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Study of interactions between sunflower genotypes and black stem (*Phoma macdonaldii*) isolates using GGE biplot approach

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Abstract: Black stem is one of the most important fungal diseases of sunflower which is caused by *Phoma macdonaldii*. This research was conducted to clarify the interactions between a few breeder lines and wild-type accessions of sunflower with seven isolates of *Phoma macdonaldii,* the causal agent, under controlled conditions. The experiment was carried out in randomized complete block design with three replications. Each replication consisted of 30 seedlings. Twenty micro liters of spore suspension was deposited at the intersection of the cotyledon petiole and hypocotyl of four-leaf-stage sunflower seedlings as well. Three, five and seven days after inoculation, seedlings were scored on a 1-9 scale for percentage of necrotic area. Highly significant differences were observed among genotypes, isolates and their interactions for disease severity 7 days after inoculation based on AUDPC (Area Under Disease Progress Curve) values. Two models of GGE biplot including Isolate-based and genotype-based models were used to study the pathogenicity of the isolates and susceptibility of sunflower, respectively. Isolate-focused biplot revealed that there was a considerable difference between virulence of isolates. Based on genotype-focused biplot model, accessions including 665 Iowa, 1012 Nebraska, 211 Illinois and 1016 Nebraska were more resistant to studied isolates of *P. macdnaldii*. Results revealed that GGE biplot approach could lead to good understanding about interactions between sunflower genotypes and *Phoma macdonaldii* isolates.

Keywords: Black stem, *Phoma macdonaldii,* Isolate-focused biplot**,** genotype-focused biplot

Introduction

Black stem, caused by the soil-borne fungus *Phoma macdonaldii*, is one of the most important diseases of sunflower in the world (Gulya *et al.,* 1997). The disease is characterized mainly by dark black, oval to long lesions on the stems of sunflower plants (Miric *et al.,* 1999). Infected plants are weak and more susceptible to lodging (Sackston, 1992). Infection during early growth stages can reduce yield by 10 to 30 percent (Miric *et al.,* 1999). The use of resistant varieties is most economical, ecologically friendly and effective method to control the disease (Nayak *et al.,* 2008). Therefore, information on the diversity in aggressiveness of plant pathogens is necessary to deploy resistance genes against the prevalent pathotypes (Ghazvini and Tekauz, 2008). Several

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research works (Roustaee *et al.,* 2000a; Rachid Al-Chaarani *et al.,* 2002; Bert *et al.,* 2004) have demonstrated partial resistance to *P. macdonaldii* in sunflower germplasm. Wild relatives of sunflower species can serve as a gene pool for resistance genes against biotic stresses. For instance, resistance genes against *Sclerotinia sclerotiorum* have been reported in wild type (Seiler and Rieseberg, 1997) and breeder lines (Davar *et al.,* 2011) of sunflower.

From breeder viewpoint, artificial infection under controlled conditions are helpful for evaluating of sunflower germplasm against the pathogen in order to differentiate resistant and susceptible individuals. Similarity in the susceptibility reaction of sunflower lines to Phoma black stem has been evidenced in both controlled and the field condition (Larfeil *et al.*, 2002).

Several methods were employed in clarifying host (genotype) by pathogen (isolate) relationship in plant disease resistant breeding programs. Recently, graphical biplot method has been introduced for visualizing host-pathogen interactions. (Yan and Falk, 2002). The biplot was originally proposed by Gabriel (1971) as a graphical tool to present results from principal component analysis (PCA). It is a scatter plot that graphically displays a rank-2 matrix by both the rows (entries) and the columns (testers). Biplot method has been efficiently used for yield stability analysis in several crops such as wheat (Kaya *et al.,* 2006), maize (Balestre *et al.,* 2009), and sorghum (Sujay *et al.,* 2012). Yan and Falk (2002) used biplot analysis for interpretation of host by pathogen interactions in barley net blotch. In another study, Darvishzadeh *et al.* (2009) determined the genetic control of partial resistance to phoma black stem in sunflower using GGE biplot analysis of a diallel mating design. Present study is aimed to evaluate the interactions of sunflower wild-type genotypes with several *P. macdonaldii* isolates using GGEbiplot analysis.

Materials and Methods

Disease assessment and aggressiveness group designation

Seven *P. macdonaldii* isolates previously collected and described by Roustaee *et al.* (2000b) were used in this study (Table 1). The seeds of wild-type accessions (*Helianthus annus* L.) and inbred lines of sunflower (Table 1) were planted in pots. Then, the response of sunflower plantlets against seven isolates of *P. macdonaldii* (Table 1) were assessed under controlled conditions (14-h photoperiod and 25 ± 1 °C/18 ± 1 °C light/dark temperature, with a light intensity of 200µ$Em^{-2}s^{-1}$ provided by NAV-T 600W lamps (Osram-Vialox) and 75-80% relative humidity). The experiment was conducted in a factorial arrangement with three replications based on randomized complete block design. Each replicate consisted of 30 seedlings. Four-leaf-stage sunflower seedlings were used for inoculation. The isolates were grown on potato dextrose agar (PDA) medium at 25 ± 1 ºC and a 12h photoperiod. A pycnidiospore suspension was prepared by flooding the 10-day-old culture plates with sterile distilled water and stirring mechanically. Twenty micro liters of suspension containing 10^6 pycnidiospores per ml in sterile distilled water, 0.5% orange juice and 0.25% v/v gelatine were put at the intersection of the cotyledon petiole and hypocotyl of sunflower plantlets (Roustaee *et al.,* 2000a). During the first 48h post inoculation, plantlets were covered with a transparent cover (Plexiglas) to provide nearly saturated humidity, favorable for fungal development. Both cotyledon petioles of each seedling were scored three, five and seven days after inoculation according to the percentage of the petiole area exhibiting disease symptoms. A score of 1 (resistant) to 9 (susceptible) was given 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.

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Table 1 Sunflower wild-type accessions and breeder lines, their origin and *Phoma macdonaldii* isolates used in this experiment.

Sunflower wild-type accessions and breeder lines			***Phomamacdonaldii* isolates**			
name	**Type**	**Origin**	**Isolates name**	**Year of collection**	**Country of origin**	**Locality**
B454/03	BL	Hungary	MP3	1996	France	Tour de Faure
1151North Dakota	W	USA	MP6	1996	France	Castanet
665Iowa	W	USA	MP8	1996	France	Saint Lys
1012Nebraska	W	USA	MP10	1996	France	Saint Pathus
211Illinois	W	USA	MA6	1997	France	Saint Lys
1016Nebraska	BL	USA	MA7	1997	France	Castanet
510Kansas	BL	USA	MF	2003	Hungry	Godolos

BL: breeder's line, W: wild type.

Data analysis

Area under the disease progress curve (AUDPC) was calculated using scores obtained at three, five and seven days after inoculation employing the formula:

$$\mathrm{AUDPC} = \sum_{i=1}^{n-1} \left(\frac{y_i + y_{i+1}}{2} \right) \left(t_{i+1} - t_i \right)$$

where Y_i is the disease score at time point i and t_i is the number of days after inoculation (Wichmann *et al.,* 2011). The non-parametric methodology of Brunner *et al.* (2002) described in detail by Shah and Madden (2004) was applied on AUDPC values and disease severity scores after 7 days post inoculation in order to test whether there were significant differences between isolates in their aggressiveness, or between genotypes in their partial resistance, and also to examine any significant genotype-isolate interactions. Graphical biplot method presented by Yan and Falk, (2002) was applied to visualize host-pathogen interactions using disease severity data scored 7 days after inoculation

Results and discussion

Analysis of variance

Results pertaining to analysis of variance manifested significant effects of genotypes, isolates and genotypes × isolates interactions on AUDPC and disease severity 7 days after inoculation (Table 2). Wild type genotype 1012 Nebraska appeared as tolerant genotype while breeder line B454/03 was the most susceptible one (Fig. 1). Several wild *Helianthus* accessions have been described as potential sources of genes conferring resistance to *S. sclerotiorum* (Seiler and Rieseberg, 1997). Significant genotypes-isolates interactions suggest that the sunflower genotypes respond differently to *P. macdonaldii* isolates (Table 2). These results are in agreement with the findings of Darvishzadeh *et al.* (2007) who have reported highly significant genotypes-isolates interactions in *P. macdonaldii*-sunflower pathosystem.

Table 2 Analysis of variance of disease scores obtained at 7 days after inoculation and AUDPC values.

Effect	**Disease score after 7 day**			**AUDPC**		
	df_N	**df_D**	**F-value**	**df_N**	**df_D**	**F-value**
Isolate	5.5	34	8.7**	5.4	38.8	8.9**
Genotype	4.8	34	39.7**	5.0	38.8	34.7**
Isolate × Genotype	15.3	34	7.2**	17.1	38.8	5.8**

df_N = numerator degrees of freedom; df_D= denominator degrees of freedom

** Significant at %1 probability level

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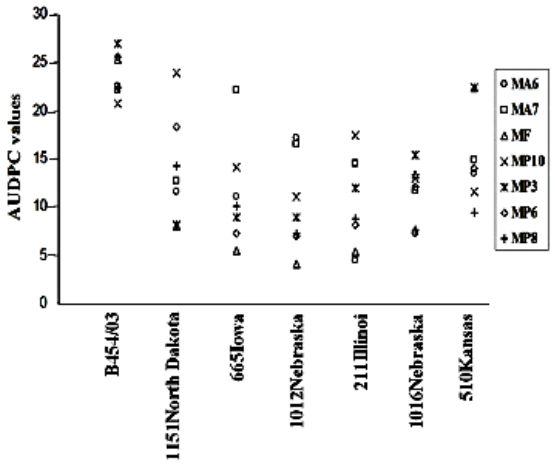

Figure 1 Area under the disease progress curve (AUDPC) values for each of seven sunflower genotypes inoculated with seven *Phoma macdonaldii* isolates. The data are mean of three replications.

Graphical analysis of host-pathogen interaction

Isolate focused biplot

Significant correlation has been observed between AUDPC values and disease severity scores 7 days after inoculation. Such a short time disease severity score can identify the sources of resistance to Phoma black stem, thus, data on disease severity 7 days after inoculation were used for graphical analysis. To identify the aggressiveness pattern of *P. macdonaldii* isolates, in biplot approach the isolates were treated as entries and sunflower genotypes as tester. Results revealed that isolates MP10 and MA7 had maximum aggressiveness on genotypes 211 Illinois and 665 Iowa, respectively (Fig. 2A). Among tested isolates, MP3 and MF equally affected '510 Kansas' genotype. Isolate MP3 had most aggressiveness on genotype '1016 Nebraska' genotype (Fig. 2A). MP10, MA6 and MA7 were significantly aggressive on '115 North Dakota' and '1012 Nebraska' genotypes (Fig. 2A). Previously, Darvishzadeh *et al.* (2007) evaluated the aggressiveness of several *P. macdonaldii* isolates on sunflower recombinant inbred and mutant lines under controlled conditions. They showed that MP3 and MA6 were the high and low aggressive isolates, respectively. Regarding complex genetic control of partial resistance to Phoma black stem in sunflower (Roustaee *et al.*, 2000a; Darvishzadeh and Sarrafi, 2007), identified that aggressive isolates could be properly used for mapping of resistance genes through phenotyping in a segregating population.

Genotype focused biplot

In order to study the resistance or susceptibility of the sunflower wild type accessions and breeder lines to various isolates, the genotypes were used as entries (Fig. 2B). Considering Fig. 2B, the first two principal component axes of the biplot accounted for 76% of the total variation of the genotype-isolate interaction and studied isolates were classified in two clusters. Regarding to lines perpendicular to the sides of the polygon (Fig. 2B), the biplot is divided into sectors. '510 Kansas' accession was most susceptible to cluster I of the isolates (MP3 and MF); '115 North Dakota' and 'B454/03' were susceptible to cluster II of the isolates (MP6, MP8, MP10, MA6 and MA7) (Fig. 2B). In accordance with these findings, Nayak *et al.* (2008) reported the efficiency of biplot in a better understanding of the host-pathogen interaction, adaptability of pathogen isolates to specific host genotypes and identification of isolates showing stable pathogenicity.

Conclusion

1- From breeder view, there is highly promising genetic variation for partial resistance to *P. macdonaldii* among studied sunflower material.

2- Among studied isolates of the fungus, MP3 has the most aggressiveness.

3- Among studied wild type accessions, accession '112 Nebraska' is the most resistant accession to the isolates.

4- GGE biplot analysis could be efficiently applied for interpretation of genotype × isolate interaction in sunflower.

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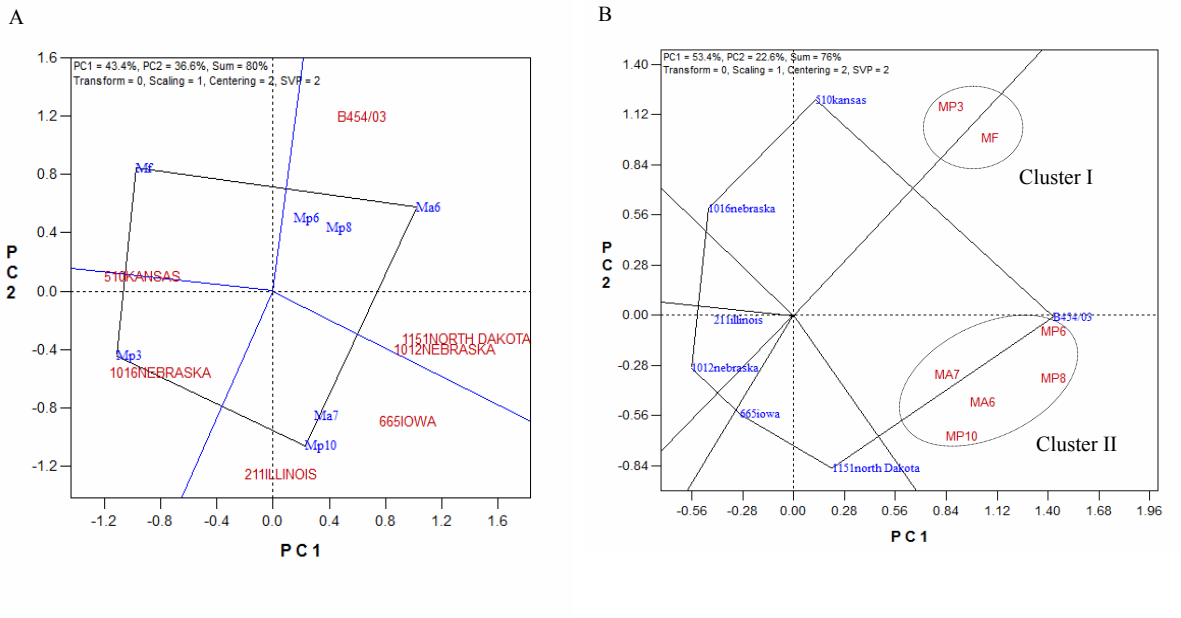

Figure 2 Biplots with *Phoma macdonaldii* isolates (A) and sunflower genotypes (B) as entries

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مطالعه برهم‌کنش بین ژنوتیپ‌های آفتابگردان و جدایه‌های مختلف بیماری ساقه سیاه (*Phoma macdonaldii*) با استفاده از روش GGE بای‌پلات

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دریافت: ۱۶ اسفند ۱۳۹۱؛ پذیرش: ۱۷ مهر ۱۳۹۲

چکیده: ساق سیاه ازجمله مهم‌ترین بیماری‌های قارچی آفتابگردان است که توسط قارچ *Phoma macdonaldii* ایجاد می‌شود. این مطالعه به‌منظور بررسی برهم‌کنش بین برخی از ژنوتیپ‌های اصلاحی و وحشی آفتابگردان با ۷ جدایه مختلف قارچ *Phoma macdonaldii* و در شرایط کنترل شده انجام گرفت. آزمایش در قالب طرح بلوک‌های کامل تصادفی و با سه تکرار انجام شد. هر تکرار شامل ۳۰ گیاهچه بود. در مرحله چهار برگی، برگ کوتیلدونی گیاهچه‌های آفتابگردان در معرض ۲۰ میکرولیتر از سوسپانسیون اسپور حاوی 10^6 پیکنیدیوسپور در هر میلی‌لیتر قرار داده شدند. سه، پنج و هفت روز پس از آلودگی، گیاهچه‌ها براساس درصد آلودگی، با اعداد ۱ الی ۹ نمره‌دهی شدند. در این مطالعه، اختلاف خیلی معنی‌دار بین ژنوتیپ‌های مورد مطالعه، جدایه‌ها و اثر متقابل آنها پس از گذشت هفت روز از آلودگی ازنظر مقادیر AUDPC (سطح زیر منحنی توسعه بیماری) مشاهده گردید. مدل‌های بر پایه جدایه و ژنوتیپ به‌ترتیب برای مطاله بیماری‌زایی و حساسیت هر یک از جدایه‌ها و ژنوتیپ‌ها استفاده گردید. نمودار بای‌پلات براساس جدایه نشان داد که بین جدایه‌ها از نظر بیماری‌زایی اختلاف وجود دارد. نمودار بای‌پلات براساس ژنوتیپ نشان داد که ژنوتیپ‌های 665 Iowa، 1012 Nebraska، 211 Illinois و 1016 Nebraska در مقابل جدایه‌های *Phoma macdonaldii* مقاومت بیشتری دارند. نتایج نشان داد که استفاده از روش GGE بای‌پلات می‌تواند فهم بهتری از برهم‌کنش بین ژنوتیپ‌های آفتابگردان و جدایه‌های *Phoma macdonaldii* بدهد.

واژگان کلیدی: ساق سیاه، *Phoma macdonaldii*، بای‌پلات براساس جدایه و بای‌پلات براساس ژنوتیپ

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