C H A P T E R 5

ADAPTABILITY AND STABILITY FOR RESISTANCE TO PHYSODERMA BROWN SPOT AND PHAEOSPHAERIA LEAF SPOT IN CORN INBRED LINES

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5.1 INTRODUCTION

Physoderma brown spot is caused by *Physoderma maydis*, occurs commonly in regions that have high temperatures and high precipitation. The first symptoms of the disease usually appear on the leaf limbs and nerves with chlorotic spots (LEÓN, 1984). According to Robertson et al. (2008), the pathogen is dormant in infected tissues or soil and produces innumerable zoospores in the presence of water. Leaf infection occurs at whorl when is present for an extended period. Occurs in a day cycle and requires a combination of light, free water and temperature between 23 and 30ºC. According to Fernandes and Balmer (1990), the brown spot is more severe in late plantings, carried out in low areas. There are a few reports in the literature regarding the quantification of this disease in corn worldwide.

Phaeosphaeria leaf spot whose etiologic agent is the fungus *Phaeosphaeria maydis* (PINTO; FERNANDES; OLIVEIRA, 1997) in association with the bacteria *Pantoeae ananas* (PACCOLA-MEIRELLES et al., 2001), occurs in the main corn producing regions in Brazil and worldwide. Under favorable conditions, it can cause premature leaf drought, reduction of the plant cycle, reduction in grain size and weight, leading to a decrease in grain yield of over 60% (FERNANDES, 2004). Leaves with 10 to 20% disease severity present a reduction in the net photosynthetic rate around 40% in susceptible cultivars evidencing the effect of the disease on photosynthesis (GODOY; AMORIM; BERGAMIN FILHO, 2001). The symptoms of this disease are related to the appearance of irregular shaped leaf stains, dark green color, that appear in the lower leaves passing to the higher leaves of the plant. Subsequently the lesions become necrotic of with straw coloration, being able to coalesce. The symptoms may present in different severities depending on the corn genotype (PACCOLA-MEIRELLES et al., 2002; REIS; CASA; BRESOLIN, 2004). Lopes et al. (2007), evaluating the genetic control of the resistance of the Phaeosphaeria leaf spot, concluded that the additive effects predominated in the control of resistance, and the characteristic has high heritability.

Loss of crops from plant diseases may result in hunger and starvation, especially in developing countries where access to disease-control methods is limited and annual losses of 30 to 50% are common for major crops (ALI; YAN, 2012). Some of these diseases were firstly considered secondary, but from 1990s onward their incidence and severity increased expressively (ARAUJO et al., 2013).

In this context, the objectives of this study were to identify resistant and susceptible inbred lines based on stability and adaptability for disease symptoms to phaeosphaeria leaf spot and physoderma brown spot, suggest resistant inbred lines aimed at producing synthetics, as well as identify the planting dates with the higher occurrence of these two diseases to use them for genetic resistance identification.

5.2 MATERIAL AND METHODS

Forty-one inbred lines were used, fourteen derived from the Isanão-VF1 population, nine from the Isanão-VD1 population, ten from the Flintisa population and eight from the Dentado population. The first two populations are brachytic, with flint and dent grains, respectively. The others have normal height, also with flint and dent grains. The inbred lines were obtained from the corn breeding program of São Paulo State University (UNESP) – Campus of Ilha Solteira – SP (Brazil), and have already been selected for general combining ability for yield.

The experiments were conducted at the Fazenda de Ensino e Pesquisa da UNESP – Campus of Ilha Solteira, located in Selvíria – Mato Grosso do Sul (MS) - Brazil (20° 20'S, 51° 23'W and the altitude of 335 m). The climate of the region, according to Köppen classification, is Aw, defined as tropical humid with a rainy season in summer and dry in winter. The average annual rainfall is 1330 mm, with the average annual air temperature of about 25°C and average humidity of 66% (CENTURION, 1982).

Forty-one experimental inbred lines were evaluated in a randomized block design with three replications in eleven planting dates (October and November 2013 and January until September 2014), with each planting being considered as an environment. Each plot was a single row 8 m in length with a spacing of 0.45 m between plots and an average of 0.4 m between plants. Planting was with normal tillage, irrigated by a center pivot, with twice the number of seeds needed and thinned at six fully developed leaves. Fertilization was done according to soil analysis with 300 kg ha^{-1} of 8-28-16 applied followed by 250 kg ha^{-1} of urea sidedress at the six-leaf stage. Temperature and relative humidity were collected from the weather station located near the experiment during all growing seasons (Figure 5).
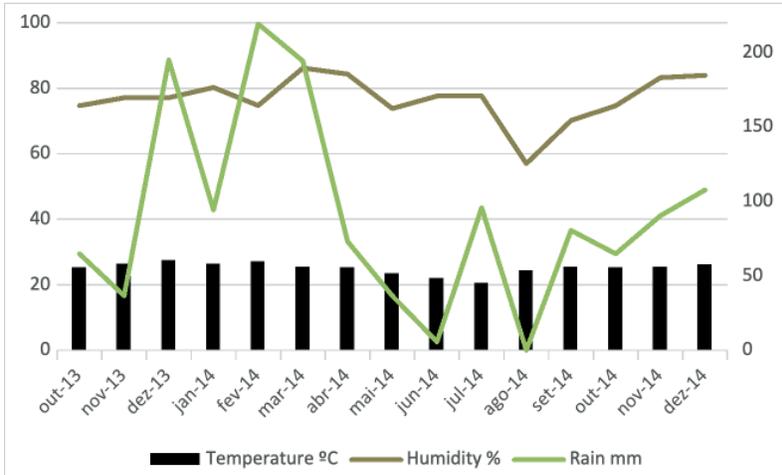
Figure 5 - Temperature, rain and humidity in Selvíria - MS,
Brazil from October 2013 until December 2014.

Source: Prepared by author

The inbred lines were evaluated for physoderma brown spot (PBS) and phaeosphaeria leaf spot (PLS). Evaluations were carried out at 30 days after silking, determining the severity of disease based on the percentage of symptoms of the plot, according to the diagrammatic scale suggested in the Agroceres Guide to Sanity (Agroceres 1996). The ratings were assigned values of 1, 2, 3, 4, 5, 6, 7, 8 and 9, corresponding to 0, 1, 10, 20, 30, 40, 60, 80 and> 80% of leaf symptoms, respectively for each plant plot, using the average plot for statistical analysis. The scores were further classified into the following disease reaction types: 1 – highly resistant; 2-3 – resistant; 4 – moderately resistant; 5 – moderately resistant/moderately susceptible; 6 – moderately susceptible; 7-8 – susceptible and 9- highly susceptible.

The original scores were transformed by $\sqrt{x+0.5}$, and a joint analysis was performed, considering each month as a season of planting and inbred lines with fixed effects and environments as random effects. The Hartley test, which is based on the ratio between the largest and smallest error mean square, was employed, considering the ratio higher than seven as an indication that the error variances were not homogeneous (PIMENTEL GOMES, 2000). To assure the homogeneity of residual variance, the degrees of freedom from residue and inbred lines x environment interaction were adjusted as recommended by Cochran (1954).

For adaptability and stability analysis, the following model, based on regression (EBERHART; RUSSELL, 1966) was used:

$$Y_{ij} = \beta_{oi} + \beta_{1i} I_j + \delta_{ij} + \varepsilon_{ij} \tag{4}$$

Where:

β_{oi} : overall average of genotype *i*;

β_{1i} : linear response of genotype *i* for environmental variation;

I_j : environmental index (j = 1, 2, …, a), being $I_j = \dfrac{Y_{.j}}{g} - \dfrac{Y_{..}}{ga}$;

δ_{ij} : deviation from regression

ε_{ij} : experimental error.

Data analysis was performed using the Genes Software, version 2015.5.0 (CRUZ, 2013).

5.3 RESULTS AND DISCUSSION

In joint analysis of variance for PBS and PLS, the F test were significant (P<0.01) for inbred lines (L), environments (E) and for inbred lines x environments (L x E) (Table 8). The significant effect of inbred lines indicates the presence of variability for the selection, the effect of environments indicates variability between the months of planting, an important fact to make the process of indicating inbred lines for developing resistant hybrids more efficiently.

Since the interaction inbred lines x environments (L x E) was significant, the analysis of adaptability and stability proposed by Eberhart and Russell (1966) was performed. The L x E interaction is fundamental for the breeding programs, since it is possible to reduce this effect from genotypes with wide adaptability or to recommend genotypes specific to certain environments (ENGELSING et al., 2012). When genotypes are compared over a series of environments, the relative rankings usually differ. This causes difficulty in demonstrating the significant superiority of any genotype. This interaction is usually present whether the genotype are pure lines, single-cross or double-cross hybrids, top crosses or any material with which the breeder may be working (EBERHART; RUSSELL, 1966). Brito et al. (2011), also found significance in the interaction L x E when evaluating 12 commercial hybrids, in three locations, for resistance to gray leaf spot and phaeosphaeria leaf spot.

The environmental indexes (I_j) and the averages non-transformed for each month of planting are shown in Table 9. The data of averages and environmental indexes showed that for PBS, in the planting date of July and August 2014, there was a higher pressure of the disease, expressing an average of 2.97 and 4.09. Despite being highest average found for PBS, inbred lines with these notes are considered resistant and moderately resistant, with 10 and 20% of the leaf area affected. For PLS the highest disease pressure occurred in the planting date of April and June, with averages of 1.88 and 2.24 respectively, notes that classify inbred lines as resistant.

Table 11 - Summary of the joint variance analysis for physoderma brown spot (PBS) and phaeosphaeria leaf spot score (PLS), for 41 corn inbred lines in 11 environments. Selvíria – MS, Brazil, 2014.

Source of variation	DF	PBS	DF	PLS
Inbred lines (L)	40	0.184**	40	0.205**
Environments (E)	10	11.365**	10	2.801**
Lx E	275	0.093**	400	0.070**
Error	585	0.054	880	0.033
Average	-	2.2	-	1.5
CV%	-	14.63	-	12.72

Note. ** - Significant by the F test ($p \leq 0.01$).
Source: Prepared by author.

Table 12 - Environmental indexes (I_j) and environmental averages of 41 corn inbred lines in 11 environments for physoderma brown spot (PBS) and phaeosphaeria leaf spot score (PLS). Selvíria – MS, Brazil, 2014.

Environment	PBS		PLS	
	Average	I_j	Average	I_j
Oct-13	2.40	-0.3538	1.36	0.2646
Nov-13	2.21	0.0342	1.07	-0.175
Jan-14	1.23	-0.2835	1.07	-0.1739
Feb-14	1.04	-0.3485	1.24	-0.1237
Mar-14	1.04	-0.3471	1.22	-0.1289
Apr-14	2.96	0.261	1.88	0.1029
May-14	2.01	-0.0257	1.82	0.0743
Jun-14	2.20	0.049	2.24	0.2182
Jul-14	2.97	0.2521	1.56	-0.0166
Aug-14	4.09	0.5393	1.54	-0.0136
Sep-14	2.88	0.2231	1.52	-0.0285

Source: Prepared by author.

For the development of PBS, the optimal temperature must be between 23 and 30ºC, while for PLS the nighttime temperature must be between 14 and 20ºC, being that for both diseases, the humidity above 60% and free water on the leaf surface is required. In this study the presence of favorable environmental conditions for the development of PBS and PLS was observed, being the months of April, June, July and August the most suitable for planting inbred lines to be evaluated for both diseases.

For the diseases analysis, the interesting thing is to find genotypes with a low average (β_0), in this case, around 1 and adaptability parameters (β_1) less than 1, because in these cases the genotypes did not increase the symptoms of the disease with the improvement of the environment. In this context, for PBS the inbred lines IVD1-9, IVD1-10, 7D, 10D and 2F can be indicated for planting in all environments (Table 10). For PLS the inbred lines 10D, 2F, 4F and 9F showed β_1 <1, indicating that these inbred lines did not increase the symptoms of the disease according to the improvement of the environment. However, these inbred lines were not stable, since the regression deviation were significant, indicating that the behavior is unpredictable, thus not being suitable for any environment. Although the inbred lines IVD1-9 and IVD1-10 presented β_1 = 1, for PLS, it can be indicated for planting in all environments.

The inbred lines IVF1-3, IVF1-6-2 and IVD1-2 may be indicated for environments with less favorable for PLS symptoms, since the disease decreases more rapidly in these environments. For PBS the inbred lines IVF1-6-1, IVF1-6-2, IVF1-6-3, IVD1-2 and 4F, may be indicated for environments with low disease pressure.

The adaptability and stability parameters is an important tool in the process of the breeder to indicate genotypes more appropriate to the studied environments. In the selection of inbred lines for the reaction to diseases in the corn crop, few or any study was developed in this context. In Figures 6 and 7 are selected inbred lines for the resistance to PBS and PLS respectively.

Reports in the literature suggest that for the reaction of corn to PLS and PBS are quantitatively inherited characteristics with the predominance of additive effects in the control of disease resistance, being more important than the effects of dominance and the epistatic effects (CARSON; STUBER; SENIOR, 2005; MOLL; THOMPSON; HARVEY, 1963; MOREIRA et al., 2009). In such cases, the inheritance of resistance and the type of response of the environment depends on the concentration of alleles for disease resistance in each genotype, the sensitivity of the encoded product by those alleles to the environmental changes and the sensitivity of the factors involved in the expression of these alleles. The results of this study demonstrated that the inbred lines presented high concentration of favorable alleles for the resistance of PBS and PLS, being favorable for the selection of inbred lines for making synthetics and hybrids. According to Brito et al. (2012), the most recommended breeding strategy would be to obtain hybrids from resistant inbred lines, taking advantage of the additive effects of the large-effect loci of the parents and the dominance that will be expressed in divergent loci of smaller effect.

Table 13 - Adaptability and stability parameters estimated, using Eberhart and Russell (1966) method, for physoderma brown spot (PBS) and phaeosphaeria leaf spot (PLS) score for 41 corn inbred lines, in 11 environments. Selvíria – MS, Brazil, 2014.

Inbred lines	Physoderma brown spot				Phaeosphaeria leaf spot			
	β_0	β_1	$\square^2_{di}$	R^2 (%)	β_0	β_1	$\square^2_{di}$	R^2 (%)
IVF1-2-1	2.6	1.15	-0.008	93.21	1.3	1.03	-0.0049	81.78
IVF1-3	2.0	0.97	-0.009	91.54	1.3	1.39[+++]	0.0010	80.53
IVF1-4	2.3	1.02	0.0008	84.96	1.7	1.35	0.0147*	64.21
IVF1-5	2.2	1.02	-0.005	89.16	1.6	1.17	0.0053	67.99
IVF1-6-1	2.9	1.49[+]	-0.0108	96.96	1.2	1.28	0.0085	68.17
IVF1-6-2	2.6	1.31[++]	-0.0009	91.23	1.4	1.48[++]	0.0008	82.54
IVF1-6-3	2.8	1.44[+]	0.0153	86.48	1.3	1.32	-0.0025	84.06
IVF1-7	2.0	0.78	-0.0019	79.73	1.3	0.95	0.0005	66.64
IVF1-8	2.1	1.12	0.0222*	76.21	1.2	0.66	0.0069	38.48
IVF1-9	2.3	1.02	-0.0059	89.76	1.2	0.86	-0.0050	75.58
IVF1-10	2.5	0.92	0.0051	79.17	1.5	1.02	0.0003	69.91
IVF1-11	2.0	0.85	-0.0094	89.72	1.5	1.05	0.0209**	46.83
IVF1-12	2.1	0.91	-0.0065	88.14	1.8	1.49[++]	0.0169**	66.94
IVF1-12-1	2.3	0.90	0.0241*	66.22	1.5	0.75	0.0268**	27.39
IVD1-2	2.7	1.23[+++]	-0.0003	89.81	1.5	1.70[+]	0.0026	84.42
IVD1-3	2.2	0.89	-0.0081	89.27	1.9	1.24	0.0070	68.63
IVD1-5	2.2	0.97	-0.0104	92.71	1.2	1.20	-0.0030	82.13
IVD1-8	2.4	1.05	-0.007	91.08	1.5	1.18	0.0051	68.88
IVD1-9	1.8	0.72[++]	-0.0079	84.06	1.5	0.81	0.0041	52.16
IVD1-10	1.8	0.72[++]	-0.0134	92.25	1.3	0.97	-0.0034	75.81
IVD1-11	2.5	1.12	0.0179*	78.17	1.4	1.03	-0.0006	72.22
IVD1-2-1	2.1	0.88	-0.0081	89.04	1.4	0.83	0.0078	48.09
IVD1-12	2.4	1.12	-0.0069	92.08	1.6	1.15	0.0355**	41.83
1D	2.1	0.92	-0.0117	93.25	1.7	0.86	0.0285**	32.00
2D	2.1	0.90	0.0135	72.39	1.9	0.65	0.0034	42.97
3D	1.9	0.80	-0.0053	83.98	1.6	1.02	0.0114*	54.37
6D	2.1	1.11	0.0167*	78.57	1.1	0.67	0.0203**	26.55
7D	2.9	0.76[+++]	0.0147	64.76	2.0	0.87	0.0319**	30.80
8D	2.4	0.88	0.0505**	53.58	1.2	0.96	0.0250**	39.09
9D	2.2	1.04	0.0106	79.42	1.3	0.87	-0.0035	71.89
10D	1.8	0.73[+++]	-0.004	79.78	1.5	0.63[+++]	0.0157**	27.57
1F	2.4	1.08	-0.0072	91.74	1.4	1.25	0.0011	76.72
2F	1.7	0.68[++]	-0.0081	83.00	1.3	0.21[+]	0.0360**	2.35
3F	2.8	1.18	-0.0043	91.31	1.5	1.13	0.0021	71.36

4F	2.7	1.35[++]	0.0111	86.54	1.9	0.37[+]	0.0510**	5.24
5F	2.1	0.94	-0.0078	89.91	1.8	0.97	0.0063	58.14
6F	2.1	0.93	0.0143	73.43	1.4	0.98	0.0049	60.43
7F	2.1	0.96	-0.0105	92.77	1.4	1.06	-0.0039	80.26
8F	2.4	1.13	-0.0024	89.38	1.3	1.05	-0.0046	81.60
9F	2.1	0.90	0.0202*	68.57	2.0	0.60[+++]	0.0207**	22.51
10F	2.5	1.11	0.0011	86.83	2.5	0.92	0.0959**	16.67
Average	**2.2**	-	-	-	**1.5**	-	-	-

(β_1) [+], [++], [+++] Differs from one, by the t test, at $p \leq 0.01$, $p \leq 0.05$ and $p \leq 0.10$, respectively.

(S^2_{di}) *, ** Differs from zero, by the F test, at $p \leq 0.05$ and $p \leq 0.01$, respectively.

(β_1): Regression Coefficient; S^2_{di}: Variance deviation regression; R^2 (%): Determination Coefficient.
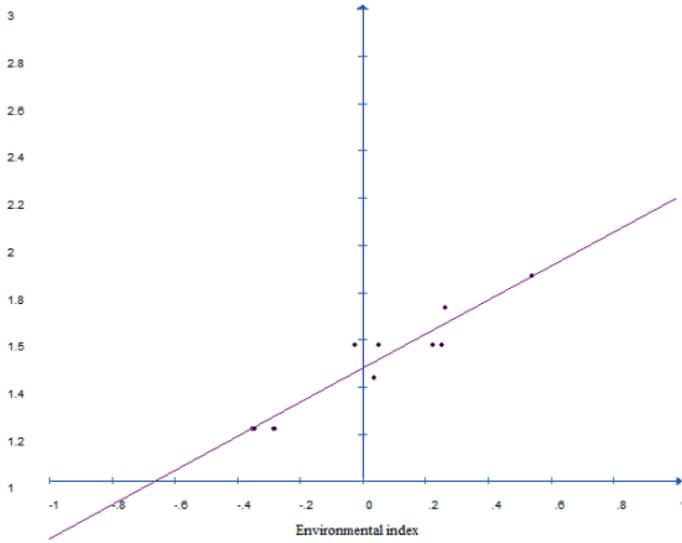
Source: Prepared by author.

Figure 6 – Regression of severity for physoderma brown spot (PBS) symptoms as a function of environmental indices for the five corn inbred lines considered more resistant, evaluate in 11 environments (October and November 2013 and January until September 2014). Selvíria - MS, Brazil, 2014.

IVD1-9

Eberhart & Russell / Finlay & Wilkinson   1.491 + 0.716Ij   ($R^2 = 84.056$)

PBS

Environmental index
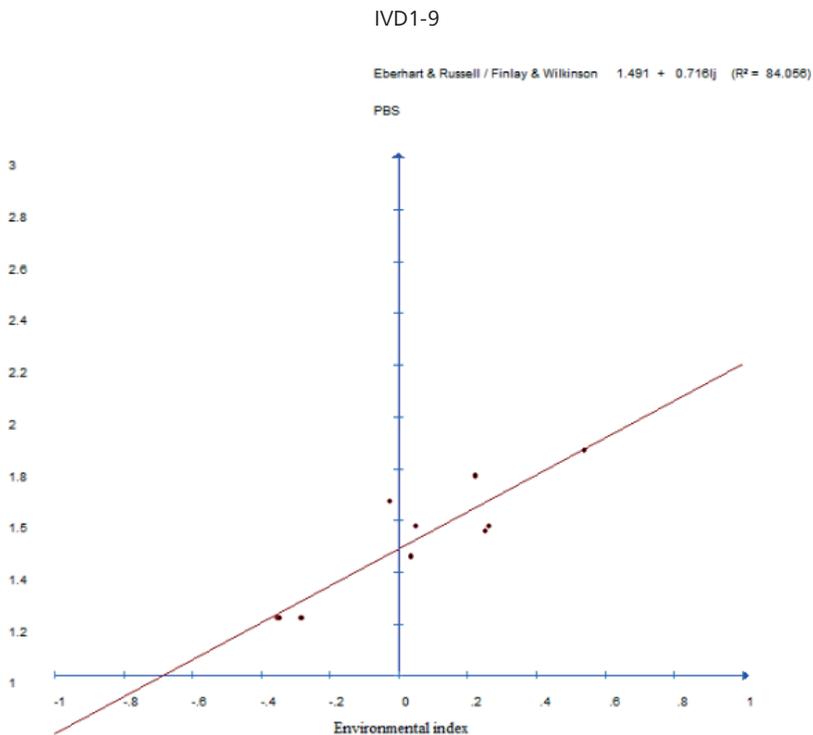
IVD1-10

Eberhart & Russell / Finlay & Wilkinson   1.479 + 0.722Ij   (R² = 92.248)

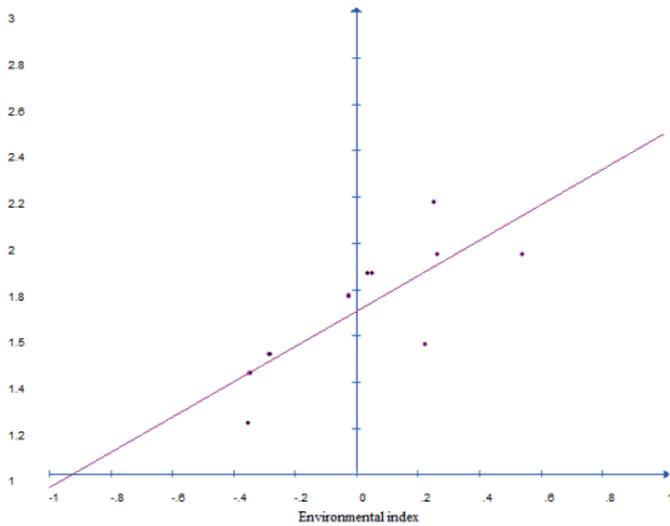PBS

7D

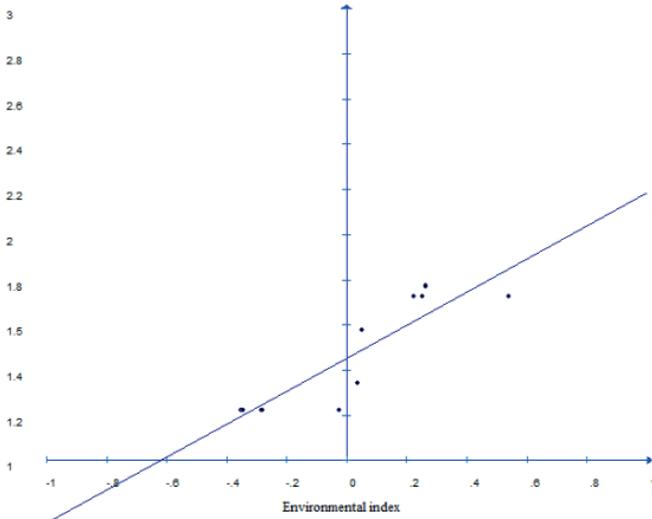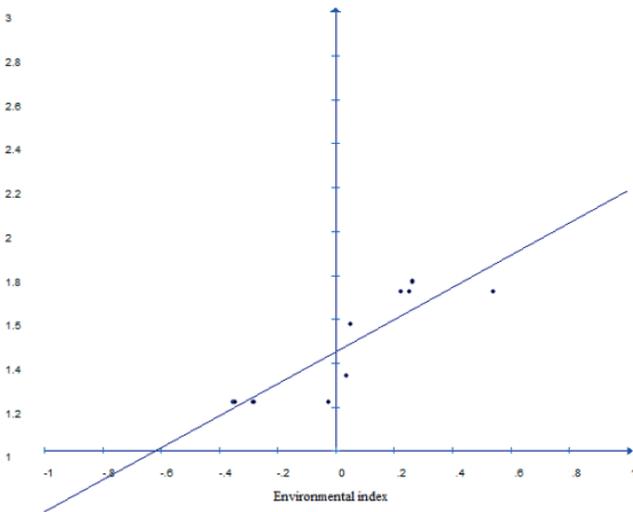Eberhart & Russell / Finlay & Wilkinson   1.709 + 0.764Ij   (R² = 64.763)

PBS

10 D

Eberhart & Russell / Finlay & Wilkinson   1.455 + 0.731Ij   (R² = 79.783)

PBS

Environmental index

2F

Eberhart & Russell / Finlay & Wilkinson   1.455 + 0.731Ij   (R² = 79.783)
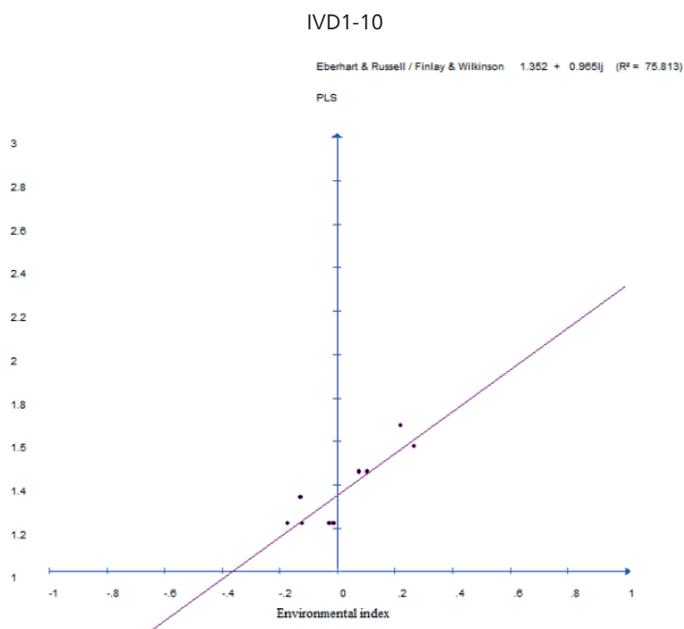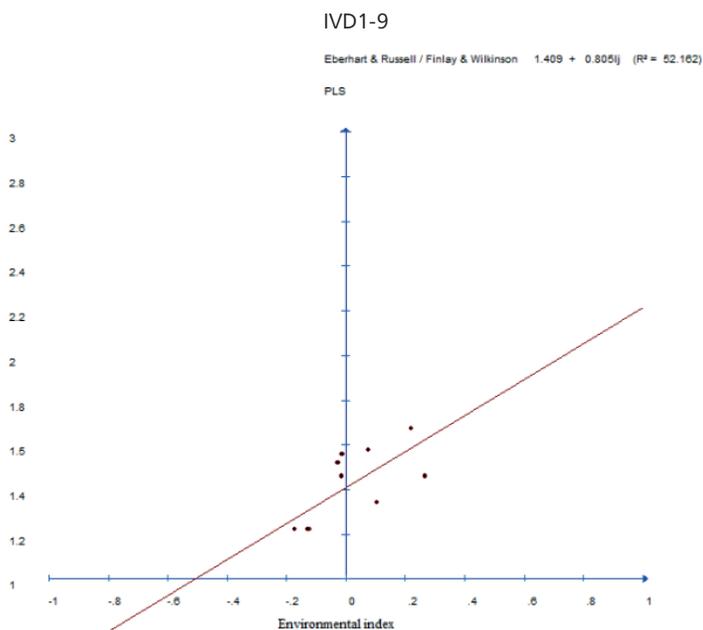
PBS

Environmental index

Figure 7 - Regression of severity for phaeosphaeria leaf spot (PLS) symptoms as a function of environmental indices for the two corn inbred lines considered more resistant, evaluate in 11 environments (October and November 2013 and January until September 2014). Selvíria - MS, Brazil, 2014.

IVD1-9

IVD1-10

5.4 CONCLUSION

The planting dates most suitable for evaluation of genotypes for genetic resistance to physoderma brown spot and phaeosphaeria leaf spot were April, June, July and August, once that showed the best information for selection. The inbred lines IVD1-9, IVD1-10, 7D, 10D and 2F may be used to form a synthetic resistant to physoderma brown spot and phaeosphaeria leaf spot.

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