CHAPTER 4

ADAPTABILITY AND STABILITY OF CORN INBRED LINES FOR RESISTANCE TO GRAY LEAF SPOT AND NORTHERN LEAF BLIGHT

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4.1 INTRODUCTION

Gray leaf spot (*Cercospora zeae-maydis* Tehon & E.Y. Daniels) and northern leaf blight (*Exserohilum turcicum* (Pass.) Leonard & Suggs) are among the foliar diseases that affect the corn crop in Brazil and worldwide. Susceptible genotypes to these diseases are responsible for causing severe losses in grain yield since they result directly in decreased photosynthetic area due to the destruction of the green tissues. A 50% reduction the catchment of incident radiation caused by the decrease in green tissue 15 days before and after female flowering may represent a reduction of 40% to 50% of grain yield (FISCHER; PALMER, 1984).

Gray leaf spot was first described in the corn crop in Illinois, United States, in 1925. In Brazil, it was described by Chupp (1953), but the disease becomes common in 2000, when epidemy were reported in production fields in the central region of the country, due to the increase of inoculum promoted by the cultural tillage, irrigation pivot and planting the second season (BRITO et al., 2007). The symptoms of gray leaf spot appear first on lower leaves, about two or three weeks before tasseling, leaf lesions are long, with a rectangular shape, and elliptical. Leaf lesions are brown and with high humidity conditions (above 90%), daytime temperatures ranging from moderate to high (22º to 32º C) and cold nights with dew, occurs dense sporulation, rendering the leaves in gray, characteristic of this disease (CASELA; FERREIRA, 2003; ROBERTSON et al., 2008).

Brito et al. (2007) evaluating 12 commercial corn hybrids for the incidence of gray leaf spot, showed that the level of damage caused by the pathogen change between planting dates and hybrids. The reduction in grain yield is mainly related to the late planting date and the use of resistant hybrids dispenses the chemical control. Silva et al. (2012) evaluating two transgenic corn hybrids in two populations (78.000 and 100.000 plants per hectare) concluded that the lower density of plants favored the increase in the severity of disease and contributing to decrease in grain yield.

Northern leaf blight is distributed worldwide and can cause yield losses of more than 60% in susceptible germplasm (RAYMUNDO; HOOKER, 1981). The disease symptoms appear about a week after beginning of infection, characterized by presenting elliptical straw lesions measuring 2.5 to 15 cm in length with well defined edges, which become dark because of fungus fructification (WORDELL FILHO; CASA, 2012). The development of northern leaf blight is favored by a temperature between 18º and 27ºC with an optimum temperature of 20ºC and presence of dew on the leaf surface (SABATO et al., 2013). In Brazil, the disease occurs strongly in the second season due to the most damage when it infects the plants in the female flowering period. According to Fernandes and Oliveira (2000), the development of *E. turcicum* is negatively correlated with the photoperiod, light intensity and the concentration of sugar in corn. These conditions are most often seen in the second season crops, which could explain the higher severity of this pathogen at the time.

Many authors describe the mechanisms of inheritance associated with northern leaf blight. The disease is controlled mainly using resistant cultivars by quantitative resistance (non-race-specific) and qualitative (race-specific). Qualitative and quantitative sources of resistance have been described (WELZ; GEIGER, 2000). The quantitative resistance conferring partial resistance in the northern leaf blight causes reduction in the development of the disease and the percentage of affected leaf area, which may affect of the epidemics, including the incubation period, latent period, intensity of sporulation, the size, number and growth rate of lesions (PARLEVLIET, 2002; CARSON; GOODMAN, 2006, HURNI et al., 2015).

The interaction between the host and pathogen is distinct in different environments; it is often possible to observe a significant interaction between genotype and environment, which may cause variation in disease severity due to the instability of resistance loci in the interaction with the environment or differences in pathogen populations between environments (CARSON et al., 2002). 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 gray leaf spot and northern leaf blight, 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.

4.2 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 4).

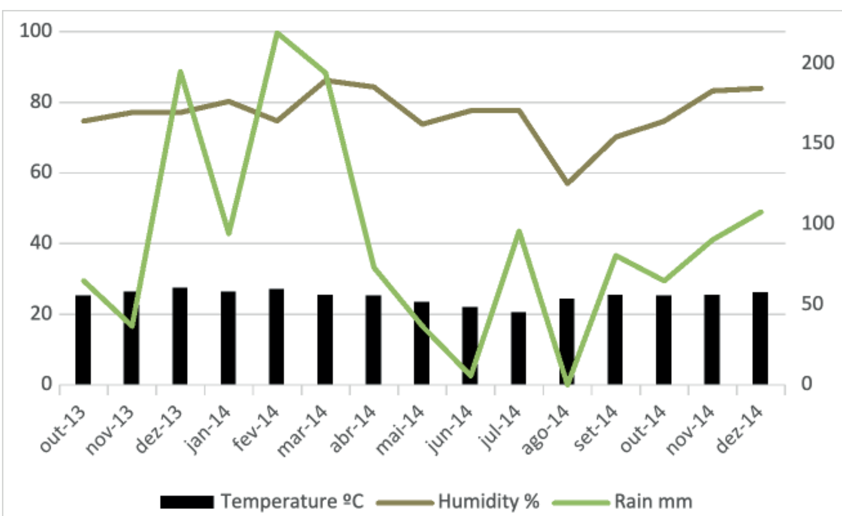

Figure 4 - Temperature, rain and humidity in Selvíria - MS, Brazil from October 2013 until December 2014.

Source: Prepared by author

The inbred lines were evaluated for gray leaf spot (GLS) and northern leaf blight (NLB). 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:

$$Y_{ij} = \beta_{oi} + \beta_{1i} I_j + \delta_{ij} + \varepsilon_{ij} \quad (3)$$

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 $I_j = \frac{Y_{.j}}{g} - \frac{Y}{ga}$;

δ_{ij} : deviation from regression

ε_{ij} : experimental error.

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

4.3 Results and discussion

The F test of joint variance analysis for GLS was significant for inbred lines (L), environments (E) and LxE interactions (P<0.01), while for NLB, the F test, was significant (P<0.01) for environments and LxE interactions (Table 5). As the LxE was significant for each disease studied, were performed the adaptability and stability analysis proposed by Eberhart and Russell (1966).

Table 7 - Summary of the joint variance analysis for Gray leaf spot score (GLS) and Northern leaf blight score (NLB), for 41 corn inbred lines in 11 environments. Selvíria – MS, Brazil, 2014.

Source of variation	DF	GLS	DF	NLB
Inbred lines (L)	40	0.267**	40	0.0723
Environments (E)	10	9.900**	10	2.240**
Lx E	333	0.134**	254	0.084**
Error	720	0.568	538	0.059
Average		2.2		1.7
CV%		14.78		16.67

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

Source: Prepared by author

The severity values for GLS and NLB were different in different months of planting. Averages and environmental indexes (I_j) for each month (Table 6) showed that in the 2013 planting dates and January, February, March and May 2014, there was less GLS pressure and, for NLB the lowest averages were observed in October 2013, January, February and March 2014. In the planting dates of August and September 2014, the highest average scores of inbred lines to the severity of both diseases were found.

According to the averages (β_0), adaptability parameters (β_1) and phenotypic stability () (Table 7) for severity of GLS, 7F, 9F and IVF1-7 presented β_1<1. The inbred lines IVF1-6-1, IVF1-6-2, IVF1-12, IVD1-2-1, IVD1-5, 5F and 6F presented β_1>1, and significantly increased the symptoms of the disease, with increasing of I_j. For the other inbred lines, the regression coefficient (β_1) was equal to 1.

Inbred lines IVD1-8 and 1F responded positively to significantly increase the I_j for NLB. The inbred line IVF1-7 with low average (1.5) for NLB and β_1<1 approached the ideal for a resistant genotype. For the other inbred lines, the average ranged between 0.57 and 1.31 and the regression coefficients were equal to 1.

Table 8 - Environmental indexes (I_j) and environmental averages of 41 corn inbred lines in 11 environments for gray leaf spot (GLS) and northern leaf blight (NLB). Selvíria – MS, Brazil, 2014.

Environment	GLS		NLB	
	Average	I_j	Average	I_j
Oct-13	1.05	-0.3725	1.6	-0.0458
Nov-13	1.25	-0.3011	1.9	0.0692
Jan-14	1.34	-0.2717	1.3	-0.1356
Feb-14	1.44	-0.25	1.1	-0.2073
Mar-14	1.76	-0.1424	1.0	-0.2233
Apr-14	2.66	0.1559	1.8	0.0576
May-14	2.01	-0.0534	1.7	0.0086
Jun-14	3.14	0.262	2.0	0.1067
Jul-14	3.09	0.2593	2.0	0.0821
Aug-14	3.58	0.3791	2.1	0.1211
Sep-14	3.37	0.3348	2.2	0.1668

Source: Prepared by author.

According to the phenotypic stability parameter for severity of GLS, the inbred lines IVF1-2-1, IVF1-3, IVF1-7, IVF1-8, IVF1-11, IVF1-12, IVD1-10, IVD1-11, 6D, 8D, 9D, 10D, 4F, 5F, 6F and 8F were considered unstable ($\square^2_{di}$ non-zero). For the severity of NLB, all variances of the deviations were less than 0.1 and all lines considered stable.

Gray leaf spot (GLS) is more severe and damaging in periods with high humidity, caused by the accumulation of water on the leaf surface and temperatures between 22º and 30ºC (BECKMAN; PAYNE, 1982). The northern leaf blight (NLB) requires temperatures between 18º and 27ºC, with the optimum at 20º C and the presence of dew on the leaf surface (SABATO et al., 2013). Throughout the period that included the first planting date to the final evaluation, the temperature conditions were favorable for the development of GLS and NLB. In the period of July to October 2014, the humidity was close to 70%, which is considered less than ideal for the development of the two diseases. Nevertheless, the severity of the GLS and NLB was largest then because the experiments were conducted under center pivot irrigation, which ensured the presence of free water in the leaves, providing the right conditions for the development of diseases. Therefore, the low averages are due to the good level of resistance of inbred lines with selection using same the standard method. Even so, small environment variations allowed verification of the best times to evaluate the resistance to both diseases are the planting dates between June and September, where I_j were positive and high (Table 6).

Following the methodology proposed by Eberhart and Russell (1966), genotypes with ideal resistance would mean average scale of symptoms around 1, regression coefficient lower than 1, and no significant regression deviations. Ideally, the disease would not consistently increase with the improvement of the environment for the disease (positive I_j), which occurred with inbred lines IVF1-7 and 9F for GLS and IVF1-7 for NLB. However, IVF1-7 it was unstable for GLS, as evidenced by the significance of the variance of the deviations and low coefficient of determination. Inbred lines IVF1-6-1 and IVD1-5 (B_1>1) had strongly increased GLS symptoms with increasing I_j and may be considered the most susceptible group, being interesting to use only in low-pressure conditions disease (negative I_j). For NLB this occurred with IVD1-8 and 1F inbred lines.

In a quantitative approach, the inheritance of resistance and the type of response to I_j depends on the concentration of alleles for disease resistance in each genotype, sensitivity of encoded product of these alleles to environmental changes and the sensitivity of regulatory factors involved in the expression of these alleles. A higher concentration of favorable alleles initially causes the genotype to resist increased disease pressure (positive I_j) and not consistently increasing the scale of symptoms even in the best condition for the disease. This effect will be maximized if the regulation of these alleles and the action mechanisms of their products are also uniformly positive, even with the change of environment. If a genotype has a good concentration of alleles for resistance, but are disabled, in control level or encoded products, their behavior will be unstable with high regression coefficient, as possibly happened with the inbred lines IVF1-6-1 and IVD1-5 for GLS and IVD1-8 and 1F for NLB. For all those unstable GLS inbred lines, one or more such effects may have also occurred.

Another aspect that can give a low response to I_j and good stability is the presence of a locus with major effect, little influenced by the environment. In another case, a locus of small effect, with alleles to increase or decrease the resistance would act as modifiers. Ramalho et al. (2012) say modifiers are smaller effect genes, able to alter the expression of other genes with greater effect. This hypothesis of the occurrence of major effect genes is true because various studies with inbred lines from multiple sources indicate that resistance is qualitatively inherited (locus with major effect) and quantitatively inherited, whereas the additive effect of genes are more important than non-additive (WARD et al., 1999; WELZ; GEIGER, 2000; JULIATTI et al., 2009; VIVEK et al., 2010; BRITO et al., 2012; VIEIRA et al., 2012; ABERA et al., 2016). Modifiers with major effect have not yet been detected in this group of inbred lines.

The results of this study showed that the inbred lines exhibit a high concentration of favorable alleles for resistance to GLS and NLB. Thus, the hybrids between these inbred lines will have good resistance, considering the additive action of these genes. If the intention is to produce a synthetic, the conditions for an inbred line to be included is stability, the lowest coefficient (or equal to one) and the desired type of grain. If the regression coefficient is equal to one, the average should be between the smaller groups.

Table 9 - Adaptability and stability parameters estimated using Eberhart and Russell (1966) method, for gray leaf spot and northern leaf spot for 41 corn inbred lines, in 11 environments. Selvíria – MS, Brazil, 2014.

Inbred lines	Gray leaf spot				Northern leaf blight			
	β_0	β_1	σ^2_{di}	R^2 (%)	β_0	β_1	σ^2_{di}	R^2 (%)
IVF1-2-1	2.0	0.83	0.0171*	63.13	1.7	1.15	0.0019	54.98
IVF1-3	2.4	1.12	0.0331**	68.17	1.6	0.99	-0.0093	65.23
IVF1-4	2.2	0.95	0.0004	80.60	1.6	0.57	-0.0061	32.28
IVF1-5	2.1	0.99	0.0002	82.18	1.8	0.76	-0.0052	44.59
IVF1-6-1	2.5	1.41^{+}	0.0030	89.05	1.7	0.87	-0.0101	61.14
IVF1-6-2	2.7	1.28^{+++}	0.0005	88.34	1.7	0.93	-0.0033	51.51
IVF1-6-3	2.5	1.24	0.0062	84.62	1.7	1.02	-0.0004	52.23
IVF1-7	3.2	0.61^{++}	0.0754**	25.87	1.5	0.46^{+++}	0.0046	15.01
IVF1-8	1.7	0.82	0.0253*	57.80	1.7	0.66	0.0154	19.99
IVF1-9	1.8	0.90	-0.0103	89.33	1.6	1.11	-0.0036	60.83
IVF1-10	1.9	0.90	-0.0069	85.75	2.1	1.02	-0.0088	65.69
IVF1-11	2.4	1.25	0.0450**	68.49	1.7	1.12	-0.0087	69.69
IVF1-12	2.9	1.25^{+++}	0.0909**	56.04	1.6	0.98	-0.0116	70.27
IVF1-12-1	1.9	0.93	0.0042	76.96	1.7	1.20	-0.0057	67.21
IVD1-2	2.1	0.79	-0.0004	75.06	2.0	0.87	-0.0007	44.20
IVD1-3	2.4	0.92	0.0032	77.31	1.8	1.26	0.0119	50.23
IVD1-5	2.4	1.30^{++}	0.0031	87.34	2.0	1.43	0.0119	56.63
IVD1-8	2.1	1.16	-0.0006	86.68	1.8	1.53^{+++}	-0.0084	80.68
IVD1-9	2.4	0.84	0.0095	68.77	1.8	0.98	-0.0019	52.01
IVD1-10	2.5	1.04	0.0523**	57.56	2.0	1.31	0.0119	52.30
IVD1-11	2.2	1.01	0.0250*	67.61	1.8	1.21	-0.0007	60.58
IVD1-2-1	2.2	1.07^{+++}	0.0015	83.43	1.9	1.23	0.0153	46.39
IVD1-12	2.1	1.10	0.0010	84.51	1.8	0.90	-0.0097	61.61
1D	2.2	1.19	-0.0041	89.50	1.7	0.87	-0.0093	59.42
2D	2.3	1.02	-0.0052	87.16	1.7	0.96	-0.0124	71.51
3D	1.9	1.12	0.0017	84.46	1.5	0.86	0.0107	32.88
6D	1.7	0.85	0.0256*	59.12	1.6	0.89	0.0176	30.12
7D	2.1	0.89	-0.0128	92.14	1.7	0.85	-0.0020	44.96
8D	2.6	0.83	0.0587**	44.45	1.6	0.65	-0.0002	30.38
9D	2.7	0.88	0.0528**	48.94	1.6	1.19	-0.0118	78.06
10D	2.0	0.95	0.0578**	51.28	1.3	0.84	-0.0124	66.04
1F	2.1	0.86	0.0123	68.19	1.9	1.63^{+++}	0.0105	64.08

2F	2.0	0.98	0.0125	73.32	1.6	0.82	-0.0097	57.47
3F	2.1	1.12	0.0042	82.83	1.7	0.73	-0.0078	47.06
4F	2.4	0.76	0.0292**	51.64	1.6	1.19	0.0019	56.91
5F	2.7	1.28^{+++}	0.0209*	78.68	1.7	1.21	-0.0109	76.81
6F	2.4	1.29^{+++}	0.0598**	65.56	1.4	0.81	0.0012	38.84
7F	1.9	0.74^{+++}	-0.0072	80.51	1.7	1.10	-0.0056	63.40
8F	2.0	1.02	0.0224*	69.33	1.6	1.16	0.0031	54.12
9F	2.1	0.51^{+}	0.0166	39.69	1.5	0.73	-0.0078	47.28
10F	2.0	0.99	0.0073	76.80	1.6	0.97	-0.0050	56.34
Average	**2.2**	-	-	-	**1.7**	-	-	-

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

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

(β_1): Regression Coefficient; $\square^2_{dii}$: Variance deviation regression; R^2 (%): Determination Coefficient.

Source: Prepared by author.

4.4 CONCLUSION

The planting dates most suitable for evaluation of genotypes for genetic resistance were August and September, as it showed the highest environmental indexes to gray leaf spot and northern leaf blight. The inbred lines IVD1-2, IVD1-3, IVD1-9, 2D and 7D, may be used to form a synthetic with dent grains for resistance to these two diseases. For synthetic flint grains, the inbred lines IVF1-7, IVF1-10, 2F, 9F and 10F can be used for resistance to both diseases.

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