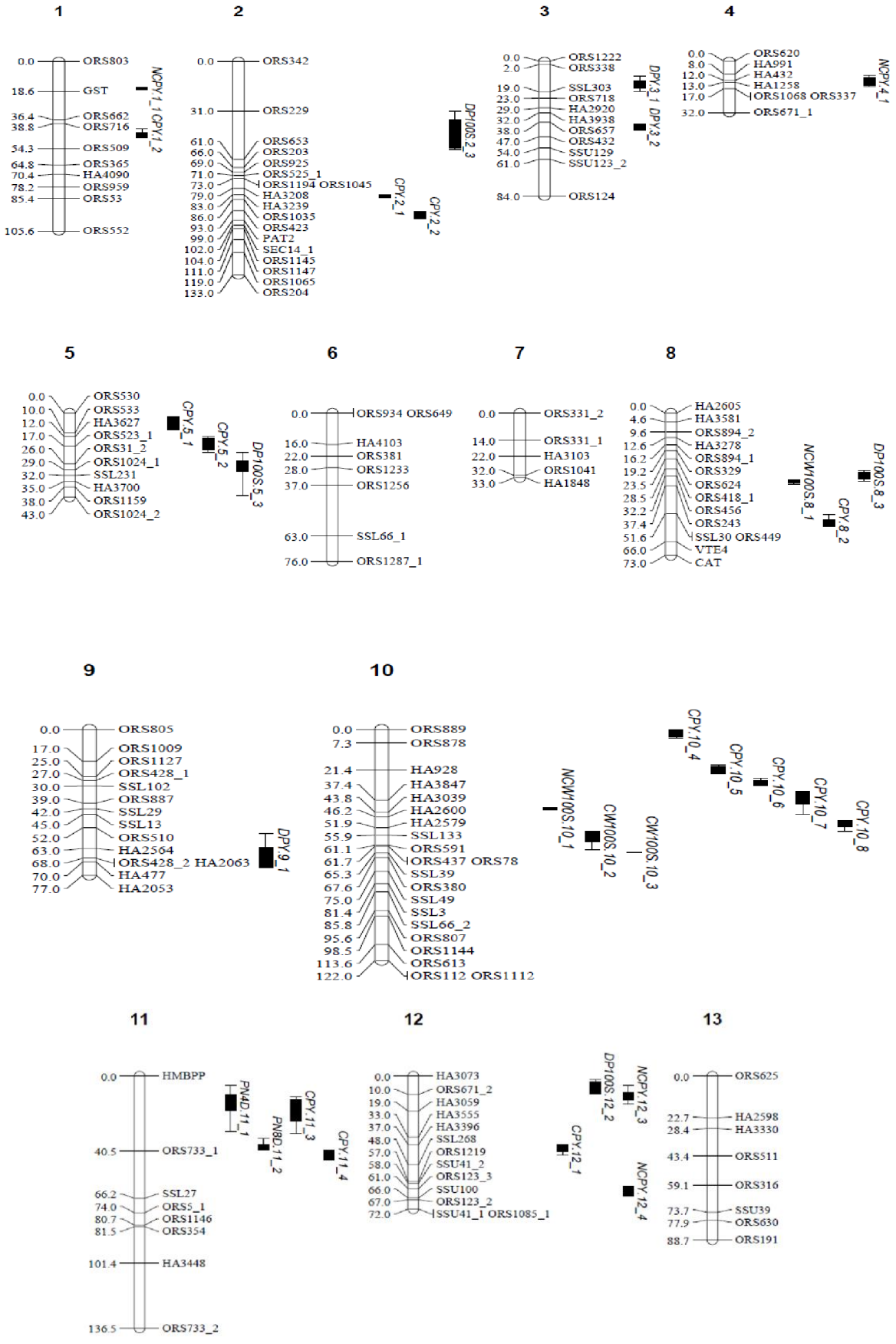

Figure 1. Genetic linkage map for sunflower based on 210 SSR and 11 SNP markers. The locations of the QTLs are presented on the right side of the linkage groups. Bars represent intervals associated with the QTLs.

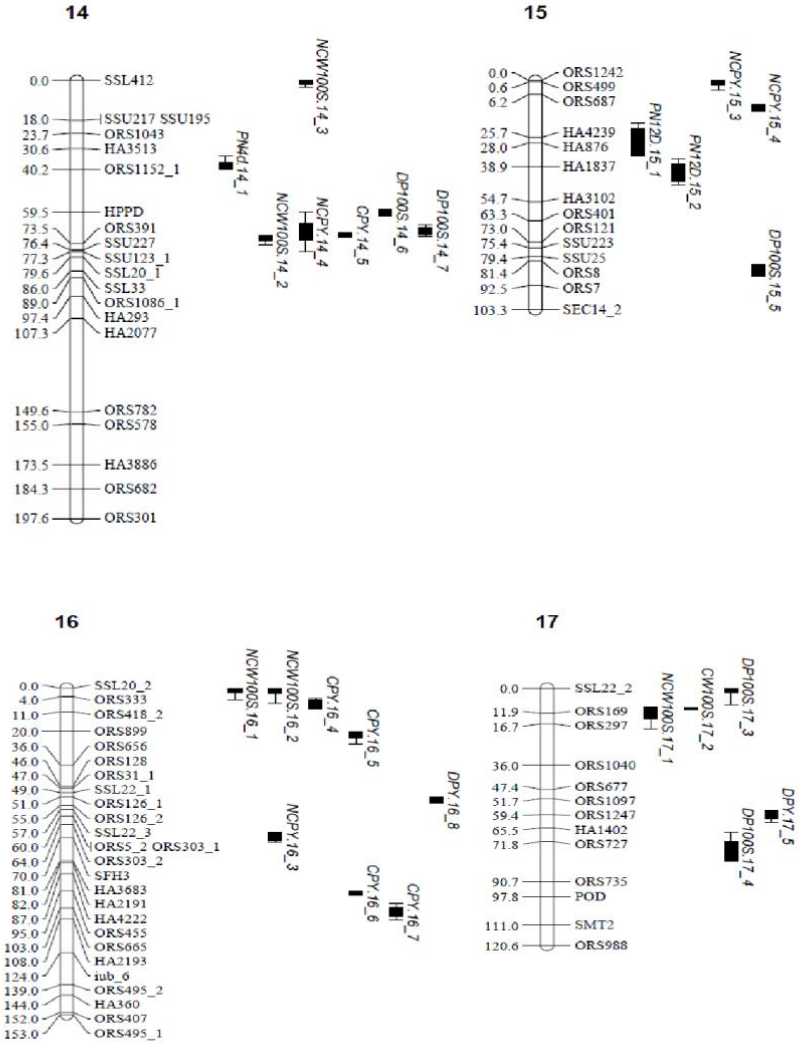

Figure 1 (Continued). Genetic linkage map for sunflower based on 210 SSR and 11 SNP markers. The locations of the QTLs are presented on the right side of the linkage groups. Bars represent intervals associated with the QTLs.

DISCUSSION

Four days post mechanical inoculation, most of the inoculated plants revealed Sclerotinia rot disease symptoms on the base of their stem. A wide range of variation was observed among genotypes for studied characters. In various studies, significant genetic variabilities have been detectedfor resistance against *S. sclerotiorum* (Micic *et al.,* 2002; Talukder *et al.,* 2014b; Seiler *et al.,* 2017; Delgado *et al.,* 2020). Transgressive segregation, as a result of accumulation of promising alleles from both paternal and maternal lines, have been observed for most of the studied characters in sunflower. Genetic gain was noticeable for all studied characters (Table 2) implying the polygenic nature of studied characters in sunflower. Paralleled with these findings, Mestries *et al.* (1998) and Davar *et al.* (2010) reported polygenic, with medium heritability for resistance to *S. sclerotiorum*.

Little is known about the QTL for resistance to *S. sclerotiorum* in sunflower (Talukder *et al.,* 2016). About 56 QTLs affecting nine characters (PN4D, PN8D, PN12D, NCW100S, CW100S, NCPY, CPY, DP100S and DPY) were detected using RILs population on different LGs except for 6, 7 and 13 groups. Four out of 56 QTLs corresponding to three characters explained >20% of the phenotypic variation (Table 2, Figure 1). The +/- signs of additive effect in detected QTLs, indicated the donation of both maternal and paternal lines and justify the transgressive segregation observed at the level of phenotype.

Regarding the literature, Davar *et al.* (2010), identified seven genomic regions associated with resistance to Sclerotina basal stem rot on the LGs 1, 2, 4, 6, 8, 14 and 17, by using a collection of RILs. These

are matched with LGs 1, 8 and 17 in the present study. Bert *et al.* (2002) detected ten genomic regions involved with head rot resistance. Three out of ten QTLs were mapped on LG 7, two on LG 8, and the remaining five were mapped on LGs 3, 5, 6, 10, and 13 that explain 9 to 20 percent of the phenotypic variation of the trait. In another study, Bert *et al.* (2004) detected five genomic regions involved in head rot resistance. They mapped two out of five QTLs on LG 9 and mapped three on LGs 6, 7, and 17 that explain 2.5 to 19.5 percent of phenotypic variation of attack percentage or index of latency. Rönicke *et al.* (2004), identified and mapped two genomic regions involved in resistance to head rot on LGs 1 and 10 that each one explains 17.1 and 10.6 percent of the phenotypic variation of the trait, respectively. Zubrzycki *et al.* (2012), detected 20 genomic regions associated with disease index (DI), disease severity (DS), and the area under the disease progress curve on LGs 10, 13, and 14. However, in some previous investigations, major effect QTLs that affect stem lesion have been detected and mapped on LGs 8 and 10 (Micic *et al.,* 2004; Micic *et al.,* 2005b). In these investigations the QTLs mapped on LG 8 explained 36.7 and 25.5 percent of the phenotypic variation, respectively and the QTL mapped on LG 10 explained 24.0 percent of the phenotypic variation (Micic *et al.,* 2004; Micic *et al.,* 2005a).

No high-resistant genotypes against Sclerotinia rot disease are available in elite sunflower germplasm (Talukder *et al.,* 2014b) and thus producing resistant genotypes is a main goal in sunflower breeding activities (Delgado *et al.,* 2020). In this research, some QTLs were identified associated with the appearance of more than one character. For instance, DP100S.8-3 (LOD=2.50) and NCW100S.8-1 (LOD=3.33) were co-localized on LG 8 at 23.50 and 28.50 cM distances from the top of the linkage group, associated with the weight of 100 seeds in non-contaminated plants (NCW100S) and 100 seeds weight loss (DP100S); NCW100S.14-2 (LOD=7.04), NCPY.14-4 (LOD=2.79), CPY.14-5 (LOD=5.06) and DP100S.pna.14-7 (LOD=5.00) were co-localized on LG 14 at 73.50-76.40 cM interval associated with the weight of 100 seeds in non-contaminated plants (NCW100S), plant yield in non-contaminated plants (NCPY), plant yield (CPY) in contaminated plants and 100 seeds weight loss (DP100S). PN4D.11-1 (LOD=3.16), PN8D.11-2 (LOD=3.68) and CPY.11-3 (LOD=2.53) were co-located on LG 11 at 0.00 and 40.05 cM were associated with the percentage of necrotic area after 4 days and 100 seeds weight loss (DP100S). QTLs: NCW100S.16-1 (LOD=6.36) and CW100S.16-2 (LOD=12.05) were co-localized on LG 16 at 0.00- 4.00 cM that were associated with per plant yield loss (DPY) and the weight of 100 seeds in non-contaminated plants (NCW100S). QTLs: NCW100S.17-1 (LOD=4.99) and CW100S.17-2 (LOD=10.01) were co-localized on LG 17 at 11.90-16.70 cM associated with per plant yield loss (DPY) and weight of 100 seeds in contaminated plants (CW100S) (Table 2; Figure 1). The co-localization of QTLs for various characters show the existence of pleiotropic phenomena or the closely linkage of QTLs. Common QTLs significantly augment the effectiveness of selection in plant breeding tries (Tuberosa *et al.,* 2002; Hittalmani *et al.,* 2003; Xu *et al.,* 2017). Previous QTL mapping studies (Mestries *et al.,* 1998; Bert *et al.,* 2002) characterized different genomic regions responsible for resistance against stem and head rot. Considerable works on quantitative resistance have been carried out, particularly in France and Argentina engaging with head rot (Vear *et al.,* 2010; Fusari *et al.,* 2012; Filippi *et al.,* 2020) and in the USA concerning stalk or root rot (Talukder *et al.,* 2014b; Talukder *et al.,* 2016).

Totally, 56 putative QTLs were identified for the studied nine PN4D, PN8D, PN12D, NCW100S, CW100S, NCPY, CPY, DP100S and DPY quantitative characters. The number of QTLs for each character ranged from 1 to 9, explaining 0.91 to 80.75% of phenotypic variation (R^2). Additive effect sign was positive for 17 QTLs, suggesting that the promising allele has been transmitted from male parent (RHA266). Major QTLs (LOD≥2.5 and R2≥10%) were identified for all of the studied characters, except for NCW100S, CW100S characters. To sum up, improving resistance in cultivars against Sclerotinia rot disease is a main goal in sunflower breeding programs especially in warm-tropical and humid area of the world. The identification of loci influencing disease resistance has the potential to facilitate the manipulation of plant architecture. This activity could enable breeders to develop improved cultivars with increased disease resistance using marker-aided selection.

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