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Resistance to powdery mildew and Cercospora leaf spot of multigerm dihaploid sugar beet lines and its inheritance in their hybrids

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Abstract

The induced haploidy through *in vitro gynogenesis* in sugar beet is effective method for the selection of valuable gene recombinations determining the expression of efficient traits of that crop. Ten years test results for the resistance to powdery mildew and *Cercospora* leaf spot of stabilized multigerm dihaploid sugar beet lines and their hybrid combinations are being reported. The performance of the tested material proves, that the multigerm dihaploid pollinators are differentiated recombinant lines with favorable combinations of genetic material, including genes responsible for the high resistance to the agents of powdery mildew and *Cercospora* leaf spot fungal diseases. The higher resistance of the dihaploid multigerm pollinators dominates in their diploid hybrid combinations. The heritage factors for higher resistance to both diseases of the maternal component dominate in the tested triploid hybrids. There are good prospects of using these pollinators in the hybrid breeding schemes.

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Introduction

The climate during sugar beet vegetation in Bulgaria favors the development of the agents of powdery mildew (*Erysiphe betae Jacz.*) and *Cercospora* leaf spot (*Cercospora beticola Sacc.*), which cause serious damages in the beet crops. Both diseases injure the foliage and upset the photosynthetic activity of plants (Nakov *et al.*, 1994; Ruppel, 1995). The severe attacks of the diseases cause abrupt root yield decrease (Keane, 1991, Smith and Campbell, 1996), the sugar content and the purity of juice are adversely affected (Krexner, 1983). There are very efficient means of chemical control of both diseases, but the diseases, especially the Cercospora leaf spot, develop fungicide resistant strains (Francis, 2002). Aiming at lower plant protection expenses and environment preservation, the creation of resistant varieties has become a basic trend in the modern sugar beet breeding (Antonov and Zakhariev, 1994).

The induced haploidy allows detection and stabilization into lines of valuable genes recombinations, determining the expression of efficient signs, including combinations of genes responsible for increased disease resistance (Zakhariev and Kikindonov, 1997). During the past years obtained through *in vitro gynogenesis* dihaploid lines are included in our breeding program.

The aim of the study is to establish the degree of resistance to powdery mildew and *Cercospora* leaf spot in some of our dihaploid multigerm lines. The test results of hybrids with these dihaploid paternal components allow determination of the inheritance of the resistance to both fungal diseases.

Material and methods

Breeding material included in the tests

The study covers hybrid combinations of eight multigerm dihaploid (doubled haploid) lines with two monogerm male sterile (MS) lines (MS 19 – diploid, and MS 316 – tetraploid).

Method of obtaining the tested lines

The dihaploid lines are obtained after induced *gynogenesis in vitro* (Slavova, 1993) and doubling the haploid regenerants from individually selected plant of the multigerm diploid population 1316. By microclonal propagation of the same plant, followed by seed reproduction, is formed the initial multigerm line 16.

Hybridization

The crosses between the parental components of the hybrids (diploid and triploid) are made in sunflower isolation, in 6:2 ratio between the plants of the MS line and the pollinator.

Field tests design

The field tests, carried out during 2003-2013, are arranged in a randomized block design, each tested variant in 4 repetitions, 3 rows spaced 45 cm apart experimental plot with 10.8 m^2 area.

Parameters of assessment

The rate of powdery mildew infection of the test field is 2.0 according to the scale of Varbanov (1977), and 2.4 is the rate of *Cercospora* leaf spot infection (Varbanov, 1978).

Visible signs of the powdery mildew are the dusty-white or whitish mycelium of the fungus on the upper leaf surface and later pale green to yellow leaves, and the symptoms of *Cercospora* Leaf Spot disease are the characteristic circular spots with necrotic, tan-colored or grayish centers and dark reddish brown border around them, on both sides of the leaf.

The resistance to powdery mildew and *Cercospora* leaf spots is determined by sight, by the visible symptoms of the respective disease, using a 6-grade scale, where:

Rating 0 – is given to the susceptible variants (100% of the plants in the experimental plot are hit by the respective pathogen);

Rating 1 – 76-99% of the plants in the plot are hit by the disease;

Rating 2 – 51-75% of the plants in the plot are affected by the disease

Rating 3 – affected 26-50% of the plants in the plot

Rating 4 – affected 1-25% of the plants

Rating 5 – given to the resistant to the pathogen variants (without visible symptoms of the respective disease in the experimental plot.

Statistical treatment of the results

Dispersion analysis (Lidanski, 1988) was used for determination of the statistical significance of the differences between the test variants.

The type of inheritance of the resistance is determined according to Genchev *et al.* (1975) by the values of the overdominance ratio d/a (the proportion between dominant and additive parameters in the hybrids).

Results and discussion

The test results for the resistance to powdery mildew (PMR) and to *Cercospora* leaf spot (CR) of multigerm dihaploid lines in conditions of moderate infections are presented on Table 1. We could outline the proved higher ratings of PMR of half of the tested Dh lines (Dh 2, Dh 3, Dh6, Dh 7) compared with the rating of the initial line 16 (3.04). Of course, the group standard (consisting of three commercial varieties) has a higher PMR rating than the initial line. But the dihaploid lines don't fall back the Standard. Even Dh 6 manifests proved higher PMR than the group standard.

Table 1. Resistance of multigerm dihaploid lines to powdery mildew and *Cercospora* leaf spot diseases.

Line	Resistance to Powdery mildew			Resistance to *Cercospora* leaf spot		
	rating	% to the initial line	% to the Standard	rating	% to the initial line	% to the Standard
Initial line 16	3.04	100.0	87.1	2.50	100.0	83.1
Dh 1	3.30	108.6	94.6	2.52	100.8	83.7
Dh 2	3.39	111.5	97.1	3.08	123.2	102.3
Dh 3	3.51	115.5	100.6	2.99	119.6	99.3
Dh 4	3.31	108.9	94.8	3.16	126.4	105.0
Dh 5	3.33	109.5	95.4	3.01	120.4	100.0
Dh 6	3.92	128.9	112.3	3.00	120.0	99.7
Dh 7	3.80	125.0	108.9	3.09	123.6	102.7
Dh 8	3.36	110.5	96.3	2.74	109.6	91.0
Standard	3.49	114.8	100.0	3.01	120.4	100.0
$P \leq 0.05$	0.38	10.8		0.10	3.3	

The diploid multigerm initial line 16 has a moderate resistance to *Cercospora* leaf spot. Only the Dh 1 line is at the same low level of resistance, all the other dihaploid lines have significantly better expressed resistance to *Cercospora* leaf spot agent than the initial line 16. The excess of their CR ratings is with very well proved statistical differences. The Dh 4 line has a 3.16 rating of resistance, which exceeds the Standard's rating significantly.

In general - the application of the dihaploidy in the breeding program allows considerable shortening of the time for creation of highly homozygous lines. The obtainment of plants from gametes, not from zygote, by induced haploidy, is an excellent possibility to find out and stabilize into lines valuable gene recombinations, determining the expression of increased disease resistance. The higher relative values of the resistance to both fungal diseases of the dihaploid lines, obtained by *in vitro gynogenesis*, are indicative for the good prospects of using this method for creation of more resistant to certain pathogens initial material for the sugar beet breeding.

The way of inheritance of the most significant morphological and economical signs is of special importance for the hybrid breeding. The results of our long standing tests allow us to determine the character of inheritance of the resistance to these two fungal diseases in diploid and triploid hybrids of the dihaploid pollinators. The PMR rating of the MS line 19 (Table 2), used as a maternal component in the diploid hybrids, is 2.88, which is comparatively high value for a diploid MS line's resistance to powdery mildew. The ratings of PMR of the dihaplod pollinators are much higher. But what impressed us most from the first years of testing, are the supreme ratings of powdery mildew resistance for the diploid hybrids of these dihaploid pollinators (the average PMR rating of the hybrids is 3.87). In such a situation it is normal to register categorical overdominance of the higher resistance of the dihaploid pollinators in their diploid hybrids (d/a=2.19). Only in the hybrid MS19 x Dh 6 the inheritance of the PMR is dominant.

Table 2. Inheritance of powdery mildew resistance in hybrids of multigerm dihaploid lines.

Hybrid	P_1rating	P_2rating	MP	F_1rating	d	a	d/a
MS 19 x Dh 1	2.88	3.30	3.09	3.85	0.76	0.21	3.62
MS 19 x Dh 2	2.88	3.39	3.14	3.99	0.85	0.26	3.27
MS 19 x Dh 3	2.88	3.51	3.19	3.63	0.44	0.32	1.38
MS 19 x Dh 4	2.88	3.31	2.95	3.65	0.70	0.22	3.18
MS 19 x Dh 5	2.88	3.33	3.11	3.69	0.58	0.23	2.52
MS 19 x Dh 6	2.88	3.92	3.40	4.00	0.60	0.52	1.15
MS 19 x Dh 7	2.88	3.80	3.34	3.96	0.62	0.46	1.35
MS 19 x Dh 8	2.88	3.36	3.12	4.16	1.04	0.24	4.33
Average for diploid hybrids	**2.88**	**3.49**	**3.19**	**3.87**	**0.68**	**0.31**	**2.19**
MS 316 x Dh 1	3.90	3.30	3.60	3.77	0.17	0.30	0.57
MS 316 x Dh 2	3.90	3.39	3.65	4.19	0.54	0.26	2.07
MS 316 x Dh 3	3.90	3.51	3.71	4.13	0.42	0.20	2.10
MS 316 x Dh 4	3.90	3.31	3.61	4.12	0.51	0.30	1.70
MS 316 x Dh 5	3.90	3.33	3.62	4.10	0.48	0.29	1.66
MS 316 x Dh 6	3.90	3.92	3.91	3.88	-0.03	0.01	-3.00
MS 316 x Dh 7	3.90	3.80	3.85	3.83	-0.02	0.05	-0.40
MS 316 x Dh 8	3.90	3.36	3.63	3.66	0.03	0.27	0.12
Average for triploid hybrids	**3.90**	**3.49**	**3.70**	**3.96**	**0.26**	**0.21**	**1.24**

The triploid hybrids in the study are received by crosses of the same dihaploid pollinators with a tetraploid MS line. The last has a very good resistance to powdery mildew – its rating is 3.90. In five of the hybrid combinations the inheritance of the high resistance of the maternal component is overdominant, and in the hybrid MS316 x Dh 1 is registered incomplete dominance of the hereditary factors for resistance of the MS line (d/a=0.70). Incomplete dominance is the way of inheritance of the Dh 7 pollinator's resistance, and intermediate inheritance is registered in the last hybrid in that group. The mean d/a value for the triploid hybrids 1.24 indicate a dominance of the higher resistance to the disease of the maternal component. Such character of the inheritance determinates the necessity of tetraploid MS lines with a very high degree of resistance to powdery mildew to be used for receiving triploid hybrids with eventually high resistance to the disease.

Most moderately and some highly tolerant varieties appear to express quantitative resistance against powdery mildew agent, and quantitative resistance refers to resistance controlled by multiple genes (Ruppel, 1995). Our test results confirm that the resistance to powdery mildew is quantitatively based. And the supreme values of the PMR of the tested hybrids are result of the accumulation of such genes for resistance from both parental components in the crosses.

According to Smith and Gaskill (1970) and other authors the inheritance of the resistance to *Cercospora* leaf spot is usually intermediate, and sometimes the resistance of the hybrid is lower than the midparents' (MP – mean value of the parents) resistance. We have also observed such inheritance of resistance in our tests with conventional triploid hybrids of sugar beet, where the pollinators are multigerm tetraploid populations. But the statement, that the polygenically controlled resistance is inherited intermediately in the hybrids is not valid in our case - with the use of dihaploid pollinators the situation is quite different and the results are in full contrast with the above mentioned authors' findings (Table 3). Data show that there is no case (with the exception of the hybrid MS316 x Dh4), where the F_1 rating of CR is lower than the midparents' rating. The inheritance of CR for the dihaploid hybrids group is overdominant (d/a=1.36), even if the correlation value of the diploid hybrids of Dh3, Dh5 and Dh 6 shows incomplete dominance of the pollinator's resistance. The overdominance of the heritage factors for CR of the MS 19 in its hybrid with Dh1 should be also noted.

Table 3. Inheritance of *Cercospora* Leaf Spot resistance in hybrids of multigerm dihaploid lines.

Hybrid	P_1rating	P_2rating	MP	F_1rating	d	a	d/a
MS 19 x Dh 1	2.84	2.52	2.68	2.94	0.26	0.16	1.63
MS 19 x Dh 2	2.84	3.08	2.96	3.12	0.16	0.12	1.33
MS 19 x Dh 3	2.84	2.99	2.94	3.00	0.06	0.08	0.75
MS 19 x Dh 4	2.84	3.16	3.00	3.23	0.23	0.16	1.44
MS 19 x Dh 5	2.84	3.01	2.92	2.95	0.03	0.09	0.33
MS 19 x Dh 6	2.84	3.00	2.92	2.98	0.06	0.08	0.75
MS 19 x Dh 7	2.84	3.09	2.97	3.32	0.35	0.13	2.69
MS 19 x Dh 8	2.84	2.74	2.79	2.88	0.09	0.05	1.80
Average for diploid hybrids	**2.84**	**2.95**	**2.90**	**3.05**	**0.15**	**0.11**	**1.36**
MS 316 x Dh 1	3.51	2.52	3.02	3.49	0.47	0.50	0.94
MS 316 x Dh 2	3.51	3.08	3.30	3.59	0.29	0.22	1.32
MS 316 x Dh 3	3.51	2.99	3.25	3.74	0.49	0.26	1.88
MS 316 x Dh 4	3.51	3.16	3.34	3.32	- 0.02	0.18	- 0.11
MS 316 x Dh 5	3.51	3.01	3.26	3.71	0.45	0.25	1.80
MS 316 x Dh 6	3.51	3.00	3.26	3.37	0.11	0.26	0.42
MS 316 x Dh 7	3.51	3.09	3.30	3.66	0.36	0.21	1.71
MS 316 x Dh 8	3.51	2.74	3.13	3.30	0.17	0.39	0.44
Average for triploid hybrids	**3.51**	**2.95**	**3.23**	**3.52**	**0.29**	**0.28**	**1.04**

The high CR ratings of the tested triploid hybrids are due to the high resistance of the maternal MS line used in the crosses. The heritage factors of the highly resistant MS line dominate in the triploid hybrids (d/a=1.04). The higher CR of the MS line is inherited with incomplete dominance (the crosses of Dh6 and Dh8), with dominance (the cross of Dh1), and with overdominance – in the crosses of Dh2, Dh3, Dh5 and Dh7. Only in the triploid hybrid of Dh4, probably because of the comparatively high resistance of the pollinator, the inheritance of CR is intermediate.

The different directions of the CR inheritance in the diploid and the triploid hybrids confirm the complicated mechanism of interaction between the genetic systems of the pathogen and the host plant. The dominance of the heritage factors for resistance to both diseases of the maternal component in the triploid crosses could be explained with the fact that the MS line is a monogerm tetraploid line, i.e. it participates with two genomes in the tested triploid hybrids. The higher resistance genes dose of the maternal component simply preconditions the higher resistance to the pathogens in its triploid hybrids.

Conclusion

The induced haploidy differentiates the initial population, and in the obtained dihaploids are stabilized unique gene recombinations for higher resistance to the fungal diseases. The higher resistance to powdery mildew and *Cercospora* leaf spot of the multigerm dihaploid pollinators is inherited overdominantly in their diploid hybrids. In the triploid hybrids of the same dihaploid pollinators dominates the higher resistance heritage factors of the tetraploid maternal component.

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