Stress-driven responses to Asian rust in soybean genotypes: Depicting the concepts of static and dynamic tolerance

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10° CBMP
CONGRESSO BRASILEIRO DE MELHORAMENTO DE PLANTAS
AGUAS DE LINDOIA/SP | 2019

1. Introduction

Asian Soybean Rust (ASR) is a serious disease for soybean production worldwide, particularly in South America. The biotic stress is caused by the biotrophic fungus *Phakopsora pachyrhizi*. The potential to reduce soybean yield is large and calculated to be approximately 33.20±5.90% across Brazillian crop regions (calculations were done using data from trials of the Brazilian network for ASR chemical control for the last five crop seasons [1]).

In spite of several control strategies being released over the years, none of them appeared to be highly effective at a reasonable cost. Hence, new approaches must be investigated to improve the arsenal used to deal with ASR. Therefore, we studied the genetic tolerance, which is the ability of a given genotype to sustain yield or quality in the presence of a stress agent (Fig. 1). As we go further in our discussion, readers will be able to see the differences underling the dynamic and static tolerance, both conceptually (Fig. 2) and through results (see R&D session).
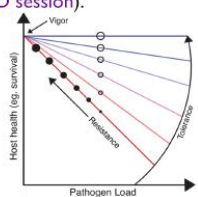
Fig. 1 Overview of host tolerance and resistance measurement [2]. Tolerance is the slope of the curve of host health over pathogen load.
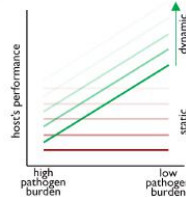
Fig. 2 A graphical depiction of the reaction norm showing the difference between static and dynamic tolerance.

2. Material and methods

Sixteen soybean elite lines were tested in eight RCBD trials with three replicates carried out across three crop seasons and two locations, yielding 384 plots. Each combination of location x year was considered a unique environment. Two identical and adjacent trials were carried out within each environment, contrasting only for the ASR occurrence (Fig. 3).

Fig. 3 Side-by-side identical RCBD trials contrasting only for ASR occurrence. This condition was achieved by applying different sets of fungicides.

We evaluated a series of agronomic traits and seed shape and size parameters through high-throughput phenotyping (Fig. 4) to measure the impact of ASR on soybean elite lines.
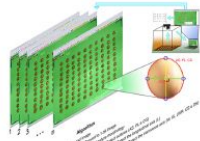
Fig. 4 High-throughput phenotyping strategy of seed shape and size parameters based on RGB imagery. 384 100-seeds samples were photographed and the images were analyzed via SmartGrain [3].

A mixed model was fitted using an unstructured variance/covariance formulation for genotype within environment effects (the **G** matrix) and heterogeneous error variances across environments for residuals effect (the **R** matrix) and block within environment (model 1).

$$y = \mu 1 + Xs + Z_1 sr + Z_2 gs + e \qquad \text{[model 1]}$$

where:

$$gs \sim MVN(0, I_g \otimes G_s) \quad \text{and} \quad G_s = \begin{bmatrix} \sigma^2_{gs1} & \sigma_{gs1gs2} & \sigma_{gs1gs3} & \sigma_{gs1gs4} \\ \sigma_{gs2gs1} & \sigma^2_{gs2} & \sigma_{gs2gs3} & \sigma_{gs2gs4} \\ \sigma_{gs3gs1} & \sigma_{gs3gs2} & \sigma^2_{gs3} & \sigma_{gs3gs4} \\ \sigma_{gs4gs1} & \sigma_{gs4gs2} & \sigma_{gs4gs3} & \sigma^2_{gs4} \end{bmatrix}$$

The stress tolerance index (STI) was used to assess the tolerance of each genotype according to the following expression: $STI = Y_s \times Y_{ns}/\bar{Y}^2_{ns}$

Stressed — Y_s, Non-stressed — Y_{ns}

The statistical analyses were performed using ASReml version 4.0 [4] and RStudio [5].

3. Results and discussion

There were no significant cross-over interactions (Fig. 5 and 9). The opposite scenario could imply the existence of overcompensation [6]. The inbreds g30, g32, g23, g29, and g35 displayed the static type of tolerance whereas the remaining genotypes revealed responsiveness to environment enhancement.
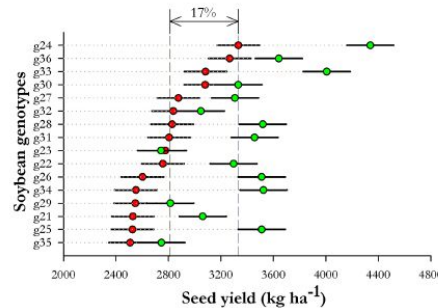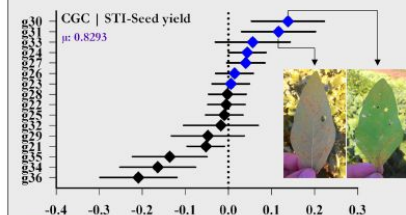
Fig. 5 Caterpillar plot revealing the differential performance of 16 soybean genotypes exposed to contrasting rust stress. Each point depicted in the graph is a BLUP (Best Linear Unbiased Predictor) computed fitting model 1.

Rust symptoms were observed on the leaves of all genotypes. Inbreds g24 (USP 04-17027) and g36 (USP 231-2222-12) yielded as much seed mass per unit of area as the average performance of all 16 genotypes in the stress-free environment (Fig. 5).

The *per se* performance in terms of tolerance is not a good predictor of the genotype's ability to transmit the characteristic to new progenies. Instead, breeders should screen the germplasm to identify the best combiners using an appropriate genetic design (see box 1).

Fig. 6 Funnel plot for Area Under the Disease Progress Curve (AUDPC).

Fig. 7 Caterpillar plot for seed yield stress tolerance index.

Fig. 8 Rust symptoms during grain filling.

Fig. 9.1 Caterpillar plot for 100-seeds weight stress tolerance index.

Box 1. Genotypes were crossed in a partial diallel scheme (8x8) yielding 64 populations. All populations were advanced to F5 generation without selection. Populations in F5 were evaluated in RCBD with four replicates in two locations. A multi-environment partial diallel model with pedigree information was fitted to obtain the breeding values of each parent. The analyses were performed using the ASReml v.4 package.
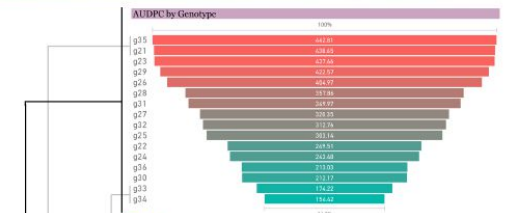Fig. 10 shows the predicted effect of GCA for seed yield stress tolerance index. Parents g30 (USP 04-17.011) and g31 (USP 231-2228-01) were the best ones at generating improved populations for tolerance to ASR. The use of such genotypes substantially increases the odds to select elite inbred lines with dynamic tolerance.
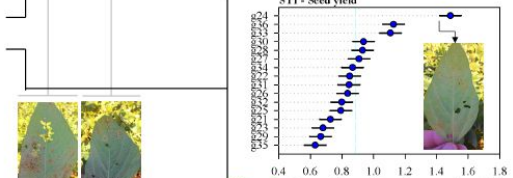
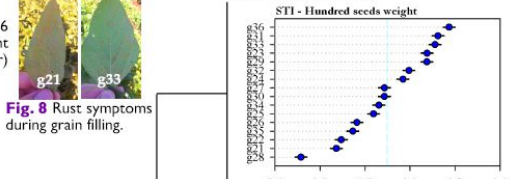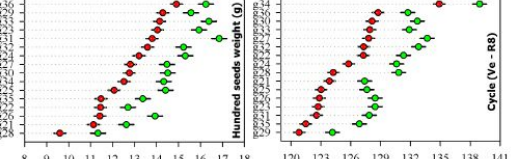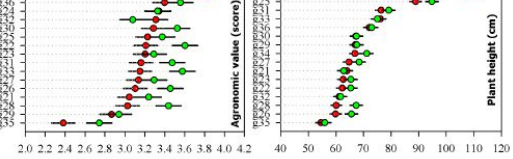**Fig. 10** Predicted general combining ability (GCA) effect of each parent plus standard error.

Fig. 9 Caterpillar plots for soybean traits showing the differential performance of each genotype under two contrasting ASR conditions. Each point depicted in the graphs is a BLUP computed fitting model 1.

It is unlikely that tolerance as any other strategy alone will solve the ASR problem faced by farms. However, this study indicates the possibility to reach high seed yield without spending resources on fungicides to control rust, which is, in fact, important for organic production. Also, the ability of soybean plants to delivery substantial yield under high pathogen burden should be investigated on its molecular bases in future studies.

4. Acknowledgments

5. Bibliography

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