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Development of diploid pollinator resistant to powdery mildew disease in sugar beet

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ABSTRACT

To develop pollinator parent resistant to powdery mildew disease, a semi- resistant population, 14442, was used. In the first year of the experiment, fifty resistant single plants were selected based on resistance index, and the seeds of half-sib families were produced. Among the 50 plants, only 39 plants produced enough seed. Harvested seeds were planted in one-row plots, replicated six times, and were evaluated for resistance to powdery mildew. Three out of the 39 half-sib families (HS.13, HS.24 and HS.35) with a lower infection (<2.5) were selected. From each of the three families, 50 plants were selected and then planted in isolated tents to develop new half-sib families. In total, 88 new half-sib families were developed. The seeds of the new families were planted in single-row plots replicated six times and were evaluated again. Among them, the half-sibs numbered 5, 17 and 22 were found to be resistant to disease compared with the other progenies. From each half-sib, 35 roots (105 roots in total) were selected for S1 production. Each of the roots was divided to four parts (clone), and cultured in an isolated cage to produce S1 seeds. Owing to the problems occurred in isolated cage, from 105 selected roots, only 13 roots could produce enough seed with good viability. Thirteen self pollinated seeds/S1 (new germplasm), were evaluated for powdery mildew resistance. Results illustrated that in each generation of selection, resistance increased with positive responses. The minimum percentage of infection (12.9) was observed in S1 plants. S1 population showed 72.6% selection response compared with first population of 14442. Due to the good resistance of S1 plants, they could be used as pollinator for producing cultivars resistant to powdery mildew disease.

Keywords: pollinator, powdery mildew, resistant, sugar beet

INTRODUCTION

Most sugar beet cultivars grown in Iran are susceptible to powdery mildew disease (Basati 2008, Basati et al. 2003). Since the peak of disease emergence occures in the late stages of sugar beet growth (first half of August), most of the farmers believe that the damages are not important so they ignore it. Results indicated that the root yield reduction was up to 25 ton and sugar loss percentage was up to 1% which shows the importance of the disease (Basati et al. 2003). Since the powdery mildew is dispersed in all sugar beet growing areas in Iran (Ahmadinejad 1973), development of resistant cultivars is necessary. Powdery mildew disease is caused by *Erysiphe betae* fungus (Weltezien 1963). The damage varies based on regional differences, and reduction in root yield depends on the time and infection severity. With the increase of mildew severity at early stages of plant growth, root yield reduction and sugar loss will increase (Ahren and Weltzien 1979, Behdad 1979). Studies carried out in Kermanshah indicated 25% decrease in root yield due to mildew infection (Basati 1998). In England, root yield decline up to 3 ton (for an average production of 45 t/ha) was reported (Asher and Williams 1992). In 1980's, the disease caused a remarkable decrease in sugar yield in US (Hills et al. 1980). At early stage of the plant growth, infection results in severe plant production decrease, reaching to

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20% reduction or higher (Asher, 1990). One time spraying against disease led to 8% increase in root yield (Dewar and Asher 1998) and the disease control resulted in 38% root yield increase (Skoyen et al. 1975). As the damage of this disease is remarkable, usage of resistant source for development of resistant cultivars is necessary. Wild species of beet (*B.maritima*) showed higher genetic variation in relation to this disease compared with sugar beet (Whiteny 1989). Among sugar beet germplasm (wild and cultivated species) the highest resistance was found in wild species of Corolinae group and *B. Coroliflora* species (Whiteny 1989).

Average resistance to this disease was identified and introduced into commercial cultivars. Higher resistance was also found in *B. maritima* species and introduced into breeding lines through backcross method. These progenies were used for determination of inheritance of resistance to disease (Lewellen and Schrandt 2001). Genetic analysis of segregating generations in kermanshah showed that one major gene and few other genes affect the disease control (Basati and Mesbah 2002). In another study in US, resistance genes were transferred from wild species of *B.maritina* to breeding lines (Lewellen and schrondt 2001) and confirmed the role of a major gene and other genes in disease control. The role of genetic resistance highlights the importance of resistant cultivar development. The goal of this study was development of pollinator parent resistant to powdery mildew for the production of resistant cultivar.

Materials and Methods

In the first year (2003), seeds of the population 14442, which had been recognised as a semiresistant population to powdery mildew (Shikholeslami and Basati 1998), were sown in a 40-row plot (10 m long). Susceptible cultivar 7233 and the population 14442 were planted side-by-side. Tables 1-3 show the infection percentage of both checks. With the onset of disease symptoms, study was conducted on cultivated plants and plants without symptoms were marked with colour label. Selection of healthy plants and determination of infection level were done using Paulus et al. (2001) method. A linear scale of 0-5 was used, in which 0, 1, 2, 3, 4, and 5 approximated 0, 10, 35, 65, 90 and 100%, respectively, of the matured leaf area covered by mildew. In this study, 200 leaves per treatment (20 plants and 10 leaves per plant) were randomly selected and scored. Using infection score, K was calculated as an infection index:

$$K= \Sigma(\text{number of leaves scored} \times \text{given score})/(\text{total number of leaves})$$

With calculation of the K in different replications of a genotype, R value was calculated as a mean of replications:

$$R= K1+ K2+K3\ldots+Kn/n$$

After calculation of the K and R, infection percentage was determined using the following equation. In this relation, value 18 is a fixed factor.

$$\text{Percent MLAD} = 100[\sin (R\times 18)]^2$$

MLAD = Mature Leaf Area Disease (%)

At the end of the season, 50 plants which were more resistant compared with other plants (with infection index lower than 3) were selected. The susceptible parental source (7233) and a resistant check (14442) were included in these tests. Selected roots were remained in the field and then transferred to an isolated plot in West Islamabad station in the second year. Plants were crossed freely and the seeds of each plant, known as a first generation of half-sib (HSF.F_1) progenies, were harvested separately. Of the 50 plants, only 39 plants had enough seed. In spring of the third year (2005), 39 half-sib families along with both susceptible and resistant checks were evaluated in the field to estimate reactions to powdery mildew disease. They were evaluated in an observation trial in single-row plots with at least 25 plants and six replications. Based on the results, three plants were selected from the best families, and from each family, 50 roots were selected. In spring of the fourth year (2006), 50 roots developed from each of the selected families, were planted in an isolated plot of Mahidasht Research Station, to be crossed with each other freely and to create a new generation of half-sib families. So, among the 150 roots in three families, only 88 new families produced enough seed. In spring of the fifth year (2007), these 88 half-sib families were again evaluated for powdery mildew resistance, from which 35 roots were selected from the best three families for S1 seed production. In the sixth year (2008), 105 roots were placed in isolated cages for S1 seed production. Each root was divided into four parts (clones) and disinfected with fungicide. Seeds derived from these roots were called S1 seeds. Among the 105 roots inside the cage, 27 plants produced enough seed. In 2011, seeds of

Table 1. Infection index and percentage of the initial 50 selected plants in 2003

Selected plants	Infection index (1-5)	Infection percentage	Selected plants	Infection index (1-5)	Infection percentage
1	2.17	39.70	26	2.00	34.54
2	2.30	43.73	27	1.50	20.6
3	2.10	37.56	28	1.90	31.5
4	2.45	48.42	29	1.89	31.3
5	2.45	48.42	30	1.68	25.36
6	2.59	52.82	31	1.80	28.7
7	2.38	46.23	32	1.84	29.8
8	2.12	38.17	33	2.33	44.66
9	2.15	39.09	34	2.10	37.56
10	2.13	38.48	35	2.12	38.17
11	2.20	40.63	36	2.11	37.87
12	2.00	34.54	37	2.16	39.39
13	2.00	34.54	38	2.20	40.63
14	2.20	40.63	39	2.35	45.29
15	2.25	42.17	40	1.53	21.3
16	2.65	54.70	41	1.87	30.7
17	2.59	52.82	42	1.90	31.5
18	1.85	30.14	43	1.89	31.3
19	2.70	56.26	44	2.00	34.5
20	2.45	48.42	45	2.10	37.5
21	2.75	57.82	46	2.20	40.6
22	2.63	54.07	47	1.56	22.15
23	2.35	45.29	48	2.20	40.6
24	2.01	34.84	49	2.22	41.24
25	2.30	43.73	50	2.63	54.07
Population 14442 (resistant check)	2.4	47.12	Cultivar 7233 (Susceptible check)	3.74	85.18

the 27 genotypes were sown in two replications had good viability and were able to germinate. Therefore, evaluation was performed on these 13 genotypes.

RESULTS

Results of 2003

Seeds of the population 14442 were planted in 40 rows, 10 m long each, and approximately 2500 plants were evaluated. For evaluation of the disease, 50 plants which had less mildew (infection

Table 2. Readings for the first cycle of evaluation of half-sib progenies resistant to powdery mildew in 2005

Half-sib number	Infection index	Infection (%)	Half-sib number	Infection index	Infection (%)
1	1.77	27.99	21	1.87	30.92
2	1.91	32.08	22	2.04	35.91
3	1.68	25.63	23	2.28	43.36
4	1.92	32.18	▸ 24	1.51	20.8
5	1.93	32.73	25	2.36	45.86
6	2.02	35.26	26	1.95	33.07
7	1.89	31.31	27	2.36	45.61
8	1.86	30.56	28	2.16	39.62
9	1.88	31.16	29	2.29	43.55
10	1.72	26.63	30	2.24	41.95
11	1.70	26.15	31	2.04	35.98
12	1.56	22.40	32	1.91	32.10
▸ 13	1.49	20.58	33	2.25	42.36
14	2.19	40.35	34	1.9	31.66
15	1.95	33.29	▸ 35	1.55	21.9
16	1.94	32.91	36	1.9	31.68
17	2.01	35.13	37	1.57	22.4
18	1.79	28.43	38	2.32	44.42
19	2.13	38.78	39	2.1	37.72
20	1.79	28.58			
Population 14442 (resistant)	2.41	47.18	Cultivar 7233 (susceptible check)	3.52	79.98

Table 3. Mean infection index and percentage of infection of new half-sib families in 2007

New half-sib number	HSF.13		HSF.24		HSF.35	
	Infection index	Infection (%)	Infection index	Infection (%)	Infection index	Infection (%)
NHSF.1	1.3	15.7	1.6	23.2	1.6	23.2
NHSF.2	1.8	28.7	1.5	20.6	1.5	20.6
NHSF.3	1.3	15.7	1.8	28.7	1.4	18.1
NHSF.4	1.3	15.7	1.6	23.2	1.9	31.5
NHSF.5	1.1	11.4	1.5	20.6	1.6	23.2
NHSF.6	1.4	18.1	1.3	15.7	1.5	20.6
NHSF.7	1.2	13.5	1.4	18.1	1.7	25.9
NHSF.8	1.3	15.7	1.5	20.6	1.8	28.7
NHSF.9	1.6	23.2	1.8	28.7	1.4	18.1
NHSF.10	1.7	25.9	1.6	23.2	1.5	20.6
NHSF.11	1.9	31.5	1.7	25.9	1.4	18.1
NHSF.12	1.2	13.5	1.5	20.6	1.6	23.2
NHSF.13	1.7	25.9	1.5	20.6	1.5	20.6
NHSF.14	1.5	20.6	1.5	20.6	1.8	28.7
NHSF.15	1.3	15.7	1.4	18.1	1.8	28.7
NHSF.16	1.5	20.6	1.4	18.1	1.6	23.2
NHSF.17	1.9	31.5	1.2	13.5	1.8	28.7
NHSF.18	1.8	28.7	1.9	31.5	1.5	20.6
NHSF.19	1.2	13.5	1.6	23.2	1.5	20.6
NHSF.20	1.3	15.7	1.3	15.7	1.5	20.6
NHSF.21	1.4	18.1	1.7	25.9	1.4	18.1
NHSF.22	1.5	20.6	1.8	28.7	1.3	15.7
NHSF.23	1.5	20.6	1.4	18.1	1.6	23.2
NHSF.24	1.3	15.7	1.6	23.2	1.5	20.6
NHSF.25	1.7	25.9	1.6	23.2	1.4	18.1
NHSF.26	1.8	28.7	1.7	25.9	1.6	23.2
NHSF.27	1.8	28.7	1.5	20.6	1.5	20.6
NHSF.28	1.9	31.5	1.9	31.5	-	-
NHSF.29	1.8	28.7	1.3	15.7	-	-
NHSF.30	1.4	18.1	1.5	20.6	-	-
NHSF.31	-	-	1.5	20.6	-	-
Mean	1.49	20.35	1.55	21.89	1.56	22.15
Resistant check	2.26	42.5		Susceptible check	3.33	75

index less than 3), and were in a better situation than the other plants were labelled using colour label. In the first week of August when mildew severity reached the peak, selected plants were recorded and scored for infection index based on Paulus et al. (2001) method. Mean infection index value for 50 plants was 48.39%. The infection index of the resistant population 14442 and susceptible check 7233 were 12.4 and 17.85%, respectively (Table 1). Selected plants remained in the field, and were planted in February 2003.

Results of 2004

Plants selected from last year in West Islamabad Station were pollinated freely and the half-sib seeds were harvested. Among 50 plants, 39 plants produced enough seed and their seeds were used for disease evaluation in 2005.

Results of 2005

A total of 39 half-sib families with two checks (14442 and 7233) were evaluated in a randomized complete block design with six replications. Each plot contained 25 plants per row. Mean infection index for 39 selected plants was 69.31%. The infection indices of the 14442 and 7233 checks were 18.47% and 98.79%, respectively. Based on individual readings, 3 families which had the least mildew (HSF24, HSF13 and HSF35) than the other families were selected and kept for new half-sib production (Table 2).

Results of 2006

As the results of 2005 shows (Table 2), 50 roots of the half-sib progenies numbered 13, 24 and 35 were selected for production of new half-sib families in 2006. In spring 2006, half-sib families were planted in an isolated cage. Finally, 88 new half-sib families (30, 31 and 27 half-sib from half-sib 13, 24 and 35, respectively) were developed and evaluated in 2007.

Results of 2007

In spring 2007, each of the new half-sib families (second cycle half-sibs) were planted in single row plots, replicated six times, and were evaluat-

Table 4. Average of infection index and rate of infection of S1 lines in 2009

No.	Genotype	Infection (%)	Average of infection index in 4 replications
1	HSF13-NHSF5.S1.5*	10	1
2	HSF13-NHSF5.S1.10	10	1
3	HSF13-NHSF5.S1.12	10	1
4	HSF13-NHSF5.S1.15	10	1
5	HSF13-NHSF5.S1.16	10	1
6	HSF13-NHSF5.S1.32	28.7	1.8
7	HSF24-NHSF17.S1.2	18	1.4
8	HSF24-NHSF17.S1.25	10	1
9	HSF24-NHSF17.S1.29	18	1.4
10	HSF24-NHSF17.S1.30	18	1.4
11	HSF35-NHSF22.S1.1	11.5	1.1
12	HSF35-NHSF22.S1.17	15.7	1.3
13	HSF35-NHSF22.S1.20	26	1.7
	Mean	15.07	1.24
	Susceptible check (7233)	59	2.8

Family No. 13 had 30 new families among which the family No. 5 was selected. Among the 35 plants of new family No. 5, S1 was produced from plant No. 5 of this family.

ed for resistance to powdery mildew. In half-sib family 13, the new half-sib number 5 had the minimum index of 1.1 and infection rate of 4.11. In family 24, the new half-sib number 17 was next with the index of 2.1 and infection rate of 5.13%, and finally the family number 35, with the new half-sib number 22 and index of 3.1 and infection rate of 7.15% had the minimum infection. Mean infection index and percentage for new half-sib families were 53.1 and 5.21%, respectively. Susceptible and resistant checks had the index of 33.3 and 26.2, and the infection rate of 75% and 5.42%, respectively. HSF13-5 (new half-sib number 5 derived from the family number 13), HSF24-17 (new half-sib number 17 derived from the family number 24) and HSF35-22 (new half-sib number 22 derived from the family number 35) were superior to the other half-sib progenies and were selected for production of S1 in next year (Table 3).

Since in each replication, only one row per genotype was planted, and the main objective of the test was to assess the disease resistance, yield and quality of the roots were not measured. In winter 2007, from each selected half-sib, 35 roots were isolated and divided into four parts and then were planted inside the cage. From 105 roots, only 13 roots produced enough seed with good viability. Therefore, in spring 2008, only 13 S1 seeds were produced. S1 seeds were sown in spring 2008. By late August, when the infection reached the peak, infection rating was conducted and S1 lines were scored (Table 4).

DISCUSSION

The population 14442 was evaluated over several years and single plant selection was carried out each year (Kolivand 1990). After individual selection, all selected plants were allowed to random mate to create new population and the derived seeds were combined. Therefore, in the preliminary population 14442, despite individual selection for several years, no more progress had been achieved for disease resistance (Basati 2002, Basati 2008), whereas, in this study, after individual selection, random-mating was conducted among the selected plants and the seeds were harvested from each plant separately. In other words, half-sib families were used to produce plants with high resistance. Using half-sib family leads the plants with undesirable traits to disappear, and also the genetic basis of the plants is broad. By using this method and selection of the best plants, in addition to moving forward to the purity of desired characters, genetic variation also exists in plants, important traits such as yield and sugar content will have likely less decrease, While if self-pollination occurs, after three generations, purity will be achieved but weak plants with undesirable traits will appear which will need several years for breeding.

In this experiment, two cycles of half-sib family selection was used which doesn't weaken the genetic basis of the plants. For achieving purity this way, 10 generations of half-sib selection should be used. Half-sib method not only maintains the genetic variation of the plants, but also fixes the disease resistance in any cycle of the half-sib selection (Ehdaee 1994). Mean infection rate in the first cycle of selection was 39.48%, which was slightly less than the initial resistant population with 47.12%. At this stage, infection rate of sus-

Table 5. Selection progress in selected families compared with the primary population 14442

Population	Average of infection percentage	Infection difference of each family compared with previous family	Infection difference in family compared with 14442	Progress percentage compared with primary population
Primary mass 14442	47.18	-	-	-
50 selected plants for producing first half-sib	39.48	7.7	7.7	16.3
First half-sib	31.69	7.79	15.49	32.8
New half-sib	21.5	10.19	25.68	54.4
S1 families	12.9	8.6	34.28	72.6

ceptible check (cultivar 7233) was about 85.18%. The lower infection of the preliminary population and selected plants compared with check represents the resistance of the primary population to powdery mildew disease. Infection rate below 50% indicated that the plant is resistant to mildew and the selections were made based on this criterion.

Populations or cultivars which have infection rate above 50%, are considered as susceptible resources because the population or the cultivar which shows the index higher than 2.5 (the index is 1-5 and any genotype with the index lower than 2.5 is considered resistant), represents more than 50% infection. Infection in the field strongly depends on environmental conditions (Asher and Dewar 2001; Asher and Williams 1991; 1992) and therefore, the infection levels in different years were quite different. However, the level of infection in the check cultivar 7233 in all years, despite of the high and low severity of the mildew, was higher than 50% (the rates in year 2003 to 2009 were 85.18, 79.98, 75 and 59%, respectively). Although in the resistant population, infection rate varied in different years but it was almost constant in the population 14442 and was approximately remained around 47%. However, in selected lines, despite the sufficient amount of inoculation to create more than 50% infection in susceptible check, , infection was considerably reducing in the selected lines. Therefore, . Infection rate reduction in selected lines (from 2003 to 2009 in the rate of 39.48, 31.69, 21.5 and 15.07%) was related to selection effect, not to the decrease of fungus inoculation rate in the environment. So, by selection of less infected plants year after year, infection level in each generation of selection decreased. When a number of plants were selected from the primary population, and the seeds were harvested and evaluated for disease resistance, infection rate in half-sib families was about 31.69%. Therefore, infection rate of the half-sib families was 7.79% lower than the initially selected seeds. As a result, the response to selection for powdery mildew disease in this experiment and this population was positive. When the selection was done among the half-sib progenies and the selected plants were used for new half-sib families production, results showed that the new half-sibs had better performance than the primary half-sibs. Mean infection rate for new half-sib population was about 21.5%, which was 10.19% lower than the primary half-sib family (Table 4). It can be observed that the response to selection in each generation was positive. Selection differential in the first 50 selected plants compared with the population 14442 was 16.3%, being 32.8%, in primary half-sib, 54.4% in new half-sib population and 72.6% in S1 population. This indicates that the single plant selection for powdery mildew resistance was quite effective. Studies (Lewellen and schrondt 2001, Basati and Mesbah 2002) showed that the number of genes controlling powdery mildew resistance, are few and only one major gene has a large effect. With a lower number of genes controlling a disease, the response to selection will be better. Therefore, in this experiment the selection efficiency was high and considerable.

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