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Uni and multivariate approaches for diallel analysis in early generation trials for soybean tolerance to rust

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ABSTRACT: Due to the high impact of Asian soybean rust (SBR) in Brazilian croplands, several studies have been conducted in order to maintain or increase the grain yield gain over years in the presence of the pathogen. The aim of this study was to define a breeding strategy applying uni and multivariate approaches for diallel analyzes in early generation trials and contrasting disease conditions. Thus, assessing genetic parameters to identify traits related to greater tolerance to soybean rust. Deploying a North Carolina design II scheme (4 elite commercial cultivars × 10 rust-tolerant experimental lines), we obtained 40 F_2 crosses that were evaluated in a randomized complete block design with 4 replicates. The crosses were conducted in two environments, contrasting only for the fungicide management (with and without rust control), enabling the estimation of the rust impact on 11 traits, divided into two groups: adaptive and reproductive. A multi-environment diallel model was applied assuming all effects as fixed in order to estimate the general (GCA) and the specific (SCA) combining abilities. The multivariate diallel analysis was performed in each rust management using MANOVA to test the genetic effects. The rust conditions were the most important source of variation for almost all evaluated traits. Using the contrasting disease conditions, we were able to identify crosses with desirable SCA and parents with GCA, which implies that tolerance can be used as a strategy to deal with SBR. The implementation and the use of multivariate diallel analysis aiming to increase the efficiency of genotype selection for rust tolerance is discussed.

Key words: *Glycine max*, *Phakopsora pachyrhizi*, multivariate diallel, North Carolina design II.

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INTRODUCTION

Soybean (*Glycine max*) is the major oil crop in the world and the fourth crop in terms of volume of production (USDA 2017). Due to its importance, several companies and research institutions have been conducting breeding programs and releasing new cultivars in all producing countries. In Brazil, the second largest soybean producer in the world, yields across the country has experienced negative impacts due to Asian soybean rust (SBR), the currently most important disease. SBR is caused by the biotrophic fungus *Phakopsora pachyrhizi*. As a consequence, the use of fungicides at multiple times per grown cycle has led to expenses as high as US$ 2 billion per year (Godoy et al. 2016).

Several strategies can be applied to reduce the pathogen population and restrict the damage for soybean production. Some strategies include the use of fungicides (Godoy et al. 2016), resistant cultivars carrying one or multiples *Rpp* (resistance to *P. pachyrhizi*) genes (Yamanaka et al. 2015), tolerance (Jarvie and Shanahan 2009; Oloka et al. 2009; Tukamuhabwa; Maphosa 2014), soybean-free period (Godoy et al. 2016), and early sowing dates. Others noncommercial strategies include transgenic events (Kawashima et al. 2016), RNA interference (Koch and Kogel 2014; Langenbach et al. 2016) and nonhost resistance (Langenbach et al. 2013). When the strategy covers genetic aspects, the use of classical breeding is essential to develop a high-performance cultivar. In this sense, the breeder must plan the crosses followed by the segregating populations assessments. A great part of the success in obtaining elite cultivars depends on how well the information available in the breeding program will be collected and used to assist in the decision-making process.

One of the most important steps in a plant breeding program is the parental selection. Regarding this, breeders have to consider several traits, since grain yield is a result of the association of multiple traits, e.g. seed weight, number of seeds per plant, and harvest index. Several research studies have reported the relevance of parental selection on the breeding success (Carpentieri-Pípolo et al. 2000; Bertan et al. 2007; Casassola et al. 2013), and many tools are available to help the selection, e.g. divergence analyses based on phenotypic, genetic, and pedigree information.

After parental selection, the crosses are made, and these can be schematized in complete diallel or North Carolina design II. Frequently, segregating populations are analyzed following a diallel design using univariate approaches (Ribeiro et al. 2007; Gavioli et al. 2008; Oliveira et al. 2014), which implies failure in considering the correlations between desirable traits. To overcome this restriction, a multivariate diallel approach can be performed, enabling the study of two or more traits simultaneously (Ledo et al. 2003). Research studies applying multivariate diallel analysis are available for several important crops, including wheat (Benin et al. 2009), sweet passionfruit (Jung et al. 2007), popcorn (Gonçalves et al. 2014), Jatropha (Teodoro et al. 2017) sweet pepper (Nascimento et al. 2010) and soybean (Amaral et al. 2004).

Tolerance can be defined as the capacity of a cultivar resulting in less yield or quality loss relative to the same disease severity when compared to other cultivars or crops (Schafer 1971). According to some researchers, tolerance against plant diseases may have several benefits and can yield valuable results (Ney et al. 2013; Tukamuhabwa and Maphosa 2014). Besides grain yield, the use of tolerance also impacts the plant × pathogen interaction complex (Kover and Schaal 2002). Genes conferring complete resistance (R-genes) tend to be surmounted by the pathogen. On the other hand, tolerance implies the absence of selection pressure over the pathogen, which contrasts with resistance and fungicide usage (Roy et al. 2000). The use of tolerance to the development process of improved plant materials is reported in the literature in various crops (Jarvie and Shanahan 2009; Roux et al. 2010; Pierre et al. 2015).

The reduction of grain yield by SBR occurs mainly through leaf loss, reduction in leaf area, decrease in dry matter accumulation and reduction in harvest index (Kumudini et al. 2010). In tolerant genotypes, the ability to maintain productivity may occur at three levels, organ, plant and crop. Some traits are influenced by source-sink relationships, an increase of assimilation in healthy tissues and by crop architecture (Ney et al. 2013). Once the losses are a result of several traits that can be evaluated in a plant-based phenotyping, the use of multivariate diallel and multivariate approaches may be an alternative as a breeding strategy to obtain tolerant genotypes to SBR. The identification of promising crosses and parents with high general combining ability and traits related with tolerance to SBR are important for plant breeding, since in this way the breeder can define the best breeding strategy.

Besides the importance of this disease, multivariate approach for soybean tolerance to rust still have not been applied. In this context, the aim of this study was to define a

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breeding strategy applying uni and multivariate approaches for diallel analyzes in early generation trials and contrasting disease conditions. Thus, assessing important genetic parameters for plant breeding to identify traits related to greater tolerance to soybean rust.

MATERIAL AND METHODS

Study location and genetic material

The experiments were carried out in Piracicaba-SP (22º 41' S; 47º 39' W; 546 m alt). The meteorological data are described in Supplementary File 1. The evaluations of rust symptoms were performed in all plots during the reproductive period of the crop, according to the canopy severity ratings, ranging from 1 to 5, 1 indicating a dark green canopy and 5 indicating noticeable yellowing throughout the canopy (Monteros et al. 2007; Harris et al. 2015).

The crosses evaluated in the F_2 generation were originated from a North Carolina design II scheme. The first group of parents consisted of 4 elite commercial cultivars and the second was 10 experimental lines selected for rust tolerance, composing the second cycle of a recurrent selection program established in 2007 (see Supplementary File 2).

Rust tolerance assessment

The experimental design was a randomized complete block with 4 replications. Each block was filled with the 40 single crosses. The sowing density was 40 seeds per single row plot (2 m × 0.5 m). The date of sowing and fungicide applications are available on the Supplementary File 1.

In order to evaluate the tolerance to SBR, the experiments were replicated in two fungicide management. In the first (RC) we controlled the rust and the late season leaf diseases, with three preventive applications of fluxapiroxade and pyraclostrobin at a dose of 58.45 + 116.55 g a.i.$\cdot$ha^{-1}, respectively. For the second management, we controlled for late season leaf diseases, except Asian rust (NRC), with three preventive applications of carbendazim at a dose of 300 g a.i.$\cdot$ha^{-1}, respectively. According to previous results, this last fungicide has no apparent effect on SBR at field conditions (Jarvie and Shanahan 2009; Araujo and Vello 2010).

Phenotyping of morphoagronomic traits

Using the scale proposed by Fehr and Caviness (1977), we estimated, in days, the period of emergence to beginning of seed development (R5), period of grain filling (beginning of seed development to beginning of maturity, GF), and the cycle (sowing to full maturity, R8). During the reproductive period, at 108 days after sowing, we evaluated the following traits by means of scores: plant architecture (1-less favorable to 5-ideal plant architecture, the ideotype considered was to be the one with the highest number of branches and with lower angulation, PA) and leaflet area estimation (1 – small to 5 – large leaflet area, LA).

At the physiological maturity stage, the following traits were measured: plant height (cm, PH); lodging (1 – all plants upright to 5 – all plants prostrate, Lo), and agronomic value (1 – less adequate to 5 – suitable plants, AV). This index is a comparative scale that gathers a series of subjective traits, such as plant architecture, plant height, vigor and number of pods. The biomass of each plot was collected, packed in bags and stored in the laboratory with air circulation until drying. Later, the material was weighed and threshed. Seed yield (kg$\cdot$ha^{-1}, SY) was estimated from the harvested material and the apparent harvest index (HI) was calculated as the ratio of seed yield over total biomass. Subsequently, 100 seeds weight (g, HSW) was determined. To attend the assumption analysis of variance, the Lo, PA and LA data were transformed to $(x)^{0.5}$.

Univariate diallel analysis

Genetic analyses were performed to estimate general combining ability (GCA), specific combining ability (SCA) and their interaction effect with fungicide management. The methodology was proposed by Griffing (1956), Model IV, adapted for partial diallel (Dhillon 1978; Vencovsky and Barriga 1992), according to the following model:

$$Y_{ijkl}= \mu+ b_{ij}+g\,I_k+ g\,II_l + S_{kl}+ f_j+ gI_k f_j+gII_l f_j+S_{kl}F_j+e_{ijkl}$$

In this model, Y_{ijkl} is the observed value relative of the klth hybrid at the ith block of the jth experiment; μ is the constant; b_{ij} is the effect of the block (i = 4); gI_k is the GCA effect for the parents of group I (k = 4), e gII_l is the GCA for the parents of group II (l = 10), S_{kl} is the SCA effect for F_2 hybrid, f_i is the effect of fungicide management (j = 2) and e_{ijkl} is the experimental error, with mean 0, normally distributed and variance σ^2. All the effects were considered fixed, except for the error.

The quadratic components for GCA and SCA were calculated using the methods of moments, according to Torres and Geraldi (2007), and are shown in the follow equations:

$$\Phi GCA_{gI}=\frac{MS_{GCAgI}-MS_E}{LJI}$$

$$\Phi GCA_{gII}=\frac{MS_{GCAgII}-MS_E}{KJI}$$

$$\Phi SCA_{Skl}=\frac{MS_{SCA}-MS_E}{JI}$$

where L is the number of parents of group I, K is the number of parents of group II, J is the number of repetition and I is the number of experiments. MS_{GCAgI}, MS_{GCAgII} and MS_{SCA} are the mean squares of GCA effects of groups I and II and SCA effects, respectively. With the quadratic components for GCA and SCA, we obtained the Baker Ratio (Baker 1978) that reveals an estimative of the additive effects regarding the genetic effects:

$$\text{Baker Ratio}=\frac{\Phi GCA_{gI}+\Phi GCA_{gII}}{\Phi GCA_{gI}+\Phi GCA_{gII}+\Phi SCA_{Skl}}$$

Correlation between traits

Traits were combined in pairs for the estimation of the Pearson's correlation coefficient in each of the fungicides management. With the correlation matrix, we calculated the variance inflation factors (VIF) and traits with VIF higher than 10 were excluded for the path analysis. Seed Yield (SY) was used as dependent variable and R8, R5, Lo, PA, LA, AV, HSW, HI and GF as explanatory.

Multivariate diallel analysis

For the multivariate diallel analysis (Ledo et al. 2003), traits were divided into two groups, adaptive and reproductive. The adaptive group consisted of R8, R5, Lo, PA, LA, AV and PH and the reproductive group by SY, HSW, HI, and GF. To simplify, we considered separate managements. The multivariate analysis of variance (MANOVA) test was utilized to verify the significance of the genetic effects, using Wilks test and the F approximation (Harris 2001). With the mean values of each genotype in each fungicide management, we performed a Principal Component Analysis (PCA), determining a minimum 70% of representation. Pearson's correlation coefficient was estimated between each principal component and the traits. Finally, the effects of GCA I, GCA II and SCA for the scores from each principal component and genotypic were estimated. The statistical analyses were performed using the software Genes (Cruz 2013) and R language (R Core Team 2016).

RESULTS

Univariate diallel analysis

No significant effects of fungicide management were observed on R5, PA, Lo, and AV, meaning that they were not influenced by the presence of the disease (Fig. 1). On the other hand, LA, PH, R8, GF, HI, HSW and SY showed significant effects for fungicide management. The highest reductions (losses) caused by rust occurred for SY (37.26%), HSW (30.85%), GF (14.80%) and HI (8.78%). The effect of GCA I and II were significant for almost all evaluated traits and significant SCA was only observed for R8, GF, HI, and HSW. The interactions between fungicide management and GCA I and II were significant for R8 and HSW, and the interaction of SCA with management was significant only for R8 (Fig. 1).

Analyses of the quadratic components for GCA and SCA revealed Baker Ratio higher than 0.75, showing a predominance of additive effects for SY, PH, PA, HSW, and R5 (Table 1). Non-additive effects were important mainly for R8, GF, and HI, with Baker Ratio of 0.495, 0.540, and 0.606, respectively.

Correlations between traits

Correlations between the 11 traits in the management of each fungicide are shown in Fig. 2. There was a differentiated response in each fungicide management. For RC, there were no significant correlations between HSW and the other traits. However, in NRC management, HSW presented high negative correlations with R5 and R8 (-0.45 and -0.42, respectively) and positive correlations with AV (0.45). For SY in RC management, there was a significant correlation only with AV (0.50). However, when in contact with rust, SY presented significant correlations with HI, R8 and AV

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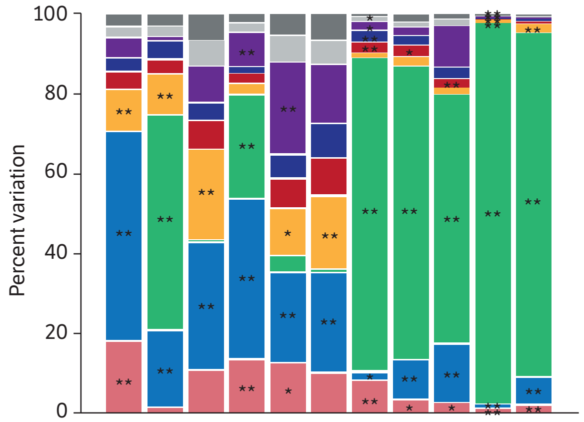
(0.38, -0.38 and 0.55, respectively). The HI showed negative correlations with R5, PH, and R8 for both management as well as GF has with R5.

The direct effects of each trait on seed yield can be observed through path analysis (Fig. 3). The coefficient of determination of each path analysis was 0.414 and 0.536 for management of the RC and NRC, respectively. Differential response between managements was observed. For RC management, the greatest direct effects occurred for AV and Lo (0.606 and 0.332, respectively). However, for NRC management, effects occur for AV, Lo, PH, R8 and PA (0.697, 0.584, -0.423, -0.287 and 0.242, respectively)

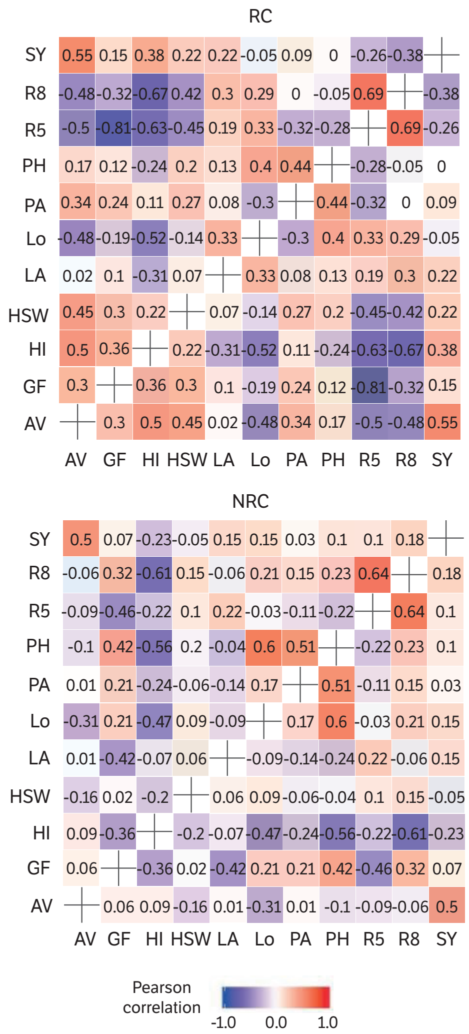


Traits	R5	LA	PA	R5	Lo	AV	R8	GF	HI	HWS	SV
RC Mean	93.63	3.46	3.92	98.1	1.56	3.33	134.85	32.13	0.41	15.67	4619.58
NRC Mean	93.79	3.79	3.89	92.22	1.51	3.32	130.34	27.99	0.37	11.97	3365.51
C.V (%)	6.74	9.73	10.32	16.09	20.16	14.53	2.76	19.42	10.54	8.59	24.48

🟥 GCA I 🟦 GCA II 🟩 F 🟧 B:F 🟥 SCA
🟦 GCA I * F 🟪 GCA II * F ⬜ SCA * F ⬛ Error

The total observed phenotypic variation is decomposed into the following elements: GCA I: general combining ability (genotypes from group one); GCA II: general combining ability (genotypes from group two); F: fungicide management, i.e. with and without rust presence; B:F: blocks within fungicide; SCA: specific combining ability; the interactions GCA I*F, GCA II*F, SCA*F; and the error term. C.V.: coefficient of variation. **, *: Significant at 1% and 5% of error probability by F-test.

Figure 1. Sources of variation for number of days to R5 (R5), leaflet area estimation (LA), plant architecture (PA), plant height (PH), lodging (Lo), agronomic value (AV), number of days from VE to R8 (R8), period of grain filling (GF), harvest index (HI), 100 seeds weight (HSW) and seed yield (SY), measured in F_2 populations from a North Carolina design II. RC mean and NRC mean indicate the average values of traits in the management with control for rust and late season leaf diseases and for late season leaf diseases, except Asian rust, respectively.



RC

	AV	GF	HI	HSW	LA	Lo	PA	PH	R5	R8	SY
SY	0.55	0.15	0.38	0.22	0.22	-0.05	0.09	0	-0.26	-0.38	
R8	-0.48	-0.32	-0.67	0.42	0.3	0.29	0	-0.05	0.69		-0.38
R5	-0.5	-0.81	-0.63	-0.45	0.19	0.33	-0.32	-0.28		0.69	-0.26
PH	0.17	0.12	-0.24	0.2	0.13	0.4	0.44		-0.28	-0.05	0
PA	0.34	0.24	0.11	0.27	0.08	-0.3		0.44	-0.32	0	0.09
Lo	-0.48	-0.19	-0.52	-0.14	0.33		-0.3	0.4	0.33	0.29	-0.05
LA	0.02	0.1	-0.31	0.07		0.33	0.08	0.13	0.19	0.3	0.22
HSW	0.45	0.3	0.22		0.07	-0.14	0.27	0.2	-0.45	-0.42	0.22
HI	0.5	0.36		0.22	-0.31	-0.52	0.11	-0.24	-0.63	-0.67	0.38
GF	0.3		0.36	0.3	0.1	-0.19	0.24	0.12	-0.81	-0.32	0.15
AV		0.3	0.5	0.45	0.02	-0.48	0.34	0.17	-0.5	-0.48	0.55

NRC

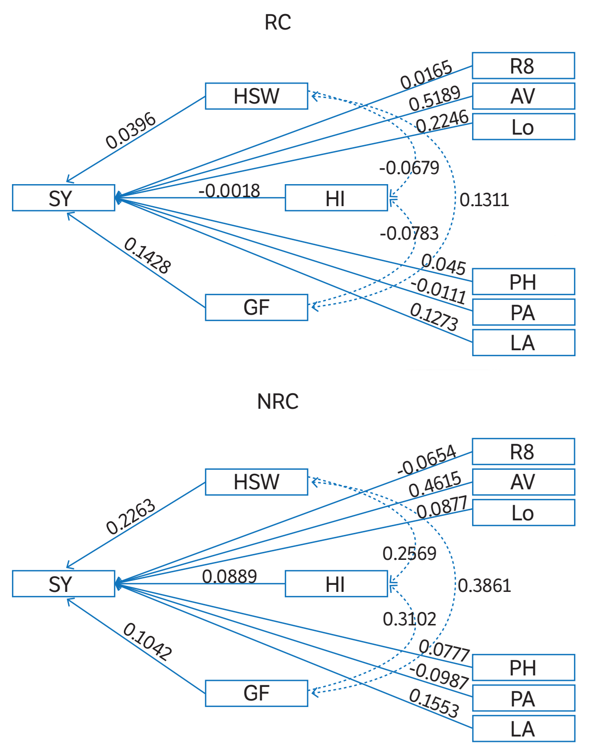
	AV	GF	HI	HSW	LA	Lo	PA	PH	R5	R8	SY
SY	0.5	0.07	-0.23	-0.05	0.15	0.15	0.03	0.1	0.1	0.18	
R8	-0.06	0.32	-0.61	0.15	-0.06	0.21	0.15	0.23	0.64		0.18
R5	-0.09	-0.46	-0.22	0.1	0.22	-0.03	-0.11	-0.22		0.64	0.1
PH	-0.1	0.42	-0.56	0.2	-0.04	0.6	0.51		-0.22	0.23	0.1
PA	0.01	0.21	-0.24	-0.06	-0.14	0.17		0.51	-0.11	0.15	0.03
Lo	-0.31	0.21	-0.47	0.09	-0.09		0.17	0.6	-0.03	0.21	0.15
LA	0.01	-0.42	-0.07	0.06		-0.09	-0.14	-0.24	0.22	-0.06	0.15
HSW	-0.16	0.02	-0.2		0.06	0.09	-0.06	-0.04	0.1	0.15	-0.05
HI	0.09	-0.36		-0.2	-0.07	-0.47	-0.24	-0.56	-0.22	-0.61	-0.23
GF	0.06		-0.36	0.02	-0.42	0.21	0.21	0.42	-0.46	0.32	0.07
AV		0.06	0.09	-0.16	0.01	-0.31	0.01	-0.1	-0.09	-0.06	0.5

The evaluated traits were: number of days from VE to R8 (R8); days to R5 (R5); period of grain filling (GF), plant architecture (PA), leaflet area estimation (LA), plant height (PH); lodging (Lo), agronomic value (AV), seed yield (SY), 100 seeds weight (HSW) and harvest index (HI). Correlation values above 0.30 and below -0.30 are significant by the t-test.

Figure 2. Pearson correlation coefficients between the traits evaluated in the management with control for rust and late season leaf diseases (RC) and for late season leaf diseases, except Asian rust (NRC).

Table 1. Quadratic components estimates for general (ϕGCAgI and ϕGCAgII) and specific (ϕSCA) combining ability for number of days from VE to R8 (R8); days to R5 (R5); period of grain filling (GF), plant architecture (PA), leaflet area estimation (LA), plant height (PH); lodging (Lo), agronomic value (AV), seed yield (SY), 100 seeds weight (HSW) and harvest index (HI), measured in F_2 crosses from a North Carolina design II.

	R5	LA	PA	PH	Lo	AV	R8	GF	HI	HSW	SY
GCAgI	5.881	-4.41E-04	8.55E-04	38.156	5.33E-03	4.17E-03	4.962	0.990	6.97E-05	0.493	66125.7813
GCAgII	7.787	2.42E-03	2.03E-03	50.662	4.94E-03	8.52E-03	0.403	1.941	2.29E-04	0.118	120680.363
SCA	2.300	1.28E-03	5.33E-04	1.750	5.73E-03	1.38E-02	5.462	2.487	1.94E-04	0.1125	20414.875
Baker Ratio	0.855	0.608	0.844	0.980	0.641	0.478	0.495	0.540	0.606	0.844	0.901



Number of days from VE to R8 (R8); days to R5 (R5); period of grain filling (GF), plant architecture (PA), leaflet area estimation (LA), plant height (PH); lodging (Lo), agronomic value (AV), seed yield (SY), 100 seeds weight (HSW) and harvest index (HI).

Figure 3. Graphic representation of the path model showing the association between SY and the direct effects of the adaptive and reproductive traits evaluated in the fungicide management with control for rust and late season leaf diseases (RC) and for late season leaf diseases, except Asian rust (NRC). The unidirectional arrow indicates the direct effects of each explanatory variable on SY, while the bidirectional arrows and dotted line indicate the correlation between two explanatory variables.

Multivariate diallel analysis

Multivariate diallel variance analyses are presented in Table 2 for management RC and NRC, individually. For the management RC, additive (GCA I and GCA II) and non-additive (SCA) effects were significant for adaptive and reproductive traits. For NRC, the additive effects (GCA I and GCA II) for the adaptive and only GCA II for the reproductive traits were significant.

For management RC, the first principal component (PCA1a) was associated with height and lodging values, while PCA2a was associated with negative values of R5 and PCA 3a with low levels of agronomic value and plant architecture (Table 3). Considering this, the desirable ideotype would be genotypes with negative values of PCA1a and PCA3a and positive values of PCA2a. The best-performing parents in

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Table 3. Pearson correlations between traits and principal components (PCA) for management with control for rust and late season leaf diseases (RC) and management for late season leaf diseases, except Asian rust (NRC). The adaptive traits were: number of days from VE to R8 (R8), days to R5 (R5), plant architecture (PA), leaflet area estimation (LA), plant height (PH), lodged (Lo) and agronomic value (AV). The reproductive traits were: period of grain filling (GF), seed yield (SY), 100 seeds weight (HSW) and harvest index (HI).

Trait	RC			NRC		
	PCA 1a	PCA2a	PCA3a	PCA1a'	PCA 2a'	PCA3a'
	Adaptive			Adaptive		
LA	-0.37	-0.3	0.26	0.32	-0.55	0.3
R5	-0.09	-0.93	-0.11	0.84	0.12	0.28
PA	0.62	0.14	-0.42	-0.48	-0.55	0.5
PH	0.89	0.15	-0.08	-0.19	-0.86	-0.3
AV	-0.3	0.2	-0.79	-0.78	-0.13	0.21
Lo	0.76	-0.07	0.39	0.63	-0.49	-0.55
R8	0.38	-0.8	-0.29	0.76	-0.19	0.48
C.P. (%)	30.58	54.65	70.63	37.85	61.42	76.76
Trait	PCA1r	PCA2r	PCA3r	PCA1r'	PCA2r'	PCA3r'
	Reproductive			Reproductive		
GF	-0.69	-0.06	0.61	-0.67	0.48	-0.42
HI	0.84	-0.07	0.02	-0.76	-0.24	-0.35
SY	-0.46	-0.59	-0.61	-0.64	-0.64	0.24
HSW	-0.31	0.83	-0.39	-0.62	0.43	0.64
C.P. (%)	37.46	63.68	86.35	45.51	67.66	86.72

C.P. = Cumulative proportion.

Table 2. Summary of multivariate North Carolina design II diallel analysis for management with control for rust and late season leaf diseases (RC) and management for late season leaf diseases, except Asian rust (NRC). The adaptive traits were: number of days from VE to R8 (R8), days to R5 (R5), plant architecture (PA), leaflet area estimation (LA), plant height (PH), lodged (Lo) and agronomic value (AV). The reproductive traits were: period of grain filling (GF), seed yield (SY), 100 seeds weight (HSW) and harvest index (HI).

S.V.	Df	RC						NRC					
		Adaptive			Reproductive			Adaptive			Reproductive		
		Wilks	Pr (>F)		Wilks	Pr (>F)		Wilks	Pr (>F)		Wilks	Pr (>F)	
GCA I	9	0.254	0	**	0.48	0	**	0.395	0	**	0.676	0.111	ns
GCA II	3	0.475	0	**	0.53	0	**	0.399	0	**	0.707	0	**
B	3	0.693	0.004	**	0.749	0.001	**	0.631	0	**	0.757	0.001	**
SCA	27	0.144	0.004	**	0.318	0.009	**	0.181	0.092	ns	0.365	0.089	ns
Error	117												

**, *, ^{ns}: Significant at 1%, 5% and nonsignificant, respectively, by approx F.

the first group were L6, L8, L10 and C01 and the crosses C01 × L01e C01 × L03 (Fig. 4).

In the management NRC and the adaptive traits, PCA1a' was associated with late cycle and low agronomic value, PCA2a' with short plants and low leaf area score and PCA3a' with upright plants and with favorable architecture. Based on this, the desirable ideotype would correspond to genotypes with low PCA1a' and high PCA2a' and PCA3a', which correspond to the parents L10, L07 and C04 with the highest contributions to GCA.

For the reproductive traits in management RC, PCA1r was associated with genotypes with a short grain filling period, PCA2r with low productivity and higher PCS and PCA3r with low SY and shorter period grain filling. Consequently, the desirable ideotype would be plants with high PCA1r and low values of PCA2r and PCA3r. The best-performing GCA parents were C03, L08 and L10 and, for SCA, the crossings L03 × C01, L04 × C02, and L08 × C03.

For the reproductive traits, PCA1r' was negatively associated with all traits, PCA2r' with low grain yield and a long period of grain filling and PCA3r' with a heavy weight of one hundred seeds. Based on this, the desirable ideotype would be genotypes with low PCA1r and PCA2r and high PCA3r values. Considering only the GCA II, the C03 was the parent that contributed the most with favorable traits.

In RC, considering short plants and favorable architecture, with early cycle and high agronomic value, the best parents were USP 14-22.006, USP 14-22.008 and USP 14-22.010 and M-SOY 7908 RR and crosses M-SOY 7908 RR × USP 14-22.001 and M-SOY 7908 RR × USP 14-22,003. However, for the selection of plants with high harvest index, productive, with heavier hundred seeds weight (HSW) and longer period

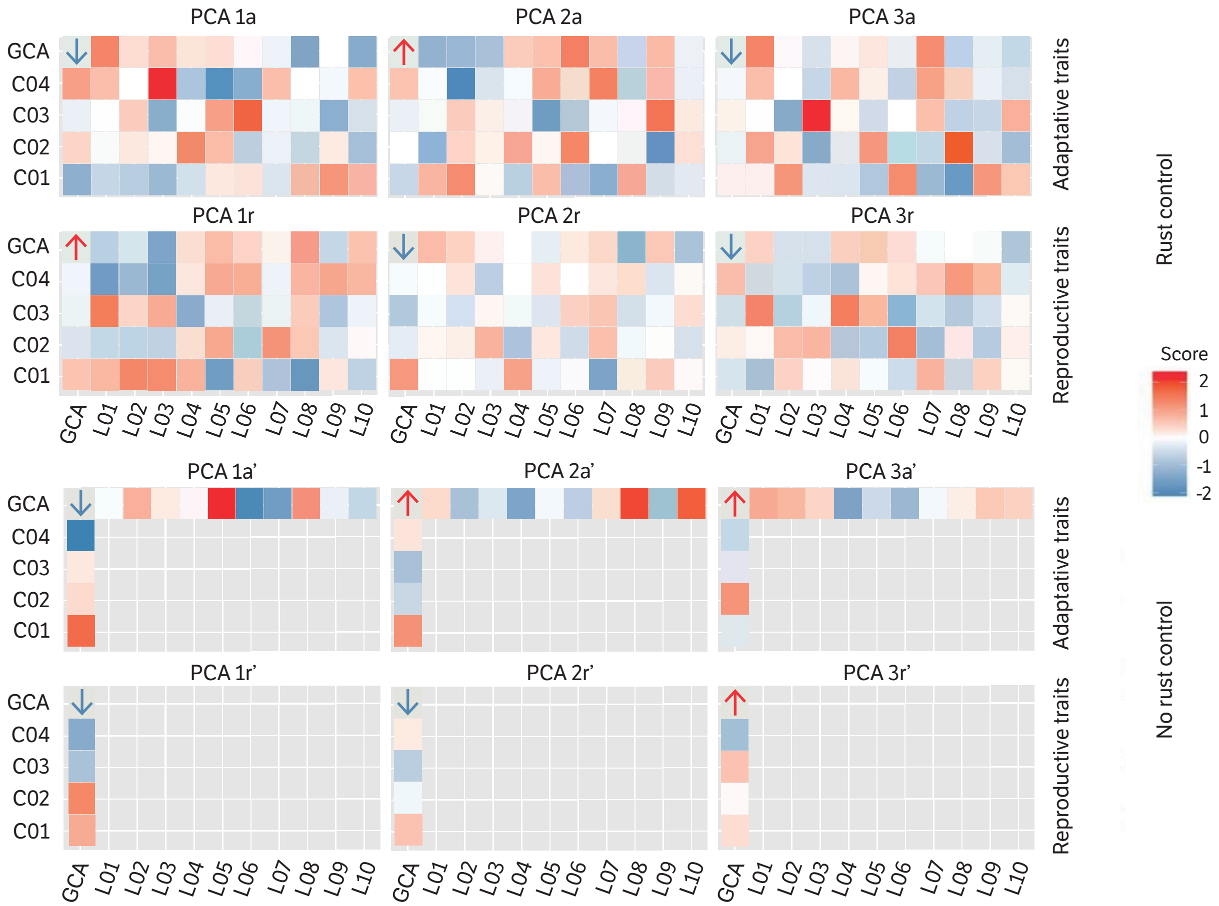

Figure 4. General and specific combining abilities of genotypes obtained by the principal components for adaptive and reproductive traits for fungicide management (A) with control for rust, whereas PCA 1a, PCA 2a, and PCA 3a are for adaptive traits and PCA 1r, PCA 2r and PCA 3r for reproductive traits; (B) with control for late season leaf diseases except rust control (PCA 1a', PCA 2a', PCA 3a' for adaptive traits and PCA 1r', PCA 2r' and PCA 3r' for reproductive traits). The directions of the arrows indicate the sign of the ideotype while the color indicates the magnitude.

grain filling, the best performing parents were V Max RR, USP 14-22.008 and USP 14- 22.010 and the crosses USP 14-22.003 × M-SOY 7908 RR, USP 14-22.004 × AS 7307 RR and USP 14-22.008 × V Max RR.

For NRC, only the effects of GCA were significant. Therefore, the parents USP 14-22.010, USP 14-22.007, and BMX Potência RR provided earlier, shorter and upright plants with favorable architecture. However, the parent V MAX RR provided additive effects for high yield genotypes, with a longer period of grain filling and heavier seeds.

DISCUSSION

The conditions of temperature and precipitation were satisfactory for the crop development (Supplementary File 1). It was possible to observe the progress of the disease as the relative humidity of the air increased, revealing that the environmental conditions were ideal for the pathogen (Godoy et al. 2016).

The mechanisms involved in the reduction of seed yield was also similar to that found in the literature, in consequence of reductions in size and seed mass and harvest index (Kumudini et al. 2008). The nonsignificant effect of fungicide management for R5 is explicated by the fact that the disease just progresses after this period. For PA and Lo, these traits are also defined before the infection period occurs. The use of VA was able to identify differences between treatments within each fungicide management, however, because it was a comparative scale, this was not significant for fungicide management.

Few traits had a significant interaction effect between GCA and SCA and fungicide management. This may be explained due to the origin of the parents from group I. This group originates from the recurrent selection program for rust tolerance, where genotypes are selected for high stability when in contact with the disease. These results were similar to those obtained by Jarvie and Shanahan (2009), who observed low interaction between rust tolerant genotypes and fungicide management.

In self-pollinated crops such as soybeans, it is expected to find a predominance of additive effects for most traits in early generations (Spehar 1999; Mebrahtu and Devine 2008). Tolerance to rust is expected to have a polygenic inheritance and a predominance of additive effects (Ribeiro et al. 2008), which is similar to those found in the present study. Dominant effects were important mainly for cycle-related traits, since few genes with major effect govern these traits, generally with dominant alleles for precocity (Suh et al. 2000; Watanabe et al. 2012).

For both correlation and path analysis, a differentiated response was observed in each management. This indicates that, for the evaluated genotypes, they behaved differently in the presence or absence of the disease. Considering NRC management, negative correlations were observed between Harvest Index and plant height (-0.56) and cycle-related traits (-0.61), implying that high and late plants contribute negatively to the biomass conversion in productivity, suggesting an unbalance in the source and drain relation. Besides that, plants with short period of grain filling tendes to be lower (0.42) and convert more dry matter into grains (-0.36).

Low coefficients of determination in path analysis may be associated with other traits not evaluated in this research, which could have contributed significantly to SY (Carvalho et al. 2002). These traits could be number of nodes on the main stem, pods per unit area and seeds per pod (Pandey and Torrie 1973; Bizeti et al. 2004). In this way, the results should be analyzed cautiously and future studies should be performed to confirm the results.

Our results indicated that agronomic value and seed yield had direct effects and correlation in the same direction and magnitude, implying that this correlation explains the true association between traits (Vencovsky and Barriga 1992). These results indicate that, even if agronomic value is a subjective visual scale, which considers the researcher's experience, indirect selection for rust tolerance was possible. It is important to emphasize that AV did not present significant differences for fungicide management in the analysis of variance but showed significant effects for genotypes (Fig. 1). In this way, AV was efficient in identifying the most productive crosses within each fungicide management, but not between experiments.

The identification of a tolerant genotype is difficult, since its phenotype is very similar to a sensitive one (Newton 2016). However, our results suggest that it is possible to identify tolerant crosses through the use of agronomic value scale. Nogueira et al. (2015) reported that the use of the number of pods is highly associated with grain yield when in contact with rust. Despite its

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accuracy, the assessment of number of pods is time-consuming and not practical in a breeding pipeline.

Low correlation between seed yield and cycle were observed. Similar results to those obtained by Melo et al. (2015), that reported the existence of significant levels of tolerance in late-maturity genotypes under long exposure to the pathogen were found. However, Kawuki et al. (2004), associated early maturing line to tolerant genotypes, since late maturing lines had greater disease severity.

The use of the multivariate diallel was efficient for a more detailed study of the GCA and SCA combinations, together with the possibility of parental selection with the intention of improving certain traits simultaneously. Similar results were obtained in the literature by several authors, proving the efficiency of this type of analysis (Jung et al. 2007; Benin et al. 2009; Teodoro et al. 2017).

CONCLUSIONS

Our research was the first insights in the study in early generation to obtain tolerant genotypes to Asian rust using several approaches. The univariate analysis was able to identify the contributions of the additive and non-additives from the several traits. The use of multivariate analysis allows a better understanding of grain yield components and other traits associated with rust tolerance. The use of visual selection via agronomic value is a useful practice to identify tolerant genotypes. The multivariate diallel analysis allows the identification of the best parents and crosses with ideal GCA and SCA, respectively, considering the ideotype.

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AUTHOR'S CONTRIBUTION

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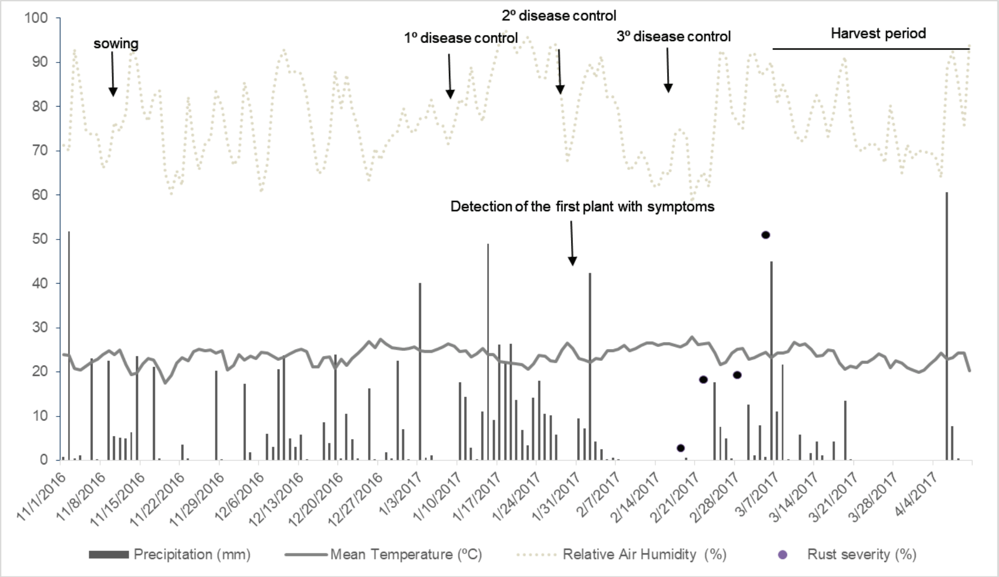
SUPPLEMENTARY FILES

Supplementary 1. Pedigree of the 10 experimental lines and the main characteristics of the four commercial cultivars. The 10 experimental lines originated the first group while the four commercial cultivars composed the second group, corresponding to the partial diallel 10x4.

Lines		Pedigree
USP 14-22.001	L01	[Conquista(6)¹ x (E96-246 x BRSMT Uirapuru)] x (Hartwig x PI 371.611)
USP 14-22.002	L02	[Conquista(6)¹ x (E96-246 x BRSMT Uirapuru)] x [Lamar x (IAC-6 x UFV-4)]
USP 14-22.003	L03	[Conquista(6)¹ x (E96-246 x BRSMT Uirapuru)] x [(IAC-Foscarin-31 x Forrest) x (Foster x FT 79-3408)]
USP 14-22.004	L04	[Conquista(6)¹ x (E96-246 x BRSMT Uirapuru)] x [(Primavera x Forrest) x (Kirby x FT-2)]
USP 14-22.005	L05	[Conquista(6)¹ x (E96-246 x BRSMT Uirapuru)] x (Soc. 81-76 x Foster) x Hartwig
USP 14-22.006	L06	(BRS 133(6)¹ x E96-246) x (Hartwig x PI 371.611)
USP 14-22.007	L07	(BRS 133(6)¹ x E96-246) x [Lamar x (IAC-6 x UFV-4)]
USP 14-22.008	L08	(BRS 133(6)¹ x E96-246) x [(IAC-Foscarin-31 x Forrest) x (Foster x FT 79-3408)]
USP 14-22.009	L09	(BRS 133(6)¹ x E96-246) x [(Primavera x Forrest) x (Kirby x FT-2)]
USP 14-22.010	L10	(BRS 133(6)¹ x E96-246) x [(SOC 81-76 x Foster) x Hartwig]

Cultivar		Characteristics				
		M.G.	G.H.	P.H.	Lodged	Note
MSOY 7908 RR	C03	7.9	Determinate	0,75	Moderately resistant	Resistance to powdery mildew, stem canker, Frogeye leaf spot and root-knot nematodes
AS 7307 RR	C01	7.3	Indeterminate	0,90	Resistant	Resistance to root-knot nematodes
V MAX RR	C04	6.2	Indeterminate	1,10	Resistant	Resistance to Cyst Nematode, stem canker, and Frogeye leaf spot
BMX POTÊNCIA RR	C02	6.7	Indeterminate	0,95	Resistant	Resistance to stem canker

¹conduction of six backcrosses for RR gene incorporation; M.G.. Maturity Groups; G.H. Growth habit; P.H. Plant height (m).



Supplementary 2. The X-axis corresponds to the timeline of the experiment, the dashed lines indicate the relative humidity of the air (%), the continuous lines refers to the average temperature (ºC) and the bars corresponds to the accumulated precipitation of the day. The dots indicate the amount of symptoms observed in the experiment (%).