Use of GGEbiplot methodology and Griffing's diallel method for genetic analysis of partial resistance to phoma black stem disease in sunflower

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Abstract

The objectives of the present study were to estimate the general combining ability (GCA) and specific combining ability (SCA) for partial resistance to phoma black stem and to identify the most promising combination for the selection of improved breeding lines. The response of five parental genotypes and their F1 hybrids to a phoma black stem isolate (MA6) were evaluated in a diallel programme under controlled growth chamber conditions. Significant GCA and SCA indicate that both additive and non additive gene effects contributed in the inheritance of partial resistance to phoma black stem, however, the Baker ratio showed that the additive genetic effects were more important than nonadditive ones. It is recommended that the GGEbiplot methodology could be an excellent tool for visualizing entry by tester (diallel) data. By using this technique to analyse black stem severity data, interaction among the sunflower genotypes in providing partial resistance to phoma black stem was clearly identified. Based on GGEbiplot presentation and Griffing's diallel analysis, the mutant line 'M6 54 1' showed the largest GCA, indicating contribution towards partial resistance, and the genotype B454/03 presented the smallest GCA, indicating contribution towards susceptibility. Our results show that the F1 hybrids 'SDR18 × B454/03' and 'M6 54 1 × B454/03' showing heterosis for partial resistance to phoma black stem come from the crosses between a susceptible genotype 'B454/03' and two partially resistant genotypes (SDR18 and M6 54 1), originated from different breeding programmes. We conclude therefore that these genotypes possess at least some different resistance genes, which were expressed in the hybrids and led to the observed effects.

Keywords: *Biplot, general combining ability (GCA),* Helianthus annuus L.*, partial resistance, phoma black stem, specific combining ability (SCA).*

Introduction

Phoma black stem caused by *Phoma macdonaldii* is one of the most important diseases of sunflower in France (Debaeke & Pérès, 2003). The disease is characterized mainly by black spots that appear on the stem at the base of leaf petioles and spread along stems. Black stem can occur at any time during the growing season, but is most common after flowering and the disease is most severe when abundant moisture is available during and after flowering. When phoma girdles the stem base, symptoms of premature ripening may occur (Donald et al., 1987) resulting in small heads and seeds that are empty or not completely filled, reducing seed and oil yields (Carson, 1991). Yield losses due to early plant senescence are moderate, varying from 0.2 to 0.7 t ha^{-1} (Debaeke & Pérès, 2003).

Infected plants are weak and may be more susceptible to lodging. Several methods such as crop rotation and chemical agents have been used to control phoma black stem. Utilization of sunflower cultivars with improved phoma black stem partial resistance in combination with appropriate crop-management practices is an economic and effective way to control phoma black stem (Roustaee et al., 2000a; Debaeke & Pérès, 2003). Genetic variability for partial resistance to phoma black stem in sunflower has been reported in both field and controlled conditions (Pérès et al., 1994; Roustaee

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et al., 2000a; Abou Al Fadil et al., 2004; Darvishzadeh & Sarrafi, 2007). Resistance to *P. macdonaldii* in the seeding and adult plants can be under the same genetic control (Roustaee et al., 2000a; Larfeil, 2003). The susceptibility of sunflower to phoma black stem is similar under field and controlled conditions (Larfeil, 2003). Inheritance of the phoma black stem resistance was described as being polygenic (Roustaee et al., 2000a; Rachid Al-Chaarani et al., 2002; Bert et al., 2004). A study on the combining ability for the phoma black stem response indicated that the variation among the crosses was primarily due to the general combining ability (GCA) and thus most of the variation is attributed to additive effects (Roustaee et al., 2000a). Improvement of the phoma black stem partial resistance by developing new varieties would benefit from knowledge on combining ability effects in potential crossing partners. For the estimation of GCA and specific combining ability (SCA) effects, the GGE biplot analysis of diallel data proposed by Yan and Hunt (2002) is highly effective and informative. GGE stands for genotype main effect (G) plus genotype by environment interaction (GE), which is the only source of variation that is relevant to cultivar evaluation. Mathematically, GGE is the genotype by environment data matrix after the environment means are subtracted. A GGE biplot, constructed from the first two principal components (PC1 and PC2) derived from PC analysis of environment-centered yield data. It was termed GGE biplot to emphasize that it displays both genotype main effect (G) and genotype $\times$ environment interaction (GE), which are two sources of yield variation that are relevant to, and must be considered simultaneously in, cultivar evaluation (Gauch & Zobel, 1996). Although the GGE biplot methodology was developed for multi-environment variety trials (MET) data analysis, it could be applied to all types of two-way data that assume an entry-by-tester data structure (Yan & Hunt, 2002). In MET data, genotypes are entries and environments are testers. In diallel data, each genotype is both an entry and a tester.

In this study, five sunflower genotypes were analysed in a diallel crossing design in order to achieve: (1) a better understanding of the phoma black stem resistance in the parental genotypes, (2) estimation of the GCA and SCA for the phoma black stem resistance in order to design an efficient plan for improvement of the existing materials, and (3) identification of the most promising combination for the selection of improved breeding lines.

Materials and methods

Genotypes and phoma isolate

Five sunflower genotypes used in this study present a high genetic variability in their susceptibilities to phoma black stem on the basis of our preliminary studies (Darvishzadeh et al., 2007). M6-54-1 is one of the mutant lines that were developed in our department by irradiation of 'AS 613' seeds with gamma rays at a dose of 75 grays (Sarrafi et al., 2000). ENSAT-B5 and ENSAT-R5 are inbred lines selected in our crossing programmes; and B454/03 and SDR18 are inbred lines introduced from Hungary and the United States Department of Agriculture (USDA), respectively. These genotypes were grown and crossed in a diallel mating system to produce 10 F_1 hybrid combinations.

P. macdonaldii isolate (MA6) was selected among the collection of seven French single pycnidiospore isolates on the basis of its aggressiveness on the five parental genotypes in our preliminary experiments (Darvishzadeh et al., 2007) and used in this programme. MA6 isolate was grown on Potato Dextrose Agar medium at $25 \pm 1^{\circ}C$ in 12 h photoperiodism ($37\ \mu E\ m^{-2}\ s^{-1}$). After a 10-day incubation period, pycniospore suspension was obtained by addition of sterile water at the surface of the culture and mechanical mixing (Roustaee et al., 2000b).

Experimental design

The responses of parental genotypes and their F_1 hybrids were evaluated in a controlled growth chamber [14 h photoperiodism and $25/18 \pm 1^{\circ}C$ light/dark temperature with a light intensity of $200\ \mu E\ m^{-2}\ s^{-1}$ provided by NAV-T 600W lamps (Osram-Vialox, Molsheim, France) under 75–80% relative humidity] (Roustaee et al., 2000b). The experiment was conducted in a completely randomized block design with three replications. Each replicate consisted of 12 plantlets of each genotype. Seeds were sterilized for 5 min in a sodium hypochlorite solution (6 chlorometric degrees) and washed in sterile distilled water 3 times. Two rows of six seeds per genotype per replication were sown in plastic containers filled with horticulture substrate (Hawita Flor, Germany). Plantlets were irrigated with a nutrient solution (NPK 6:3:6 and micronutrients; Substral, Boulogne Billancourt, France). Twenty microliters of spore suspension containing 10^6 pycniospores/ml in sterile water, 0.5% orange juice, and 0.25% gelatine were deposited at the intersection of cotyledon petiole and hypocotyl of two-leaf-stage sunflower plantlets. During the first 48 h following contamination, the containers in

which plants were grown were covered with a transparent cover (Plexiglas) to maintain nearly saturated humidity, favorable for pathogen development. Both cotyledon petioles of plantlets were scored seven days after inoculation according to the percentage of the petiole area exhibiting disease symptoms (necrosis). Score was ranged from 1 (resistance) to 9 (susceptible) in relation to the proportion of petiole area showing necrosis as proposed by Roustaee et al. (2000a) where: 1 = 0–5%, 2 = 6–10%, 3 = 11–20%, 4 = 21–30%, 5 = 31–40%, 6 = 41–60%, 7 = 61–80%; 8 = 81–99%, and 9 = 100%, with necrosis spreading down the stem.

Statistical analysis

Disease severity scores were multiplied by −1 before analysis so that a higher value means better resistance (W. Yan, Personal communication, 2007). The phoma black stem severity data were subjected to analysis of variance (ANOVA). ANOVA was performed using the general linear model (GLM) procedure in the SAS software (SAS Institute, Cary, NC).

Genetic analysis of partial resistance to phoma black stem was performed by GGEbiplot methodology presented by Yan & Hunt (2002), using the GGEbiplot software (Yan, 2001).

Data were also analysed using Griffing's method 2 model I (Griffing, 1956), using the SAS program for Griffing's diallel analysis (Zhang et al., 2005). The hypothesis that GCA estimates of the parents and SCA estimates of the hybrids equalled zero was tested by a two-tailed *t*-test. The Newman–Keuls test was used for comparing mean performance of parents and F_1s. The percentage of heterosis when F_1s were compared with the mid-parents were also determined and compared using the least significant difference (LSD) method. Compared with Griffing's methods, GGEbiplot is more informative because a) it is graphical, and b) it allows visualization not only of the parents but also of the crosses. Griffing's methods, however, allow testing for significance as all conventional methods do (Yan & Hunt, 2002).

Table I. Analysis of variance and combining abilities for partial resistance of sunflower lines and their F_1 hybrids to phoma black stem disease.

Source of variation	DF[1]	MS[2]	*F* value	*P* value
Variance analysis:				
Total	44	4.219		
Block	2	0.023	0.11	0.891
Genotype	14	12.835	61.11	<0.0001
Residual	28	0.210		
Diallel analysis:				
GCA[3]	4	37.750	539.286	<0.0001
SCA[4]	10	2.871	41.014	<0.0001
Error	28	0.07		
$2S^2_{gca}/2S^2_{gca}+S^2_{sca}$	0.96			

Coefficient of determination (R^2) of the model is 0.97. Coefficient of variation (CV) is 11.22.
[1]DF = degrees of freedom. [2]MS = Mean of squares. [3]GCA = general combining ability. [4]SCA = specific combining ability.

Result and discussion

Genetic variability

Analysis of variance presented in Table I showed highly significant differences among parents and F_1 hybrids for disease tolerance score, indicating genetic control of partial resistance to phoma black stem. Mean-square values of GCA and SCA were also significant, indicating that both additive and nonadditive gene effects contributed significantly in the inheritance of partial resistance to phoma black stem (Table I). The relative importance of general and specific combining ability in determining progeny performance was assessed according to the $2S^2_{gca}/2S^2_{gca}+S^2_{sca}$ ratio presented by Baker (1978). However, the Baker ratio was nearest to 1:1, showing that additive gene effects were more important than nonadditive ones in controlling partial resistance to phoma black stem. Mean disease severity summarized in Table II showed high differences among parental lines for partial resistance to phoma black stem. M6-54-1, SDR18, and ENSAT-R5 showed partial resistance to phoma black stem whereas ENSAT-B5 and B454/03 were more susceptible to it. F_1 hybrids showed a continuous range for susceptibility from partially resistant to highly susceptible (Table II). These results confirmed the genetic variability for partial resistance to *P. macdonaldii* reported earlier in both field and controlled conditions by several research works (Pérès et al., 1994; Roustaee et al., 2000a; Rachid Al-Chaarani et al., 2002; Abou Al Fadil et al., 2004; Bert et al., 2004; Darvishzadeh & Sarrafi, 2007).

General and specific combining ability of the entries

The biplot for the sunflower black stem severity data explained 99% (82% and 17% by the first two principle components (PC1 and PC2), respectively) of the total variation of GCA and SCA (Figure 1). Based on projection onto the ATC abscissa genotype B (M6-54-1, partially resistant mutant line) showed the largest and the genotype E (B454/03, the most

Table II. Mean disease severity scores[1] (above diagonal), SCA effects and heterosis values (below diagonal), and GCA effects of sunflower lines and their F_1 hybrids for partial resistance to phoma black stem.

	Testers						
Entries	A	B	C	D	E	Mean	GCA[4]
A		[2] -2.37^{abc}	-2.60^{bdc}	-2.70^{dc}	-5.37^{f}	-2.90^{dc}	0.810^{***}
B	[3] -0.546^{*} [5] (5.19^{ns})		-2.10^{abc}	-1.47^{a}	-5.20^{f}	-1.60^{ab}	1.453^{***}
C	0.373^{ns} (-18.75^{ns})	0.230^{ns} (-17.65^{ns})		-4.00^{e}	-6.70^{g}	-3.50^{ed}	0.301^{***}
D	1.135^{***} (-44.33^{*})	1.725^{***} (-65.08^{*})	0.344^{ns} (-22.33^{ns})		-6.67^{g}	-6.8^{g}	-0.561^{***}
E	-0.089^{ns} (5.23^{ns})	-0.565^{*} (16.85^{ns})	-0.913^{***} (24.07^{ns})	-0.017^{ns} (-5.44^{ns})		-7.3^{g}	-2.004^{***}

[1]Disease tolerance scores based on a 1–9 scale. A score of 1 to 9 was given in relation to the proportion of petiole area showing necrosis, where: 1 = 0–5%, 2 = 6–10%, 3 = 11–20%, 4 = 21–30%, 5 = 31–40%, 6 = 41–60%, 7 = 61–80%; 8 = 81–99%, and 9 = 100%, with necrosis spreading down the stem. Original data were multiplied by –1 so that a higher (more positive) score is more desirable (resistance).
[2]Average disease tolerance scores of 36 plantlets per genotype challenged by phoma black stem isolate, seven days after petiole inoculation. Means followed by the same letter are not significantly different ($P = 0.05$) according to the Student–Newman–Keuls (SNK) test. *, and *** = Significant at 0.05, and 0.01 probability level respectively. n.s. = Not significant. [3]SCA = Specific combining ability. [4]GCA = general combining activity. [5]MPH (%) = 100(F1 − MP)/MP; percentage of heterosis when F1 is compared with mid parents (MP); LSD ($P = 0.05$) = 44%. A = SDR18, B = M6 54 1, C = ENSAT R5, D = ENSAT B5, E = B454/03.

susceptible genotype of Hungary origin) showed the smallest GCA effects. The ranking of the genotypes for GCA was: B > A > C > D > E, which is consistent with the order of B > A > C > D > E based on Griffing's diallel analysis (Table II). A larger GCA effect indicates a contribution towards resistance, while a smaller GCA effect represents the opposite (Deglene et al., 1999). Therefore, the parents with largest GCA effects contain suitable additive genes to be used to develop partial-resistance cultivars to phoma black stem. Because the biplot displays both GCA and SCA, and because GCA 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 represent the tendency of the entries to produce superior hybrids with specific testers. The ranking of the genotypes for SCA was: D > B > A > E ≥ C. Entry D (ENSAT-B5, susceptible genotype) showed the highest SCA effect (largest projection onto the ATC ordinate), whereas Entry C (ENSAT-R5, partially resistant genotype) had the smallest SCA effects (smallest projection onto the ATC ordinate). Entry D had the highest SCA because it interacts positively with testers A and B and negatively with itself. Based on projection onto ATC abscissa two heterotic groups are suggested by Figure 1A: Genotype D, as one group, and Genotypes A and B as the other group. Therefore, two crosses, that is, [D] × [A or B], are expected to show heterosis for partial resistance defined as better than both parents. Entries C and E, which are located near the ATC abscissa, did not seem to belong to any of the groups.

Best tester for GCA

An ideal tester is defined as a tester that has the longest vector (the most discriminating) and zero projection onto the ATC ordinate (the most representative of the testers) (Yan & Hunt, 2002). Therefore, genotype C was the best tester in this dataset (Figure 1B). The speculation is that the GCA effects of the entries should be reasonably assessed by the performance of their hybrid with genotype C. Ranking of the genotypes for GCA based on the actual values of the hybrids with ideal tester (C) is B > A > C > D > E (Figure 1B), which is consistent with the order of B > A > C > D > E based on the GCA effects of Griffing's diallel method (Figure 1B and Table II).

Best hybrids

The polygon view of a biplot provides the best way for visualizing the interaction patterns between entries and testers and to effectively interpret a biplot (Yan & Hunt, 2002). The biplot in Figure 1C was divided into 3 sectors, with entries B, D, and E as the vertex entries, and are referred to as sector B, sector D, and sector E, respectively. None of the testers was located in sector E, meaning that entry E was not the best mating partner with any of the genotypes. A single tester, B, fell in sector D, indicating that entry D was the best mating partner with B. Moreover, since genotype D as a tester, and was not in sector D, the cross B × D must be better, and show heterotic

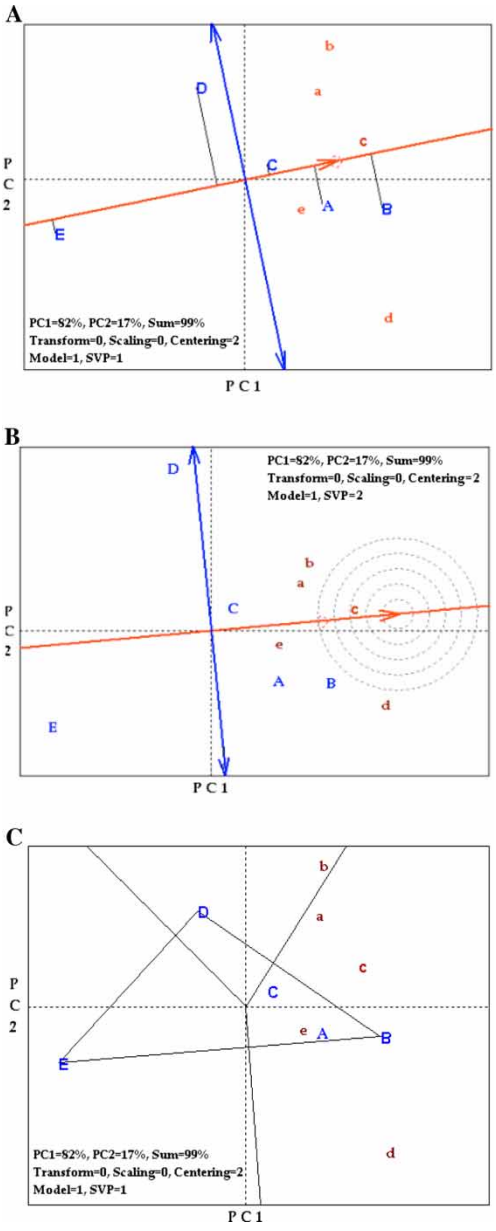

Figure 1. Biplot based on diallel data of five sunflower genotypes with varying resistance to phoma black stem. (A) average tester ordination view, (B) ideal tester, (C) polygon view. Codes of genotypes are: A = SDR18, B = M6 54 1, C = ENSAT R5, D = ENSAT B5, E = B454/03. Genotypes are labelled with upper case letters when viewed as entries and with lower case letters when viewed as testers. Exact positions of the entries and testers are at the beginning of the labels. The circle in (A) indicates the average tester.

character. Testers A, C, D, and E fell in sector B, indicating that entry B was the best mating partner with these genotypes. This confirms the largest GCA of genotype B as a vertex of the sector in which four out of five testers were located. Since genotype B as a tester was not in sector B, all crosses between genotype B and above-mentioned genotypes (A, C, D, and E) should be heterotic. Furthermore, the genotypes A and C as entry are also in this sector (B). Thus, the cross between each of these two genotypes (A and C) and genotype B would not result in heterosis. As mentioned earlier, entry E was not the best mating partner with any of genotypes. Consequently, the cross (B × D) must be the best of all possible combinations, which was identified also by Griffing's diallel method (Table II).

Hypothesis concerning the genetic relationships among the genotypes

Assuming that heterosis results from the accumulation of different dominant gene loci, entries B and D each would appear to carry at least one unique dominant gene. In the other part, since genotypes A, C, and B are in the same group as the group of similar genotypes, some dominant resistance genes present in entry B may also be present in entries A and C. However, partial resistance to phoma black stem is certainly controlled by a large number of genes because the additive genetic effect was found to be more important than the nonadditive effect (Figure 1A, Table I). Rachid Al-Chaarani et al. (2002) using recombinant inbred lines (RILs) and Bert et al. (2004) using F_2-F_3 families found seven and four quantitative trait loci (QTLs) controlling partial resistance to phoma black stem, respectively. They reported an additive gene effect for partial resistance to phoma black stem, which shows also the polygenic nature of resistance to this disease. Our biplot presentation shows that among genes controlling partial resistance to phoma black stem at least two have dominant effects.

In conclusion, the significant GCA and SCA effects indicate the contribution of both additive and nonadditive genetic components in controlling phoma black stem partial resistance. Although both GCA and SCA were significant, however, the Baker ratio reflects a relative greater importance of the variation due to GCA than to SCA. This is in agreement with a previous report from a factorial cross indicating GCA is more important than SCA for phoma black stem resistance (Roustaee et al., 2000a). Based on biplot presentation and Griffing's diallel analysis, the mutant line 'M6-54-1' showed the largest GCA, indicating contribution towards partial resistance, and the genotype B454/03 presented the smallest GCA, indicating contribution towards susceptibility. Our results show that the F_1 hybrids 'SDR18 × B454/03' and 'M6-54-1 × B454/03' showing heterosis for partial resistance to phoma black stem come from the crosses between a susceptible genotype 'B454/03' and two partially resistant genotypes (SDR18 and M6-54-1), originating from different breeding programmes. We

conclude therefore 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 presented by biplot and Griffing's diallel analyses. Our present study demonstrated that the GGEbiplot methodology, which to our knowledge is the first for this particular disease of sunflower, could be an excellent tool for visualizing entry by tester (diallel) data. By applying this technique to analyse black stem severity data, interaction among the sunflower genotypes in providing partial resistance to phoma black stem was clearly identified.

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