CBAB
CROP BREEDING AND
APPLIED BIOTECHNOLOGY

Breeding maize for resistance to fall armyworm (*Spodoptera frugiperda*) in Argentina: genetic and environmental effects

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ABSTRACT - *Pests can strongly affect the maize yield in tropical regions. Objective of this work was to evaluate the performance for fall armyworm resistance in a set of maize populations tested under different environments and to determinate the possibility of introducing some of these populations into maize breeding programs. The trials were carried out in three naturally pest-infested environments. The populations were evaluated for plant (PH) and ear height (EH), grain yield (Y), and fall armyworm-resistance (FAR). Significant differences among the populations were observed for Y and FAR, besides a significant genotype x environment interaction. The genotype x environment interaction for FAR could be explained by the erratic performance across the environments presented by one of the tolerant populations. Our results attested that at least one of the tested populations could be included into the maize breeding program.*

Key words: *Zea mays*, pest resistance, breeding, exotic germplasm.

INTRODUCTION

Maize is considered one of the most important commodity crops in the world and sometimes shows different sensitivity degrees to environmental stresses (Llanos Company 1984). While research and genetic improvement have lifted some of these restrictions to maize production, others such as soil and pests still remain limiting factors, according to Giaveno et al. (2001). Among the insects that affect maize production, the fall armyworm (*Spodoptera frugiperda* Smith) is considered one of the most important pests because it indirectly affects grain yield (Alvarez and Miranda Filho 2002). Larvae that feed on maize leaves reducing the photosynthetic area are responsible for the fall armyworm damage and cause an indirect effect on maize yield. According to Cruz (1995), an important attack of fall armyworm could produce a grain yield reduction from 15 to 34%, depending on the stage of plant development in Brazil. Pest control could be accomplished using insecticide application or resistant cultivars. Several authors (Lara et al. 1984, Wiseman and Widstrom 1992) reported the feasibility of utilization of resistant genotypes to control *S. frugiperda* infestations. Despite the great genetic variability in maize, little information is available on the germplasm or the metabolic pathway utilized by these sources. According to Constabel (1999), some plants show a set of biochemical defense mechanisms with the capacity of detaining, poisoning, or starving pests or other herbivores that

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feed on them. This defense strategy involves the rapid accumulation of proteins or phytochemicals that posses the capacity of preventing or reducing herbivore damage. The use of proteins as defense mechanism has an advantage over phytochemicals because proteins are encoded by a single gene, which can be isolated and used to obtain genetically engineered crops (Estruch et al. 1997). Nevertheless, this situation could lead to a less durable resistance. For phytochemicals, the complexity of the metabolic pathways determines the presence of gene complexes resulting in a quantitative, more durable resistance.

The presence of a set of mechanisms based on a wound-induced serine proteinase (Cordero et al. 1994) or cysteine proteinase in maize leaves (Pechan et al. 2000) and secondary defense metabolites such as DIMBOA (Frey et al. 2000) and caffeic acid (Capellades et al. 1996) have been reported.

Maize germplasm can be evaluated for resistance to fall armyworm in the field by natural or artificial infestations. Natural infestations evaluate the genotypes by using regional specific races of the insect and proved to be useful in environments where the insect populations remain at a high level every year. On the other hand, artificial infestation assures approximately the same number of larvae per plant and thus increases the trial precision (Alvarez and Miranda Filho 2002). Several authors reported the feasibility of the evaluation of fall armyworm-resistance in maize under natural (Lara et al. 1984, Marques et al. 1988, Alvarez and Miranda Filho 2002) and artificial (Viana and Guimarães 1994, Alvarez and Miranda Filho 2002) infestation.

Another important aspect to be considered in a breeding program is the nature of the genetic bases of the trait. For fall armyworm-resistance, the presence of a quantitative trait with important additive genetic effects has been reported (Guimarães and Viana 1994).

Maize breeding for insect resistance can center on two aspects. One is the achievement of transgenic genotypes by genetic engineering. This technique allows the introduction of a single gene from the same or other species. Genetic improvement, on the other hand, can offer resistant genotypes selected from natural resistance sources. Focusing on the last alternative, the objective of this work was to evaluate the performance of a set of tropical maize populations under different environmental conditions tested under natural fall armyworm infestation and to determine the possibility of introducing some of these populations into breeding programs.

MATERIAL AND METHODS

The field trials were carried out using a set of maize genotypes of different agronomic characteristics and climatic origins (Table 1). Genotype 1 is a transgenic commercial temperate hybrid that incorporates the Bt technology and was therefore used as tolerant check in the experiments performed in Esperanza, Argentina. This genotype was eliminated from the set of materials in the trial carried out in Piracicaba, Brazil, since a specific legislation in this country regulates the utilization of transgenic genotypes in field research.

Genotype 2 is a tropical population with high susceptibility to fall armyworm that had been used as susceptible check. Genotype 3 and 4 are tropical populations selected for fall armyworm by Embrapa (Brazilian Agronomic Research Enterprise) and the Federal University of Pernambuco, Brazil, respectively. Genotype 5 is an experimental composite developed by crossing of selected tropical germplasm at the Faculty of Agronomy, Esperanza, Argentina.

Table 1. Set of genotypes used in the field trials

Genotype	Name	Type	Cycle	Origin
1	Pioneer P35R58	Hybrid	Temperate	Argentina
2	Piranão	Population	Tropical	Brazil
3	CMS14C	Population	Tropical	Brazil
4	São José	Composite	Tropical	Brazil
5	Experimental FCA	Composite	Tropical	Argentina

The trials were conducted at two sites (Table 2). Two experiments were carried out under temperate-subtropical climate in the experimental field of the National Littoral University in the city of Esperanza (lat 31° S and long 61° W), province of Santa Fe, Argentina. The other trial was carried out under tropical environmental conditions in the experimental field of the Department of Genetics of the University of São Paulo, in Piracicaba (lat 22° S and long 47° W), São Paulo, Brazil. Two sowing dates were used in Esperanza to determine the effect of a lagged sowing date on pest incidence. In Piracicaba, only one sowing date was used due to the natural presence of *S. frugiperda*. Fertilization and weed control were performed according to Giaveno and Ferrero (2003).

Table 2. List of environments used in field evaluations

Environment	City	Country	Climate	Sowing date
1	Esperanza	Argentina	Temperate-subtropical	Early
2	Esperanza	Argentina	Temperate-subtropical	Late
3	Piracicaba	Brazil	Tropical	Late

Populations were evaluated for fall armyworm resistance (FAR), plant (PH) and ear (EH) height, and grain yield (Y) in a set of experiments with three replications. In these experiments, plots with two rows, 5 m long and spaced 0.7 m apart with 50 plants each, were used.

The parameter FAR was measured using a modification of visual scoring. The modified scale includes only three tolerance

levels and the plants received values of 0 (without symptoms), 2.5 (intermediate - partial whorl damage), and 5 (susceptible - complete whorl damage). The damage done by fall armyworm was assessed at the phenological stage of six fully expanded leaves (V6) (Bleiholder et al. 1992). PH and EH were measured as the distance from the soil surface to the first branch of the tassel and the first ear, respectively. Six plants grown under competitive conditions were collected from each plot for Y determination. Ears were harvested by hand and the grain samples weighed and stove-dried at 65 °C until achieving constant weight and then corrected to 14% standard moisture to obtain Y.

Data analysis derived from a single environment was performed using a hierarchical model that included the sample effects within genotypes. Environments were compared by a statistical model that includes genotype x environment effects. Analysis of variance in both situations was performed using the ANOVA procedure of software SAS (SAS Institute Inc 1994).

RESULTS AND DISCUSSION

Single environment analysis

Experiment 1

For the trial carried out in environment 1 (Esperanza, early sowing date), genotype differences (P < 0.01) were observed for all traits with the exception of FAR (Table 3). For the other traits, the differences were explained by the performance of Genotype 1. This genotype is a temperate commercial hybrid and hence both PH, EH and Y were very different from the values of the other typically tropical populations. These observations appear to be similar to those reported under similar field conditions by Giaveno and Ferrero (2003).

Plant height (Table 3) showed cultivar differences varying from the lowest PH values of 1.47 m of Genotype 1 to the highest 2.13 m presented by Genotype 4. The PH values of Genotype 1 were similar to those reported under similar conditions by several other authors (Fossati 2000, 2001, Giaveno and Ferrero 2003) who observed PH values for temperate hybrids below 2.0 m. The PH values above 2.0 m in the set of tropical populations were consistent with those reported by Giaveno and Ferrero (2003).

Significant cultivar differences were observed for ear height - EH value (Table 3), varying from 0.56 m in Genotype 1 to 1.19 m presented by Genotype 4. The highest values presented by the tropical populations that showed a EH above 1 m could be considered an undesirable characteristic due to the negative association with lodging and harvest loss. As expected, PH and EH showed a positive association in all environments and thus the tropical populations presented the highest PH and EH values.

The excellent climatic conditions in this environment resulted in good grain yield (Y) values. Significant genotype differences (P < 0.01) were observed in this trial and, as expected, the highest Y values were observed for the commercial hybrid (Table 3). Under these conditions, Genotype 1 showed an average value of Y of 6.9 t ha^{-1}. The best Y value among the tropical populations was presented by Genotype 5 (6.09 t ha^{-1}) because this is a composite obtained in our breeding program by hybrid crossings selected under similar environmental conditions. The worst mean value of Y was observed for Genotype 3 that showed low adaptability to the regional climatic conditions. These performances appear to be analogous to those reported by Fontanetto et al. (1998) for tropical hybrids under similar environmental conditions. According to Giaveno and Ferrero (2003), tropical unselected genotypes can show low values of Y due to the failure in homeostatic capacity when sown under very different environmental conditions. In such cases, these populations are considered to be exotic germplasm and introgressive crosses could be carried out to introduce these populations into the breeding program (Hallauer and Miranda Filho 1988). In literature, several authors reported on the use of exotic germplasm in introgressive crossing (Miranda Filho 1992, Giaveno and Ferrero 2003).

Non-significant differences (P > 0.05) among genotypes were observed for FAR (Table 3). Contrary to the expected, the transgenic hybrid (Genotype 1) showed little differences when compared to the susceptible check (Genotype 2). This apparent contradictory performance could be explained by the reduced presence of the insect in the field.

Table 3. Means of the traits plant (PH) and ear (EH) height, grain yield (Y), and fall armyworm resistance (FAR) at Esperanza, Argentina, early sowing date

Genotype	PH**	EH**	Y**	FAR
	m	m	t ha^{-1}	value
Pioneer P35R58	0.56 C	1.47 C	6.91 A	0.00 A
Piranão	0.96 B	1.79 B	4.27 C	0.11 A
CMS14C	0.92 B	1.81 B	3.83 C	0.09 A
São José	1.19 A	2.13 A	4.86 BC	0.11 A
Experimental FCA	1.04 AB	1.90 B	6.09 AB	0.03 A
VC (%)	14.35	17.60	19.30	24.30

**P<0.01
Means with the same letter are not significantly different by Tukey´s test VC is the variation coefficient of the trial for each trait

Experiment 2

For the trial carried out in environment 2 (Esperanza, late sowing date) the delayed sowing date caused alterations in all traits. Cultivar differences (P < 0.01) were therefore observed for both PH and EH (Table 4). As emphasized for environment 1, the differences observed among genotypes were due to the contrasting performance of Genotype 1 when compared to the

tropical ones. In the broad sense, non-important alterations in PH and EH were observed between environments 1 and 2.

Significant genotype differences (P < 0.01) were observed for Y and the best Y values were observed for Genotype 5 (Table 4), in contrast to the reported for environment 1. Under these conditions, Y values varied from the best mean presented by Genotype 5 (5.28 t ha^{-1}) to the lowest of Genotype 3 (2.71 t ha^{-1}).

When the performance of the genotypes in this environment was compared to that observed in environment 1, we observed that the delayed sowing date was responsible for the poor use of the nutritional status. The negative effects on Y produced by the delayed sowing date were reported by Tollenaar et al. (1992) and could be explained by the reduced kernel number per ear (Cirilo and Andrade 1994) or number of ears per plant (Andrade et al. 1996). Under these conditions, the central area of Santa Fe, Argentina, presents an important reduction in radiation, air temperature, and soil water status, especially during the grain filling period. The climatic characteristics observed in this environment induced the presence of many pests such as fall armyworm (Willink et al. 1991, Sosa 2002). Therefore, cultivar differences (P < 0.01) were observed for FAR due to the excellent performance of the transgenic hybrid (Genotype 1, Table 4) that contrasted with the susceptible population (Genotype 2). While Genotype 1 showed values of 0 (without symptoms) in all samples, Genotype 2 showed values closed to 1. Opposite to the expected, Genotype 4 showed a mean value of 1.03, the worst FAR performance. This population has been reported as source of fall armyworm-resistance (Alvarez and Miranda Filho 2002). This loss of resistance could be explained by the presence of different strains of the insect in Esperanza in

Table 4. Means of the traits plant (PH) and ear (EH) height, grain yield (Y), and fall armyworm-resistance (FAR) in Esperanza, Argentina, late sowing date

Genotype	PH**	EH**	Y**	FAR**
	m	m	t ha^{-1}	value
1	0.56 C	1.42 C	4.32 BC	0.00 D
2	1.13 B	1.88 B	3.57 B	0.91 B
3	1.35 A	2.18 A	2.71 C	0.45 C
4	1.19 AB	2.13 A	4.05 B	1.03 A
5	1.04 B	1.90 AB	5.28 A	0.55 C
VC%	13.40	15.30	18.50	16.25

**P < 0.01
Means with the same letter are not significantly different by Tukey´s test VC is the variation coefficient of the trial for each trait

relation to the Brazilian region where this population had been selected.

Experiment 3

In the experiment carried out in Piracicaba, Brazil, the temperate transgenic hybrid (Genotype 1) was not included in

the set of tested materials in awareness of the law of biosafety that control the use of genetically manipulated germplasm.

Opposite to observations in the other environments, no significant differences (P > 0.05) among populations were observed for PH and EH (Table 5). In the other environments, the differences were explained based on the contrasting performance shown by the commercial hybrid and the tropical populations.

In this environment, Genotype 4 and 5 showed the highest means of PH (2.5 m) while Genotype 2 presented the lowest mean (1.80 m). A similar tendency was observed for EH (Table 5) where the Genotypes 3, 4, and 5 showed values of EH above 1 m. Grain yield (Y, Table 5) showed population differences (P < 0.01) varying from the highest Y of 4.6 t ha^{-1} of Genotype 5 to the lowest of 2.10 t ha^{-1} presented by Genotype 2, evidencing an increase in productivity of 119%.

Under the tropical environmental conditions found in Piracicaba, Brazil, characterized by a warm and rainy climate, the large fall armyworm populations were present at a high level during the maize growing season. According to Table 5, the tested

Table 5. Means of the traits plant (PH) and ear (EH) height, grain yield (Y), and fall armyworm-resistance (FAR) in Piracicaba, state of São Paulo, Brazil

Genotype	PH	EH	Y**	FAR**
	m	m	t ha^{-1}	value
1	-	-	-	-
2	1.80 A	0.80 A	2.10 C	2.98 A
3	2.30 A	1.05 A	3.70 B	1.12 B
4	2.50 A	1.20 A	4.40 A	1.27 B
5	2.50 A	1.10 A	4.60 A	1.68 B
VC%	19.70	17.50	15.70	22.10

Significance levels: NS (P > 0.05), * (P < 0.05) and ** (P < 0.01) Tukey´s test. Means with the same letter are not significantly different VC is the variation coefficient of the trial for each parameter

populations showed significant differences (P < 0.01) with FAR values varying from 1.12 (Genotype 3) to 2.98 (Genotype 2).

Joint analysis

The three trials were analyzed together with the objective to study the presence of genotype x environment interactions for the evaluated traits. The genotype performance showed a tendency of modification by environments in the broad sense. Y values, for example, decrease from environment 1 to 3, while FAR showed the opposite tendency.

According to Table 6, all evaluated traits showed significant differences (P < 0.01) among environments, genotypes, and for the interaction, confirming the previously established climatic and genetic differences. Significant differences among environments could be explained based on the climatic differences of the sites. While Esperanza, Argentina, has a temperate-sub tropical climate, Piracicaba, Brazil, has a typical

tropical environment. Genotype differences were induced by the contrasting performance of the susceptible check (Genotype 2) with the worst performance in all sites except for Y and FAR in environment 2, compared to the resistant ones. An interesting fact was observed for the genotype x environment interaction: in environment 2, the population previously considered resistant (Genotype 4) showed the worst performance for FAR, worse also than the susceptible check (Genotype 2).

The presence of an association between some traits was observed, which was positive (0.7) between PH and EH and negative (-0.5) between Y and FAR. These associations could be considered expected, but when data were analyzed for each genotype (Figure 1) we observed that the susceptible check (Genotype 2) showed a strong depression of Y as FAR increases, especially in the environment with a stronger presence of fall armyworm. Under this condition, a resistant population (Genotype 5) showed a moderate depression of Y.

Table 6. F values of joint ANOVA for plant (PH) and ear (EH) height, grain yield (Y), and fall armyworm-resistance (FAR)

Source of Variation	Traits			
	EH	PH	Y	FAR
Environments	5.55 **	49.15 **	27.30 **	300.7 **
Genotypes	7.56 **	20.18 **	19.80 **	35.9 **
Genotypes x Environments	12.29 **	16.42 **	9.18 **	21.6 **
VC (%)	14.08	8.82	20.62	23.91

*P < 0.05, and **P < 0.01
VC is the variation coefficient of the trial for each trait
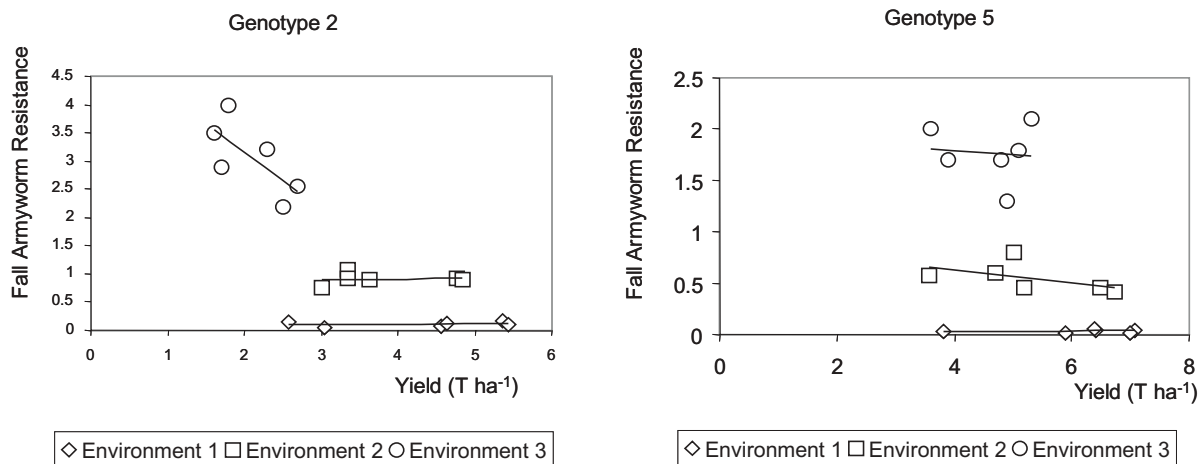
Figure 1. Negative association between fall armyworm-resistance and yield shown by the susceptible check (Genotype 2) and one of the tolerant populations (Genotype 5)

CONCLUSIONS

Our results showed that the tested tropical populations, especially Genotype 5 and 3, were productive and showed good performance regarding fall armyworm-resistance and adaptability to the environmental conditions of central Santa Fe, Argentina. These populations, especially the composite Experimental FCA, could be incorporated by introgressive crossings into the maize breeding program.

Melhoramento de milho para resistência à lagarta do cartucho (*Spodoptera frugiperda*) na Argentina: efeitos genéticos e ambientais

RESUMO - *Nas regiões tropicais as pragas podem afetar fortemente a produção do milho. O objetivo deste trabalho foi avaliar a resistência à lagarta do cartucho em um conjunto de populações ensaiadas sob diferentes condições ambientais e investigar a possibilidade de incorporar algumas delas nos programas de melhoramento. Os experimentos foram conduzidos em três ambientes sob infestação natural. As populações foram avaliadas para altura de plantas (PH) e espigas (EH), rendimento de grãos (Y) e resistência à lagarta do cartucho (FAR). Para Y e FAR foram observadas diferenças significativas entre populações e para a interação genótipos x ambientes. Para FAR, a interação pode ser explicada pelo comportamento instável entre ambientes apresentado por uma das populações tolerantes. Os resultados obtidos comprovam que pelo menos uma população poderia ser incorporada no programa de melhoramento.*

Palavras-chave: *Zea mays*, resistência, melhoramento, germoplasma exótico.

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