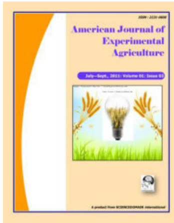

American Journal of Experimental Agriculture
1(4): 214-225, 2011

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Diallel Cross Analysis in Sugar Beet (*Beta vulgaris* L.): Identification of the Best Parents and Hybrids for Resistance to Bolting and *Cercospora* Leaf Spot in Sugar Beet Monogerm O-type Lines

Mohsen Niazian[1*], Khodadad Mostafavi[2], Seyed Habib Shojaei[2], Elaheh Fayyaz[3] and Ali Shahbazi[4]

[1]Department of Plant Breeding, College of Aboureyhan, University of Tehran, Iran.
[2]Department of Agronomy and Plant Breeding, Karaj Branch, Islamic Azad University, Karaj, Iran.
[3]Department of Agronomy and Plant Breeding, Bauali Sina University, Hamedan, Iran.
[4]Department of Horticultural Crop Production, Abouraihan, University of Tehran, Tehran, Iran.

Research Article

Received 26th June 2011
Accepted 14th July 2011
Online Ready 16th July 2011

ABSTRACT

Aims: In order to identify the best parents and hybrids for resistance to Bolting and *Cercospora* leaf spot 9 sugar beet O-type lines in format method of Diallel 9×9 were crossed.
Study design: 9 Sugar beet O-type lines in format method of 9×9 4 Diallel crossing was performed using II Griffing's method were crossed and with four control treatments in a triple lattice design with three replicates.
Place and Duration of Study: Safiabad Agricultural Research Center, Dezful, Iran during 2008-2009 growing season.
Methodology: Analysis of combining ability by using Griffing's method II Diallel crossing scheme after elimination of the control treatments. KWS scale from 1 to 9 (1= healthy plants and 9 = maximum injury) was used to estimation of resistance to *Cercospora* disease.
Results: General combining ability of O-types was significant for potassium, alpha amino

**Corresponding author: Email: nizianm@yahoo.com;*

nitrogen and alkalinity at 1% and for resistance to Bolting and *Cercospora*, molassed sugar, root yield, sugar yield and white sugar yield at 5% probability levels. Also, specific combining ability was significant for resistance to Bolting, potassium, root yield and sugar yield at 1% and for resistance to *Cercospora* and white sugar yield at 5% probability levels.
Conclusion: Additive and non-additive gene effects control the expression of resistance to *Cercospora* and white sugar yield. Also, resistance to Bolting was found to be mainly determined by the non-additive gene effects. The best parent and hybrid for resistance to *Cercospora* were RR607 and RR607 × 452, respectively. Furthermore, the best parent and hybrid for resistance to Bolting were 7173-36 and 436 × 436, respectively. Also the best parent for double resistance to *Cercospora* and bolting were RR607 and RR607×436 and RR607×7112-36 is the best hybrids.

Keywords: Sugar beet; Cercospora; double resistance; general combining ability; specific combining ability;

1. INTRODUCTION

Sugar beet (*Beta vulgaris* L.) is one of the most important sugar crops. It is a biennial plant, a member of the Chenopodiaceae, and like many others in the family is a halophyte (able to grow on saline soils) (Elliot and Weston, 1993). It is essentially a crop of temperate regions, the great majority being grown between 30° and 60°N (e.g. from Cairo to Helsinki) in Europe, Asia, North America and North Africa, with a relatively small amount grown in South America (Chile and Uruguay) (Cooke and Scott, 1993). In southwest Iran Sugar beet seeds are sown in the field from February until May. Sugar beet in autumn sowing areas, like southern Spain, northern Italy and southwest of Iran, remain in the field during the winter months. The plants then encounter the moderately low temperatures of the season (Sadeghian and Johansson, 1993).

One of the genetic analysis methods that illuminate the control method of quantitative traits is Diallel crossing. Diallel cross designs are frequently used in plant breeding research to obtain information on genetic effects for a fixed set of parental lines or estimates of general combining ability (GCA) and specific combining ability (SCA) variance components and heritability for a population from randomly chosen parental lines (Topal et al. 2004). The four methods of Griffing (1956) have usually been used to obtain genetic information on the basis of data from only 1 year or one location, although multiple environment data were suggested to provide more reliable genetic information on material tested (Zhang and Kang, 1997). In addition, the diallel cross technique was reported to provide early information on the genetic behaviour of these attributes in the first (F1) generation (Chowdhry et al., 1992).

Major soil-borne fungal diseases in sugar beet include *Rhizoctonia solani*, *Aphanomyces cochlioides*, *Fusarium* spp., and *Verticillium dahliae*. Above-ground, sugar beet leaves are attacked by *Erysiphe betae* (mildew), *Uromyces betae* (rust), and the leaf spotting pathogens *Ramularia beticola* and *Cercospora beticola* (Vereijssen, 2004). *Cercospora beticola* Sacc., severely infect sugar beet plants worldwide (Georgopoulos and Dovas, 1973; Smith and Ruppel, 1974) and causes great reduction in sugar yield up to 43% (Shane and Teng, 1992).

Loss of sugar in beet roots occurs as new leaves are grown to replace those heavily damaged by *Cercospora* leaf spot (Steinkamp et al., 1979; Vereijssen et al., 2003). Losses are manifested as reduction in root weight, lower sugar content, and increased impurities leading to a loss of sugar to molasses (Smith and Martin, 1978).

The Kleinwanzlebener Saatzucht (KWS) scale (Shane and Teng, 1992) for CLS is described for both single leaves and whole plants. Description of disease categories is broad, but the KWS scale is used by many plant breeders for rapid assessment of resistance to *Cercospora* leaf spot in sugar beet breeding lines (Shane and Teng, 1992). Resistance to *Cercospora* leaf spot in sugar beet has been described as quantitatively inherited and rate limiting with respect to disease development (Rossi et al ., 1999; Smith and Gaskill, 1970). Sadeghian and Johansson (1993), by doing a factorial mating design (N.C. design II) to determination the genetic basis of bolting and stem length in sugar beet full-sibs, reported that bolting resistance seemed to be dominant to the bolting susceptibility, and Narrow sence heritability estimated for bolting was generally very large (0.93 to 0.96), which suggests that early generation selection for bolting resistance in a sugar beet population would be successful.

Guan et al., (1994), stated that bolting phenomenon involved several genes that one of them is themain and others are subsidiary.

The objective of the current study was to estimate GCA, SCA, and heritability of resistance to *Cercospora beticola* leaf spot disease and bolting traits of sugar beet o-type lines and identification the best parents and hybrids for resistance to Bolting and *Cercospora* leaf spot with the use of diallel crossing.

2. MATERIAL AND METHODS

In this study 9 sugar beet O-type lines including 7173, 474, 452, 261, 436-104, SB-FIROZ, RR607, 436 and 7112-36 in format method of Diallel 9×9 were crossed. Diallel crossing was performed using II Griffing's method. Number of treatments was p(p+1)/2= (9×10)/2=45 (36 F1 hybrid and 9 hybrid as parent) that together with four control treat (total treatments 45 + 4 = 49 treat) in a triple lattice design with three replicates were cultured at Safiabad Agricultural Research Center, Dezful, Iran during 2008-2009 growing season under natural infection of disease of Cercospora condition. Each plot in each replication was included two lines with 5 meter in length.

Resistance to traits of *Cercospora* and bolting in F1 hybrid population that derived from 9*9 Diallel crossing design was evaluated with control treatments. KWS scale from 1 to 9 (1= healthy plants and 9 = maximum injury) was used to estimation of resistance to *Cercospora* disease. The KWS scale is based on a set of the drawings and descriptions distributed by the Kleinwanzlebner Saatzucht Company of Einbeck, Germany, for rating disease intensity (Shane and Teng, 1992). Resistance to *Cercospora beticola* estimated at two stages: 15 and 30 may 2009 (15 day interval). Percentage of plants that bolted at harvest time in each plot was used for evaluation of resistance to bolting. The SQRT transformation was used for raw data set obtained from KWS scale. Technological traits that measured in this experiment was including: potassium (K), molassed sugar (MS), alpha amino (N) and alkalinity (ALC). Also normality test was calculated for investigated traits by using SAS software (Ver 9.1).

According to obtained result, logarithmic transformation was used for alkalinity (ALC) and alpha amino (N) traits, and reverse transformation was used for molassed sugar (MS).

Finally analysis of variance for general and specific combining ability was carried out according to Griffing's (1956) by using Griffing's method II Diallel crossing design after elimination of the control treatments.

3. RESULTS AND DISCUSSION

3.1 Analysis of Variance of Diallel Crosses

Results of analysis of variance showed that, there were significant differences among 49 genotypes for resistance to Bolting and *Cercospora*, potassium, alkalinity, root yield, sugar yield, white sugar yield, molassed sugar and alpha amino nitrogen traits (Table not reported).

Table 1 shows the analysis of diallel crosses in genotypes in terms of desired traits. Analysis of combining ability indicated that general combining ability of 45 genotypes was significant for potassium, alpha amino nitrogen and alkalinity at 1% and for resistance to Bolting and *Cercospora*, molassed sugar, root yield, sugar yield and white sugar yield at 5% probability levels. Also, specific combining ability was significant for resistance to Bolting, potassium, root yield and sugar yield at 1% and for resistance to Cercospora and white sugar yield at 5% probability levels. Also the SCA variances are significant only for traits resistance to bolting, root yield, sugar yield and white sugar yield ($P < 0.05$).

Our study showed that additive and non-additive gene action (dominant and epistasis) plays an important role in the genetic control of resistance to *Cercospora beticola* and bolting, but in resistance to bolting non-additive effects is more. A low narrowsense heritability of resistance to bolting is also estimated (Table 4).

3.2 Estimation the General and Specific Combining Ability for Resistance to *Cercospora* and Bolting

Results of general combining ability for resistance to *Cercospora* indicated that, 7172-36 o-type had the highest general combining ability (positive and significant at 5%), and the lowest general combining ability is related to RR 607 genotype (negative and significant at 1% probability levels) (Figure 1b).

According to obtained results, it suggests that RR607 genotype can be useful to apply in breeding programs for resistance to *Cercospora* disease.

Results of specific combining ability for resistance to *Cercospora* indicated that, most SCA related to hybrid obtained from selfing RR 607 o-type(positive and significant at 1%), and the lowest specific combining ability is related to hybrid obtained from 452 and RR607 o-types cross (negative and significant at 1% probability levels) (Table 2).

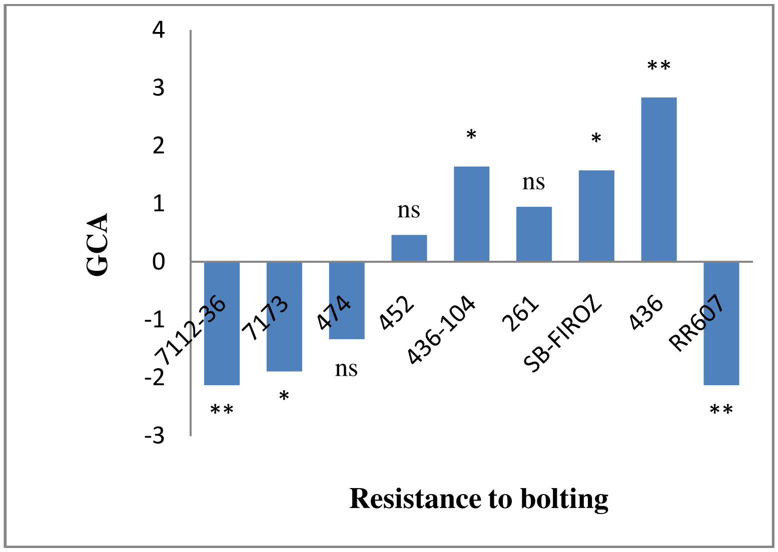
For resistance to bolting trait the highest general combining ability was for 436 genotype (positive and significant at 1%) and the lowest general combining ability is related to RR 607 and 7112-36 genotypes (negative and significant at 1% probability levels) (Figure 1a). SCA variance contains dominance epistasis (Griffing, 1956).

Table 1. Analysis of variance of diallel crosses for some agronomic and technologic traits in sugar beet

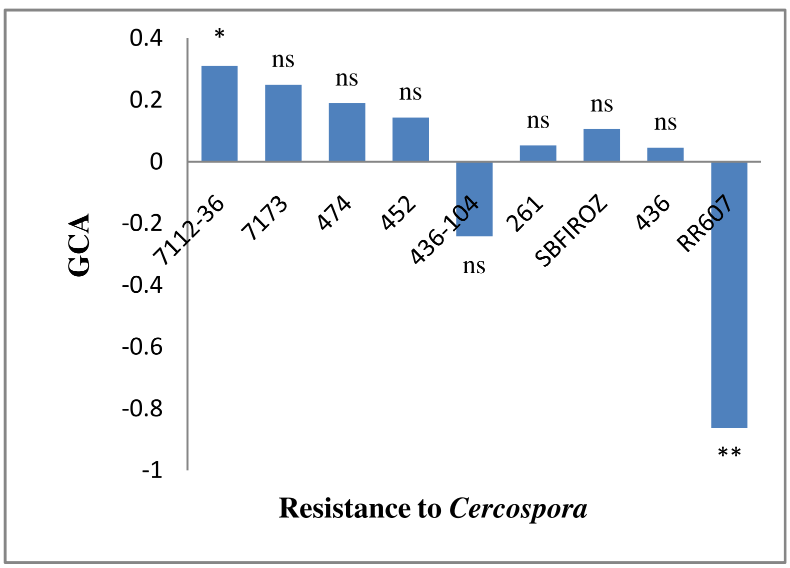
	MS					
Traits variance Std err (var)	GCA (df=8)	SCA (df=36)	Error (df=88)	$F=\frac{V_{GCA}}{V_{SCA}}$	$t=\frac{\delta^2 gca}{\sqrt{var(\delta^2 gca)}}$	$t=\frac{\delta^2 sca}{\sqrt{var(\delta^2 sca)}}$
R.C	4.27*	1.48*	0.85	2.88*	1.30 ^{ns}	1.67 ^{ns}
Variance	0.08	0.2	0.85	----	----	----
Std err (var)	0.06	0.12	0.12	----	----	----
R.B	118.68*	52.39**	21.6	2.26*	1.094^{ns}	2.41*
Variance	2	10.26	21.6	----	----	----
Std err (var)	1.83	4.25	3.25	----	----	----
K	1.22**	0.21**	0.11	5.58**	1.63 ^{ns}	1.92 ^{ns}
Variance	0.03	0.03	0.11	----	----	----
Std err (var)	0.01	0.01	0.01			----
N	0.03**	0.07 ^{ns}	0.07	4.76**	1.57 ^{ns}	0.00
Variance	8.2×10^{-4}	0.00	7.8×10^{-3}	----	----	----
Std err (var)	5.2×10^{-4}	6.9×10^{-4}	0.11	----		----
ALC	0.02**	3×10^{-2ns}	3×10^{-2}	7.48**	1.72 ^{ns}	0.28 ^{ns}
Variance	6.4×10^{-4}	8.4×10^{-5}	3×10^{-3}	----	----	----
Std err (var)	3.7×10^{-4}	2.9×10^{-4}	4.5×10^{-4}	----	----	----
MS	0.01*	0.06^{ns}	5×10^{-3}	2.93*	1.29^{ns}	0.40 ^{ns}
Variance	3.5×10^{-4}	2.2×10^{-4}	5.3×10^{-3}	----	----	----
Std err (var)	2.7×10^{-4}	5.4×10^{-4}	8×10^{-4}	----	----	----
RY	605.81*	203.81**	99.38	2.97*	1.31 ^{ns}	2.07*
Variance	12.18	34.81	99.38	----	----	----
Std err (var)	9.29	16.77	14.98	----	----	----
SY	13.6*	5.23**	2.25	2.60*	1.21 ^{ns}	2.32*
Variance	0.25	0.99	2.25	----	----	----
Std err (var)	0.2	0.42	0.33	----	----	----
WSY	8.24*	3.48*	1.51	2.36*	1.13 ^{ns}	2.30*
Variance	0.144	0.65	1.51	----	----	----
Std err (var)	0.12	0.28	0.22	----	----	----

**, **: significant at the 5 and 1% of probability level, respectively; ns = not significant, T(t) (df=88) α=0.05=1.98, α=0.01=2.617. RC: Resistance to Cercospora; RB: Resistance to Bolting; K: Potassium; N: Alpha Amino; ALC: Alkalinity; MS: Molassed Sugar; RY: Root Yield; SY: Sugar Yield; WSY: White Sugar Yield; GCA: General Combining Ability; SCA: Specific Combining Ability.*

Bolting is influenced by genetic, environmental and physiological factors and genes with additive effects and epistasis also effective in this phenomenon (Sadeghian and Johansson, 1993). Jullife et al. (1993) in study of bolting by use the incomplete diallel method in 9 S1 line in sugar beet stated that Additive effects of genes that have important and significant non-additive effects and where it is involved in the incidence epistasis.



(a)



(b)

Fig. 1. General combining ability of 9 sugar beet O-type lines for resistance to Bolting (a) and *Cercospora* (b)

Table 2. Specific combining ability of 9 sugar beet O-type lines for resistance to *Cercospora beticola*

P	7112-36	7173	474	452	261	436-104	SB-Firoz	RR607	436
7112-36	-0.01 ns	0.04ns	0.107ns	0.81ns	-0.42ns	-0.54ns	0.85ns	-1.25ns	-0.41ns
7173	0.04ns	-0.55ns	-0.91ns	0.88ns	-0.36ns	-0.06ns	1.16*	-0.11ns	0.47ns
474	0.107ns	-0.91ns	0.06ns	0.19ns	-0.05ns	-0.42ns	0.14ns	0.61ns	0.2ns
452	0.81ns	0.88ns	0.19ns	0.06ns	-0.006ns	0.28ns	-0.05ns	-1.3**	-0.91ns
261	-0.42ns	-0.36ns	-0.05ns	-0.006ns	1×10^{-3}ns	0.46ns	0.03ns	0.08ns	0.25ns
436-104	-0.54ns	-0.06ns	-0.42ns	0.28ns	0.46ns	0.75ns	-0.58ns	-0.11ns	-0.52ns
SB-Firoz	0.85ns	1.16*	0.14ns	-0.05ns	0.03ns	-0.58ns	-0.93*	0.03ns	0.28ns
RR607	-1.25ns	-0.11ns	0.61ns	-1.3**	0.08ns	0.11ns	0.03ns	1.66**	-1.24*
436	-0.41ns	0.47ns	0.2ns	-0.91ns	0.25ns	-0.52ns	0.28ns	-1.24*	0.51ns

**, **: significant at the 5 and 1% of probability level, respectively; ns = not significant.*

Table 3. Specific combining ability of 9 sugar beet O-type lines for resistance to Bolting

P	7112-36	7173	474	452	261	436-104	SB-Firoz	RR607	436
7112-36	2.03 ns	1.01 ns	-1.2 ns	2.49 ns	1.56 ns	-2.22 ns	-3.04 ns	-0.41 ns	-2.25 ns
7173	1.01 ns	-0.88 ns	-1.44 ns	-0.27 ns	4.16 ns	-3.28 ns	-1.5 ns	-0.65 ns	3.75 ns
474	-1.2 ns	-1.44 ns	-2.00ns	0.32 ns	1.24 ns	4.83 ns	0.96 ns	-1.2 ns	0.49 ns
452	2.49 ns	-0.27 ns	0.32 ns	-2.65 ns	-1.39 ns	-5.76 ns	-2.6 ns	4.55 ns	7.97*
261	1.56 ns	4.16 ns	1.24 ns	-1.39 ns	-1.56 ns	2.12 ns	-6.29*	2.55 ns	-0.82 ns
436-104	-2.22 ns	-3.28 ns	4.83 ns	-5.76 ns	2.12 ns	-5.84**	11.43**	-4.18 ns	8.76**
SB-FIROZ	-3.04 ns	-1.5 ns	0.96 ns	-2.6 ns	-6.29*	11.43**	0.59 ns	-0.23 ns	0.11 ns
RR607	-0.41 ns	-0.65 ns	-1.2 ns	4.55 ns	2.55 ns	-4.18 ns	-0.23 ns	1.09 ns	-2.6 ns
436	-2.25 ns	3.75 ns	0.49 ns	7.97*	-0.82 ns	8.76**	0.11 ns	-2.6 ns	-7.71**

**, **: significant at the 5 and 1% of probability level, respectively; ns = not significant.*

Since the RR607 parent terms of both traits resistant to *Cercospora* and bolting, has the lowest general combining ability (Figure 1). Therefore be stated that in this research is to identify a parent that it can use in cross programs for increasing resistance to *Cercospora* and bolting simultaneously. Because it parent generally (General combining ability) if crossed by other parents led to improve both traits resistance to *Cercospora* and bolting. This represents the additive effects genes in it parent for both traits increasing resistance to *Cercospora* and bolting.

For resistance to bolting highest estimate of SCA from crosses of 436×436-114 parents (positive and significant at 1%) and the lowest SCA estimate from SB-Firoz×261 negative and significant at 5% probability levels) (Table 3).

The best hybrid for double resistance to *Cercospora* and bolting is 436×RR607 hybrid which have negative SCA for both treat.

3.3 Estimation of Heritability (h2 ns) and Additive, Dominance and Error Variance with Use of the Griffing Method

For resistance to bolting, resistance to *Cercospora*, root yield, sugar yield and white sugar yield traits, percent of dominant variance more than from additive variance was estimated (Table 4). Estimated values indicated that non-additive genes effects of controlling these traits are more. But for technological traits, such as Potassium and Alkalinity, additive genes effects were involved in controlling these features in all genotypes. High additive gene effects for a specific trait will increase success in selections for that trait (Tosun et al., 1995).

The additive and dominance genetic variances of resistance to *Cercospora* accounted for 25.76% and 36.18% and for resistance to Bolting accounted for 18.22% and 53.2% of the total variance, respectively (Table 4).

In the one study in order to determine the Parental effect on yield and quality and division of genetic variance and estimation of gene function in root yield, sugar content, and Components of quality of sugar beet in sugar beet hybrids, reported that non-additive gene action is more important for root yield, while for sugar content and Components of quality of root, the additive gene action is more important (Antonov, 1985).

Srivastava et al. (1986) by examining the combining ability and Combination of genetic variance components for root yield trait in sugar beet, showed that Genes with non- additive effects works in controlling these traits are more and for potassium, alpha amino nitrogen, alkalinity and molassed sugar treat, additive effects of genes more than non- additive effects of genes was estimated.

3.4 Heterosis Estimation Relative to High Parent (Heterobeltiosis) for Resistance to *Cercospora* and Bolting

For resistance to Bolting the highest heterosis is related to hybrid genotypes derived from cross genotypes 436×436-104 and the lowest heterosis related to hybrid resulting from cross SB-FIROZ × 261. Since heterosis SB-FIROZ × 261 negative, so can be use this genotype as a hybrid with high resistance to the Bolting (Table 5).

Table 4. Heritability (h^2_{ns}) and additive, dominance and error variance as percent to total phenotypic variance for some traits in sugar beet

Character	Phenotype		Error		Dominance		Additive		H^2_n
	%	varinace	%	variance	%	variance	%	variance	
Resistance to *Cercospora*	100	0.749	38.05	0.285	36.18	0.271	25.76	0.193	25.76
Resistance to Bolting	100	25.19	28.57	7.2	53.2	13.4	18.22	4.59	18.22
Potassium	100	0.15	25.06	0.038	29.73	0.045	45.39	0.06	45.39
Alpha amino nitrogen	100	0.004	59.09	0.002	0.00	0.00	40.9	0.0018	40.9
Alkalinity	100	0.0025	38.75	0.001	4.26	0.0001	56.97	0.0014	56.97
Molassed sugar	100	0.0028	61.88	0.0017	10.13	0.0002	27.97	0.0008	27.97
Root yield	100	106.42	31.12	33.12	42.71	45.46	26.15	27.83	26.15
Sugar yield	100	2.62	28.59	0.751	49.35	1.29	22.04	0.57	22.04
White sugar yield	100	1.68	29.89	0.505	50.62	0.85	19.47	0.329	19.47

Table 5. Heterosis by cross of 9 sugar beet O-type lines, relative to high parent (Heterobeltiosis) for resistance to Bolting

Parents	7112-36	7173	474	452	261	436-104	SB-FIROZ	RR 607
7173	-0.78							
474	-2.45	0						
452	2.56	0.03	1.18					
261	0.05	2.89	0.53	-0.31				
436-104	-.049	-0.98	7.69	-1.93	4.39			
SB-FIROZ	-7.34	-5.56	-2.54	-4.31	-7.52	10.9		
RR 607	-2.45	-1.56	-1.51	4.62	1.04	-2.12	-4.53	
436	0.49	6.74	4.04	13.01	2.62	15.28	0.77	0.14

Number which is normal underlined is low heterosis and number by bold underlined is high heterosis.

Table 6. Heterosis by cross of 9 sugar beet O-type lines, relative to high parent (Heterobeltiosis) for resistance to *Cercospora beticola*

Parents	7112-36	7173	474	452	261	436-104	SB-FIROZ	RR 607
7173	0							
474	0	-0.91						
452	0.66	0.91	0.08					
261	-0.66	-0.16	-0.25	-0.16				
436-104	-1.08	-0.33	-0.91	-0.16	0			
SB-FIROZ	0.66	1.58	0	-0.16	0.08	-1		
RR 607	-2.41	-0.66	-0.5	-2.41	-0.83	-1.5	-0.66	
436	0.16	0.16	-0.16	-1.33	-0.25	-1.33	-0.16	-2.66

Number which is normal underlined is low heterosis and number by bold underlined is high heterosis.

For resistance to *Cercospora* disease, the highest heterosis is related to SB-FIROZ × 7173 hybrids, and the lowest heterosis related genotypes from RR607 × 436 crosses (Table 6). So can be use RR607 × 436 hybrids for highest resistance to *Cercospora beticla* disease.

Also hybrids RR607 × 7112-36 have a negative heterosise for both traits resistance to Bolting and *Cercospora*, and also this genotype have a negative SCA for both traits resistance to Bolting and *Cercospora* (Tables 2 & 3), and can be used this hybrid for double resistance to *Cercospora Beticola* and Bolting.

4. CONCLUSION

The best parent and hybrid for resistance to *cercospora* were rr607 and rr607 × 452, respectively. Furthermore, the best parent and hybrid for resistance to bolting were 7173-36 and 436 × 436, respectively. Also the best parent for double resistance to *Cercospora* and bolting were rr607 and rr607×436 and rr607×7112-36 is the best hybrids**.**

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