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ORIGINAL ARTICLE

Inheritance and molecular mapping of powdery mildew (*Golovinomyces orontii*) resistance gene(s) in sunflower (*Helianthus annuus* L.)

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Abstract
Sources of resistance to powdery mildew incited by *Golovinomyces orontii* have been identified in wild sunflowers and few exotic lines. The present investigation has been undertaken to study the inheritance of powdery mildew resistance and to map the quantitative trait loci (QTLs) governing resistance to powdery mildew in a multiple disease resistance line, TX16R (PI 642072). The inheritance was observed as a continuous distribution in a set of 264 F_2 population and 93 recombinant inbred lines (RILs) of a cross between a highly susceptible accession PS 2023 and TX16R. Screening of the two population sets was done with 484 sunflower-specific SSR primers of which 175 primers showed polymorphism between the parents. Based on the phenotyping and genotyping data, the linkage map was constructed with 93 RILs. The map spanned 1200 cM and included 64 markers distributed along the 17 sunflower chromosomes in the haploid set. Quantitative trait loci (QTL) analysis identified three genomic regions for resistance to powdery mildew, two of which mapped on chromosome 10 and one on chromosome 5. This is the first report on mapping of powdery mildew resistance in sunflower and paves the way in fine mapping and introgression of resistance for powdery mildew in sunflower through marker-assisted breeding.

Keywords *G. orontii* · Powdery mildew · Quantitative trait loci · Sunflower

Introduction

Sunflower is one of the major sources of vegetable oil in the world with an area of 26.66 million hectares and production of 51.95 million tons. The major constraint for profitable production of sunflower across the globe including India is the vulnerability of the improved cultivars to biotic and abiotic stresses throughout the crop growth stages. The important diseases that cause significant yield losses in sunflower are downy mildew [*Plasmopara halstedii* (Farl.) Berl. & De Toni], *Sclerotinia sclerotiorum* (Lib.) de Bary (Gulya et al. 2013), rust (*Puccinia helianthi* Schwein.) (Shtienberg and Zohar 1992), chlorotic mottle virus (SuCMoV) (Lenardon et al. 2001), sunflower necrosis (SND) (Bhat and Reddy 2016), *Alternaria* leaf spot [*Alternariaster helianthi* (Hansf.) Tub. and Nish.] (Carson 1985), powdery mildew (*Golovinomyces orontii* (Castagne) V.P. Heluta), and Phomopsis (*Phomopsis helianthi* Munt.-Cvetk., Mihaljc. & M. Petrov) (Roustaee et al. 2000). In India, the major diseases prevalent in sunflower growing areas are SND, *Alternariaster* leaf spot, powdery mildew, and downy mildew (Basappa and Santhalakshmi Prasad 2005; Reddy et al. 2013). The changing climatic conditions and increased incidence of diseases like powdery mildew, *Alternaria* leaf spot, and SND had drastically reduced the sunflower area under cultivation and production in India.

The changing disease scenario has become a constant challenge to the breeders and crop protection researchers for the deployment of suitable management strategies. The pathogen *G. orontii* has been reported to cause powdery mildew disease in the tropical and subtropical regions. During the last decade, powdery mildew has emerged as a severe problem and has spread rapidly to all sunflower cultivation regions in India (Reddy et al. 2013; Sujatha et al. 2015). Powdery mildews have a worldwide distribution,

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but higher intensity on sunflower is reported in the tropical regions (Zimmer and Hoes 1978; Diaz-Franco 1980; Gulya et al. 1997; Anyanga and Biruma 2010). The disease is reported to cause yield losses up to 13% in Mexico (Diaz-Franco 1980), 25% in USA between 1983 and 1989 (Gulya et al. 1991), and 30–74% in India depending on the disease severity (Dinesh et al. 2010; Sujatha et al. 2015).

A few studies reported the inheritance of resistance to powdery mildew in sunflower. Jan and Chandler (1988) reported the contribution of a partially dominant gene for resistance. Two genes controlling inheritance were proposed by Rojas-Barros et al. (2006). Christov (2008) reported the existence of two types of inheritance in wild *Helianthus* species. In one type, the inheritance was controlled by a single dominant gene, whereas in the other, it was polygenic. Similarly, the existence of both single and polygenic inheritance was reported by Naggayya (2013). All these studies were based on either a low number of individuals in the population (Jan and Chandler 1988; Rojas-Barros et al. 2006; Christov 2008) or displayed irregular disease frequency distribution. In sunflower, several reports exist in the mapping of resistance genes for important diseases like downy mildew (Vear et al. 2008; Mulpuri et al. 2009; Liu et al. 2019), rust (Liu et al. 2019), and sclerotinia (Micic et al. 2005; Fusari et al. 2012; Talukder et al. 2014, 2016). So far, there appear to be limited reports on mapping the powdery mildew resistance in sunflower, although inheritance and mapping studies related to powdery mildew resistance were reported in other economically important crops. Different kinds of molecular markers were employed for mapping the QTLs for powdery mildew resistance in other crops (Reddy and Sujatha 2019). However, all these crops differ from sunflower in their causative agents for powdery mildew, genome size, and the number of linkage groups. Lettuce is the crop that belongs to Asteraceae family and attacked by powdery mildew (caused by *Golovinomyces cichoracearum*), and four QTLs for powdery mildew resistance were mapped on LG-1, 2, 7 (Simko et al. 2013). Lettuce ($2n = 18$) has nine linkage groups, whereas sunflower ($2n = 34$) has 17 linkage groups.

A resistant line, TX16R (PI 642072) which confers resistance to rust, downy mildew, and sunflower mosaic virus from USDA-ARS at North Dakota Agricultural Experimental Station, Fargo has been identified and registered (Jan and Gulya 2006). This line is also found to possess resistance to powdery mildew (Reddy et al. 2013, 2018). The downy mildew (Pl_{33}) and rust resistance (R_{16}) genes in TX16R were mapped on the linkage groups 4 and 13, respectively (Liu et al. 2019). The present investigation has been undertaken to study the inheritance of powdery mildew and genetically map powdery mildew resistance gene(s) using a population derived from the cross involving a highly susceptible line (PS 2023) and the resistant line, TX16R.

Materials and methods

Plant material

A population of 264 F_2 individuals was produced from the cross between the highly susceptible (PS 2023) and resistant accessions (TX16R), and scored for powdery mildew disease incidence. These individuals were further advanced through single seed descent for the development of recombinant inbred F_6 lines (RILs). Both F_2 and RIL populations were used for inheritance studies. A population of 93 F_6-derived RILs was used for genotyping of powdery mildew resistance gene(s). TX16R is originally derived from a population of wild *Helianthus annuus*. In *Helianthus* species and also the cultivated sunflower, autogamy is a major issue. Consequently, in each generation, a few populations were lost due to seed set failure under selfing, and finally, a set of 93 RILs representing different levels of resistance to powdery mildew were stabilized.

Powdery mildew disease assessment

The powdery mildew responses were recorded in both F_2 and RILs at natural infection in field conditions. Both field trials were conducted at the Indian Institute of Oilseeds Research, Hyderabad. The highly susceptible genotype PS 2023 was used as infector row at every 10th row of the plot. Each F_6-derived RIL was sown in rows with 10–15 plants per row. The disease was scored at the maturity stage of the plants. The disease reaction was assessed based on disease intensity on the leaves and extent of spread on the plant as depicted in Supplementary Fig. 1. Disease was scored on a 0–9 scale (0 = immune; 1 = highly resistant, 2 = resistant; 3–4 = moderately resistant; 5–9 = susceptible/highly susceptible) according to Reddy et al. (2013).

DNA extraction

Genomic DNA was isolated from young leaves of parents and their RILs using CTAB method with minor modifications (Doyle and Doyle 1990). The plant material was ground in liquid nitrogen, then homogenized in 20 ml of extraction buffer (2% CTAB, 20 mM EDTA, 2% PVP, 1.4 M NaCl, 100 mM Tris–HCl pH 8.0 and 1% β-mercaptoethanol) and incubated at 65 °C for 1 h. The supernatant was twice extracted with chloroform:isoamyl alcohol (24:1 v/v), treated with RNase A (100 µg/ml), and incubated at 37 °C for 30 min. The DNA was precipitated with isopropanol and washed twice with 70% ethanol. The pelleted DNA was

air-dried and resuspended in 500 µl in sterile MilliQ water and stored at − 20 °C. The DNA concentration was determined electrophoretically using a known amount of DNA as standard.

Simple sequence repeat analysis

A total of 484 SSR primers were used to assess the parental polymorphism and 64 polymorphic primers (Suppl. Table 1) that were distributed evenly in all the 17 linkage groups of sunflower were used in linkage map construction and QTL analysis. PCR reaction mixture contained 7.5 mM dNTPs, 3.75 pM each of the forward and reverse primers, 1 U *Taq* DNA polymerase, 1X PCR buffer with 1.5 mM $MgCl_2$ (Bangalore Genei, India), 50 ng of template DNA, and sterile distilled water to a final volume of 25 µl. PCR amplification was carried out in a thermal cycler (Eppendorf, USA) using the following amplification conditions: 5 min at 94 °C for the initial denaturation, followed by 45 cycles consisting of 40 s of denaturation at 94 °C, 1 min of annealing at 62 °C (varied from primer to primer), and 1.5 min of extension at 72 °C with a final extension at 72 °C for 5 min. The PCR amplified products were resolved by electrophoresis on 3.0% agarose (Bangalore Genei, India) gel in 1 X Tris–acetate-EDTA buffer at 100 V for 3 h and visualized with ethidium bromide staining. The gel images were recorded using the Syngene G-Box gel documentation system.

Construction of molecular linkage map

A linkage map was constructed using QTL IciMapping software (Meng et al. 2015). The map distances were calculated based on the Kosambi's function (Kosambi 1944). Data generated after genotyping of 93 RILs by polymorphic SSR markers were tested using the χ^2 goodness-of-fit test for a 1:1 segregation ratio.

QTL mapping

QTL mapping was performed with single-marker analysis, interval mapping, and inclusive composite interval mapping function of QTL IciMapping software v3.3 (https://www.isbreeding.net/software/?type=detail&id=14). Single marker analysis served as the primary method of detecting associations between markers and the target trait. Two or more closely linked markers that showed significant association were assumed to identify the same QTL. To determine the precise location of the putative QTLs, interval mapping and composite interval mapping functions were used. Inclusive Composite Interval Mapping (ICIM) (Wang 2009) was used to confirm QTLs and estimate the phenotypic variation explained (PVE), as implemented in the integrated software QTL IciMapping for building genetic linkage maps and mapping quantitative trait genes (available from https://www.isbreeding.net).

Results

Inheritance of powdery mildew resistance

The levels of resistance or susceptibility to powdery mildew was tested in F_2 and RILs of the cross PS 2023 and TX16R and their parental lines based on visual inspection and scores on a 0–9 scoring scale of powdery mildew (Suppl. Figure 2 and Suppl. Table 2). Powdery mildew disease score distribution of both F_2 and RILs exhibited continuous variation (Fig. 1). The continuous distribution of disease scores in both populations confirms the polygenic control of resistance. These observations led to conclude the quantitative

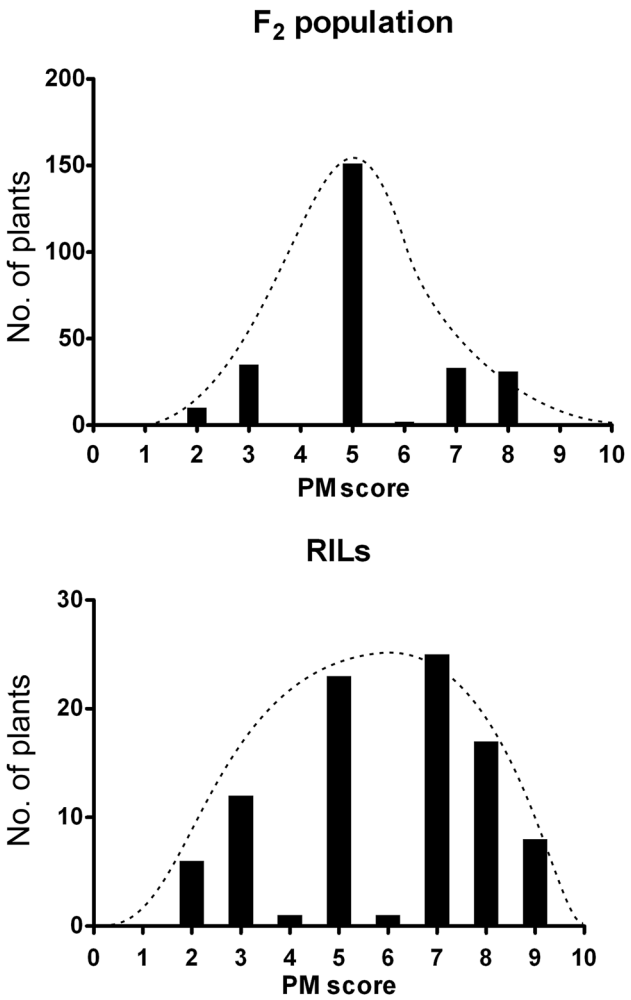

Fig. 1 Graphical representation of frequency distribution of powdery mildew disease scores in F_2 and recombinant inbred line (RILs) populations derived from a cross between PS 2023 (susceptible parent) and TX16R (resistant parent)

inheritance of powdery mildew resistance in sunflower in the TX16R accession.

Linkage map construction

The two parents, PS 2023 and TX16R, were screened for molecular polymorphism using 484 SSR markers. A total of 175 primers showed polymorphism among the two parents (Suppl. Figure 3a, b). The polymorphism between PS 2023 and TX16R was 36%. Data obtained from a set of 64 polymorphic SSR markers were selected for Linkage map construction (Suppl. Table 3). The map consisted of 17 linkage groups corresponding to the 17 chromosomes. These 64 markers were evenly distributed on 17 linkage groups (haploid set of chromosomes in sunflower). The constructed maps represented 17 linkage groups spanning 1200 cM.

QTL analysis

QTL analysis resulted in the identification of three QTLs, distributed in three genomic regions on two chromosomes. One QTL was identified on LG-5 (LOD = 3.61) (Fig. 2) and two QTLs were identified on LG-10 with LOD scores of 7.11 and 4.38 (Fig. 3). Thus, these QTLs are involved in the quantitative resistance to powdery mildew (Table 1). The three QTLs have an LOD score of 31.80, 57.12, and 23.82% of phenotypic variation in the mapping population

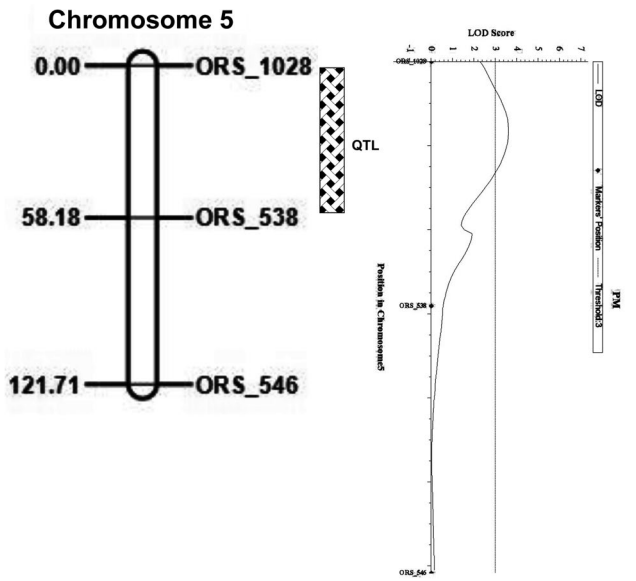

Fig. 2 Position of the QTL on linkage group-5 (LG-5) for powdery mildew resistance in the linkage map derived from the 93 RIL population obtained from the cross PS 2023 and TX16R. The position of QTL involved in resistance to powdery mildew is represented by checkered box. The distance between the markers is indicated in cM, and the LOD significance threshold of 3.0 is also shown

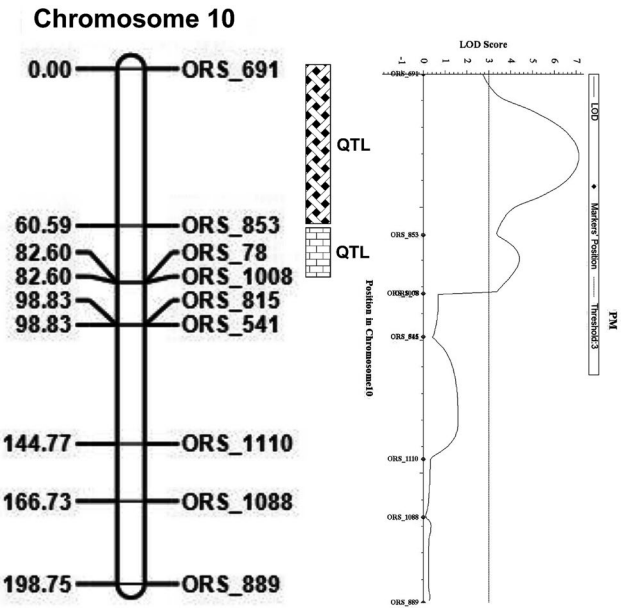

Fig. 3 Position of the two QTLs on linkage group-10 (LG-10) for powdery mildew resistance in the linkage map derived from the 93 RIL population obtained from the cross PS 2023 and TX16R. The positions of QTLs associated with resistance to powdery mildew are represented by checkered boxes. The distance between the markers is indicated in cM, and the LOD significance threshold of 3.0 is also shown

for resistance to powdery mildew. All three QTLs showed a positive additive effect (Table 1).

Discussion

Powdery mildew has become one of the major problems in sunflower cultivation in the tropics and subtropics. Earlier reports were confined to the determination of inheritance of resistance to powdery mildew; however, the corresponding resistance gene(s) were not mapped. One of the main objectives of the present investigation was to study the inheritance of powdery mildew resistance in sunflower and map the genes responsible for resistance to powdery mildew using an appropriate mapping population.

Information on the inheritance of resistance to powdery mildew in sunflower is available, and inheritance pattern varied with the resistant donor. The previous studies had reported partial dominance (Jan and Chandler 1988), digenic control (Rojas-Barros et al. 2006), single dominant, and polygenic inheritance of resistance (Christov 2008; Nagayya 2013) to powdery mildew. In the present investigation, continuous range of resistance on F_2 suggests quantitative inheritance of powdery mildew resistance in TX16R. The non-normal distribution for RILs could be due to loss of resistant alleles due to self-sterility of plants carrying

Table 1 QTLs for resistance to powdery mildew (LOD > 3.0)

Trait	Chromosome	Position cM	Left marker	Right marker	LOD	PVE%	Additive
PMR	5	16	ORS 1028	ORS 538	3.61	31.00	1.36
PMR	10	31	ORS 691	ORS 853	7.11	57.12	1.77
PMR	10	70	ORS 853	ORS 78	4.38	23.82	1.08

PMR powdery mildew resistance, *LOD* likelihood of odd, *PVE* phenotypic variation explained

TX16R genome. The low number of resistant lines was due to the cross of a resistant (TX16R) line with the highly susceptible line PS 2023.

There are no reports on mapping genes for resistance to powdery mildew in sunflower. QTLs governing powdery mildew resistance were identified in several crops like apple, barley, cucumber, peach, grapevine, melon, mung bean, oat, pepper, roses, ryegrass, tomato, water melon, and wheat (Reviewed in Reddy and Sujatha 2019). These studies have identified both qualitative and quantitative resistance in different crops suggesting the existence of multiple mechanisms of resistance to powdery mildew. In sunflower also, both qualitative and quantitative inheritance of resistance to powdery mildew is reported (Jan and Chandler 1988; Rojas-Barros et al. 2006; Christov 2008; Nagayya 2013).

In the present study, three QTLs in three genomic regions of two chromosomes, viz., LG-5 and LG-10, were found to be linked to genes conferring resistance to powdery mildew in sunflower. Quantitative resistance to downy mildew in sunflower was reported on LG-10 (Vear et al. 2008). The simple sequence repeat marker ORS 853 was found to be the flanking marker for both the QTLs present in LG-10 and this region indicates that it is an important region for marker-assisted selection (MAS) for downy mildew resistance. Both powdery mildew and downy mildew are biotrophic fungi that attack sunflower. Also, QTLs for *Sclerotinia* rot resistance were detected on LG-10 (Gentzbittel et al. 1998; Bert et al. 2001; Ronicke et al. 2005; Micic et al. 2005; Talukder et al. 2016). In addition to disease resistance, LG-10 is a region that is reported to be associated with branching found in most restorer lines (Rojas-Barros et al. 2008; Mandel et al. 2013; Nambeesan et al. 2015), and probably, the QTLs associated with powdery mildew resistance could be linked to the recessive branching gene, as well. All the three QTLs identified for powdery mildew resistance were additive. These QTLs together explained most of the total phenotypic variation with only additive effect.

Since quantitative resistance is probably more durable (Johnsen 1983; Lindhout 2002), it would be of great importance to deploy such resistance against powdery mildew in sunflower. In the present study, the genomic regions close to the QTLs controlling powdery mildew resistance in sunflower were identified which would accelerate the breeding programs aimed at introgression of resistance from TX16R to elite breeding lines through marker-assisted selection. TX16R is reported to be resistant to rust, downy mildew, and virus (Jan and Gulya 2006), and molecular mapping studies resulted in the identification of markers linked to rust and downy mildew (Liu et al. 2019). The RILs involving PS 2023 and TX16R could be screened for rust, downy mildew, and powdery mildew, and promising lines combining resistance to rust, downy mildew, and powdery mildew could be selected. However, for fine mapping of the genes, a large number of markers, appropriate populations, and multilocation screening of the RILs are warranted.

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Compliance with ethical standards

Conflict of interest The author(s) declare no conflict of interest.

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