Analysis of Resistance to Cercospora Leaf Spot and Bolting in Sugar Beet as Winter Crop Using Griffing's Diallel Method and GGE Biplot

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ABSTRACT

In order to study the genetics of resistance to Cercospora leaf spot (CLS) and bolting in sugar beet, nine parental lines and their F_1 hybrids were formed in a diallel cross pattern. The genotype main effect plus genotype x environment interaction (GGE) biplot and Griffing's method II diallel scheme were used for analysis. Combining ability, gene action and heterosis were estimated for these two important traits. The biplot displayed the most important entry by tester patterns of the data and allowed the information to be extracted visually. Using this technique, different genetic aspects were clearly visualized. The biplot analysis indicated that the first two principal components explained 74% (59% and 15% by PC1 and PC2, respectively), and 75% (42.36% and 32.64% by PC1 and PC2, respectively) of the variation for resistance to CLS and bolting, respectively. Based on GGE biplot presentation and Griffing's diallel

analysis, RR607 showed the largest negative General Combining Ability (GCA), indicating contribution towards resistance to CLS, and Genotype 436 presented the largest positive GCA, indicating contribution towards susceptibility. Also 7112-36 and RR607 showed the largest negative GCA for bolting percentage, indicating contribution towards resistance to bolting, and 436 presented the largest positive GCA, indicating contribution towards susceptibility to bolting. The results indicated that the SB-FIROZ × 261 F_1 hybrid showing negative heterosis for bolting percentage, so these genotypes possess at least some different resistance genes, which were expressed in the hybrids and led to the observed effects. Results were coherently presented by biplot and Griffing's diallel analyses. However, the GGE biplot method is recommended because of its powerful capability to visualize the results of entry by tester (diallel) data.

Additional Key Words: Bolting, Cercospora leaf spot, general combining ability (GCA), GGE biplot, specific combining ability (SCA), winter beet

Abbreviations: ATC = Average tester coordinate, CLS = Cercospora leaf spot, GCA = General combining ability, GGE = Genotype main effect plus genotype x environment interaction, MPH = Mid-parent heterosis, RCBD = Randomized complete block design, SCA = Specific combining ability, SREG = Site regression.

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 able to grow on saline soils (Elliot and Weston, 1993). In southwest Iran, sugar beet is generally sown in October and harvested in June as in other autumn sowing areas, such as southern Spain and northern Italy, to take advantage of moderately low temperatures of the winter season (Sadeghian and Johanson, 1992). The advantage of these areas is the presence of already established sugar factories for processing the tropical sugar beet. However, in each region, the planting pattern, agronomic practices and development of new bolting resistant varieties should be considered for cultivation of sugar beet in the future (Fathollah-Taleghani et al., 2010).

One of the important fungal diseases on sugar beet worldwide is Cercospora leaf spot (CLS) caused by *Cercospora beticola* Sacc. (Vereijssen, 2004). CLS at high levels may cause reduction in sugar yield up to 43% as plants consume sugar to produce new leaves (Georgopo-

lus and Dovas, 1973; Steinkamp et al., 1979; Shane and Teng, 1992; Smith and Martin, 1978; Vereijssen, et al., 2003). Some breeders used a numerical scale (0-10) for evaluation of resistance to CLS, in which 0 indicates healthy plants and 10 represents maximum damage (Abasi et al., 2002; Smith and Martin, 1978). Other scales are those of Kleinwanzlebener Saatzucht (KWS) that have individual plant rating scales consisting of (1-9), (1-6) and (1-5), which have been used by some researchers for evaluation of resistance to CLS (Shane and Teng, 1992; Panella and Frese, 2000; Abbasi et al., 2002; Khan and Khan, 2003; Khan et al., 2008). Resistance to CLS in sugar beet has been described as quantitatively inherited and rate limiting with respect to disease development (Smith and Gaskill, 1970; Smith and Ruppel, 1974; Rossi et al., 1999).

A promising strategy to increase the yield potential of sugar beet is to grow it as a winter crop thus extending its vegetative period (Kopisch-Obuch et al., 2009). However, this has not been achieved because sugar beet starts bolting after prolonged exposure to cold followed by long days (vernalization) (Kopisch-Obuch et al., 2009). Sadeghian and Johansson (1992) used a factorial mating design (N.C. design II) to determine the genetic basis of bolting and stem length in sugar beet full-sibs, and reported that bolting resistance seemed to be dominant to bolting susceptibility. They also reported that narrow-sense heritability estimated for bolting was generally very high (0.93 to 0.96), suggesting that early generation selection for bolting resistance in a sugar beet population would be successful. Guan et al. (1994) stated that the bolting phenomenon involved several genes including a major gene and some minor genes.

One genetic analysis to elucidate the control method of quantitative traits is diallel crossing (Orazizadeh et al., 2002). Diallel cross designs are frequently used in plant breeding research to obtain information on genetic effects for a fixed set of parental lines, to estimate general and specific combining abilities (GCA and SCA), variance components, and heritability for a population from randomly chosen parental lines (Topal et al., 2004). The four methods of Griffing (1956) usually are used to gain genetic information on the basis of data from only one year or one location (Zhang and Kang, 1997). In addition, the diallel cross technique was reported to provide early information on the genetic behavior of these attributes in the first (F_1) generation (Chowdhry et al., 1992). The genotype main effect plus genotype x environment interaction (GGE) biplot analysis of diallel data proposed by Yan and Hunt (2002) is a highly effective and informative method to estimate GCA and SCA effects. Although the GGE biplot methodology was developed for multi-environment variety trials (MET) data analysis, it is applicable to all types of two way data that assume an entry-by-tester data structure (Yan and Hunt, 2002). In a diallel data set, each genotype is both an entry and a tester (Darvishzadeh et al., 2009). Applying GGE biplot allows

breeders to visualize those varieties that show the GCA and SCA effects of each parent; the best testers; the heterotic groups; and genetic constitutions of parents with regard to the trait under investigation (Yan and Hunt, 2002). The model used for biplot analysis of diallel data is tester-centered principal component analysis.

The objectives of the current study were to estimate GCA and SCA for *Cercospora beticola*-induced leaf spot (CLS) and bolting resistance in the parental genotypes and to identify the best parents, hybrids, and testers for resistance to bolting and CLS by using GGE biplot of diallel crossing data.

MATERIALS AND METHODS

Nine sugar beet lines, 7173, 474, 452, 261, 436-104, SB-FIROZ, RR607, 436 and 7112-36 (Breeding materials of the Sugar Beet Seed Institute, SBSI, Karaj, Iran), were crossed in a 9×9 diallel format. Diallel crossing was performed using Griffing's method II (Table 1). The number of treatments was 45 (where $(p(p+1))/2 = (9\times10)/2$; 36 F_1 hybrids (Table 2) and 9 lines as parents = P) which, together with four control treatments (for a total of 49 treatments), were grown at Safi-

Table 1. Sugar beet varieties used in the experiments. Included are parents used in the 9×9 diallel crossing and GGE biplot analysis and check varieties selected for response to CLS and bolting behavior.

No.	Name of Variety/ Pedigree	Distinctive features *(Entry ♀, Tester ♂, Seed)*[†Z]
1	7112-36	Cytoplasmic male sterility, O-type, monogerm
2	7173	Cytoplasmic male sterility, O-type, monogerm
3	474	Cytoplasmic male sterility, O-type, monogerm
4	452	Cytoplasmic male sterility, O-type, monogerm
5	261	Cytoplasmic male sterility, O-type, monogerm
6	436-104	Cytoplasmic male sterility, O-type, monogerm
7	SB-FIROZ	Cytoplasmic male sterility, O-type, monogerm
8	RR607	Cytoplasmic male sterility, O-type, monogerm
9	436	Cytoplasmic male sterility, O-type, monogerm
10	Monotuno	Check- resistant to bolting
11	Palma	Check- resistant to CLS
12	Rasoul	Check- resistant to CLS and bolting
13	9597	Check-susceptible to CLS and bolting

[†]Features include, leaf spot response (resistant or susceptible to Cercospora leaf spot (CLS).

Table 2. Sugar beet F_1 hybrids used in the experiments.

Hybrid number	Parents	Hybrid number	Parents
1	7112-36×7173	19	SBFIROZ×7173
2	474×7112-36	20	RR607×7173
3	452×7112-36	21	436×7173
4	261×711236	22	SBFIROZ×261
5	436-104×7112-36	23	452×261
6	SBFIROZ×7112-36	24	436×261
7	RR607×7112-36	25	436-104×261
8	436×7112-36	26	RR607×261
9	261×474	27	436-104×452
10	7173×474	28	SBFIROZ×452
11	SBFIROZ×474	29	RR607×452
12	452×474	30	436×452
13	436×474	31	436×SBFIROZ
14	436-104×474	32	436-104×SBFIRO
15	RR607×474	33	RR607×SBFIROZ
16	452×7173	34	RR607×436-104
17	261×7173	35	436×436-104
18	436-104×7173	36	RR607×436

abad Agricultural Research Center, Dezful, Iran, in a triple lattice design with three replications during the 2008-2009 growing season under natural CLS infection. Each plot consisted of two rows of 6 m length. Resistance to CLS and bolting in the F_1 hybrid populations, derived from the 9×9 diallel crossing design, was evaluated. The KWS 1-9 rating scale (1= healthy plants and 9 = maximum damage) was used to quantify resistance to CLS (Table 3, Shane and Teng, 1992; Panella and Frese, 2000; Abbasi et al., 2002; Khan and Khan, 2003; Khan et al., 2008). 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 severity. Resistance to CLS was estimated in ten plants per plot at two stages of plant growth on May 15 and 30 in 2009, and averages of the ten plants scores were considered as a plot score. A test of normality was carried out for the investigated traits using SAS software (Ver 9.1). Because the distribution of raw data for CLS score was not normal, a square root transformation was used to normalize the data distribution. The percentage of bolted plants at harvest in each plot was used to evaluate resistance to bolting. Analysis of variance of the raw data was done using SAS software (Ver 9.1). The analysis of variance showed that the relative efficiency of the lattice design compared with a randomized complete block designs (RCBD) was 107 percent; therefore, the analysis of variance and genetic analysis were done based on

Table 3. Categories of the Kleinwanzlebner Saatzucht scale for rating disease intensity of Cercospora leaf spot (CLS) in sugar beet (Shane and Teng, 1992).

Stage[†]	Leaf	Whole Plant
1	Healthy leaf.	Whole plant healthy.
3	Spots on the outer leaves.	First symptoms of disease, spots on outer leaves.
5	Spots joining together to form areas of dead leaf.	Disease spreads, spots joining together to form areas of dead leaf.
7	Greater part of leaf brown and dead with only the lower part of the leaf still alive.	Whole plant diseased, large parts of the outer leaves dying.
9	Leaf and leaf stalk dead and dried up.	Whole plant diseased, outer leaves dead, inner leaves severely damaged, regrowth of new leaves.

[†]Stages 2, 4, 6, and 8 are derived by interpolation.

RCBD. Genetic analysis of resistance to CLS and bolting was performed using GGE biplot methodology as presented by Yan and Hunt (2002), using MINITAB (Ver. 15) and EXCEL software.

Mathematical Model for GGE biplot

Yan et al. (2001) compared two site regressions (SREG) models, SREG2 and $SREG_{M+1}$, that can be used to generate GGE biplots. The SREG2 model consisted of PC1 and PC2 derived from environment-centered data, referred to as primary and secondary effects, respectively; the $SREG_{M+1}$ model used regressions of environment-centered data against genotype main effects as the primary effect, and PC1 derived from the residual of the regressions as the secondary effect. The SREG2 model had the advantage of explaining more variation, whereas the $SREG_{M+1}$ model had the advantage of explicitly indicating the average yield and stability of the genotypes and the representativeness and discriminating ability of the environments. However, through axis rotation, the SREG2 biplot also can indicate the approximate average yield and stability of the genotypes and the representativeness and discriminating ability of the environments. This allows the advantages of both models to be combined reasonably. Therefore, we used the SREG2 model for diallel data analysis according to Yan and Hunt (2002) by using the average data of three replications (Tables 7 and 8). When GGE biplot is applied to diallel data, the terms average yield and stability of the genotypes correspond to the GCA and SCA of the parents, respectively. Note that in conven-

tional diallel analyses, SCA is associated with crosses rather than parents (Yan and Hunt, 2002).

The SREG2 model is written as:

$$Y_{ij} - \beta_j = \lambda_1 \xi_{i1} \eta_{j1} + \lambda_2 \xi_{i2} \eta_{j2} + \varepsilon_{ij} \quad [1]$$

where Y_{ij} is the genotypic value of the combination (pureline parent or F_1 hybrid) between Entry i and Tester j for the trait of interest; β_j is the mean of all combinations involving Tester j; λ_1 and λ_2 are the singular values for PC1 and PC2, respectively; ξ_{i1} and ξ_{i2} are the PC1 and PC2 eigenvectors, respectively, for Entry i; η_{j1} and η_{j2} are the PC1 and PC2 eigenvectors, respectively, for Tester j; and ε_{ij} is the residual of the model associated with the combination of Entry i and Tester j. Because in diallel cross data each genotype is an entry and a tester, i and j can refer to the same or different genotypes. When i=j, the combination is a pureline rather than a hybrid. In some statistical software such as the Statistical Analysis System (SAS Institute, 1996), the singular values are usually combined with their respective row (entry) eigenvectors so that equation [1] looks like:

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$$Y_{ij} - \beta_j = (\lambda_1 \xi_{i1}) \eta_{j1} + (\lambda_2 \xi_{i2}) \eta_{j2} + \varepsilon_{ij}.$$

To display PC1 and PC2 in a biplot, it is rearranged as

$Y_{ij} - \beta_j = \xi^*_{i1} \eta^*_{j1} + \xi^*_{i2} \eta^*_{j2} + \varepsilon_{ij}$, where $\xi^*_{i1} = \lambda_k^{1/2} \xi_{i1}$ and $\eta^*_{jk} = \lambda_k^{1/2} \eta_{jk}$, with k=1 or 2. This singular value partitioning method is called symmetrical scaling (Yan and Hunt, 2002). Raw data also were analyzed using the Griffing's method II mixed B model (Griffing, 1956), using the DIALLEL software (Burow and Coors, 1994) after elimination of the control treatments. GGE biplot was used to compare results with Griffing's method and to visualize the results.

RESULTS

Analysis of variance showed highly significant differences among parents and F_1 hybrids for disease resistance score and bolting percentage (Table 4). Mean square values of GCA were also significant for both traits. For resistance to bolting, mean square value of SCA was also significant (Table 4). Baker's ratio ($2\sigma^2_{gca} / 2\sigma^2_{gca} + \sigma^2_{sca}$) (Baker, 1978) for resistance to CLS was near to 1, but this ratio was lower than 0.5 for resistance to bolting (Table 4).

According to Griffing's diallel analysis, the largest positive GCA for KWS scale and percentage of bolted plants was related to entry 436, whereas the largest negative GCA for this trait was related to entry RR607 (Tables 5 and 6, respectively). The largest negative SCA for infection by CLS was related to 436 × 7173 (Table 5). Our results show that the SB-FIROZ × 261 F_1 hybrid demonstrated negative het-

Table 4. Analysis of variance and combining abilities for resistance of sugar beet lines and their F_1 hybrids to CLS and bolting.

Sources of Variation	DF	MS	
		Resistance to CLS	*Resistance to bolting*
Variance analysis:			
Block	2	0.021^{ns}	84.28^{*}
Genotype	48	$^{**}0.263$	$^{**}308.14$
Error	96	0.69	23.92
Diallel analysis:			
Block	2	0.201^{ns}	68.44^{*}
GCA	8	19.62^{**}	118.68^{**}
SCA	36	1.87^{ns}	52.39^{**}
Error	88	1.51	21.6
$2\sigma^2_{gca} / 2\sigma^2_{gca} + \sigma^2_{sca}$		0.84	0.36

* & **: significant at 5 and 1% probability level, respectively;
^{ns} = not statistically significant.

erosis for percentage of bolted plants (Table 6). Also, SB-FIROZ × 436-104 F_1 hybrid showed the highest positive SCA for percentage of bolted plants (Table 6). Means of the diallel tables for resistance to CLS and bolting, which were used in GGE biplot, are shown in Tables 7 and 8, respectively. Results from the symmetrically scaled PC1 and PC2 scores are listed in Tables 9 and 10, which were used to construct the biplots.

The biplot for the CLS severity data explained 74% (59% and 15% by the first two principal components, PC1 and PC2, respectively), and for bolting percentage explained 75% (42% and 32% by the first two principal components, PC1 and PC2, respectively) of the total variation of GCA and SCA (Figures 1 and 2). Based on the projection onto the ATC abscissa, entry 436 showed the largest, and entry RR607 revealed the smallest GCA effects (Figure 1a). The GCA rankings of the entries for resistance to CLS, from resistant to susceptible, were RR607 > 261 > 436-104 > 7112-36 > SB-FIROZ > 452 > 474 > 7173 > 436 (Figure 1a), which was consistent with the GCA order of the parents based on Griffing's diallel analysis (Table 5). For resistance to bolting, entry 436 showed the largest, and entries 7173, RR607 and 7112-36 showed the smallest GCA effects (Figure 2a). The GCA rankings of the entries for resistance to bolting, from resistant to susceptible, were 7173 > RR607 ≥ 7112-36 > 452 > 474 > 436-104> 261 > SB-FIROZ >436.

Because the biplot displays both GCA and SCA, and because GCA

Table 5. SCA effects (above diagonal), heterosis value (below diagonal), and GCA effects of sugar beet lines and their F_1 hybrids for resistance to CLS. Negative numbers indicated reduction in the KWS scale, which signifies more resistance to CLS.

	Testers ♂									
Entries ♀	**7112-36**	**7173**	**474**	**452**	**261**	**436-104**	**SB-Firoz**	**RR607**	**436**	**GCA[§]**
7112-36	0.86[ns†]	0.31 [ns]	1.00 [ns]	-1.06 [ns]	-1.06 [ns]	-1.21 [ns]	0.64 [ns]	-0.66 [ns]	0.30 [ns]	-0.175 [ns]
7173	(-0.78)[‡]	0.18 [ns]	-0.33 [ns]	0.57 [ns]	-0.16 [ns]	-0.80 [ns]	0.95 [ns]	0.59 [ns]	-1.49[*]	0.74[**]
474	(0.44)	(-0.75)	-0.12 [ns]	0.42 [ns]	-0.10 [ns]	0.60 [ns]	-0.82 [ns]	0.46 [ns]	-0.97 [ns]	0.51[*]
452	(-1.70)	(-0.01)	(0.38)	0.11 [ns]	-0.03 [ns]	-0.47 [ns]	0.85 [ns]	-0.59 [ns]	-0.59 [ns]	0.34 [ns]
261	(-2.34)	(-1.67)	(-1.07)	(-1.08)	0.67 [ns]	-0.91 [ns]	-0.59 [ns]	0.55 [ns]	0.97 [ns]	-0.58[**]
436-104	(-2.21)	(-1.96)	(-0.01)	(-1.17)	(-2.31)	1.04 [ns]	-0.99 [ns]	1.18 [ns]	0.51 [ns]	-0.23 [ns]
SB-Firoz	(-0.03)	(0.03)	(-1.20)	(0.40)	(-1.22)	(-1.8)	0.03 [ns]	-0.60 [ns]	0.49 [ns]	0.01 [ns]
RR607	(-2.92)	(-1.9)	(-1.48)	(-2.63)	(-1.10)	(-1.20)	(-2.21)	-0.32 [ns]	-0.30 [ns]	-1.57[**]
436	(-1.02)	(-1.91)	(-1.62)	(-0.72)	(-0.77)	(-0.87)	(-0.65)	(-3.03)	0.19 [ns]	0.95[**]

[*] & [**]: significant at 5 and 1% probability level, respectively; [ns] = Not statistically significant.
[†]SCA = specific combining ability.
[‡]MPH(%) = 100(F1-MP)/MP; MPH = percentage of heterosis when F_1 is compared with mid parents (MP).
[§]GCA = general combining ability.

Table 6. SCA effects (above diagonal), heterosis value (below diagonal), and GCA effects of sugar beet lines and their F_1 hybrids for resistance to bolting. Negative numbers indicated reduction in the average percent of bolted plants, which signifies more resistance to bolting.

	Testers ♂									
Entries ♀	**7112-36**	**7173**	**474**	**452**	**261**	**436-104**	**SB-Firoz**	**RR607**	**436**	**GCA[§]**
7112-36	2.03[ns†]	1.01 [ns]	-1.20 [ns]	2.49 [ns]	1.56 [ns]	-2.22 [ns]	-3.04 [ns]	-0.41 [ns]	-2.25 [ns]	-2.12 [ns]
7173	(-0.78)[‡]	0.88 [ns]	-1.44 [ns]	-0.27 [ns]	4.16 [ns]	-3.28 [ns]	-1.50 [ns]	-0.65 [ns]	3.75 [ns]	-1.89[*]
474	(-2.45)	(0.00)	-2.00 [ns]	0.32 [ns]	1.24 [ns]	4.83 [ns]	0.96 [ns]	-1.20 [ns]	0.49 [ns]	-1.33[*]
452	(2.56)	(0.03)	(1.18)	-2.65 [ns]	-1.39 [ns]	-5.76 [ns]	-2.60 [ns]	4.55 [ns]	7.97 [**]	0.46 [ns]
261	(0.05)	(2.89)	(0.53)	(-0.31)	-1.56 [ns]	2.12 [ns]	-6.29 [ns]	2.55 [ns]	-0.82 [ns]	0.94 [ns]
436-104	(-0.49)	(-0.98)	(7.69)	(-1.93)	(4.39)	-5.84 [**]	11.43 [**]	-4.18 [ns]	8.76 [**]	1.64 [*]
SB-Firoz	(-7.34)	(-5.56)	(-2.54)	(-4.31)	(-7.52)	(10.90)	0.59 [ns]	-0.23 [ns]	0.49 [ns]	0.01 [ns]
RR607	(-2.45)	(-1.51)	(-1.51)	(4.62)	(1.04)	(-2.12)	(-4.53)	1.09 [ns]	-2.60 [ns]	-2.12 [**]
436	(0.49)	(6.70)	(4.04)	(13.01)	(2.62)	(15.28)	(0.77)	(0.14)	-7.71 [**]	2.83 [**]

[*] & [**]: significant at 5 and 1% probability level, respectively; [ns] = not statistically significant.
[†]SCA = specific combining ability.
[‡]MPH(%) = 100(F1-MP)/MP; MPH = percentage of heterosis when F_1 is compared with mid parents (MP).
[§]GCA = general combining ability.

Table 7. Means of disease score for parents and their F_1 hybrids for resistance to CLS which were used for GGE biplot.

	Testers ♂								
Entries ♀	**7112-36**	**7173**	**474**	**452**	**261**	**436-104**	**SB-Firoz**	**RR607**	**436**
7112-36	5.66	5.66	5.66	6.33	5.00	4.58	6.33	3.25	5.83
7173	5.66	5.00	4.58	6.33	5.00	5.00	6.58	4.33	5.83
474	5.66	4.58	5.50	5.58	5.25	4.58	5.50	5.00	5.50
452	6.33	6.33	5.58	5.42	5.25	5.25	5.25	3.00	4.33
261	5.00	5.00	5.25	5.25	5.17	5.33	5.25	4.33	5.42
436-104	4.58	5.00	4.58	5.25	5.33	5.33	4.33	3.83	4.33
SB-Firoz	6.33	6.58	5.50	5.25	5.25	4.33	4.33	4.33	5.50
RR607	3.25	4.33	5.00	3.00	4.33	3.83	4.33	5.00	3.00
436	5.83	5.83	5.50	4.33	5.42	4.33	5.50	3.00	5.66

Table 8. Means of percentage of bolted plants for parents and their F_1 hybrids for resistance to bolting which were used for GGE biplot.

	Testers ♂								
Entries ♀	**7112-36**	**7173**	**474**	**452**	**261**	**436-104**	**SB-Firoz**	**RR607**	**436**
7112-36	2.45	1.67	0.00	5.50	5.05	1.96	1.07	0.00	3.13
7173	1.67	0.00	0.00	2.97	7.89	1.14	3.05	0.00	9.38
474	0.00	0.00	0.00	4.13	5.53	9.81	5.88	0.00	6.67
452	5.50	2.97	4.13	2.94	4.68	1.01	4.16	7.56	15.95
261	5.05	7.89	5.53	4.68	4.99	9.39	0.90	6.14	7.62
436-104	1.96	1.14	9.81	1.01	9.39	2.12	19.33	0.00	17.92
SB-Firoz	1.07	3.05	5.88	4.16	0.90	19.33	8.42	3.89	9.20
RR607	0.00	0.00	0.00	7.56	6.14	0.00	3.89	1.52	2.78
436	3.13	9.38	6.67	15.95	7.62	17.92	9.20	2.78	2.63

Table 9. Principal component scores used for generating the biplot for resistance to CLS.

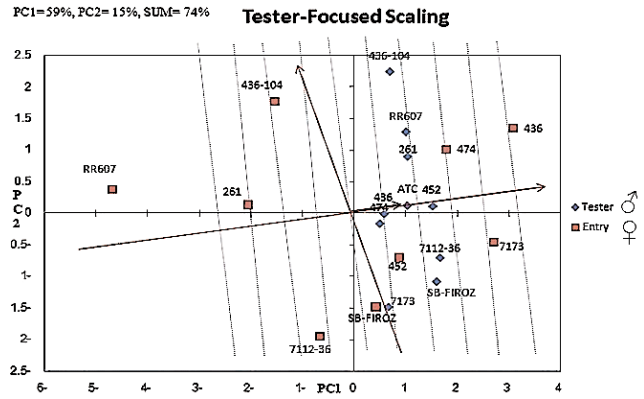
	Entries ♀		Testers ♂	
Genotype	**x- axis**	**y- axis**	**x- axis**	**y- axis**
	ξ_{i1}^*	ξ_{i2}^*	η_{j1}^*	η_{j2}^*
7112-36	-0.6496	-1.9544	1.6687	-0.7001
7173	2.7194	-0.4617	0.6736	-1.4789
474	1.7924	1.0072	0.5068	-0.1620
452	0.8770	-0.7111	1.5276	0.1128
261	-2.0491	0.1266	1.0427	0.8965
436-104	-1.5338	1.7636	0.7021	2.2391
SB-Firoz	0.4337	-1.4871	1.6047	-1.0775
RR607	-4.6789	0.3720	1.0111	1.2880
436	3.0890	1.3448	0.5894	-0.0097

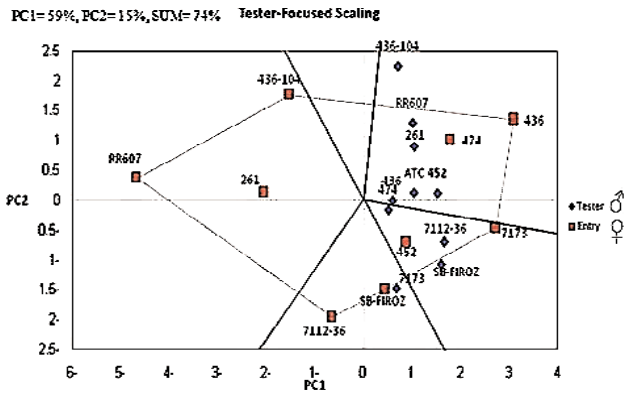
and SCA are orthogonal, if the projections of the entries onto the ATC abscissa approximate their GCA effects, as just demonstrated, then projection of the entries onto the ATC ordinate must approximate their SCA effects, which represents the tendency of the entries to produce superior hybrids with specific testers. The ranking of the entries for SCA for CLS resistance, from resistant to susceptible, was 7112-36 > 436-104 > SB-FIROZ > 436 > 452 > 474 > 7173 > RR607 > 261.

Table 10. Principal component scores used for generating the biplot for resistance to bolting.

	Entries ♀		Testers ♂	
Genotype	**x- axis**	**y- axis**	**x- axis**	**y- axis**
	ξ_{i1}^*	ξ_{i2}^*	η_{j1}^*	η_{j2}^*
7112-36	-3.2095	-9.0870	0.1247	0.7680
7173	-7.5803	-4.0194	6.5780	1.1161
474	0.9314	-2.1541	2.7006	9.4464
452	-7.5527	2.4628	8.2066	-3.5822
261	3.9808	-1.9957	-2.2385	1.3554
436-104	-8.8934	16.2355	18.2068	4.9270
SB-Firoz	10.1397	5.5191	0.0062	14.8658
RR607	-4.4200	-8.2252	1.6845	0.8940
436	16.6041	1.2641	-7.2907	12.5941

Figure 1. GGE-Biplot based on diallel data of nine sugar beet genotypes with varying resistance to CLS. (A) Average tester ordination view, (B) polygon view, (C) ideal tester. Genotypes are represented as squares when used as entries (♀) and with diamonds when used as testers (♂). The end of the ATC arrow in (A) indicates the average tester coordinate.





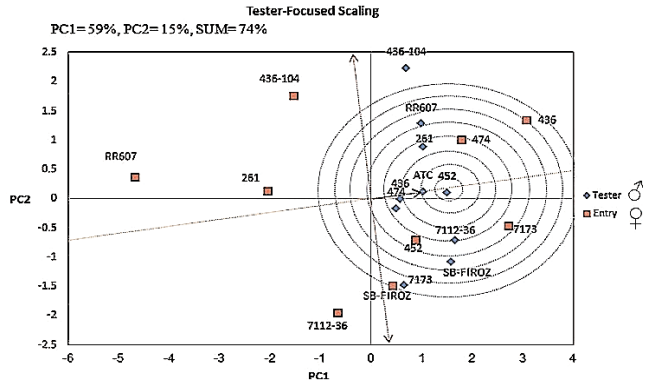
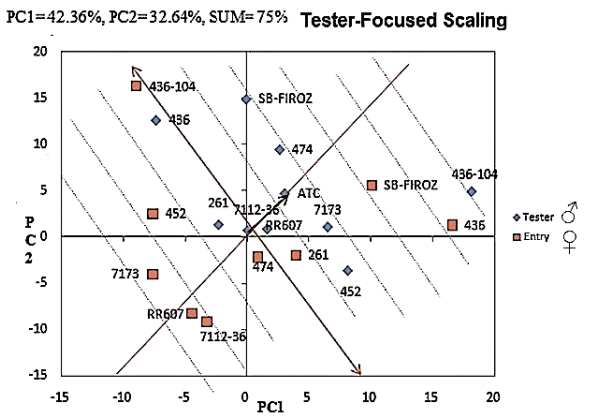
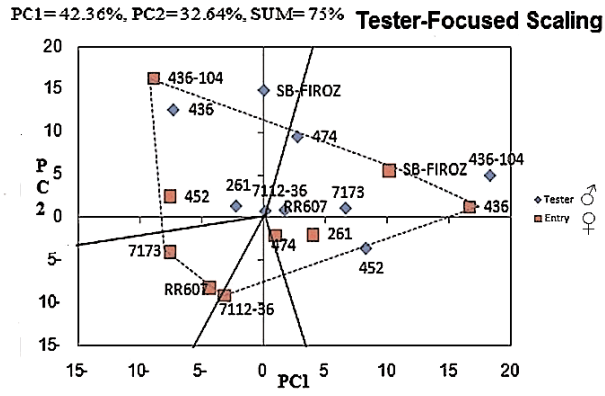
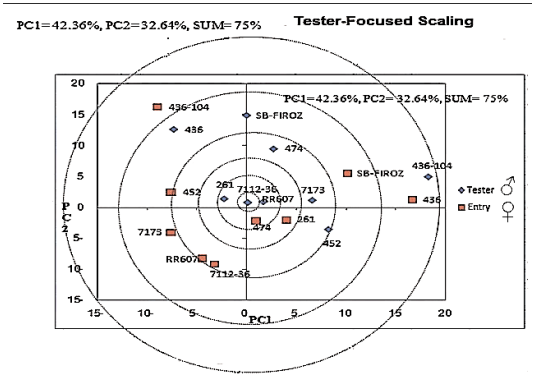


Figure 2. GGE-Biplot based on diallel data of nine sugar beet genotypes with varying resistance to bolting. (A) Average tester ordination view, (B) polygon view, (C) ideal tester. Genotypes are represented as squares when used as entries (♀) and with diamonds when used as testers (♂). The end of the ATC arrow in (A) indicates the average tester coordinate.







Entry 7112-36 showed the highest SCA effect (largest projection onto the ATC ordinate), whereas entry 261 had the smallest SCA effect (smallest projection onto the ATC ordinate) (Figure 1a). The ranking of the entries for SCA for percentage of bolting, from resistant to susceptible, was 436-104 > 436 > 452 > SB-FIROZ ≥ 261 > 7173 > 7112-36 > 474 > RR607. Entries 436-104 and 436 showed the highest SCA effect (largest projection onto the ATC ordinate), where for entry 436-104, SCA was positive and for entry 436 SCA was negative. Entry RR607 had the smallest SCA effect (smallest projection onto the ATC ordinate) (Figure 2a).

Based on projection onto ATC abscissa (Figure 1a), the two heterotic groups for resistance to CLS were: genotypes 436, 474, 436-104, 261 and RR607 as one group, and genotypes 7173, 452, SB-FIROZ and 7112-36 as the other group. The two heterotic groups for resistance to bolting were 436-104, 452 and 7173 as one group, and genotypes 436, SB-FIROZ, 261, 474 and 7112-36 as the other group (Figure 2a). The RR607 entry which was located near the ATC abscissa did not seem to belong to either of the groups.

The biplot for resistance to CLS (Figure 1b) was divided into 5 sectors, with entries 436, 436-104, RR607, 7112-36 and 7173 as the vertex entries, referred to as the 436, 436-104, RR607, 7112-36, and 7173 sectors, respectively. For resistance to bolting, the biplot (Figure 2b) was divided into 4 sectors, with entries 436, 0436-104, 7173 and 7112-36 as the vertex entries, referred to as the 436, 436-104, 7173 and 7112-36 sectors, respectively.

Ranking of the genotypes for best tester (male parent) for resistance to CLS was 452 > 436 > 474 ≥ 261 > 7112-36 > SB-FIROZ > RR607 > 7173 >436-104 (Figure 1c). Ranking of the genotypes for best tester (male parent) for resistance to bolting was 7112-36 > RR607 > 261 > 7173 > 474 > 452 > SB-FIROZ ≥ 436 > 436-104 (Figure 2c).

DISCUSSION

Significant differences among parents and F_1 hybrids for disease resistance score and percent bolting indicated the type of genetic control of resistance to CLS and bolting. The significant GCA effect for resistance to CLS indicated the contribution of additive genetic components in controlling resistance to CLS. Also, the significant GCA and SCA effects indicated that the contribution of both additive and non-additive genetic components were important in controlling resistance to bolting in sugar beet in the genotypes studied (Jullife et al., 1993).

SCA variance indicates dominance and epistasis effects (Griffing, 1956). Hybrids with higher SCA show higher heterosis in diallel crosses. Sadeghian and Johansson (1992) reported that the phenomenon of bolting was influenced by genetic, environmental, and physiological factors, and that genes with additive effects and epistasis

also affected this phenomenon. Our study showed that additive, non-additive and epistatic gene effects have an important role in expression of the bolting trait in sugar beet.

The relative importance of general and specific combining abilities in determining progeny performance was assessed according to Baker's ratio (Baker, 1978). Although both GCA and SCA were significant, Baker's ratio reflected a relatively greater importance of the variation due to SCA than GCA for resistance to bolting. But additive gene effects were more important than non-additive ones in controlling resistance to CLS, because, although Baker′s ratio for resistance to bolting was lower than 0.5, for resistance to CLS it was closer to 1. If Baker′s ratio is near to 1, the additive gene effects are more important than non-additive ones in controlling those traits (Baker, 1978; Darvishzadeh et al., 2009). Also, selection has no effect on non-additive genetic variance because it cannot contribute a lasting response to selection (Darvishzadeh et al., 2009).

The GCA ranking of the genotypes for resistance to CLS and bolting was approximately coincident with the ranking order of the parents based on Griffing's diallel analysis. A little difference between the GCA ranking from diallel analysis and biplot for resistance to bolting was expected, because the GGE biplot only accounted for about 75% of variation of the diallel data, whereas Griffing's diallel analysis was based on all of the data variation.

The parents with the largest negative GCA effects contain suitable additive genes to be used to develop cultivars resistant to CLS and bolting. Based on biplot results and Griffing's diallel analysis, it is suggested that line RR607 could be used for resistance to CLS. Lines 7112-36, RR607 and 7173 would be useful for resistance to bolting in sugar beet (tropical beet) as a winter crop, which is needed because in tropical regions, sugar beet remains in the field during winter and winter cold temperatures can lead to bolting.

The polygon view of a biplot provides the best way to visualize the interaction patterns among entries (female parents) and testers (male parents) and to effectively interpret a biplot (Yan and Hunt, 2002). An interesting property of this polygon view of biplot is that testers falling into the same sector share the same best mating partner, which is the entry at the vertex of the polygon in that sector. Testers that fall in different sectors have different best mating partners (Yan and Hunt, 2002). For resistance to CLS, none of the testers were located in 436-104 or RR607 sectors, meaning that entries 436-104 and RR607 were not the best mating partners with any other genotypes in this study. Also, a single tester, 7173, fell in the 7112-36 sector, indicating that entry 7112-36 was the best mating partner with 7173. Moreover, because genotype 7173, as a tester, was not in sector 7173, the 7173 × 7112-36 cross should be better and show heterosis. SB-FIROZ, 7112-36 and 474 as testers fell in the 7173 sector, indicating that the entry 7173 was the best mating partner for these

genotypes. Testers 436-104, RR607, 261, 452 and 436 fell in the 436 sector, indicating that entry 436 was the best mating partner with these genotypes (Yan and Hunt, 2002). This confirmed the largest GCA (positive) of the entry 436, which was a vertex of the sector in which five out of nine testers were located.

For resistance to bolting, none of the testers were located in the 7173 and 7112-36 sectors, meaning that the entries 7173 and 7112-36 were not the best mating partner for any of the genotypes. Testers SB-FIROZ, 436 and 261 fell in the 436-104 sector, indicating that entry 436-104 was the best mating partner with these genotypes. Testers 452, 436-104, 7173, 474, RR607 and 7112-36 fell in the 436 sector, indicating that entry 436 was the best mating partner for these genotypes. This confirmed the largest GCA (positive) of entry 436, which was a vertex of the sector in which six out of nine testers were located. Because genotype 436 was not in sector 436 as a tester, all crosses between genotype 436 and the above-mentioned genotypes (452, 436-104, 7173, 474, RR607 and 7112-36) should be heterotic. Furthermore, genotypes SB-FIROZ, 261 and 474 were also in this sector (436) as entries. Thus the cross between each of these three genotypes (SB-FIROZ, 261 and 474) and genotype 436 would not result in heterosis. Consequently the cross (436 × 436-104) must have the highest positive SCA of all possible combinations, which also was identified as such by Griffing's diallel method.

Darvishzadeh et al. (2009) used the GGE biplot methodology and Griffing's diallel method for genetic analysis of partial resistance to Phoma black stem in five sunflower genotypes. They stated that the genotypes possessed at least some different resistance genes, which were expressed in the hybrids and led to the observed effects (Darvishzadeh et al., 2009). In our investigation, it seems that different resistance genes for CLS and bolting were present in the genotypes, which was what led to different expression of resistance and susceptibility in hybrids.

An ideal tester is defined as a tester that has the longest vector (the most discriminating) vector and zero projection onto the ATC ordinate (the most representative of the testers) (Yan and Hunt, 2002). Based on these criteria, genotype 452 was the best tester in this dataset for resistance to CLS. For resistance to bolting, genotype 7112-36 was the best tester in this dataset. Yan and Hunt (2002) studied seven genotypes of wheat for resistance to Fusarium head blight using the GGE biplot method and the varieties were divided into two heterotic groups. In our study, the GGE biplot method divided genotypes in two heterotic groups for resistance to CLS and bolting, similar to the results of that previous study.

GGE biplot is considered a graphical method, which allows visualization not only of the parents but also of the crosses. Griffing's methods, however, allow testing for significance as do all conventional methods (Yan and Hunt, 2002).

CONCLUSIONS

In conclusion, the significant GCA and SCA effects indicate the contribution of both additive and non-additive genetic components in controlling both resistance to CLS and bolting. Based on the biplot presentation and Griffing's diallel analysis, entry RR607 showed the largest negative GCA for both Cercospora resistance (using the KWS scale) and percentage of bolted plants, so it can be suggested as a resistant genotype for both the CLS and bolting traits in tropical beet. Also 452 and 7112-36 were the best testers for resistance to CLS and bolting, respectively. We conclude that these genotypes possess at least some different resistance genes, which were expressed in the hybrids and led to the observed effects. This was coherently shown in the biplot and Griffing's diallel analysis. This study demonstrated that the GGE biplot methodology could be an excellent tool for visualizing entry by tester (diallel) data. By applying this technique to analyses of CLS and bolting data, interaction among the genotypes in providing partial resistance to CLS and bolting was clearly identified.

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