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#### Introduction

Asian Soybean Rust (ASR) is the major disease affecting soybean production in Brazil. In spite of existing alternative options for its control, fungicide remains the leading strategy. Genetic resistance has been modestly used to cope with ASR while researches on tolerance continue scarce. Tolerance is defined as the host's ability to maintain fitness regardless of the pathogen load (Fig.1) [1, 2]. Here, we investigate the tolerance as a strategy to sustain fitness (i.e., grain yield) in the presence of ASR using germplasm from a recurrent selection program. Therefore, this research aimed to understand the important features of this defense mechanism, how the disease affects important traits and the relationship among them and discuss the applications of tolerance for soybean breeding.

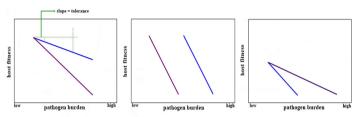


Fig.1 Reaction norms.

## **Material** and Methods

The trials included 768  $\rm F_{5:6}$  lines and were carried out in 2016/17 crop season in two ASR conditions (Fig.2).

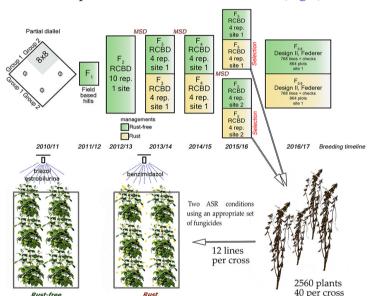


Fig. 2 Breeding workflow.

Table	1. Traits.		$\begin{bmatrix} a_{11} & a_{22} & a_{13} & \cdots & a_{1n} \\ a_{21} & a_{22} & a_{23} & \cdots & a_{2n} \end{bmatrix}$	**************************************
SY	Seed yield		a <sub>11</sub> a <sub>22</sub> a <sub>23</sub> a <sub>34</sub>	Constitution of the second
AV	Agronomic value		a <sub>n1</sub> a <sub>n2</sub> a <sub>n3</sub> a <sub>nn</sub>	9 9 9 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
HSW	hundred seed weight	AS PL	- 1 44	
Cycle	days from emergency to R8	CS L		
SF	Seed filing period	O W		
DaysR7	days from emergency to R7			
PH	plant height		1 1	
LOD	Lodging	. —		21
AS	Seed area	1		SASSON .
PL	perimeter length			
L	seed length		0.55550	Airmann Co
W	seed width		000000	
LWR	length-to-width ratio	ll .		
CS	circularity		Fig. 3 High-throu	ghput phenotyping o
DS	Distance IS and CG	II.	seed shape [3]	

The raw data was analyzed using the following spatial model [4]:

$$y = f(u, v) + X_b \beta_b + X_\tau \tau_\tau + Z_g c_g + Z_r c_r + Z_c c_c + \varepsilon$$

### Rust symptoms

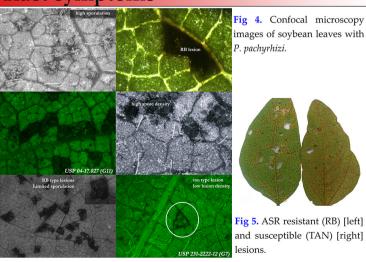


Fig 6. Field view

### Results and Discussion

Table 2. Effective dimensions associated with the spatial trend, the row and column random factors, the genetic random factor, and the residuals.

Trait	Effective dimensions $(ED_k)$											
	Additive trends		Interaction tre			Total	Random effects		- Total	N	$H_g^2$	
	$f_u(u)\dagger$	$f_v(v)$	$vh_u(u)$	$uh_v(v)$	$f_{u,v}(u,v)$	Total	$c_r$	$c_c$	$c_g$	Total		
General tra												
$\operatorname{SY} \P$	0.01	1.9	5.2	0.0	5.2	12.3	34.1	10.9	340.2	397.5	753	0.4
	$0.0^{2}$	3.0	1.3	1.4	2.7	8.4	30.4	13.8	205.8	258.4	765	0.2
	0.0	5.0	0.0	0.0	0.0	5.0	24.6	10.5	361.8	401.9	761	0.4
AV	0.9	5.2	2.7	3.2	6.0	18.0	20.4	3.3	335.3	377.0	763	0.4
LICW	0.0	5.2	1.2	1.9	3.1	11.4	2.7	0.0	650.0	664.1	760	0.8
HSW	2.5	0.0	2.4	0.0	2.4	7.3	8.1	8.8	633.8	658	761	0.83
0.1	1.2	3.1	0.0	0.0	0.0	4.3	12.6	2.4	653.4	662.7	761	0.80
Cycle	1.9	2.2	8.6	8.0	16.6	37.3	6.4	13.3	589.9	646.9	763	0.77
SF	0.0	3.3	0.9	3.0	3.8	11.0	9.0	9.2	590.1	619.3	760	0.78
SF	1.8	5.6	5.5	6.2	11.6	30.7	8.7	10.5	381.5	431.4	763	0.50
Days -R7-	0.0	2.9	2.1	2.7	4.9	12.6	14.5	10.2	644.2	681.5	762	0.8
	1.0	0.0	7.3	6.8	14.2	29.3	0.0	14.6	597.1	641	763	0.7
PH	1.9	6.8	0.2	1.9	2.1	12.9	4.0	4.9	332.7	354.5	761	0.4
PH	1.5	6.8	5.9	5.7	11.6	31.5	0.0	8.7	356.6	396.8	763	0.4
LOD	0.8	5.3	1.7	3.8	5.5	17.1	16.1	11.7	362.4	407.3	761	0.4
LOD	1.7	6.3	7.5	7.1	14.7	37.3	0.0	7.6	438.2	483.1	763	0.5
High-throu	ghput pheno.											
AS	0.0	3.3	0.0	0.6	0.6	4.3	0.3	7.8	605.1	617.7	760	0.80
110	0.0	0.8	3.2	3.4	6.5	13.9	2.4	12.7	605.1	634.1	763	0.79
$\operatorname{PL}$	0.0	3.0	0.0	0.5	0.5	4	0.9	7.7	585.2	597.8	760	0.7
	0.0	0.7	3.6	3.8	7.4	15.5	2.2	13.5	579.4	610.6	763	0.7
L	0.0	3.0	0.0	0.0	0.0	3.0	2.5	7.1	608.2	620.8	760	0.8
	0.0	0.5	4.2	4.2	8.4	17.3	0.0	14.4	581.3	613	763	0.7
W	0.0	3.6	0.2	1.8	2.0	7.6	0.0	9.9	557.4	574.9	760	0.7
	0.0	1.3	2.6	3.5	6.1	13.7	9.0	14.2	563.3	600.0	763	0.7
LWR	0.7	2.6	3.3	4.8	8.1	19.5	2.2	6.7	605.4	633.8	760	0.8
	0.0	4.2	9.6	8.6	18.2	40.6	0.0	13.6	491.7	545.9	763	0.6
CS	2.1	4.0	5.1	5.2	10.3	26.7	0.0	14.0	166.2	206.9	760	0.2
	0.0	2.0	16.9	12.0	28.9	59.8	0.0	17.4	251.6	328.8	763	0.33
DS	0.8	0.0	2.7	3.2	5.9	12.6	4.3	12.4	376.3	405.6	760	0.5
	1.3	4.2	2.2	1.4	3.6	12.7	0.0	6.6	443.0	462.3	763	0.5

The smooth gradient across both field trials captured variations due to what seems to be irregular fertility (Fig. 7). Do not account for spatial variability could lead to underestimates of heritability, affecting selection response and genetic gain [4, 5].

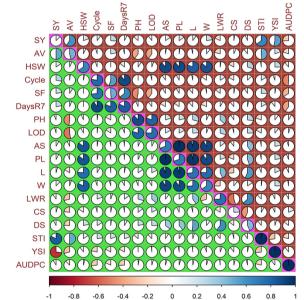


Fig. 8 Genetic correlation (Pearson' method). Matrix pairwise genetic correlations in the rust (upper half, red) and rust-free (lower half, green) environments. The diagonal (pink background) depicts the correlation between vectors of the same trait but evaluated in contrasting rust conditions.

We found a negative correlation between tolerance and fitness in the absence of the pathogen, which implies "allocation costs" (Fig. 9). In this scenario, the biological costs to sustain tolerance may not be translated into increased fitness in the absence of ASR.

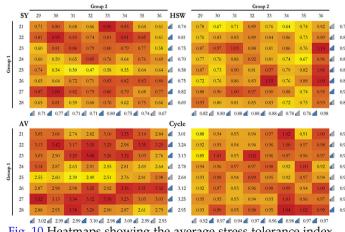


Fig. 10 Heatmaps showing the average stress tolerance index (STI) for four traits. Each cell in the heatmap represents the average performance of 12 lines. STI is calculated as in the following equation:  $STI = (Y_s \times Y_{ns})/(\bar{Y}_{ns})^2$ 

The spatial variation was more intense in the ASR trial, requiring more parameters to model the field trend and the generalized heritability was negatively impacted in 10 out of 15 traits, compared to the ASR-free trial (Table 2). The genetic signal ( $c_{\circ}$ ) was the main source of variation across the field layout in both rust conditions. Effective dimension of genotype was 1.7x lower in the ASR condition.

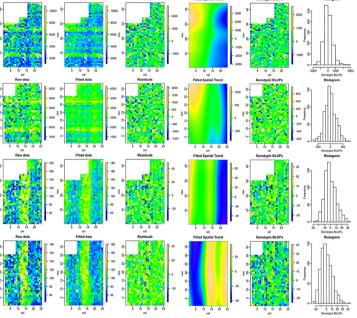


Fig. 7 Raw yield data, fitted values (including all terms, fixed and random), residuals' spatial plot, fitted spatial trend and genotypic BLUPs. First and second row are results for SY in ASR-free and ASR conditions, respectively. Third and fourth rows are results for PH for ASR-free and ASR conditions, respectively.

There was a high correlation between SY and AV in both ASR environments [o≥0.01] (Fig. 8). SF was also correlated with SY in both ASR conditions [o≥0.01]. In theory, no differential disease symptoms and severity can be visualized between tolerant and susceptible genotypes [1]. However, correlations between AUDPC with SY, Cycle, SF, DaysR7 suggests that selection under stress added quantitative resistance in the selected inbreds.

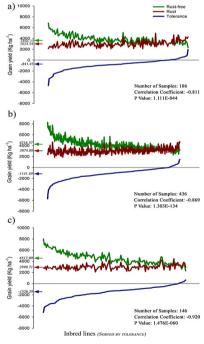


Fig. 9 Correlation between tolerance to ASR and fitness in the non-stressed environment. al 186 lines with less than 130 days; b | 426 lines with growing cycle between 130 and 140 days; and c | 146 lines with more than 140 days.

Despite the evidence of allocation costs, sufficient genetic variability was found, allowing the selection of agronomic superior lines even under high pathogen burden. Parents 22, 27, 30, and 33 are good combiners in terms of increasing tolerance with high performance in the non-stressed environment (Fig. 10).

Subsequent studies should address marker information to obtain a further understanding of the genetic architecture of tolerance to ASR.

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