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Understanding maize genotype behavior under stunt complex pressure

Entendendo o comportamento de genótipos de milho sob pressão do complexo dos enfezamentos

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ABSTRACT

The stunt complex, transmitted by *Dalbulus maidis*, has become one of the main maize diseases in recent years in Brazil and Latin America. Genetic resistance is one of the best ways to control and prevent plant diseases due to its efficiency, low environmental impact, and reduced need for phytosanitary products. We sought to identify maize genotypes tolerant to the stunt complex and quantify the levels of damage caused by the disease in commercial hybrids and native varieties. A two-year field experiment was conducted to evaluate the maize stunting. We used an augmented block design with 60 genotypes and 3 test hybrids, arranged in 4 blocks. We assessed the incidence and severity of stunting between stages R2 and R3. We evaluated the agronomic characteristics of the different genotypes to compare them with the effects caused by the stunt complex and each genotype's tolerance. We found more than 1,000 kg ha^{-1} of losses for commercial and native hybrids due to the stunt complex. Regression and multivariate analyses showed results that prove the effect of the maize stunt complex in reducing productivity, which allowed the classification of the evaluated genotypes into tolerance and resistance levels. The genotypes GNZ 7788 VIP3, Sempre 10A40 VIP3, DKB 360 PRO3, FS 575 PWU, NK Defender VIP3, and MG 540 PWU proved to be the best genotypes to serve as resistance sources to the stunt complex. The results emphasized the importance of genetic improvement in adding resistance/tolerance traits to breeding programs and increasing maize productivity and global sustainability.

Index terms: Mollicutes; spiroplasma; phytoplasma; *Dalbulus maidis*; tolerance; breeding.

RESUMO

O complexo dos enfezamentos, transmitido por *Dalbulus maidis*, tornou-se uma das principais doenças do milho nos últimos anos no Brasil e na América Latina. A resistência genética é uma das melhores formas de controlar e prevenir as doenças devido à sua eficiência, baixo impacto ambiental e redução de produtos fitossanitários. Neste trabalho identificamos genótipos de milho tolerantes ao enfezamento e quantificamos os níveis de danos causados em híbridos comerciais e crioulos. Um experimento de campo de dois anos foi conduzido para avaliar o impacto do enfezamento. Foi utilizado um delineamento em blocos aumentados com 60 genótipos e 3 híbridos testemunhas. Avaliamos a incidência e a severidade do enfezamento em R2 e R3. Avaliamos as características agronômicas dos genótipos para compará-las com os efeitos causados pelos enfezamentos e a tolerância de cada genótipo. Encontramos mais de 1.000 kg ha^{-1} de perdas em híbridos comerciais e crioulos devido ao complexo dos enfezamentos. As análises de regressão e multivariada apresentaram resultados que comprovam o efeito do enfezamento do milho na redução da produtividade, o que permitiu a classificação dos genótipos avaliados em níveis de tolerância e resistência. Os genótipos GNZ 7788 VIP3, Semper 10A40 VIP3, DKB 360 PRO3, FS 575 PWU, NK Defender VIP3 e MG 540 PWU provaram ser os melhores genótipos para servir como fontes de resistência ao complexo dos enfezamentos. Os resultados enfatizaram a importância do melhoramento genético na adição de características de resistência/tolerância aos programas de melhoramento e no aumento da produtividade do milho e da sustentabilidade global.

Termos para indexação: Mollicutes; spiroplasma; fitoplasma; *Dalbulus maidis*; tolerância; melhoramento.

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Introduction

Brazil produced 112.8 million tons of maize in the 2021/2022 harvest (Companhia Nacional de Abastecimento - Conab, 2022). Maize is grown in Brazil's micro-regions and serves as the basis for feeding cattle, pigs, and poultry. It is also used to supply the industry and the energy sector; its main application is feed material (Ranum, Peña-Rosas, & Garcia-Casal, 2024). Vian, Santi and Amado (2016) attribute the increase in Brazilian maize productivity to the quality of fertilizers, the optimization and use of agricultural mechanization, development of genetically superior hybrids in terms of productivity, and precision in irrigation and agriculture, which improves resource management.

Maize crops can be found at all times of the year in Brazil due to their great versatility, high demand, and the possibility of growth in several crops. These characteristics favor the maintenance of pest and pathogen populations that can generate

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phytosanitary threats to the crop over time (Oliveira & Frizzas, 2022, Canale et al., 2023, Oliveira, Oliveira, & Barros, 2023).

The late stunt complex is the most important maize disease in the Americas and the Caribbean; it causes severe plant development deficits and can result in losses of up to 100% of productivity (Jones & Medina, 2020, Egito et al., 2024). Two types of maize stunting are known: pale stunting and red stunting, caused by the prokaryote *Spiroplasma kunkelii* Whitcomb (Maize Stunt Spiroplasma) and by phytoplasma (Maize bushy stunt phytoplasma), respectively. Both pathogens are transmitted in a persistent propagative manner by the maize leafhopper (*Dalbulus maidis* DeLong and Wolcott) (Homoptera: Cicadellidae) (Whitcomb et al., 1986; Lee, Davis, & Gundersen-Rindal, 2000; Firrao et al., 2004), a pest that can negatively impact maize production through direct damage during feeding and possible transmission of phytopathogens, as expressed by Jones et al. (2021) and Faria et al. (2022). Symptoms caused by mollicutes are reduction in plant size, ear height and leaf area, multi-spike, and malformation of ears and grains, which affects yields. However, these symptoms' expression varies according to the maize genotype since the diseases can occur in association with multiple infections from other pathogens (Galvão, Sabato, & Bedendo, 2021, Egito et al., 2024).

When infected by red stunt or pale stunt, maize can show symptoms 30 days after infection, but, normally, the symptoms in the field are more evident from the peaking stage onwards (Galvão, Sabato, & Bedendo, 2021). Environmental temperature directly affects the maize leafhopper's reproduction: temperatures above 20 °C shorten the cycle from egg to adult, and temperatures below 20 °C prolong this cycle (Marín, 1987). Therefore, in climatic conditions with high temperatures and low amplitude, populations of *D. maidis* can grow more rapidly, which favors the occurrence of the maize stunt complex.

Despite using other plant species as shelter in the off-season, *D. maidis* only reproduces on maize and teosinte (Ribeiro & Canale, 2021). Therefore, eliminating remaining plants is important to reduce the population of *D. maidis* in the region during the off-season. In southern Brazil, especially in Paraná's western region, there have been significant losses in the productivity of maize grown in the second harvest (2019/20) due to phytosanitary problems, especially stunting; this has been worrying farmers, technical assistance, and the Paraná Agribusiness Defense Agency.

The occurrence of pests and diseases in the maize crop leads to increased chemical pesticide application rates, destabilizing the environment and increasing production costs. Therefore, sources of resistance to diseases and pests need to be found, thus developing resistant varieties that can guarantee high productivity and reduce costs. However, there is little information on the susceptibility of maize cultivars to pests, especially on the susceptibility of maize genotypes to the stunt complex (Tozetti, Osuna, & Banzatto, 1995; Zurita et al., 2000, Faria et al., 2022). When choosing a crop, the characteristics of the materials most adapted to regional conditions must be noted, along with their production potential, stability, disease resistance, suitability for the production system in use, and soil and climate conditions (Troyer 2006).

The most appealing disease management strategy is the use of genetically resistant cultivars since they require no additional cost to the grower and have no negative impact on the environment (Boyd et al., 2013, Faria et al., 2022). In genetic improvement, a hybrid's resistance to diseases is proportional to the number of resistant strains that go into its synthesis (Dong et al., 2024). In this paper, we aimed to evaluate commercial maize genotypes and native maize for their resistance/tolerance level to the stunt complex, its impact on productivity, and to identify sources of resistance with potential for use in genetic improvement.

Material and Methods

Location and experimental design

The field experiments were conducted in two sites during the 2020/21 harvest: in the municipality of Palotina, (experimental farm of the Federal University of Paraná - UFPR Palotina, 24°20'45.0"S 53°45'11.3"W) and Entre Rios do Oeste (24°43'41.3"S 54°14'18.1"W), both located in Paraná, Brazil (privately owned area). However, the experiment was affected by the adverse weather conditions that year. We evaluated the incidence and severity of the stunt complex, but the data was not considered for statistical analysis. In the 2021/22 harvest, the experiment was carried out on UFPR Palotina's experimental farm in the municipality of Palotina. We assessed the plots' incidence and stunting severity, as well as the levels of chlorophyll a and b, and total chlorophyll. The yield variables were analyzed in 31 of the 60 genotypes to quantify the damage caused by the stunt complex on these variables.

The experiment's region has an average annual temperature ranging from 19 °C to 23 °C; the hottest month (January) varies from 23 °C to 26 °C and the coldest month (June) varies from 14 °C to 18 °C. Average annual rainfall ranges from 1,700 mm to 2,200 mm; the wettest quarter is December-January-February (historical average ranging from 460 mm to 580 mm), and the least rainy quarter is June-July-August (historical average ranging from 260 mm to 420 mm) (Gomes et al., 2020).

We used Federer's augmented block design with 60 regular genotypes (45 commercial hybrids and 15 native varieties from UFPR's genetic improvement collection, Table 1). The choice of corn genotypes used in this work was based on the importance of these hybrids for the corn market, in addition to being the most cultivated genotypes in the western region of the state of Paraná. The design was divided into four blocks (with 15 plots), and the control plants common to the blocks were the commercial hybrids: Agroceres 9000 PRO3, Syngenta NK Feroz VIP3, and Syngenta NK Supremo VIP3. The plots were made up of 4 rows 5 m long and 0.9 m apart; the two central rows — except for 0.5 m at the ends of each plot — were considered the useful area. Five seeds were sown per linear meter with an average population density of 55,500 plants per hectare. Cultivation was carried out per the requirements for growing maize, following Sangoi (2001) recommendations, without disease control. Sowing and top-dressing fertilizations were carried out according to the soil analysis of the experimental area.

Table 1: Name and characteristics of the genotypes used to study resistance to the stunt complex.

Nº	Genotype	Transgenic	Type[1]	Cycle[2]	STU[3]	Company
1	MG 408 PWU	Yes	SH	E	NI	Morgan
2	UFPR 123	No	Native	NI	NI	-
3	FS 12B082PW	Yes	NI	NI	NI	Forseed
4	STINE 9801-20VIP3	Yes	SH	E	MT	Stine
5	UFPR 120	No	Native	NI	NI	-
6	NK DEFENDER VIP3	Yes	MSH	E	NI	Syngenta
7	NK SUPREMO VIP3	Yes	SH	E	NI	Syngenta
8	UFPR 141	No	Native	NI	NI	-
9	P-3310 VYHR	Yes	SH	SE	MT	Pioneer
10	FS 700PWU	Yes	SH	E	NI	Forseed
11	PRE 10A40VIP3	Yes	SH	SE	MR	Sempre
12	UFPR 119	No	Native	NI	NI	-
13	P 30R520VYHR	Yes	NI	NI	NI	Pioneer
14	ROXO CASSOL	No	Native	NI	NI	-
15	AG 8480PRO3	Yes	SH	E	NI	Agroceres
16	UFPR 26	No	Native	NI	NI	-
17	MG 464PWU	Yes	NI	NI	NI	Morgan
18	GNZ 7740VIP3	Yes	NI	E	NI	Geneze
19	ROXO POP 2	No	Native	NI	NI	-
20	AG 9000PRO3	Yes	SH	SE	T	Agroceres
21	UFPR 108	No	Native	NI	NI	-
22	PRE 20A12VIP3	Yes	SH	E	T	Sempre
23	GNZ 7720VIP3	Yes	SH	E	NI	Geneze
24	FS 575PWU	Yes	SH	E	NI	Forseed
25	ROXO POP 1	No	Native	NI	NI	-
26	NK STATUS VIP3	Yes	SH	E	NI	Syngenta
27	NK-EXPERI-1 VIP3	Yes	NI	NI	NI	Syngenta
28	UFPR 149	No	Native	NI	NI	-
29	AS 1800PRO3	Yes	SH	SE	MT	Agroeste
30	UFPR 144	No	Native	NI	NI	-
31	DKB 360PRO3	Yes	SH	E	NI	Dekalb
32	UFPR 76	No	Native	NI	NI	-
33	GNZ 7788VIP3	Yes	NI	SE	T	Geneze
34	B 2782PWU	Yes	SH	E	MT	Brevant
35	B 2702PWU	Yes	SH	SE	MT	Brevant
36	UFPR 57	No	Native	NI	NI	-
37	PRE 20A38VIP3	Yes	SH	E	T	Sempre
38	AS 1633PRO3	Yes	SH	E	NI	Agroeste
39	UFPR 101	No	Native	NI	NI	-
40	NK 488VIP3	Yes	SH	SE	NI	Syngenta
41	FS 670PWU	Yes	SH	E	NI	Forseed
42	NK FEROZ VIP3	Yes	DH	E	NI	Syngenta
43	NK 422VIP3	Yes	SH	SE	NI	Syngenta
44	NK 555VIP3	Yes	SH	E	NI	Syngenta
45	UFPR 19	No	Native	NI	NI	-
46	FS 505PWU	Yes	SH	E	NI	Forseed
47	NK 467VIP3	Yes	SH	SE	NI	Syngenta
48	STINE 9602-20VIP3	Yes	SH	E	MS	Stine
49	MG 593PWU	Yes	SH	SE	NI	Morgan
50	UFPR 73	No	Native	NI	NI	-
51	FS 564PWU	Yes	SH	E	NI	Forseed
52	MG 540PWU	Yes	SH	E	NI	Morgan
53	UFPR 145	No	Native	NI	NI	-
54	PRE 20A44VIP3	Yes	SH	E	MS	Sempre
55	P 3858PWU	Yes	SH	E	NI	Pioneer
56	AS 1844PRO3	Yes	SH	E	MT	Agroeste
57	UFPR 102	No	Native	NI	NI	-
58	NK 520VIP3	Yes	SH	E	NI	Syngenta
59	PRE EXPERI-2 VIP3	Yes	NI	NI	NI	Sempre
60	UFPR 31	No	Native	NI	NI	-

[1] Hybrid type: SH = Simple Hybrid; MSH = Modified Simple Hybrid; DH = Double Hybrid; NI = No Information; [2]Cycle: E = Early; SE = Semi-Early; NI = No Information; [3]Crop Stunting: T = Tolerant; MT = Moderately Tolerant; MS = Moderately Susceptible; MR = Moderately Resistant.

Assessment of *D. maidis* infestation

We found the presence of *D. maidis* at the V6 stage in all evaluated areas' plots. For this analysis, we used the plastic bag method, adapted from Waquil (1997), which consists of quickly bagging the cartridge of the maize plant with a 10-liter plastic bag. The plant was then cut up, and the sample was transported to the laboratory. The samples were stored in a freezer at -15 °C for approximately three hours. Then, the maize leafhoppers were identified and counted using a magnifying glass, and separated into nymphs and adults (number of leafhoppers per cartridge).

The leafhopper count (data not shown) showed the presence of the insect vector in all genotypes for at least one experimental site. However, quantifying the number of insects was only a qualitative parameter to verify their presence in the experimental area; this assured us that the maize genotypes would be exposed to the main vector that transmits mollicutes.

Incidence and severity of stunting

The incidence of maize stunting was assessed 90 days after emergence (approximately between stages R2-R3). It was calculated as the ratio between the number of plants with stunting symptoms (red stripes on the leaves, white streaks, reduced plant height, sprouts, and spike proliferation) and the total number of plants in the two central rows of each plot (Silva et al., 2021). Data were reported as the percentage (%) of plants with stunting. Due to the difficulty in differentiating the symptoms of maize stunting types (pale and red) in the field, and because both diseases can occur on the same plant, we adopted the term "stunting" for both diseases. Genotypes with a significantly lower incidence of stunting than others were considered more resistant, and vice versa.

The intensity of the symptoms and damage caused by stunting can vary depending on the age at which the plant was infected. We assessed the severity of the disease individually on each plant, using a score scale (from 1 to 6) suggested by Silva et al. (2021), referring to the average plant symptoms in the plot (Table 2).

Table 2: Score scale to evaluate the severity of maize stunting according to Silva et al. 2021

Note	Description
1	Absence of symptoms
2	Plants with less than 25% of their leaves showing symptoms, i.e., reddish, yellowish leaves, or chlorotic stripes on their insertion.
3	Plants with 25% to 50% of their leaves showing symptoms.
4	Plants with 50% to 75% of their leaves showing symptoms.
5	Plants with more than 75% of their leaves showing symptoms.
6	Plants with early death caused by stunting.

Because the severity assessment was carried out per plant, we adopted weights for the notes: notes 1, 2, and 3 were assigned a weight of 1; notes 4 and 5 were assigned a weight of 2; and note 6 was assigned a weight of 3. We used the following Equation (1) to analyze the severity index:

$$\text{Severity index} = \frac{\sum \text{weights x number of plants wiht symptoms}}{\text{Number of evaluated plants}} \quad (1)$$

All plants with stunting symptoms were marked with red spray paint (diseased plants), while the healthy plants remained unidentified.

Determining production variables

The ears of all maize in the plots' useful rows were harvested, and the ears of diseased plants (marked with red spray paint) were separated from the healthy ones (unmarked) to determine the yield variables. Before harvesting, we measured chlorophyll a, b, and total levels in the diseased and healthy plants at the R2 stage using a portable chlorophyll meter (chlorofiLOG CFL-1030 model, Falker Automação Agrícola Ltda.) with five measurements per plot in the center of each ear's leaf.

We evaluated the following variables of the ears from diseased and healthy plants after the harvest: i) ear length (EL) in centimeters; ii) ear diameter (ED) in millimeters; iii) number of rows per spike (NRS); and iv) number of grains per row (NGR). After evaluating these variables, the ears were threshed using a manual thresher. The moisture content of the grains in each plot was assessed before evaluating the weight of 100 grains (W100) and yield (kg ha^{-1}). These data were obtained considering the standard humidity of 13%. The potential yield (PY) was obtained by dividing the total grain weight of the healthy ears by the number of healthy ears and then multiplying this value by the total number of ears. The real yield (RY) was obtained from the sum of the grains' weight in the healthy and diseased ears. The damage estimate (D) was obtained by subtracting the PY from the RY (Reis, Casa, & Bresolin, 2004).

Statistical analysis

The data was subjected to the homogeneity of variance and normal distribution tests (Kolmogorov-Smirnov and Shapiro-Wilk). They were then subjected to analysis of variance (ANOVA), considering genotypes and situation (healthy/diseased) as factors. We applied the Scott-Knott test to split the genotypes within each situation (healthy/diseased). To assess it, we applied the LSD (Least Significant Difference) test. A significance level of 5% probability ($P \leq 0.05$) was considered for all tests. All the analyses were carried out using the Sisvar software (Ferreira, 2014).

We also carried out a regression analysis considering incidence (%) and severity (%) as independent variables, and the average yield (kg ha^{-1}) as the dependent variable. We used the equation with the highest R^2 as the criterion for fitting the best regression model using the R Core Team package, Kolde (2019).

We used a multiple regression analysis to evaluate the factors that affect maize yields. Three scenarios were evaluated considering: i) only the average yield of healthy plants; ii) only the average yield of diseased plants; and iii) only the average yield (healthy and diseased). The independent variables used to model yield (kg ha^{-1}) were: incidence (%), severity (%), length (cm), and diameter (mm) of ears, number of rows per spike (NRS), number of grains per row (NGR), weight of 100 grains (W100) (g), and damage level (Real Yield minus Potential Yield). The variables had to meet a significance level of 5% to be added to the model ($P \leq 0.05$). We used the software Jamovi (Version 1.6) and The Jamovi project (2022) for the multiple regression analyses.

We carried out a principal component analysis (PCA) to arrange the genotypes according to diseased and healthy plants. For this analysis, the variables used were standardized ($\mu = 0$, $\sigma = 1$) to ensure the proportionality of the effects. The FactoMineR and Factoextra packages (Kassambara & Mundt, 2020) from R Core Team (2022) were used to plot the graphs of individual genotypes grouped by the incidence of stunting (diseased and healthy) and the contribution of the variables analyzed.

We used the heatmap R package (version 0.7.7) to build the heatmap. The clustering was built by K-means analysis with the tidyverse (Wickham et al., 2019) and Factoextra (Kassambara & Mundt, 2020) packages, and the dendrogram was built with the heatmap package (Kolde, 2019).

Results and Discussion

We found statistical differences between the genotypes evaluated for the incidence ($F = 9.559$ and $P < 0.0000$) and severity ($F = 12.233$ and $P < 0.0000$) of stunting (Figure 1). There was a high incidence of stunting for the genotypes UFPR-76, NK 488VIP3, NK 422VIP3, 9602-20VIP3, SEMPRE 10A40PWU, UFPR-26, AS 1800PRO3, AS 1844PRO3, FS 564PWU, and UFPR101, while the genotypes B 2702PWU, FS 505PWU, SEMPRE 10A40VIP3, DKB 360PRO3, FS 57PWU, NK DEFENDER VIP3, MG 540PWU, and BNZ 7788VIP3 showed the lowest incidence rates, with emphasis on GNZ 7788VIP3, which did not present any stunting symptoms (Figure 1A). The UFPR 26 and UFPR 76 genotypes also showed the highest stunting severity among the evaluated ones (Figure 1B). All genotypes with the lowest incidence had lower severity indices since the Pearson correlation between these variables was positive and significant ($r = 0.58$; $P < 0.004$). There was no statistical difference in the incidence (Figure 1A) of stunting between the native and commercial groups; however, the native groups were generally less tolerant (t-test; $P < 0.05$) in terms of severity (Figure 1B).

Approximately 13% of the evaluated genotypes had a low stunting incidence (less than 15% of plants with symptoms) (Figure 1A). These genotypes may be important for future breeding programs as sources of resistance, in addition to being important for choosing the best commercial hybrids for planting in the western region of Paraná and even for other regions of Brazil. Stunting severity was low in approximately 68% of the genotypes (Figure 1B).

When assessing the stunting incidence in this study's genotypes, we found infected plants at levels ranging from 0 to 100% (Figure 1A); this demonstrates the great heterogeneity of the materials available for cultivation since, under the same environmental and temporal conditions, the response to pathogens is different between genotypes. The various reactions to diseases exhibited by plants indicate which materials can serve as reference for identifying sources of genetic resistance to stunting. A study by Costa et al. (2019) showed that, under the same conditions, some maize hybrids can have a stunting incidence of less than 10%, while others can reach more than 65%. Since commercial maize hybrids have different degrees of resistance to diseases of the maize stunt complex, sowing hybrids that are more resistant to these pathosystems can also reduce the risk of production losses (Oliveira & Frizzas, 2022, Faria et al., 2022).

The commercial hybrid GNZ 7788 VIP3 did not show any symptoms of stunting, and we found no other studies involving the analysis of the occurrence of the stunt complex for this genotype in the literature. Therefore, this hybrid showed great potential for future work on stunting resistance, as it may have a certain level of resistance or not show any visual symptoms at all. Furthermore, this hybrid can also be extremely useful for farmers with recommendations for planting in the western region of Paraná and even for other regions of Brazil with high incidences of leafhoppers. However, there is a possibility that the genotype does not directly express the symptoms and still suffers losses in yield, which may be linked to the genetics that limit yield. Cota et al. (2018) also found this with the hybrid RB 9108 PRO, one of the hybrids that produced the least — even though it had an intermediate damage score. We observed this with the NK STATUS VIP3 genotype, which showed a low level of incidence of the stunt complex (Figure 1) but had one of the lowest yields (2,500 kg ha^{-1}, Figure 5).

Around 13% of the evaluated genotypes had low stunting incidence, which corroborates other studies that show that commercial maize hybrids do not have good resistance to the stunt complex (Costa et al., 2019; Jones & Medina, 2020; Oleszczuk et al., 2020, Faria et al., 2022). As demonstrated by Oliveira et al. (2002), different hybrids with equal stunting incidence rates can respond differently to infection: some develop better and show lower yield losses, while others are completely compromised. Genotypes with similar levels of incidence and severity of symptoms can produce different yields (Hidalgo, Castañón, & Rodríguez, 1998; Oliveira et al., 2002, Costa et al., 2023). This has been described as host tolerance, or the ability to obtain yield despite the damage caused by maize stunting (Oleszczuk et al., 2020). According to Jones and Medina (2020), more comprehensive studies of the maize stunt complex are needed; those could pave the way for the discovery of new molecular targets for the genetic control of pests, addressing both the insect vector and the phytopathogen.

When comparing the groups of native and commercial maize, the commercial hybrids showed a lower incidence of the disease and are better adapted to resist the stunt complex. However, the search for sources of resistance must consider each genotype individually to advance research and technology. Therefore, it is not possible to dismiss the analysis of native maize, especially those that remained productive after infection.

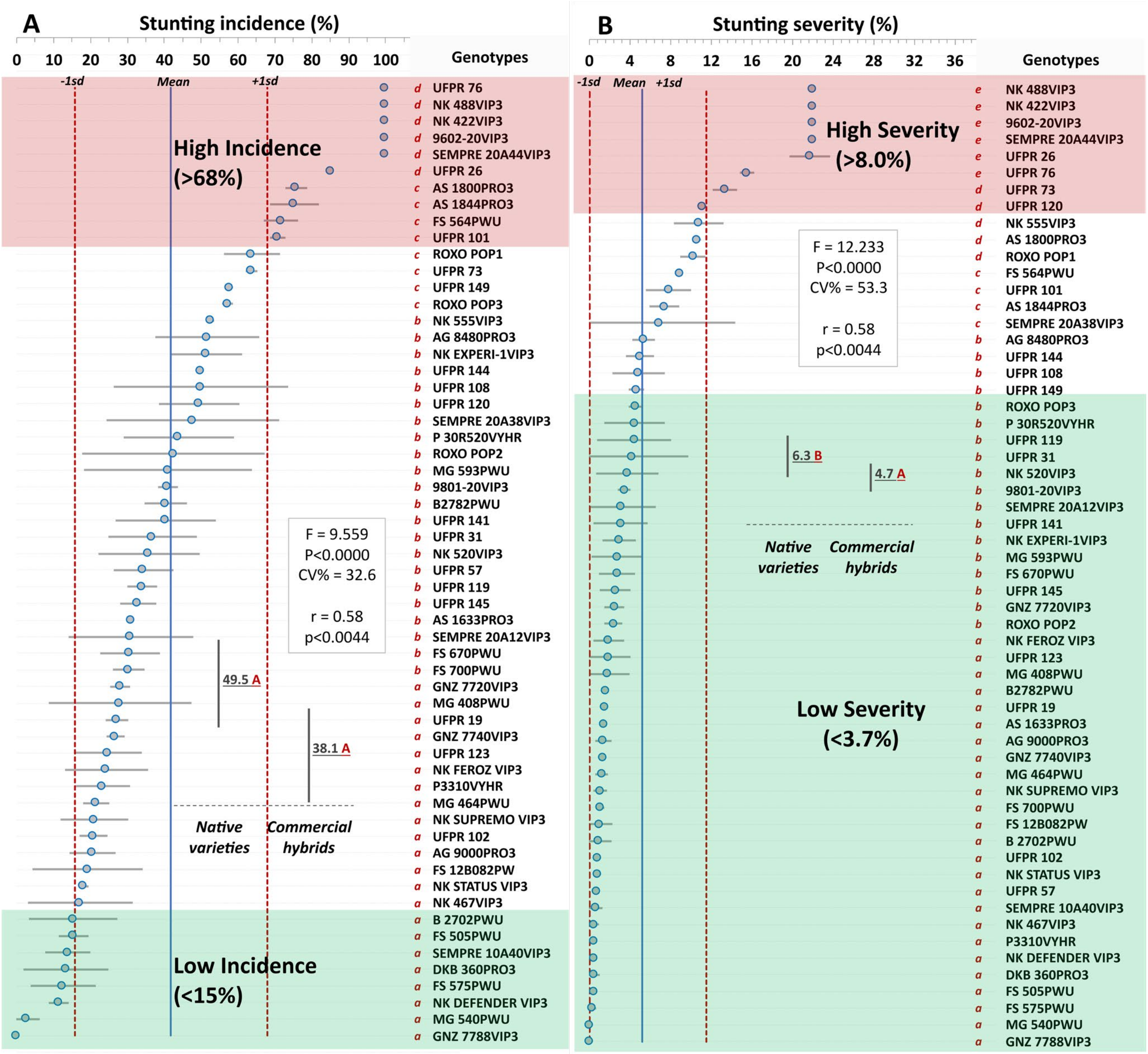

Figure 1: Incidence and severity of stunting in maize genotypes. The genotypes' averages are represented by the gray circles, followed by the standard deviations (horizontal bars). The vertical line in blue represents the average incidence (A) and severity (B). The dashed vertical lines represent the standard deviations (positive: +1sd; and negative: -1sd). The vertical bars represent the standard deviations of the genotype groups (Native and Commercial). Different letters are significant by the t-test ($P < 0.05$). Genotypes followed by other letters indicate a significant difference using the Scott-Knott test ($P < 0.05$). "r" represents the Pearson correlation between Incidence and Severity.

There was a statistical difference between the genotypes for the chlorophyll a (Figure 2A), b (Figure 2B), and total (Figure 2C) levels. In general, diseased plants had lower chlorophyll a, b, and total levels than healthy plants in the t-test ($P < 0.05$); however, each genotype had a specific response. Chlorophyll levels were lower for diseased plants than healthy ones, indicating metabolic impacts on contaminated plants that reduce their photosynthetic capacity and limit their development and production.

According to Junqueira, Bedendo and Pascholati (2011), the incidence of maize stunting can cause changes in plant tissue compounds such as proteins, phenolic compounds, and chlorophyll, resulting in morphological changes. This can indicate their levels of resistance/tolerance to the stunt complex since our study demonstrated significant variation for 24 genotypes among those evaluated (Figure 2).

The stunt complex caused a reduction in EL and ED traits (Figure 3A and 3B), as shown by the significant difference ($P < 0.05$) between diseased and healthy plants, for 45% and 52% of the genotypes, respectively. These characteristics are directly related to production parameters as they make up the structure of the commercial product, resulting in a yield reduction. The average EL of healthy plants was 16.32 cm, while diseased plants showed an 18.4% reduction in EL (Figure 3A). Stunting also decreased ED by approximately 13.2% (Figure 3B). There was no statistical difference between the native and commercial groups for these traits.

For the NRS and NGR variables, stunted plants showed an average reduction of 6.5% and 19.2%, respectively (Figure 3C and 3D). The greatest reductions in NRS were observed for the genotypes ROXO POP2 (-17%), UFPR 57 (-12%), NK STATUS VIP3 (-14%), and DKB 360PRO3 (-9%). However, for NGR, the incidence of plant stunting caused more pronounced reductions, as seen for the genotypes UFPR 57 (-49%), AS 1800PRO3 (-43%), UFPR 108 (-41%), UFPR 141 (-34%), DKB 360PRO3 (-31%), NK STATUS VIP3 (-26%), and NK DEFENDER VIP3 (-23%). There was no statistical difference between the native and commercial groups for these traits. Lower ear development (as assessed by EL, ED, NRS, and NGR) results in a smaller quantity of grains. This, added to the reduced grain weight, turns the productive impacts significant for commercial cultivation. Among the evaluated genotypes, native maize showed greater reductions for these characteristics.

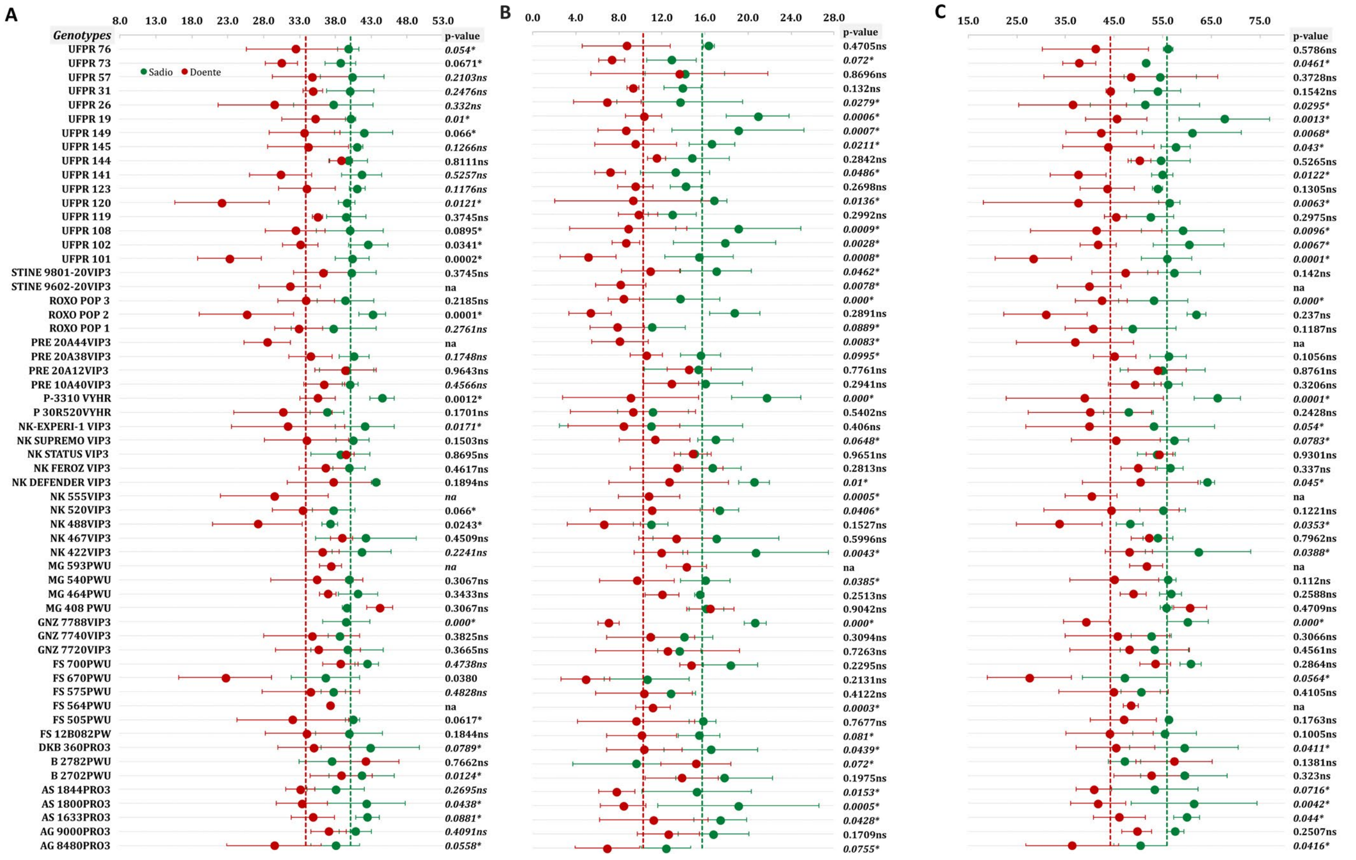

Figure 2: Chlorophyll a (A), b (B), and total (C) levels of the evaluated genotypes. The averages are represented by circles (red = diseased, green = healthy) followed by horizontal bars (standard deviation). The dashed vertical lines represent the average diseased (red) and healthy (green) plants.

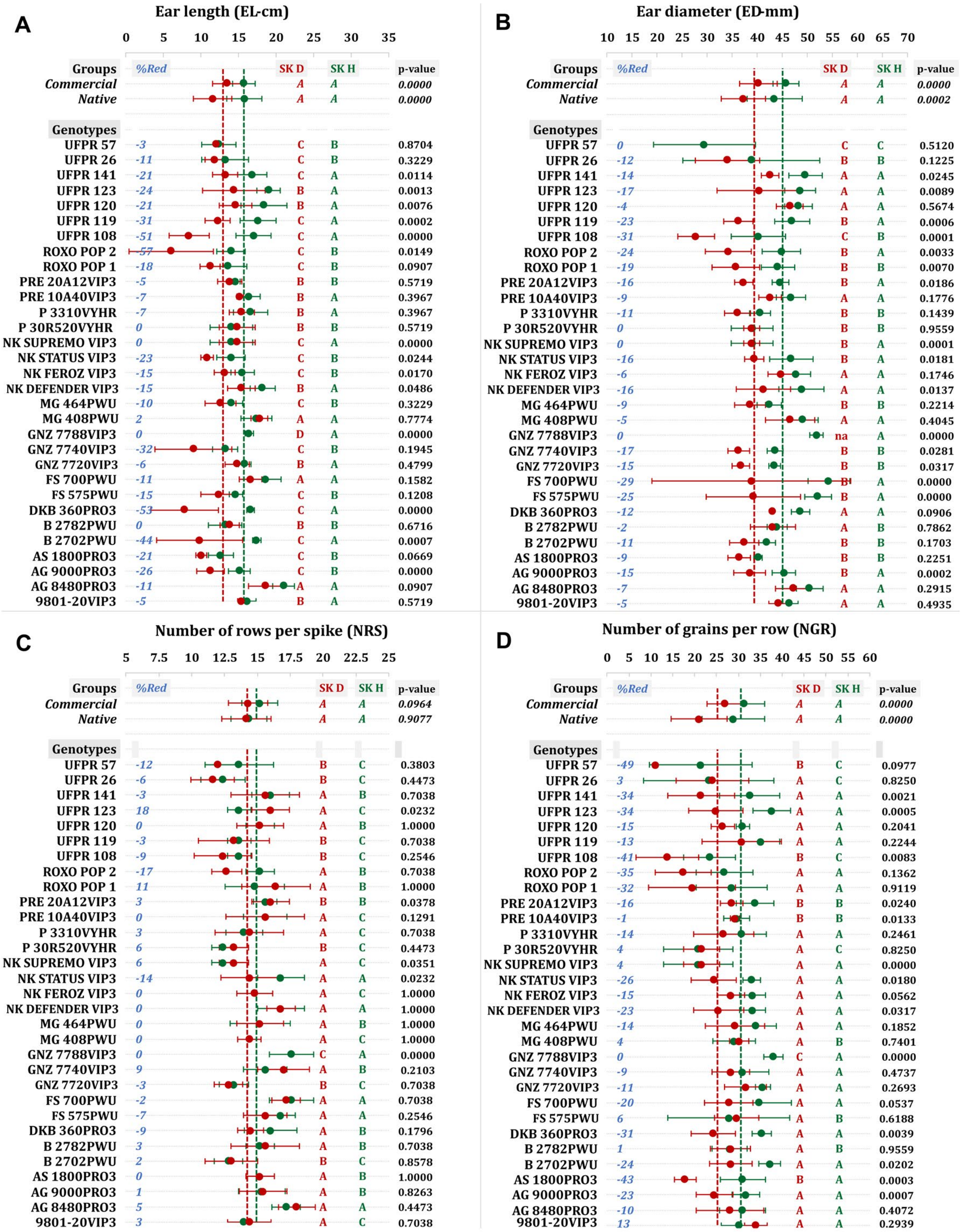

Figure 3: Average genotypes' evaluated performance, considering the parameters A) ear length (cm), B) ear diameter (mm), C) number of rows per spike, and D) number of grains per row. The averages are represented by circles (red = diseased, green = healthy) followed by horizontal bars (standard deviation). The dashed vertical lines represent the average diseased (red) and healthy (green) plants. Genotypes followed by different letters (SK D: diseased plants; SK H: healthy plants) indicate a significant difference using the Scott-Knott test ($P < 0.05$). p-value: indicates a significant difference using the t-test ($P < 0.05$) to compare diseased and healthy plants within genotypes. %Red: indicates the percentage reduction of each variable (%Red = [(Diseased/Healthy)*100].

For the W100 variable (Figure 4), the genotypes UFPR 26, UFPR 108, and FS 575PWU differed statistically (by the Scott-Knott test, $P < 0.05$) and were the plots with the highest weights among the healthy plants; the genotypes P 30R520 VYHR, MG 464PWU, GNZ 7740VIP3, and AS 1800PRO3 had the lowest weights among the healthy plants. Genotype B 2782 PWU had the highest W100 for diseased plants, and genotypes ROXO POP1 and P 30R520 VYHR had the lowest weights among diseased plants (Figure 4). The commercial hybrids differed statistically (by t-test, $P < 0.05$) between diseased and healthy plants regarding W100, with a higher value for healthy plants. There was also a statistical difference (by t-test, $P < 0.05$) in the healthy plants between the commercial and native groups: the native groups had the highest W100. Evaluating the percentage reduction in W100, the genotypes UFPR 108 and GNZ 7740VIP3 had over 50% reduction in weight due to stunting, and genotypes UFPR 57, GNZ 7788 VIP3, and B 2782 PWU had no reduction in weight due to stunting. The average reduction in W100 caused by stunting was 28.8% (Figure 4).

Figure 5 shows the yield reduction for diseased plants compared to healthy plants for the commercial genotype group (-333 kg ha^{-1}) and the native genotype group (-871 kg ha^{-1}). The genotypes with the greatest yield damage caused by stunting were UFPR 26, AS 1800PRO3, ROXO POP1, and UFPR 120, with estimated yield reductions of 2,664, 1,204, 1,086, and 1,081 kg ha^{-1}, respectively. The genotypes GNZ 7788VIP3 and PRE 20A12VIP3 showed no reductions in productivity due to the stunt complex. The GNZ 7788VIP3 genotype did not have any plants with stunting incidence (Figure 1A) and was the most productive among those evaluated (Figure 5). The average yield reduction caused by the stunt complex was 11.6% (Figure 5). Regarding the 2021 harvest, except for hybrids P3310 VYHR and AS 1800 PRO3, the plots generally had lower yields than the diseased plants in the 2022 harvest (Figure 5).

The yield of the 2022 harvest was higher than the yield of the previous harvest for all the materials studied, except for P 3310 VYHR (Figure 5). This result can be attributed to environmental conditions, since a significant water shortage and high temperatures compromised the plants' development. According to Sabato et al. (2020), temperatures influence the infection potential and latency period of *S. kunkelii* and MBS-phytoplasma. This indicates that high temperatures increase plant infection speed, while maintaining low temperatures increases the latency period of the organisms.

D. maidis can survive mild winters on remnant maize plants or other winter grasses. This is indicated by Carloni et al. (2021) as a factor that corroborates the rapid recovery of the vector population with rising temperatures, facilitating the infection of maize in the early stages, which causes greater damage. Oleszczuk et al. (2020) investigated components of resistance to maize stunting and identified differences in their levels of resistance and target organism, whether for the insect vector or the pathogen.

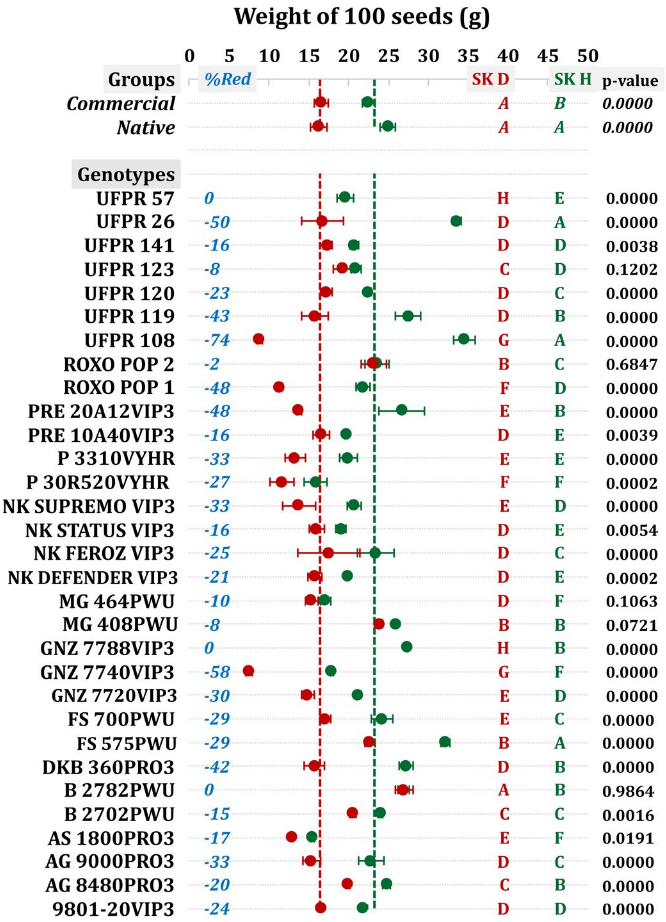

Figure 4: Weight of 100 grains of the evaluated genotypes. The averages are represented by circles (red = diseased, green = healthy) followed by horizontal bars (standard deviation). The dashed vertical lines represent the average of diseased (red) and healthy (green) plants. Genotypes followed by different letters (SK D: diseased plants; SK H: healthy plants) indicate a significant difference using the Scott-Knott test ($P < 0.05$). p-value: indicates a significant difference using the t-test ($P < 0.05$) to compare diseased and healthy plants within genotypes. %Red: indicates the percentage reduction in W100 (g) of diseased plants (%Red = [(W100 Diseased/W100 Healthy)*100].

The incidence and severity of the stunt complex negatively affected ($P < 0.05$) the average yield of the evaluated maize genotypes (Figure 6). This corroborates Cota et al. (2018), who found that, although yield is a characteristic of each genotype, grain yield correlated negatively with the severity of stunting, with differences in disease tolerance between hybrids.

The incidence of the stunt complex affected yield more significantly compared to the symptoms' severity (Figure 6), as seen by Oliveira et al. (2003), who made estimates of yield losses based on levels of incidence of the stunt complex. Toffanelli and Bedendo (2001), when evaluating the incidence of stunting, found that, for the most susceptible hybrids, a reduction of up to 98% in grain yield was observed. There is a need to avoid contaminating the crop with the stunt complex since this characteristic is dominant over yield, and its absence excludes the need for severity/tolerance control. The commercial hybrids NK 488 VIP3, NK 422 VIP3, 9602 20 VIP3, and SEMPRE 20A44 VIP3, which make up the group with the highest incidence levels, are also the genotypes with the lowest severity indices, except for GNZ 7788 VIP3, which had no incidence. This group also suffered little impact in its yield, with losses of between 80 and 256 kg ha^{-1}, while the more susceptible materials had up to 2,664 kg ha^{-1} in losses.

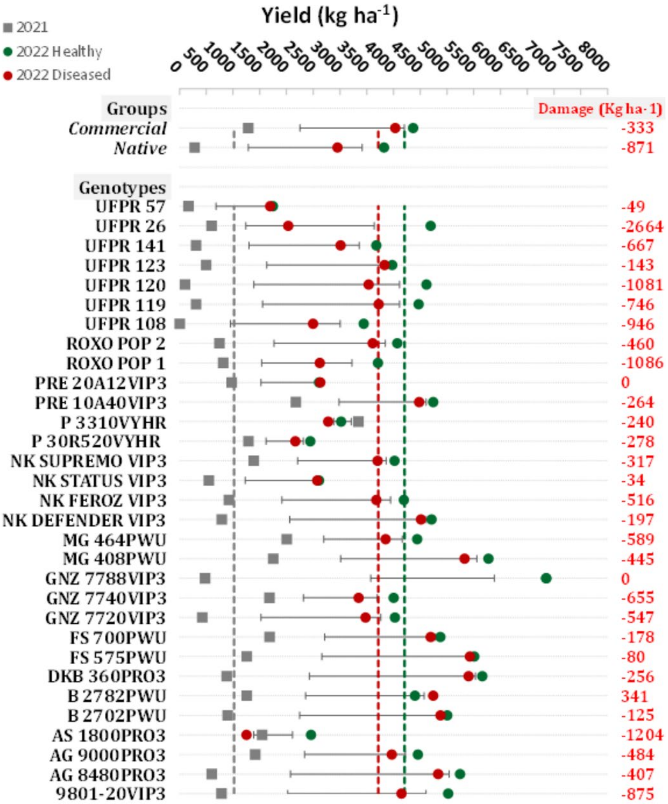

Figure 5: Average productivity of the evaluated genotypes in the 2021 and 2022 harvests (second harvest). The averages are represented by circles (red = diseased, green = healthy) followed by horizontal bars (standard deviation). The dashed vertical lines represent the average of diseased plants (red), healthy plants (green), and the 2021 average (gray). Damage indicates reduced Productivity (kg ha^{-1}) of diseased plants (Damage = Real Yield minus Potential Yield).

Considering that all the hybrids were exposed to the same population of insect vectors at the same time, the differences between the productive and developmental effects are due to specific characteristics of each hybrid and may be related to genetic characteristics that define a preference for the insect vector. Therefore, further studies are needed to clarify the specific metabolic outcome of each genotype. Another condition that influences the differences in the incidence of the disease complex, as cited by Cota et al. (2018), is the sowing season: the later the planting, the greater the likelihood of the vector population being contaminated with mollicutes or spiroplasmas, just as the first plantings of the harvest receive the first migratory population.

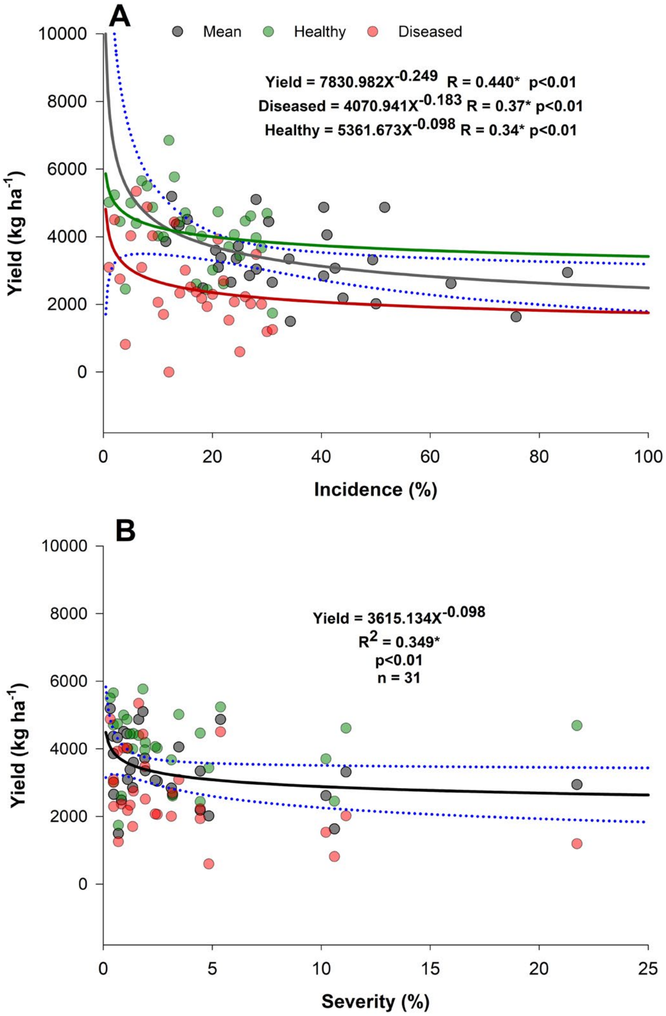

Figure 6: Average yield as a function of the stunting incidence (A) and severity (B) percentages. For both situations (incidence and severity), a non-linear Power equation was estimated as a function of average productivity. Blue dotted lines indicate the estimated equation’s 95% confidence interval (CI).

Multiple regression analysis showed that the stunting incidence negatively affected ($P < 0.05$) the yield of healthy and diseased plants, and the average yield of the genotypes (Table 3).

The EL and W100 variables contributed positively ($P < 0.05$) to the productivity of healthy plants, while EL, NRS, and

W100 positively influenced ($P < 0.05$) the average yield and the diseased plants' yield of the evaluated genotypes. All the multiple regression models showed significant adjustments ($P < 0.01$; Table 3). The multiple regression model to predict the diseased plants' yield was Y = -3,490.3 -20.8INC + 77.2EL + 198.2NRS + 181.5W100, which explained 82.1% of the observed variability (Tables 3 and 4). The adjusted model was Y = -3,490.3 -20.8INC + 77.2EL + 198.2NRS + 181.5W100 for the average yield of the genotypes.

Table 3: Analysis of variance of the multiple regression model for yield (kg ha^{-1}) of healthy, diseased, and average maize plants, using the following independent variables: incidence (%), severity (%), W100_D[1] (g), W100_H[2] (g), EL[3] (cm), NRS[4], and Damage (RY - PY).

Term[5]	Estimate	SE[6]	t-value	P≥|t|
Healthy Plants				
Intercept	-198.148	1.230.114	-0.161	0.873
Incidence	-17.461	7.670	-2.276	0.031
EL	172.704	68.963	2.504	0.019
W100	76.009	31.375	2.423	0.023
Damage	-0.31	0.107	-2.896	0.008
Diseased Plants				
Intercept	-3,490.3	1,038.11	-3.36	0.003
Incidence	-20.8	5.27	-3.94	< 0.001
EL	77.2	35.1	2.2	0.038
NRS	198.2	65.87	3.01	0.006
W100	181.5	22.95	7.91	< 0.001
Average Yield				
Intercept	-4,479.6	1,264.13	-3.54	0.002
Incidence	-14.7	5.61	-2.62	0.015
EL	117.6	49.81	2.36	0.026
NRS	243	61.1	3.98	< 0.001
W100	155.9	29.04	5.37	< 0.001

[1] Weight of 100 grains from diseased plants. [2] Weight of 100 grains from healthy plants. [3] Ear length. [4] Numbre of Row per Spike. [5] Variables that did not show significant effect (P>0.05) on Yield (kg ha^{-1}) were excluded from the model. [6] SE = Standard error.

The yield reduction was impacted mainly by the incidence of the stunt complex (Table 4). The characteristics that contributed the most to the yield reduction were ear length, number of rows per spike, and weight of 100 grains. As shown in Figure 3, for some genotypes the incidence of the disease led to an isolated increase in some of them, such as the number of rows per spike and the number of grains per row. However, for all genotypes, there was a reduction in the weight of 100 grains (Figure 4), length, and ear diameter (Figure 4), and, consequently, a reduction in yield. This indicates physiological alterations in ear formation and grain filling caused by the stunt complex.

Using PCA analysis, the genotypes were separated according to the distinct groups of diseased and healthy plants, demonstrating that stunting significantly affects maize crop's productivity. The first two axes explain approximately 72.2% of the total variation (Figure 7A). The first axis (PCA1) explains 55.2% of the variation and was negatively correlated with the variables PY, RY, and NGR (Figure 7B). The second axis (PCA2) explains approximately 17% of the variation and was correlated with the variables NRS, EL, ED, and W100. There is a high correlation between the RY and PY variables with the GNZ 7788VIP3 genotype (Figure 7A and 7B), in which there was no stunting incidence. However, these same variables (RY and PY) were negatively correlated with the genotypes UFPR 57 and UFPR 108 from the diseased plant group (high stunting incidence). Furthermore, the variables evaluated in this study showed a high correlation with the healthy plants of the evaluated genotypes (Figure 7A and 7B), while the correlation was lower in the diseased plants of these genotypes. The RY and PY variables contributed the most to the variability found in this study (Figure 7B).

The heatmap shows the association between the genotypes and the investigated characteristics (Figure 7C); this allowed the separation of the variables assessed for healthy and diseased plants into distinct groups. Additionally, the genotypes were separated in four groups, where the genotypes GNZ 7788VIP3 (commercial hybrid) and UFPR 57 (native) formed isolated groups (Figure 7C). It was not possible to separate the genotypes into commercial and native groups. The commercial hybrids MG 464PWU, 9801-20VIP3, PRE 10A40VIP3, DKB 360PRO3, and NK DEFENDER VIP3 showed the highest correlations with the yield variables RY and PY among plants with a high stunting incidence. Hybrid B 2782PWU showed the highest

Table 4: Adjustment measures of the models evaluated by multiple regression.

Model	R	R^2	R^{2}Ajust	AIC	BIC	RMSE	Anova for the models			
							F	Gl1	Gl2	P
Healthy	0.768	0.590	0.527	506	515	702	9.35	4	26	< 0.001
Diseased	0.920	0.847	0.821	450	458	459	33.1	4	24	< 0.001
Average	0.845	0.714	0.670	488	496	519	16.2	4	26	< 0.001

correlation with W100 among the diseased plants (Figure 7C). Among the healthy plants, the hybrid GNZ 7788VIP3 showed high correlations with the yield variables RR and RP.

Figure 7A groups the hybrids by stunting symptoms in two groups: one with a higher incidence of symptoms and lower yields (on the right) and another with a lower incidence of symptoms and higher yields (on the left). This corroborates Oliveira et al. (2002), who demonstrated heterogeneous behavior among commercial hybrids. Therefore, there are genotypes on the market that can serve as sources of resistance to the stunt complex, ensuring that hybrids with a good level of resistance are planted by farmers whenever the disease occurs. We highlight the GNZ 7788 VIP3 hybrid, which showed no symptoms of the stunt complex and the highest yield with 6,853 kg ha^{-1}.

Our work provides valuable contributions on the real impact of the stunt complex on maize crops. The results emphasize the importance of genetic improvement to add resistance/tolerance traits to breeding programs, contributing to increased maize yield and global sustainability. Future work should also be carried out to quantify or relate the types of associated stunting at the molecular and field levels. Therefore, further studies are needed to clarify the specific metabolic outcome of each genotype. In addition, work should be carried out to associate the population level with the incidence and severity of stunting.

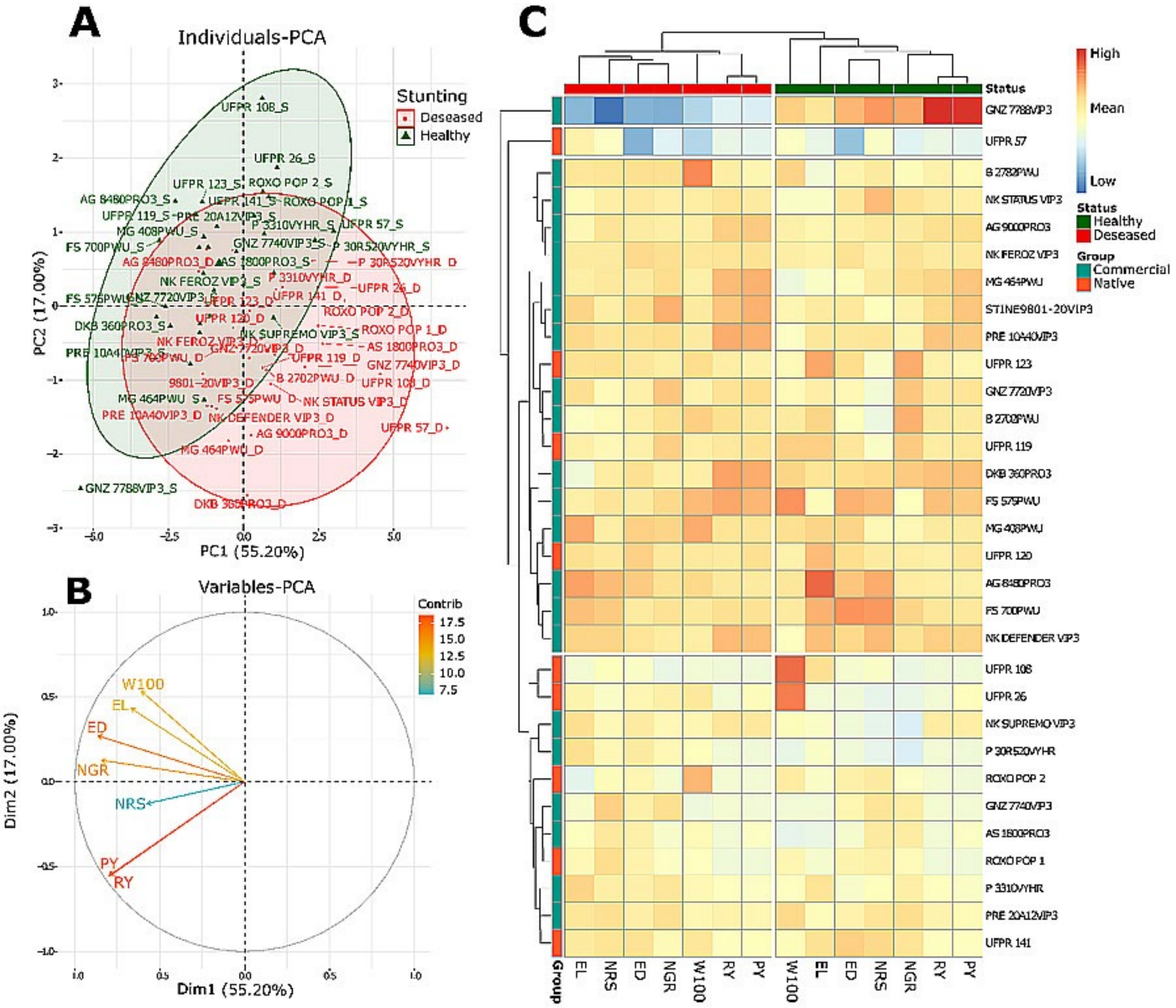

Figure 7: Principal component analysis (PCA) and heatmap for the genotypes and variables evaluated. A) PCA analysis of individual genotypes grouped by stunting incidence (diseased and healthy). B) PCA analysis showing the contribution of the variables analyzed. C) Heatmap of genotypes and yield-related variables. Annotations at the top of the heatmap show the grouping of genotypes (Group: Commercial and Native) and production variables (Status: diseased and healthy plants). NRS: Number of Rows per Spike. EL: Ear length. W100: Weight of 100 grains. ED: Ear diameter. NGR: Number of grains per row. PY: Potential yield. RY: Real yield.

Conclusions

The yield reduction was impacted by the incidence of the stunt complex. The study showed that stunting caused productive damage and significantly reduced the agronomic traits evaluated. The hybrids with the highest tolerance to the stunt complex were GNZ 7788 VIP3, which showed no symptoms of the disease; B 2702 PWU, FS 505 PWU, Sempre 10A40 VIP3, DKB 360 PRO3, FS 575 PWU, NK Defender VIP3, and MG 540 PWU showed low incidence of the disease and higher yield.

Author Contribution

Conceptual idea: Missio, R. F.; Dudek, G.; Methodology design: Dudek, G.; Dal Molin, L. M.; Data collection: Dudek, G.; Dal Molin, L. M.; Missio, V. C.; Data analysis and interpretation: Missio, R. F.; Dudek, G.; Luchese, A. V., and Writing and editing: Dudek, G.; Missio, R.F; Missio, V. C.

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