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8 **Evaluation of popcorn germplasm for resistance to pink stem borer**

9 **attack**

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18

1 **Abstract**

2

3 Popcorn adapted to Spanish conditions could be an interesting and profitable
4 alternative to field corn. However, little is known about breeding popcorn
5 germplasm for adaptation to Spain. Pink stem borer, *Sesamia nonagrioides* Lef. is
6 the main insect pest affecting popcorn quality and yield under Spanish
7 conditions. The objectives of the study were the search for sources of
8 resistance to pink stem borer among popcorn germplasm, and to study the
9 genetics of the resistance to pink stem borer attack. Eight breeding populations
10 along with a five-inbred line diallel and two popcorn commercial checks were
11 evaluated under pink stem borer infestation in two years. Significant
12 differences were found among general combining ability (GCA) effects for
13 days to silking, pink stem borer tunnel length, general appearance of the ear,
14 kernel moisture, and yield. Specific combining ability (SCA) effects were found
15 to be significant for yield, and ear damage. Therefore, heterotic patterns among
16 popcorn materials should be taken into account to generate new popcorn
17 hybrids that are not only more productive but also have higher kernel quality.
18 Breeding popcorn populations BSP4APC0 and PSPW1C1 could be base
19 germplasms in a breeding program for obtaining parental inbreds of healthy-
20 kernel popcorn hybrids. New inbred lines could be generated from the cross
21 BP1 x BP2 that would have improved GCA and SCA effects for pink stem
22 borer resistance when crossed to South American inbreds.

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1 The use of popcorn germplasm adapted to Spanish conditions could be an
2 interesting and profitable alternative to field corn production. However,
3 breeding of popcorn germplasm for adaptation to Spanish conditions has not
4 been done. Although large kernel expansion and good quality flakes are the
5 most important breeding traits, agronomic characteristics such as yield,
6 standability, and resistance to pests should be improved (Ziegler 2001).

7 Corn borer pests are distributed world wide (Dicke and Guthrie 1988).
8 In the Mediterranean area the pink stem borer, *Sesamia nonagrioides* Lef., is the
9 main maize pest (Anglade 1961). Pink stem borer larvae prefer attacking stems
10 rather than ears, but for specialty corns intended for human consumption, ear
11 damage might be the major constrain. When attacking popcorn varieties, corn
12 borers affect agronomic traits as well as quality traits (Thomas et al. 1960,
13 Jarvis et al. 1990).

14 The search for maize varieties resistant to pink stem borer attack has
15 been mainly focused on field corn (Malvar et al. 1993, 2004, Cartea et al. 1994,
16 Butrón et al. 1999a, Soengas et al. 2004) and sweet corn (Velasco et al. 1999a,
17 b) germplasm. Malvar et al. (2004) evaluated one popcorn population,
18 ESP0070441, along with several field corn populations for resistance to stem
19 and ear damage by the pink stem borer and found that ESP0070441 was as
20 resistant as the most resistant field corn population. Butrón et al. (2005)
21 evaluated four popcorn inbred lines (BP1, BP2, IADS28 and IADS69) and 44
22 field corn inbreds. BP1, BP2 and IADS28 did not differ significantly from the
23 field corn inbred line that presented the lowest percentage of damaged stem

1 and BP2 was among those with the least ear damage (Butrón et al. 2005),
2 suggesting that it is possible to increase stem and ear resistance of popcorn
3 varieties without crossing to field corn varieties. Among field and sweet corn
4 germplasm, general combining ability for stem and ear traits related to the pink
5 stem borer and yield under infestation were significant, while specific
6 combining ability was significant for yield (Butrón et al. 1998, 1999b, Velasco
7 et al. 2002).

8 Evaluation of available popcorn germplasm for resistance to pink stem
9 borer could identify sources of resistance for a breeding program addressing
10 development of popcorn hybrids adapted to European conditions. In addition,
11 knowledge of the genetics involved in popcorn resistance to pink stem borer
12 would help to determine the most appropriate breeding approach. The present
13 study intends to study whether this rule can be extended to popcorn
14 germplasm. The objectives of the present study were: i) search for sources of
15 resistance to pink stem borer among popcorn germplasm, and ii) study the
16 genetics involved in popcorn resistance to pink stem borer attack.
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2 **Materials and methods**

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4 Eight popcorn populations (BSP1C4, BSP2C1, BSP4APC0, BSP5C0,
5 BSP7SAC0, BSP8SGC0, BSPW1C1, and HPSGDS-2), two commercial
6 hybrids (Poper and TBS-121), and 10 hybrids resulting from a five inbred line
7 (BP1, BP2, IADS28, IADS69, and IADS91) diallel were evaluated under pink
8 stem borer infestation in 2002 and 2003. Popcorn varieties were arranged in a
9 randomized complete block design with three replications. Each variety was
10 planted in a two-row plot, 0.80 m between rows and 0.21 m between two-
11 kernel hills within rows. After thinning the final density was about 60 000
12 plants ha⁻¹.

13 When approximately 50% of maize plants were silking, ten plants per
14 plot were infested with pink stem borer eggs, obtained following the insect
15 rearing protocol developed by Eizaguirre (1989) from wild pink stem borer
16 larvae collected in the fall. Infestations were made as described by Butrón et al.
17 (1998). The stems of infested plants in each plot were dissected at harvest and
18 tunnel lengths (cm) were recorded. General appearance of infested ears in each
19 plot was scored on a 9- point subjective rate (9 = no kernel damage, 8 = up to
20 10% of kernels damaged, 7 = 11% to 20% of kernels damaged, 6 = 21% to
21 30% of kernels damaged, 5 = 31% to 40% of kernels damaged, 4 = 41% to
22 50% of kernels damaged, 3 = 51% to 60% of kernels damaged, 2 = 61% to
23 70% of kernels damaged, and 1= more than 80% of kernels damaged). In each

1 plot, the number of days to silking and percentage of grain moisture at harvest
2 were recorded. Additionally, two important agronomic traits (i.e. stem lodging
3 (percentage of infested plants that were broken below the main ear) and grain
4 yield (Mg ha^{-1} at 140 g kg^{-1} moisture content)) that could be affected by corn
5 borer attack were recorded on infested plants.

6 Individual and combined analyses of variance were computed. Year
7 and replication were considered random factors and genotype a fixed factor.
8 Genotype sums of squares were orthogonally partitioned into ‘populations’
9 (including hybrid checks), ‘diallel’ and ‘populations *vs* diallel’. Variation among
10 hybrids of the diallel was further partitioned into General combining ability
11 (GCA), and specific combining ability (SCA) effects using Griffing’s Method 4,
12 Model I (fixed effects) (Griffing 1956). The diallel was analyzed using a
13 program developed by Zhang and Kang (1997). Simple correlation coefficients
14 among traits were calculated. Comparisons of means were accomplished using
15 Fisher’s protected least significant difference method (LSD). All analyses were
16 made using SAS, version 9.1 (SAS Institute 2000).
17

Results

The combined analyses of variance showed significant differences among genotypes for days to silking, tunnel length, kernel moisture, and yield (data not shown). For general appearance of the ear and stem lodging, the year $\times$ genotype interaction was significant (data not shown). Variation among genotypes was mostly due to differences within each germplasm group, populations and diallel, and no difference was found between groups. There were significant differences among general combining ability (GCA) effects for all characters except lodging. Specific combining ability (SCA) effects were significant for general appearance of the ear and yield.

Diallel hybrids BP2 $\times$ IADS69, BP2 $\times$ IADS91, and IADS28 $\times$ IADS91 along with the two hybrid checks, Poper and PBS-121, and the population BSPW1C1 did not significantly differ from the least stem damaged genotype, BP1 $\times$ IADS91, and showed significantly less tunnel length on the stem than the most stem damaged genotype (Table 1). The hybrid IADS28 $\times$ IADS91 did not differ from the hybrid checks for yield under infestation conditions. The population BSP4APC0 was also among the highest yielding and earliest genotypes, but was not among the most resistant to the pink stem borer.

In the diallel analysis, IADS28 had the most favorable GCA effects for general appearance of the ear and yield but the least favorable GCA effects for

1 tunnel length, while IADS91 had the most favorable GCA effects for tunnel
2 length but the least favorable GCA effects for general appearance of the ear
3 and yield (Table 2).

4 SCA effects for general appearance of the ear of crosses BP1 ×
5 IADS91 and BP2 × IADS69 were significantly different from zero (Table 3),
6 but these crosses yielded significantly less than crosses IADS28 × IADS91 and
7 IADS28 × IADS69.

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9 **Discussion**

10

11 The importance of GCA and SCA effects on traits related to popcorn attack by
12 the pink stem borer differed from results obtained in previous studies
13 involving field (Butrón et al. 1998, 1999a, b) and sweet (Velasco et al. 2002)
14 corn, in which no significant SCA effects for general appearance of the ear
15 were found. Therefore, the present work showed the importance of
16 considering heterotic patterns to generate popcorn hybrids with higher yield
17 and kernel quality.

18 There is a general lack of public information about heterotic patterns
19 among popcorn germplasm because popcorn breeding is mostly done by
20 private seed companies. Burnham Larish and Brewbaker (1999) showed the
21 existence of hybrid vigor among three basic heterotic groups: Yellow Pearl,
22 South American and Supergold. For yield and general appearance of the ear,

1 SCA effects were, in general, significant and positive in crosses between inbred
2 lines belonging to two different germplasm groups, one formed with BP1,
3 BP2, and IADS28 and the other with IADS69, and IADS91. As IADS28 is a
4 Yellow Pearl inbred while IADS69 and IADS91 belong to the South American
5 heterotic group, inbreds BP1 and BP2 seem closer to Yellow Pearl than to
6 South American popcorn germplasm.

7 Populations BSP4APC0 and BSPW1C1 are promising as base
8 germplasm in a popcorn breeding program because they have high yield under
9 infestation, are significantly earlier than the hybrid checks and have less kernel
10 moisture than the hybrid check TBS-121. In addition, synthetic BSP4APC0
11 showed popping expansion equal to that of BSP1C1, a line closely related to
12 BSPW1C1 (Ziegler et al. 1987). Therefore, obtaining promising inbred lines
13 from these populations could easily be done and future efforts could be
14 devoted to defining heterotic patterns among popcorn populations. This will
15 help breeders derive potentially successful popcorn hybrids; particularly for
16 populations developed from unknown commercial hybrids such as BSPW1C1
17 (Ziegler 2001). Inbreds developed from the synthetic BSP4APC0 (related to
18 the Amber Pearl type) are expected to perform well in crosses to South
19 American and Supergold inbreds (Burnham Larish and Brewbaker 1999).

20 Synthetic BSP1C1 was categorized as partially resistant to both
21 generations of the European corn borer (Ziegler et al. 1987). Therefore,
22 resistance genes to pink stem borer present in BSPW1C1 could be also
23 involved in resistance to European corn borer. Resistance to both corn borers

1 was not completely independent among sweet (Velasco et al. 1999) and field
2 corn (unpublished data) germplasm. The other two synthetics previously
3 evaluated as resistant to the European corn borer, BSP2C1 and BSP1C4, were
4 not among the most resistant genotypes to pink stem borer, pointing out the
5 need for testing for resistance to both corn borer species (Malvar et al. 2004).

6 There was a lack of concordance between favorable GCA effects for
7 ear traits and tunnel length, showing the difficulty of developing materials
8 combining both, stem resistance and good ear quality characteristics. The good
9 performance of the cross IADS28 $\times$ IADS91 under pink stem borer attack
10 could be due to the favorable additive effects supplied by inbred parents since
11 the cross between these inbred lines did not show significant SCA effects for
12 any trait, and IADS28 and IADS91 had the best GCA effects for ear
13 characteristics and tunnel length, respectively. Based on the significant SCA
14 effects of BP1 $\times$ IADS91 and BP2 $\times$ IADS69 for general appearance of the ear,
15 we propose to develop new inbred lines with good GCA and SCA effects
16 when crossed to South American inbreds from the cross BP1 $\times$ BP2. BP1
17 could contribute to precocity and high heterosis when crossed to IADS91
18 while BP2 could reduce kernel moisture and contribute to high heterosis when
19 crossed to IADS69. Neither BP1 nor BP2 had unfavorable GCA effects for
20 tunnel length when compared to IADS28.

21 In conclusion, genetics involved in popcorn ear resistance to pink stem
22 borer are slightly different from those involved in field and sweet corn

1 resistance. Populations BSP4APC0 and PSPW1C1 seemed promising as base
2 germplasm in a popcorn breeding program for obtaining parental inbreds for
3 healthy-kernel popcorn hybrids. Further, new inbred lines could be generated
4 from the cross BP1 x BP2 with improved GCA and SCA effects for resistance
5 to pink stem borer when crossed to South American inbreds.

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8

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1 Table 1. Means for popcorn populations and hybrids evaluated under
 2 infestation with pink stem borer eggs in two years.

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1	BP1 × IADS28	71.5	54.5	7.0	28.1	60.3	3.9
2	BP1 × IADS69	71.5	52.4	6.0	29.1	14.0	2.5
3	BP1 × IADS91	71.8	38.8	6.5	30.8	29.7	3.1
4	BP2 × IADS28	74.0	64.5	6.7	29.5	69.2	3.9
5	BP2 × IADS69	75.8	43.6	6.7	27.3	28.2	2.8
6	BP2 × IADS91	76.0	50.1	5.9	29.2	40.7	2.7
7	IADS28 × IADS69	73.3	66.2	6.8	31.0	41.3	4.0
8	IADS28 × IADS91	75.7	41.3	6.6	34.0	31.3	4.2
9	IADS69 × IADS91	80.0	46.4	4.9	25.7	19.0	1.1
10	LSD	2.8	14.7		4.2		0.8

¹ General appearance of each infested ear per plot was taken on a 9- point
 subjective rate (9 = non damaged kernels, 8 = up to 10% of kernels damaged,
 7 = 11% to 20% of kernels damaged, 6 = 21% to 30% of kernels damaged, 5
 = 31% to 40% of kernels damaged, 4 = 41% to 50% of kernels damaged, 3 =
 51% to 60% of kernels damaged, 2 = 61% to 70% of kernels damaged, and 1=
 more than 80% of kernels damaged)

1 Table 2. General combining ability effects for traits related to damage by *S.*
2 *nonagrioides* from a diallel of five inbred lines of popcorn grown for two years.

Inbred	General					Yield
	Days to silking	Tunnel length	appearance of the ear	Kernel moisture		
BP1	-3.64*	-0.46	0.09	-1.18		0.05
BP2	0.02	3.72	0.08	-1.88*		-0.01
IASD28	-0.48	6.44*	0.59*	-0.06		1.14*
IADS69	1.58*	0.50	-0.31	0.54		-0.69*
IADS91	2.52*	-10.19*	-0.45*	2.58*		-0.49*
LSD ($p<0.05$)	1.73	9.93	0.56	2.00		0.52

15 * Significantly different from zero ($p<0.05$).

16

1 Table 3. Specific combining ability effects for grain yield under infestation with
2 *S. nonagrioides* eggs (above the diagonal) and general appearance of the ear
3 (below the diagonal) from a diallel of five inbred lines of popcorn grown for
4 two years.

	BP1	BP2	IADS28	IADS69	IADS91
BP1		-0.02	-0.43	0.04	0.40
BP2	-0.32		-0.38	0.34	0.06
IASD28	-0.06	-0.29		0.44	0.36
IADS69	-0.17	0.64*	0.19		-0.82*
IADS91	0.55*	-0.03	0.15	-0.67*	

13 * Significantly different from zero ($p<0.05$).

14 LSD ($SCA_{ij} - SCA_{ik}$) for grain yield and general appearance of the ear were 0.74
15 and 0.79, respectively ($p<0.05$).

16 LSD ($SCA_{ij} - SCA_{kl}$) for grain yield and general appearance of the ear were 0.52
17 and 0.57, respectively ($p<0.05$).

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