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## Combining ability for Fusarium head blight resistance in wheat (*Triticum aestivum* L.)

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### ABSTRACT

Fusarium head blight (FHB) caused by *Fusarium graminearum* reduces wheat (*Triticum aestivum* L. em. Thell) grain yield and end-use quality worldwide. Three each of FHB susceptible ('Nekota', '2137', and 'Harding') and resistant ('Ning 7840', 'ND2710' and 'BacUp') parents were included in a partial diallel mating design (Griffing's Method 4, Model 1). The F<sub>4:5</sub> progeny was evaluated for healthy index, undamaged kernels, and deoxynivalenol (DON) content following artificial inoculation and mist-irrigation in 2006 and 2007. General combining ability (GCA) was highly significant ( $P < 0.01$ ) for healthy index; whereas specific combining ability (SCA) and GCA-by-year interaction were not significant. The combining ability ratio and narrow-sense heritability were 0.95 and 0.83, respectively. The Genotype, Genotype-by-Environment (GGE) biplot analysis showed that the ND2710/BacUp combination had the best healthy index and undamaged kernels; whereas Ning 7840 contributed resistance to DON accumulation. The results indicated additive gene effects mainly control that healthy index. Thus, genetic gain in developing resistance in wheat can be achieved through selection.

**Key Words:** *Fusarium head blight; diallel; combining ability; heritability; GGE biplot.*

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### INTRODUCTION

Fusarium head blight (FHB), caused by *Fusarium graminearum* Schwabe [teleomorph *Gibberella zeae* (Schwein) Petch], is a major wheat production constraint in warm and humid growing areas (Stack and McMullen, 1985; Tuite et al., 1990). This disease decreases grain

yield and reduces quality (McMullen et al., 1997; Nightingale et al., 1999). *Fusarium* species produce mycotoxins (trichothecenes) in infected kernels, which are harmful to humans and animals (Hagler et al., 1984). Among the trichothecenes, deoxynivalenol (DON) is of major importance because it causes the refusal of feed, emesis, and decreased weight gain in non-ruminants. Due to concerns about DON toxicity in humans, the U.S. Food and Drug Administration has issued a requirement of no more than 1 µg g<sup>-1</sup> in finished flour products (FDA, 2010).

Plant breeding programs use different mating systems to study the inheritance of quantitative traits. The diallel is a mating design in which the genotypic variance is partitioned to estimate general combining ability (GCA) and specific combining ability (SCA) of the parents (Griffing, 1956). Estimation of GCA and SCA helps to identify superior parents, which can be utilized for hybrid and cultivar development. The diallel design has also been used to estimate gene action, heterosis, and inbreeding depression involved in determining quantitative traits (Gardner and Eberhart, 1966). Hence, the design assists plant breeders in devising efficient and effective breeding programs.

Inheritance studies on FHB resistance have been inconsistent, where heritability estimates have varied greatly and both additive and non-additive gene effects have been found important. Singh et al. (1995) reported narrow-sense heritability estimates of 0.66 to 0.93; whereas, broad-sense and realized heritability estimates were reported to range from 0.05 to 0.89 (average 0.39) in F<sub>2</sub> and 0.00 to 0.96 (average 0.23) in F<sub>3</sub> wheat populations (Snijders, 1990b; Snijders, 1990c). Bai and Shaner (1994) reported that both additive and non-additive gene effects were important for resistance to FHB in wheat. But some studies considered additive gene effects to be more important than non-additive gene effects (Snijders, 1990a; Snijders, 1990b; Bai et al., 2000). Reports of transgressive segregants in FHB resistance studies also highlighted the importance of additive gene effects (Liu and Wang, 1991).

The biplot is a method of analyzing data where results are visually interpreted (Gabriel, 1971). Yan et al. (2000) described Genotype, Genotype-by-Environment (GGE) biplot based on the site regression (SREG) model to analyze multi-environment data where the first two principal components from the data were used to display a graph of genotype main effect (PC1 – primary effects) and genotype-by-environment interaction (PC2 – secondary effects). Yan and Hunt (2002) showed that the GCA of parents and SCA of the crosses in a diallel were analogous to ‘average yield’ and ‘stability’ of genotypes, respectively, for a multi-environment testing dataset.

Understanding the heritability and interrelationships among disease index (type I and type II resistances) (Schroeder and Christensen, 1963), *Fusarium* damaged kernels (FDK) (type III resistance) (Mesterhazy, 1995) and DON content (type V resistance) (Mesterhazy, 1995) are important in FHB resistance breeding. The objectives of this study were to (1) determine the heritability of FHB resistance using Griffing’s and biplot methods of diallel analysis in a set of winter and spring wheat germplasm; (2) examine the relationship among different types of FHB resistance evaluation; and (3) compare early generations (F<sub>1</sub> and F<sub>2</sub>) and F<sub>4:5</sub> diallels.

## MATERIALS AND METHODS

### PLANT MATERIAL

Parents for the diallel were three winter, one facultative, and two spring wheat genotypes. The relative resistance of these parents to FHB, as measured by severity varied from 72.5 to 12.1% (Table 1). A partial diallel consisting of only F<sub>1</sub> crosses (Griffing’s Method 4) was made in 2002. The single seed descend (SSD) method was used to advance the populations from F<sub>2</sub> to F<sub>4</sub>. From 20 to 50 F<sub>4:5</sub> lines for each of the fifteen crosses were evaluated.

Table 1. Pedigree and Fusarium head blight (FHB) severity under greenhouse evaluation of the diallel parents based on three seasons of greenhouse study in 2003 and 2004. Disease reaction arbitrarily assigned on a scale of 1-100%: R = Resistant (1-20%); MR = Moderately resistant (21-40%); MS = Moderately Susceptible (41-60%) and S = Susceptible (>60%).

Parent	Pedigree	Growth habit	FHB Severity (%)	Disease reaction
A Nekota	Bennett/TAM 107	Winter	42.2	MS
B 2137	W2440/W9488//2163	Winter	72.5	S
B Harding	Brule//Bennett/Chisholm/3/Arapahoe	Winter	54.7	MS
D Ning7840	Aurora/An Hui 11 (F2)//Sumai3	Facultative	12.1	R
E ND2710	Sumai3/Wheaton//Grandin	Spring	22.1	MR
F BacUp	Nuy Bay/2375//Marshall	Spring	39.4	MR

#### EXPERIMENTAL DESIGN

Prior to planting, ten seedlings for each line were vernalized for eight wk at 4°C. The F<sub>4.5</sub> lines were transplanted to a field study (Agronomy Farm (44°16'14" N, 96°46'18" W), South Dakota State University, Brookings, SD) characterized by a Barnes series (fine-loamy, mixed, superactive, frigid Calcic Hapludolls) soil type (Millar, 2004; Malo, 2003) in May 2006 and 2007. Lines were planted in a randomized complete block experimental design with two replicates. The experimental unit was a 25 cm x 40 cm single row plot.

#### INOCULUM PREPARATION

An aggressive *Fusarium graminearum* isolate (Fg4 isolate) was multiplied and its macrospores harvested as described by Zhang et al. (2001). In brief, the isolate was evenly spread on lactic acid (1.6 ml L<sup>-1</sup>) half strength potato dextrose agar (PDA) media. The plates were incubated at 25°C in a 12 h dark and light cycle for one wk. Macroconidia were harvested in sterile water and the conidial suspension was adjusted to 70,000 spore ml<sup>-1</sup> with distilled water. TWEEN 80 (Fisher Scientific International Inc., Pittsburgh, Pennsylvania, U.S.A.), a wetting agent, was added to the suspension at the rate of 400 µl L<sup>-1</sup>.

Ten *Fusarium* isolates were used to produce artificial inoculum. Corn kernels that filled one-fourth of a steel tray (50 x 29 x 6 cm) were soaked in water for 12 h. The water was subsequently drained and the kernels autoclaved for 45 min. For each isolate, agar from two previously colonized PDA plates was cut into approximately 1-cm<sup>2</sup> pieces and spread on each tray. Subsequently, inoculated corn was incubated at room temperature for 14 d and the inoculum was sun-dried inside a glasshouse for 5 d and afterwards, stored at 4°C.

#### INOCULUM APPLICATION

Inoculum was applied as infected corn kernels in the field at the rate of 1.25 g plot<sup>-1</sup> (0.25 m by 0.4 m) when wheat was at the jointing stage [Growth Stage (GS) 31; Zadoks et al., 1974] and the process was repeated at one wk intervals until the heading (GS 59). The field nursery was mist-irrigated (0.4 L h<sup>-1</sup>) on the same day the first inoculum was spread in the field. The mist irrigation was a 2 minute duration every 28 min from 7:00 to 19:00 hours. The nursery was mist-irrigated daily until disease symptoms were observed for the latest maturing population.

At heading, a conidial suspension of 70,000 spore ml<sup>-1</sup> was applied to spikes using a back pack sprayer (0.5 L min<sup>-1</sup> at 207 kPa). The suspension was again sprayed one week later to assure the inoculation of late tillers.

#### DISEASE AND MYCOTOXIN ASSESSMENT

Disease incidence (type I resistance) and severity (type II resistance) were recorded 21 d after the first conidial suspension application. Disease ratings for each entry were averaged

over a plot. Disease incidence was measured as the percentage of number of spikes infected over total spikes. Disease severity was measured as the percentage of infected spikelet(s) within the spike on a 0 to 9 scale (Stack and McMullen, 1985: 0=no disease, 1=7%, 2=14%, 3=21%, 4=33%, 5=50%, 6=66%, 7=79%, 8=90% and 9=100% disease severity). Disease index was calculated as the product of disease incidence and disease severity. Percentage of healthy index was calculated as the reciprocal of disease index, according to the following formula:

$$\text{Disease Index\%} = \frac{\text{Disease Incidence\%} \times \text{Disease Severity\%}}{100}$$

$$\text{Healthy Index\%} = (100 - \text{Disease Index})\%$$

The percent shriveled and bleached kernels was calculated for each cross after hand harvesting and threshing inoculated spikes from the field study. The number of *Fusarium* damaged kernels (FDK) is considered a measure of Type III resistance to FHB (Mesterhazy, 1995). A reciprocal of FDK, referred to as the percent undamaged kernels, was used in the biplot analyses:

$$\text{Percent of undamaged kernels} = (100 - \text{FDK})\%$$

Mycotoxin concentrations were measured in crosses as a means of determining type V resistance to FHB (Mesterhazy, 1995). The inoculated spikes from each plot were harvested by hand and threshed by hand. Kernels from individual lines were bulked for each cross. A 20 g sample from each cross was ground in a coffee-grinder and sent to North Dakota State University in Fargo, North Dakota, U.S.A. for determination of mycotoxin content. The mycotoxins deoxynivalenol (DON), 15-acetyl-deoxynivalenol (15-ADON) and nivalenol (NIV) were analyzed using the gas chromatography/electron capture detection method according to Tacke and Casper (1996). Both 15-ADON and NIV were less than 0.5  $\mu\text{g g}^{-1}$  and they are not discussed in this study.

#### STATISTICAL AND GENETIC ANALYSES

The diallel analysis was based on Griffing's Method 4 (Griffing, 1956), where one set of crosses ( $F_1$ ) [ $p(p-1)/2$  entries] was used to estimate general combining ability (GCA) and specific combining ability (SCA). Data were analyzed with the Diallel-SAS program of Zhang and Kang (1997). The general linear model for Griffing's Method 4 and Model 1 is:

$$Y_{ijk_y} = \mu + a_y + b_{ky} + v_{ij} + (av)_{ij_y} + e_{ijk_y}$$

$$v_{ij} = g_i + g_j + s_{ij} \quad \text{where } i \neq j,$$

where  $Y_{ijk_y}$  is the observed disease reaction value ( $i$  and  $j$  = parents;  $k$  = replication;  $y$  = year),  $\mu$  is the population mean,  $a_y$  is year effect,  $b_{ky}$  is the replication within year effect,  $v_{ij}$  is the genotype effect,  $g_i$  is the GCA effect for the  $i^{\text{th}}$  parent,  $g_j$  is the GCA effect for the  $j^{\text{th}}$  parent,  $s_{ij}$  is the SCA effect for the cross between  $i^{\text{th}}$  and  $j^{\text{th}}$  parents,  $(av)_{ij_y}$  is the interaction between genotypes and year, and  $e_{ijk_y}$  is the residual effect.

Narrow-sense heritability was calculated from expected variance components of GCA and SCA effects from the ANOVA according to the following formula:

$$h_n^2 = \frac{\sigma_A^2}{\sigma_A^2 + \sigma_D^2 + \left(\frac{\sigma_E^2}{2k}\right)}$$

where  $\sigma_A^2$  is the additive variance,  $\sigma_D^2$  is the dominance variance,  $\sigma_E^2$  is the error variance and  $k$  is the number of replications.

The combining ability ratio was obtained according to Baker (1978) as follows:

$$\text{Combining ability ratio} = \frac{2\sigma_{GCA}^2}{2\sigma_{GCA}^2 + \sigma_{SCA}^2}$$

where  $\sigma_{GCA}^2$  is the variance of GCA and  $\sigma_{SCA}^2$  is the variance of SCA.

Using SAS version 9.0 (SAS Institute Inc., NC), Pearson's correlation coefficients were computed to estimate the association between healthy index, undamaged kernels, and DON content.

The GGE biplot software was used to generate biplot figures for healthy index, undamaged kernels, and DON content (Yan and Kang, 2003). Each parent was considered both an entry and a tester. A two-way matrix of entries and testers was generated from the mean values for hybrids, where rows were entries and columns were testers. The biplot model is as follows:

$$\hat{Y}_{ij} - \mu - \beta_j = \lambda_1 \xi_{i1} \eta_{j1} + \lambda_2 \xi_{i2} \eta_{j2} + \varepsilon_{ij}$$

where  $\hat{Y}_{ij}$  is the expected value of the combination between entry  $i$  and tester  $j$ ;  $\mu$  is the grand mean;  $\beta_j$  is the mean of all combinations involving tester  $j$ ;  $\lambda_1$  and  $\lambda_2$  are the singular values for PC1 and PC2, respectively;  $\xi_{i1}$  and  $\xi_{i2}$  are the PC1 and PC2 eigenvectors, respectively, for entry  $i$ ;  $\eta_{j1}$  and  $\eta_{j2}$  are the PC1 and PC2 eigenvectors, respectively, for tester  $j$ ; and  $\varepsilon_{ij}$  is the residual of the model associated with the combination of entry  $i$  and tester  $j$ . When  $i \neq j$ , the combination is a hybrid.

## RESULTS AND DISCUSSION

### GENETIC ANALYSIS

Due to a significant correlation between years ( $r = 0.55$ ,  $P < 0.05$ ) and a non-significant homogeneity for variance test (Levene's test), data were pooled over the years for the healthy index. There was a significant variation among the crosses for healthy index (Table 2). Partitioning of the crosses variance showed highly significant GCA mean squares and non-significant SCA mean squares. A highly significant SCA  $\times$  Year interaction was found, whereas the GCA  $\times$  Year interaction was non-significant. Estimates of GCA and SCA effects for healthy index are shown in Table 3 and means for the healthy index of the crosses is shown in Table 4. Positive and negative values for GCA and SCA indicate that parents were contributing resistance and susceptibility, respectively. The combining ability ratio was 0.95 and narrow sense heritability was 0.83 for healthy index.

Table 2. Analysis of partitioned genotypic variance for percentages of healthy index of F<sub>4:5</sub> evaluated in a mist-irrigated field in 2006 and 2007, using Griffing's Method-4, model 1.

Source	df	MS
Year	1	5185
Block(Year)	2	1476*
Genotype	14	5492
General combining ability (GCA)	5	11593**
Specific combining ability (SCA)	9	580
Genotype $\times$ Year	14	1556**
GCA $\times$ Year	5	736
SCA $\times$ Year	9	1978**
Experimental Error	1716	335

\*, \*\* Significant at 0.05 and 0.01 probability level, respectively.

### BIPLOT

The GGE biplot software generated average tester coordinate (ATC) and polygon views. We interpreted the diallel data based on these two biplots as explained by Yan and Hunt (2002) and Yan and Kang (2003). In the ATC view, the average of PC1 and PC2 of the tester is called the average tester and marked with a small circle. In the diallel study, entry distances

on the ATC abscissa, a horizontal line passing through the origin and the average tester, approximate the GCA effects, which is the value of its hybrid with the average tester. The entries with positive GCA are those represented on the same side of the average tester circle from the origin. Similarly, the projection of the entries on the ATC ordinate, a perpendicular line to ATC abscissa from the origin, approximates the SCA effects. The higher the distances of an entry from the origin on either direction of ATC ordinate, the larger is the SCA effect of that entry.

The polygon view of the biplot describes the interaction between the entry and tester. Joining the outermost entries, which become the vertices of the polygon, from the origin, draws the polygon. Perpendicular lines drawn from the origin to the sides of the polygon divide it into different entry sectors. Any tester(s) falling in such a sector forms a superior hybrid, exhibiting heterosis, from a cross between the tester and the vertex entry. In any sector, if the tester and vertex entry of the same genotype exist together, then the parent must be superior to any hybrid formed with the vertex entry.

#### HEALTHY INDEX

The biplot explained 90.4% (PC1 = 76.9% and PC2 = 13.5%) of the total variation for percentages of healthy index (Fig. 1a). Entries e and f had positive GCA effects as they were on the positive end of the ATC abscissa, which indicated that the entries contributed to resistance in their progenies. Entries a, b, c and d were on the negative side of the ATC abscissa suggesting that they had negative GCA effects and contributed to susceptibility in their offspring. The ranking of entries based on Griffing's GCA effects were  $e > f > d > c > b > a$  (Table 3), which was similar to the ranking suggested by biplot analysis as shown in Fig. 1a. Based on biplot analysis, the optimal entry is the one that lies at or closest to the center of the concentric ring in Fig. 1a. Entry e was closest to the ideal entry. This entry also had the highest positive GCA effects (Table 3). The SCA effects of the entries were estimated based on the projection of the entries on the ATC ordinate. Entry e projected high on the ATC ordinate from the biplot origin, so it had large SCA effects compared to entries a, b, c, d, and f (Fig. 1a).

The ideal tester in the diallel should be highly discriminating of all entries and at the same time, be representative of all testers (Yan and Hunt, 2002). The tester view generated from the biplot showed that D was the closest to the ideal tester (data not shown).

Table 3. Estimates of general combining ability (GCA) and specific combining ability (SCA) effects for percentages of healthy index of  $F_{4.5}$  evaluated in a mist-irrigated field in 2006 and 2007. The  $LSD_{0.05}$  for testing differences between GCA effects = 17.93

Parent	Nekota	2137	Harding	Ning7840	ND2710	GCA+
Nekota	-	-	-	-	-	-7.09**
2137	0.80	-	-	-	-	-3.44**
Harding	2.57	1.78	-	-	-	-2.70**
Ning7840	-2.33	1.24	-1.86	-	-	-1.39
ND2710	1.38	-3.40**	-0.94	0.82	-	8.79**
BacUp	-2.43	-0.36	-1.55	2.14	2.20	5.83**
$LSD_{0.05}$ for SCA			19.64			
SE for SCA			10.02			
$LSD_{0.05}$ for GCA						11.58
SE for GCA						5.91

\*\* Significant at 0.01 probability level.

Based on the ideal tester, GCA effects of the entries can be assessed by the performance of the hybrids they form with genotype D. A high correlation ( $r = 0.98$ ) was found between Griffing's GCA effects of the parents and the F<sub>1</sub> hybrid between each entry and tester D. This result highlighted that genotype D was a good tester.

None of the testers fell on sector a, indicating that entry a did not produce a highly resistant hybrid with any of the tested genotypes (Fig. 1b). Since tester D was the farthest and on the opposite end to sector a, entry a produced the least resistant hybrid with tester D (Table 4). Testers A, B, C, D and F fell in sector e and so crosses e/[A, B, C, D and F] produced superior resistant hybrids. Similarly, tester E fell in sector f and the hybrid f/E would be expected to exhibit heterosis for FHB resistance. Because tester E was not in sector e and tester F was not in the sector f, hybrid e/F exhibited higher resistance than E/E and F/F (E and D pure lines). The most resistant hybrid among all possible combinations was the cross E/F because e/F and f/E were the best mating partners in sector e and f, respectively. The cross E/F had the highest healthy index (69.1%) and was the most resistant of all parental combinations (Table 4).

Table 4. Mean of percentages of healthy index (MHI), percentages of undamaged kernel (MUdK) kernels and deoxynivalenol (MDON) content of F<sub>4:5</sub> evaluated in a mist-irrigated field in 2006 and 2007.

Population		MHI	MUdK	MDON
			%	µg g <sup>-1</sup>
A/B	Nekota/2137	42.6	44.8	4.5
A/C	Nekota/Harding	45.1	46.2	6.9
A/D	Nekota/Ning7840	41.5	49.2	3.3
A/E	Nekota/ND2710	55.4	50.9	3.5
A/F	Nekota/BacUp	48.6	57.1	4.7
B/C	2137/Harding	47.9	51.0	6.4
B/D	2137/Ning7840	48.7	56.2	4.3
B/E	2137/ND2710	54.2	53.7	5.7
B/F	2137/BacUp	54.3	49.9	5.8
C/D	Harding/Ning7840	46.4	44.0	4.3
C/E	Harding/ND2710	57.5	54.2	5.2
C/F	Harding/BacUp	53.9	52.7	4.6
D/E	Ning7840/ND2710	60.5	54.9	1.5
D/F	Ning7840/BacUp	58.9	55.6	2.2
E/F	ND2710/BacUp	69.1	64.3	1.9
	SE	1.9	1.4	0.4

#### UNDAMAGED KERNEL

The first two principal components described 64.2% (PC1 = 45.3% and PC2 = 18.9%) of the total variation for the percent healthy index (Fig. 2a). Entries e and f had positive GCA effects, whereas a, b, c and d had negative GCA effects. The order of the entry based on GCA effects was: f>e>d>b>a>c. Entry f was an important contributor as it was located closer to the center of the concentric circle and expected to contribute high resistance in its progenies. Based on the projection of the entries towards the ATC ordinate, entries b, f and e were expected to have high SCA effects (Fig. 2a). However, entry b did not have high SCA effects,

as sector b did not have any tester (Fig. 2b). Tester A exhibited the highest % undamaged kernels as it was closest to the ideal tester (data not shown).

In the polygon view, none of the testers fell in sectors a, c and b, which indicates that entries a, c and b did not produce any highly resistant hybrid (Fig. 2b). In sector e, entry e would produce a more resistant hybrid with tester F (e/F) compared to the E/E pure line. Similarly, sector f contained testers A, B, C, D and E. The hybrids f/[A, B, C, D and E], therefore, would be superior to the F/F pure line for resistance. Since the e/F and f/E hybrids were superior to the pure line in sectors e and f, respectively, hybrid E/F would represent the cross most likely to optimize resistance. As predicted, this cross had the highest percent of undamaged kernels (Table 4).

#### DON CONTENT

The first two principle components described 69.5% and 14.1% (total = 83.6%), respectively, of the total variation for DON content. Fig. 3a shows the GCA effects along the ATC abscissa and SCA effects along the ATC ordinate. Entries d, e and f had positive GCA effects; whereas, entries a, b and c had negative GCA effects. The ranking of entries based on GCA effects was  $d > e > f > a > b > c$ . Among the entries, d was at the center of the concentric ring, suggesting that it was the ideal parent. Entry d was the farthest on the positive side of the ATC abscissa and the optimal entry for resistance. Based on Griffing's diallel analysis, entry d also had the highest GCA effects, indicating its ability to contribute a high level of resistance to FHB in its offspring. In contrast, entry c was the farthest on the negative side of the ATC abscissa, and it exhibited the highest negative GCA effect, indicating that it would likely contribute susceptibility to its progenies. Based on the entries projection on the ATC ordinate, entries b and c were expected to have high SCA effects and entry d was expected to have low SCA effects. However, entry d produced more resistant hybrids; whereas, entries b and c did not produce very resistant hybrids (Fig. 3b). Tester E was the most resistant as it was closest to the ideal tester (Fig. not shown).

The polygon view of DON content showed five sectors, namely f, d, a, b and c (Fig. 3b). None of entries a, b, c and f formed highly resistant hybrids, as there was no tester in sectors a, b, c and f. Entry d produced more resistant hybrids with testers A, B, C, E and F, as they were in sector d. Nevertheless, sector d also contained tester D, which indicated that the D/D pure line was superior in resistance to the hybrids d/[A, B, C, E and F]. Hence, the D/D pure line was expected to have the lowest DON content.

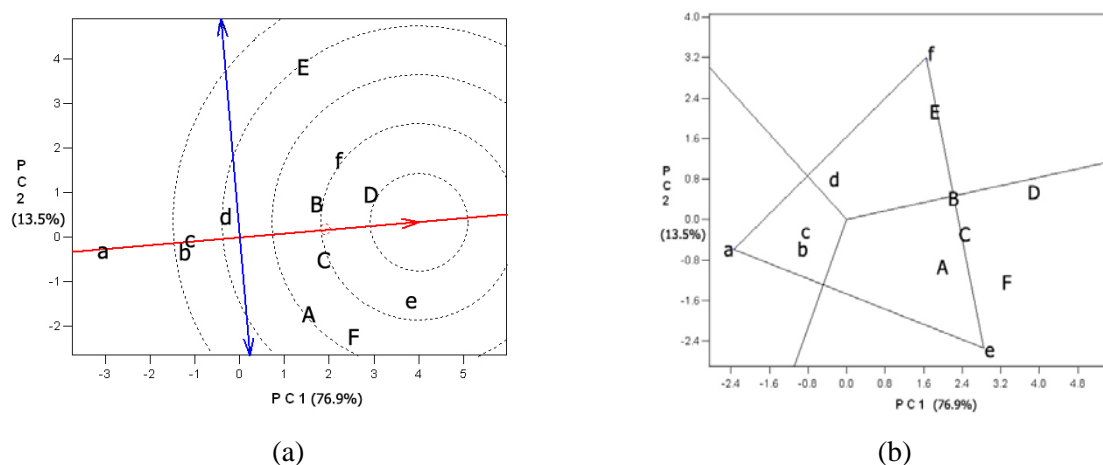


Figure 1. Biplot showing (a) average tester coordinate (ATC) view and (b) polygon view of six parents for percentages of healthy index in the  $F_{4.5}$  evaluated in a mist-irrigated field in 2006 and 2007. Uppercase letters are testers and lowercase letters are entries of six parents. Parents are: A = Nekota, B = 2137, C = Harding, D = Ning7840, E = ND2710 and F = BacUp.



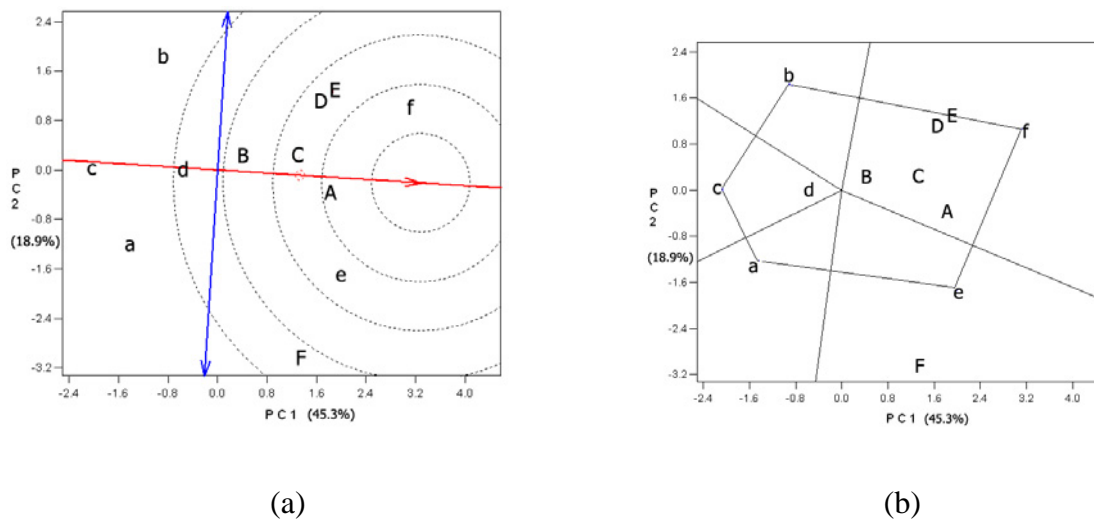


Figure 2. Biplot showing (a) average tester coordinate (ATC) and (b) polygon view of six parents for percentages of undamaged kernels in the  $F_{4:5}$  evaluated in a mist-irrigated field in 2006 and 2007. Uppercase letters are testers and lowercase letters are entries of six parents. Parents are: A = Nekota, B = 2137, C = Harding, D = Ning7840, E = ND2710, and F = BacUp.

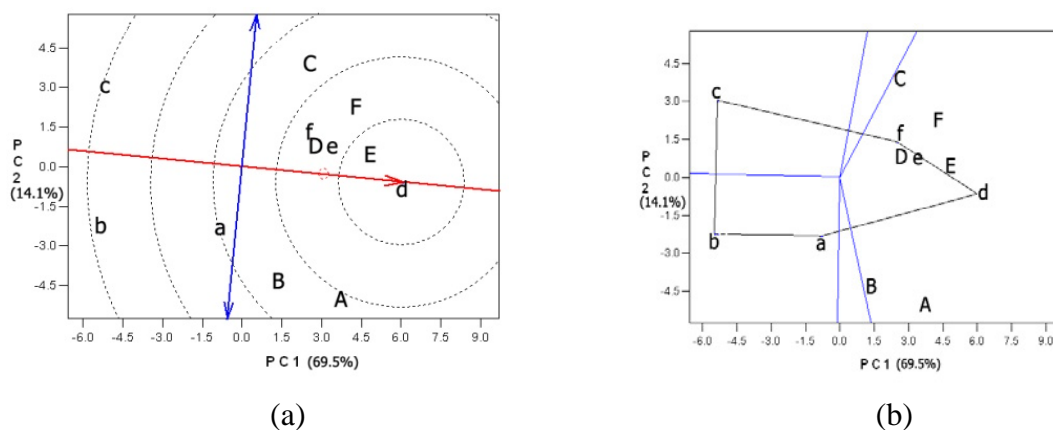


Figure 3. Biplot showing (a) average tester coordinate (ATC) and (b) polygon view of six parents for deoxynivalenol (DON) content in the  $F_{4:5}$  evaluated in a mist-irrigated field in 2006 and 2007. Uppercase letters are testers and lowercase letters are entries of six parents. Parents are: A = Nekota, B = 2137, C = Harding, D = Ning7840, E = ND2710, and F = BacUp.

## DISCUSSION

The ANOVA method according to Griffing's and principal component analysis method of GGE biplot gave similar results. The biplot described a large portion of the total variation ranging from 64.2% to 90.4% for percent healthy index, percent undamaged kernels, and DON content. This indicates its reliability in predicting resistance due to FHB.

While both GCA and SCA mean squares were significant using greenhouse early generation ( $F_1$  and  $F_2$ ) data for healthy index (Malla et al., 2009), only the GCA mean squares were significant using the present study field-grown  $F_{4:5}$  population. A significant GCA variance indicates that additive gene action was important and parents differed in the level of the resistance to FHB they contributed to the progeny. The combining ability ratio for healthy index was 0.95. According to Baker (1978), when combining ability ratio approaches unity, GCA alone can predict the performance of the parents. Thus, the GCA scores in the present study could be used to predict the performance of the parents. Narrow-sense

heritability was high for healthy index, which is consistent with Bai et al. (2000) and Singh et al. (1995), who calculated high narrow sense heritability estimates for wheat of 0.80 to 0.91 and 0.66 to 0.93, respectively. The high combining ability ratio and narrow sense heritability emphasizes the importance of additive gene action to healthy index.

A moderate negative correlation was observed between healthy index and DON content ( $r = -0.53$ ,  $P < 0.05$ ) as well as undamaged kernels and DON content ( $r = -0.48$ ,  $P = 0.07$ ). However, a high positive correlation was observed between healthy index and undamaged kernel ( $r = 0.77$ ,  $P < 0.01$ ). A high negative correlation was also observed between healthy index and DON content for the same parental diallel set in the  $F_1$  ( $r = -0.71$ ,  $P < 0.01$ ) and  $F_2$  ( $r = -0.84$ ,  $P < 0.01$ ) generations in a greenhouse study (Malla et al., 2009). Based on these correlations, selection for one trait should result in some genetic gain for other traits related to resistance.

In the biplot analysis, resistant parents E and F exhibited positive GCA effects for all three resistance traits, indicating that the parents contributed resistance and improved healthy index, lowered the number of undamaged kernels, and reduced DON content. The resistant parent D had positive GCA effects for DON content indicating its ability to contribute resistance to its progenies. Cross E/F exhibited the highest level of resistance as determined by healthy index and undamaged kernels. Parent E was derived from 'Sumai3', a landrace carrying a Chinese source of FHB resistance, and parent F was derived from 'Nuy Bay', a variety with a Japanese source of FHB resistance. These results differed from tests on early generation ( $F_1$  and  $F_2$ ) diallels, where cross D/E was superior for percent healthy index (Malla et al., 2009). Parent D was superior to all crosses with regard to DON content. Parent D was also derived from 'Sumai3'. In an early generation ( $F_1$  and  $F_2$ ) diallel study, pure lines D and E were superior to all crosses for DON content (Malla et al., 2009). These results suggested that of these parental lines D should be best parent for breeding to reduce DON content. Parents A, B and C contributed susceptibility with negative GCA effects for all three traits. Mean greenhouse estimates of FHB severity, even when the range of differences had been maximized, were not good predictors of best parents to be used in breeding.

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