Line selection and correlation between traits of soybean genotypes under high naturally occurring stink bug infestation

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ABSTRACT. The soybean crop is undoubtedly important to not only Brazil but also for most parts of the globe, as economic and social dependency upon this crop becomes evident. However, the scenario of the soybean crop production has faced challenges with environmental changes, which have escalated the incidence of pests. Due to the abundance of stink bugs, especially *Euschistus heros* in tropical regions, they can considerably impact the productivity of the crop compromising total grain yield and seed quality. Therefore, this research was aimed to evaluate soybean lines with desirable agronomic traits under high natural infestation of *Euschistus heros* and to access the genotypic and phenotypic correlations between important agronomic traits for soybean breeding. Twenty-three soybean F8 lines developed by the Soybean Breeding Program of Federal University of Uberlândia and four cultivars (Msoy 8527, UFUS Xavante, Msoy 8787, and UFUS Milionária) were evaluated under randomized complete block design with three repetitions during the growing season of 2015/2016 and 16 agronomic traits were accessed. There was genetic variability for all traits but Asian soybean rust severity at 1% probability level through F test. For all traits in the study but the total number of pods and Asian soybean rust severity, the

coefficient of genotypic determination was superior to 70%, indicating that the most part of phenotypic variability was due to genetic differences among the genotypes in study. Grain yield was demonstrated to be a useful trait for indirect selection of soybean genotypes with resistance to brown stink bugs. Also, early cycle genotypes, heavy seeds and a higher number of pods revealed a positive correlation with grain yield over the same conditions. The lines G1, G2, and G24 are very promising genotypes as they have shown valuable agronomic traits for stink bug resistance.

Key words: *Glycine max*; *Euschistus heros*; Damaged seeds; Breeding

INTRODUCTION

As the second largest worldwide producer of soybeans, the cultivated area in Brazil during the growing season of 2016/2017 was estimated at 33.91 million of hectares and an overall production at 114 million tons of grains (Conab, 2017). The soybean crop reached approximately 56% of the total land occupied with grain crops in Brazil over the same period, because of its versatility, profitability, and huge potential for animal and human feed (Conab, 2017).

Current issues in the productive scale of the crop have been escalated by global warming and climate change that make the agricultural scenario of cropping production even more prone to failure (Nelson et al., 2014). Consequently, raises in abiotic and biotic stresses, such as the higher incidence of bugs can damage the crop by reducing the potential of soybean grain yield, which can lower the overall production to very low levels and pose an additional threat to food security (Bebber et al., 2014).

Breeding programs have a crucial role in developing new cultivars and assessing the potential of soybean lines that show valuable parameters for selection, such as resistance to main pest and diseases of the crop, high standards of grain yield, heavy seeds, efficiency of water and nutrient uptake, among others (Ramalho et al., 2012).

Stink bugs represent a hazard to many crops, especially to soybeans as they can considerably impact the productivity of the crop by the occurrence of stunted plants, empty pods, and foliar retention, which can compromise total grain yield and seed quality (Guedes et al., 2012). Among the soybean stink bug complex, the species *Euschistus heros,* known as the brown stink bug, is one of the most damaging pests (Souza et al., 2017) as it is generally associated with the highest population density among other species of stink bugs; therefore, it has a greater potential to cause injuries to soybeans (Kuss et al., 2012).

Phenotypic and genotypic correlations are indeed important in this scenario as they can establish a helpful association among agronomic traits for breeding programs. Furthermore, since many agronomic traits of interest are of quantitative nature, which is influenced by more than two genes, it can be hard to identify the lines that may stand out basing only on one trait (Cruz et al., 2012).

Those types of correlations can infer a positive or negative linear relationship among two agronomic traits (Cruz et al., 2012). Correlation studies on soybeans, mainly under field conditions with natural infestation of stink bugs, have suggested significant positive correlations among important agronomic traits (Rocha et al., 2014; Moreira, 2015;

Rocha et al., 2015). Consequently, to achieve such approach, identifying lines that have tolerance the attack of stink bug complex, as well as have desirable phenotypic and genotypic correlations is very important for the success of soybean cultivars (Kurasch, et al. 2017).

This research is aimed at selecting soybean lines with desirable agronomic traits under high natural infestation of *Euschistus heros*, evaluating the genotypic and phenotypic correlations between important agronomic traits for soybean breeding, as well as estimating important genetic parameters for the soybean crop.

MATERIAL AND METHODS

The experiment was carried out during the growing season of 2015/2016 in an experimental area located at Capim Branco farm (18º52'S; 48º20'W and 805 m of altitude), belonging to the Federal University of Uberlândia, Uberlândia, Minas Gerais, Brazil. The meteorological data for the experimental field during the vegetation season of the experiment can be seen in Figure 1.
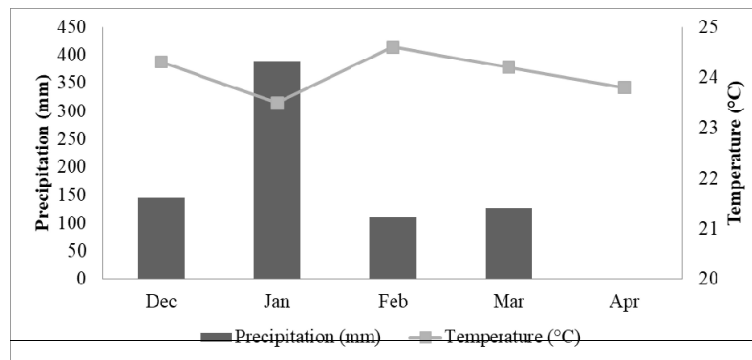
Figure 1. Temperature and rainfall averages at Capim Branco farm in Uberlândia MG, growing season 2015/16, from December 2015 to April 2016. Source: Meteorological station of Capim Branco farm and National Institute of Meteorology (INMET).

Climatic conditions such as the rainfall and temperature were classified as satisfactory during the development of soybeans according to the soybean crop necessity of water and temperature, which depends on the genotype and it varies from 450 to 800 mm of water per cycle with temperatures oscillating between 20°C and 30°C (Empresa Brasileira de Pesquisa Agropecuária - Embrapa, 2014).

The experimental field is situated on a dystrophic Dark Red Latosol. The soil preparation for seeding was done over conventional tillage system with one plowing and two harrowing. Before sowing, the field was grooved and fertilized according to soil analysis (Table 1).

Table 1. Chemical characteristics of the soil, samples collected from 0 to 20 cm depth, in Uberlândia, MG.

pH in H_2O	O.M (dag kg^{-1})	P Meh^{-1} (mg dm^{-3})	K$^+$	Ca^{2+}	Mg^{2+}	Al^{3+}	H$^+$ and Al$^+$	V (%)
					cmol$_c$ dm^{-3}			
6.2	2.5	16.6	0.4	3.4	1,3	0	1.5	77

O.M: Organic matter; V: Base saturation.

It was evaluated 23 soybean F8 lines developed by the Soybean Breeding Program of Federal University of Uberlândia and four cultivars (Msoy 8527, UFUS Xavante, Msoy 8787, and UFUS Milionária) under randomized complete block design with three repetitions.

The experimental plot was formed by 4 soybean plant rows with 5 m length, spaced at 0.5 m within rows. The useful area was composed of the 2 central lines, wherein 0.5 m from each edge was discarded, resulting in a useful plot of 4 m^2.

Before sowing, the seeds were treated with fungicide Carbendazim and Tiram (Protreat®) and inoculated with liquid in oculant containing *Bradyrhizobium japonicum.* The sowing occurred manually on December 5th, 2015, adopting 16 seeds per linear meter as an average. Regarding crop operations for weed control, pre-emergent and post-emergent herbicides were used. Firstly, the herbicide application of S-Metolachlor (Dual Gold®) was done right after sowing and Haloxyfop-P-Methyl (Verdict®) 20 days after sowing. Meanwhile, complementary weeding was done as often as necessary. Also, at the 30th day after emergence, 100 mL.ha^{-1} dose of cobalt and molybdenum (Nectar®) was applied, which is approximately 3 g of cobalt and 22 g of molybdenum per hectare (Embrapa, 2014).

In order to control *Phakopsora pachyrhizi* fungi, field application of Trifloxystrobin and Prothioconazole (Fox®) was done in a dose of 0.4 L.ha^{-1}, as well as Strobilurin and Triazol (APROACH® PRIMA) were applied in a dosage of 0.3 L.ha^{-1}. Furthermore, to control pests, two applications, one with Acephate (Achero®), 0.4 kg.ha^{-1} dose, and another with Thiamethoxam and Lambda-Cyhalothrin (Platinum Neo®), 200 mL.ha^{-1} dose, was done. However, the population of brown stink bugs was higher than the control level, which is 2 adults or third instar nymphs per linear meter, during all reproductive stage (Corrêa-Ferreira; Panizzi, 1999).

By means of visual observation, measurements were performed in accordance with the stages of soybean development proposed by Fehr and Caviness (1977) and the following traits were accessed:

a) Plant height at flowering (PHF) and at maturity (PHM): the distance, in centimeters, from the soil surface up to the end of the main stem was measured when plants were at reproductive stage R1 and R8, respectively.

b) Number of nodes on the main stalk at flowering (NNF) and at maturity (NNM): the number of nodes on the main stem was counted when 50% of the plot was identified at the R1 and R8 stage, respectively.

c) Number of days to bloom (NDB) and to maturity (NDM): These parameters are defined as the number of days from the emergence to flowering, when 50% of plants in the useful plot have at least one flower fully opened (R1), and 95% of the pods of the useful area are ripe (R8), respectively.

d) Number of seeds per pod (NSP): the average number of seeds per pod from randomly collected plants from each useful plot was counted after harvesting.

e) Total number of pods per plant (TNP): the average number of pods was counted in five randomly collected plants of the useful plot.

f) Number of empty pods (NEP): the average number of empty pods (without at least one seed) caused by the attack of stink bugs was randomly sampled in each useful plot.

g) One hundred seed weight (HSW): after plants of the useful area were harvested and processed, a hundred-seed weight was determined according to the methodology proposed by Rules For Seeds Analysis (Ministério da Agricultura, Pecuária e

Abastecimento - Brasil, 2009). The weight of each sample was adjusted to a moisture content of 13% according to the formula below:

$$PF = PI \frac{100\text{-}UI}{100\text{-}UF} \qquad (Eq.1)$$

Where:
PF: corrected weight of the sample;
PI: initial weight of the sample;
UI: initial moisture content of the sample;
UF: final moisture of the sample (13%).

h) Asian soybean rust severity (SEV): the Asian soybean rust severity was measured according to the Godoy diagrammatic scale from the average of three samplings, the first occurred 20 days after the first fungicide application followed by the second, which was done seven days after the second fungicide application (Godoy et al., 2006).

i) Grain yield (Y): Accomplished through harvesting, threshing, and weighing the soybean seeds from the useful plot. Data obtained (grams per useful plot) were transformed into kg ha^{-1}.

j) Stink bug damage to seeds: The stink bug damage to soybean seeds was visually classified in percentages according to the criteria proposed by Panizzi et al. (1979). The classification was based on a sample size of 50 seeds per each experimental plot randomly collected and sorted into four different groups, which were: A) healthy seeds, without discoloration; B) slightly damaged, normal form, discoloration caused by punctures; C) damaged seeds, deformed, partially wrinkled seeds with discoloration caused by punctures; D) highly damaged seeds, completely deformed and discolored seeds (Figure 2).
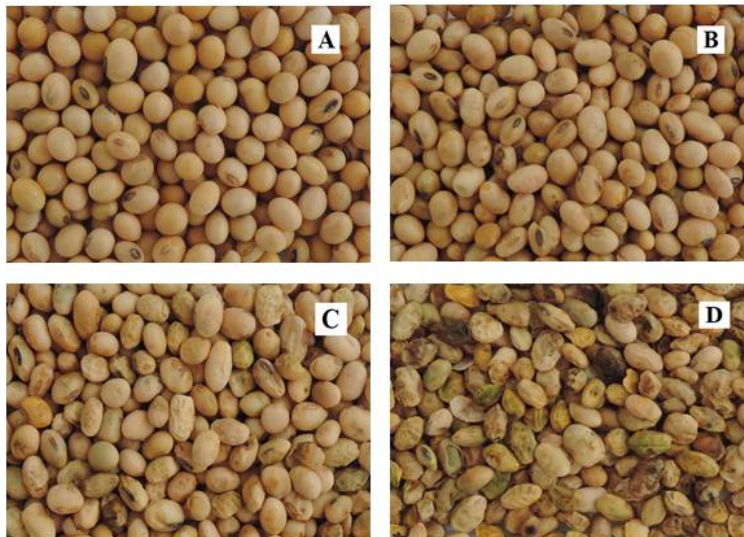
Figure 2. Visual classification criteria for stink bug damage to seeds of soybean genotypes. A) Healthy seeds; B) slightly damaged seeds; C) damaged seeds; D) highly damaged seeds. Each group represents a joint sample collected from more than one genotype.

For statistical analysis, having met all the ANOVA presuppositions such as casualization, variance homogeneity, residual normality, and block additivity for the agronomic traits evaluated using SPSS software, analysis of variance was carried out for the assessed parameters with the Sisvar program (Ferreira, 2014). When differences in the effect of treatment were observed by F test (P < 0,05), it was used Scott-Knott test for grouping the averages.

After tabulating all the data, phenotypic and genotypic correlation analyses were done adopting the effect of genotype as fixed, conforming to the following estimators:

Phenotypic correlation

$$r_p = \frac{PMGxy}{\sqrt{MSGx\ MSGy}} \qquad \text{(Eq. 2)}$$

Where:

r_p: estimator of phenotypic correlation;
PMGxy: average product associated with the effects of genotypes for x and y traits;
MSGx: mean square among the genotypes for the trait X;
MSGy: mean square among the genotypes for the trait Y.

Genotypic correlation

$$r_g = \frac{(PMG_{xy} - PMR_{xy})/r}{\sqrt{\hat{\phi}_{g(X)}\hat{\phi}_{(Y)}}} = \frac{\hat{\phi}_{g(XY)}}{\sqrt{\hat{\phi}_{g(X)}\hat{\phi}_{(Y)}}} \qquad \text{(Eq. 3)}$$

$$\hat{\phi}_{g(X)} = \frac{MSG_x - MSE_x}{r} \qquad \text{(Eq. 4)}$$

$$\hat{\phi}_{g(Y)} = \frac{MSG_y - MSE_y}{r} \qquad \text{(Eq. 5)}$$

Where:

r_g: estimator of genotypic correlation;
$\hat{\phi}_{g(XY)}$: estimator of genotypic covariance;
$\hat{\phi}_{g(x)}$ e $\hat{\phi}_{g(Y)}$: estimators of the quadratic components associated with genotypic variability for characters X and Y, respectively.

The phenotypic correlation significance was estimated by t-test with n-2 degrees of freedom, where the "n" corresponds to the number of genotypes evaluated. The significance of genotypic correlations was evaluated through the *bootstrap* method with 5000 simulations. Those statistical analyses were accomplished by the Genes program (computational application in genetics and statistics) (Cruz, 2016).

RESULTS AND DISCUSSION

For all agronomic traits, the existence of genetic variability was observed at 1% probability level by F test, with the exception of Asian soybean rust severity (Table 2). The

coefficient of variation (CV) fluctuated from 2.54 % (NDM) to 54.13 % (HDS) (Table 2). In another study with soybeans, the coefficient of variation (CV) ranged from 19.88 to 51.27%. These high CV values, beyond genetic variability, can be explained as some genotypes are more affected than others by the stink bug attack (Rocha et al., 2014).

Table 2. Genotypic mean square, genotypic determination coefficient, quotient between genotypic and environmental variation coefficients, and coefficient of variation of soybean agronomic traits evaluated from 27 genotypes over the growing season of 2015/2016, Uberlândia- MG.

Traits	MSG	H^2(%)	CV/Cve	CV(%)
Number of days to bloom (NDB)	74.32**	84.04	1.32	6.28
Number of days to maturity (NDM)	64.67**	85.37	1.39	2.54
Plant height at flowering (PHF)	239.33**	88.27	1.58	7.19
Plant height at maturity (PHM)	281.64**	92.64	2.04	5.22
Number of nodes at flowering (NNF)	4.13**	89.50	1.68	5.28
Number of nodes at maturity (NNM)	9.65**	87.31	1.51	6.93
Total number of pods (TNP)	747.82**	63.16	0.75	28.19
Number of empty pods (NEP)	307.27**	72.93	0.94	22.83
Number of seeds per pod (NSP)	0.09**	94.95	2.50	2.93
One hundred seed weight (HSW)	5.81**	80.23	1.16	10.91
Asian soybean rust severity (SEV)	117.07[ns]	32.38	0.40	38.16
Grain yield (Y)	2280853.64**	74.20	0.98	31.77
Healthy seeds (HS)	296.57**	91.45	1.89	37.65
Slightly damaged seeds (SDS)	558.78*	69.46	0.87	31.49
Damaged seeds (DS)	452.37*	68.43	0.85	38.04
Highly damaged seeds (HDS)	602.16**	91.10	1.84	54.13

*, **: significant at 5% and 1% probability level, respectively, by F test; [ns]: non-significant.

The existence of phenotypic variability that is mainly attributed to genetic causes is crucial for establishing an outstanding base for selection (Cruz et al., 2012). In advanced generations through genetic breeding, the H^2 parameter is called coefficient of genotypic determination as the genotypes are already fixed and pre-selected (Vasconcelos et al., 2012).

For all the traits in the study, with exception of the total number of pods (63.16%), Asian soybean rust severity (32.38%), slightly damaged seeds (69.46%), and damaged seeds (68.43%), the coefficient of genotypic determination was superior to 70%, indicating that the most part of phenotypic variability was due to genetic differences among the genotypes (Ola Moreira et al., 2015). Similar results were accomplished by Glasenapp et al. (2015), which found H² values superior to 70% for some traits in the study. Besides, most of the evaluated traits (NDB, NDM, PHF, PHM, NNF, NNM, NSP, HSW, HS, and HDS) showed a quotient between the genotypic and environmental variation coefficient higher than 1, which is another indicator that suggests successful possibility of selection (Cruz et al., 2012).

The number of days to bloom and maturity are important parameters as the longer time soybean genotypes need to complete their cycle, the more time of exposure to the attack of stink bugs there is (Rocha et al., 2015). Therefore, in a natural occurring infestation scenario early genotypes tend to escape from the attack of pests by a host evasion type of resistance, and consequently, maintain higher grain yield by shortening the most susceptible stages of the crop, R5 to R7 (Corrêa- Ferreira et al., 2013; Rocha et al., 2015; Fernandes et al., 2017).

One indirect symptom that is also caused by the attack of stink bugs and may have increased the life cycle of genotypes is the foliar retention that can delay the

physiological maturity, impairing harvesting of the crop (Silva et al., 2013). Also, it is important that soybeans should not exceed a height of 100 cm according to Sediyama et al. (2015) in order to avoid large lodging indexes, as well as to enhance mechanical harvesting efficiency. In the current experiment, only one line reached over 100 cm height at maturity (G23 – Table 3).

Table 3. Soybean agronomic traits evaluated among 27 soybean genotypes during the growing season of 2015/2016 in Uberlândia- MG.

Genotypes	Traits					
	NDB	NDM	PHF	PHM	NNF	NNM
G1	55.4 b	116.0 a	62.1 d	75.1 d	13.3 b	17.4 b
G2	58.7 c	116.0 a	66.4 d	77.5 d	13.8 a	16.0 b
G3	60.3 c	124.5 c	81.2 b	81.4 c	13.1 b	15.3 c
G4	57.5 c	116.5 a	68.4 c	75.8 d	12.8 b	16.2 b
G5	58.7 c	116.0 a	87.8 a	98.1 b	13.0 b	16.4 b
G6	47.0 a	127.5 c	63.1 d	97.3 b	11.7 c	21.6 a
G7	60.3 c	127.5 c	82.9 b	94.0 b	13.4 b	17.1 b
G8	58.7 c	118.5 b	83.7 b	91.1 b	14.6 a	16.6 b
G10	47.7 a	118.0 b	70.8 c	88.4 b	11.3 c	14.4 c
G11	49.0 a	116.0 a	83.6 b	97.7 b	11.5 c	15.5 c
G12	56.0 b	117.0 a	72.2 c	81.3 c	13.0 b	16.7 b
G13	57.0 b	120.0 b	69.2 c	75.7 d	12.7 b	15.0 c
G15	55.3 b	121.0 b	74.3 c	91.9 b	11.8 c	16.7 b
G17	51.0 a	123.5 c	69.4 c	89.5 b	11.4 c	15.7 b
G18	48.3 a	120.0 b	57.2 d	71.2 d	10.6 c	13.3 c
G19	57.0 b	116.0 a	73.7 c	90.6 b	12.7 b	14.5 c
G20	58.7 c	120.5 b	80.9 b	92.2 b	13.9 a	16.2 b
G21	60.3 c	123.5 c	80.5 b	84.1 c	13.6 a	14.6 c
G22	53.7 b	126.5 c	73.9 c	88.7 b	12.5 b	16.3 b
G23	54.3 b	130.0 c	96.1 a	112.9 a	14.8 a	17.2 b
G24	46.3 a	120.0 b	62.0 d	75.3 d	10.8 c	13.3 c
G25	46.3 a	114.0 a	63.3 d	72.8 d	11.1 c	14.0 c
Msoy 8527	50.0 a	120.0 b	76.8 b	92.7 b	11.4 c	15.2 c
G27	55.3 b	120.5 b	74.1 c	87.5 b	12.8 b	17.1 b
UFUS Xavante	54.3 b	127.5 c	71.4 c	92.5 b	13.0 b	14.3 c
Msoy 8787	62.0 c	128.0 c	75.3 c	94.5 b	11.3 c	17.4 b
UFUS Milionária	62.0 c	126.0 c	69.0 c	82.8 c	10.9 c	16.0 b

L' Means followed by the same lowercase letter (column) belong to the same group at 5% level of significance by Scott and Knott test. NDB and NDM: number of days to bloom and to maturity, respectively; PHF and PHM: plant height at flowering and at maturity (cm), respectively; NNF and NNM: number of nodes on the main stalk at bloom and at maturity, respectively.

Regarding the number of nodes, both at flowering and at maturity, a higher number of nodes are a valuable soybean characteristic, as they will potentially become reproductive nodes. According to some authors, for soybean plant to accomplish a large productive potential, it would need an average of 17 to 18 nodes on the main stalk (Sediyama et al., 2015). In the current study, most of genotypes achieved numbers of nodes at maturity close to this average, including the genotypes G1, G7, G8, G12, G15, G23, G27, Msoy 8787, and the genotype G6 that overcame those values with an average of 21.6 nodes on the main stalk at maturity (Table 3). In another study, number of nodes at soybean maturity oscillated from 12.03 to 16.61 (Perini Junior et al., 2012).

According to Camâra (1998), a soybean plant can reach up to 400 pods; nevertheless, typical Brazilian cultivars usually have an average of 30 to 80 pods per plant. As it can be seen in Table 4, all genotypes, except G1, have shown averages in between this interval.

Table 4. Soybean agronomic traits evaluated among 27 soybean genotypes during the growing season of 2015/2016 in Uberlândia- MG.

Genotypes	Traits					
	TNP	NEP	NSP	HSW	SEV	Y
G1	114.3 a	52.5 c	2.2 c	10.4 a	15.4 a	4113.1 a
G2	69.7 a	30.8 a	2.2 c	10.9 a	14.9 a	4426.6 a
G3	40.2 a	37.7 b	2.5 b	10.9 a	23.0 a	1993.6 c
G4	52.6 a	34.4 b	2.7 a	10.3 a	22.5 a	3253.6 b
G5	33.5 a	24.7 a	2.6 a	10.4 a	35.4 a	2197.4 c
G6	59.5 a	54.8 c	2.1 d	8.4 b	10.2 a	1062.5 c
G7	53.9 a	44.3 c	2.2 c	9.5 a	30.7 a	1487.5 c
G8	50.1 a	25.7 a	2.1 d	11.1 a	15.8 a	2316.8 c
G10	49.2 a	30.4 a	2.3 c	11.6 a	26.7 a	2616.2 b
G11	54.2 a	39.9 b	2.2 c	10.7 a	28.2 a	2705.2 b
G12	57.1 a	40.0 b	2.4 b	10.7 a	21.7 a	2571.4 b
G13	61.6 a	41.5 b	2.3 c	9.4 a	31.8 a	2836.0 b
G15	67.1 a	54.8 c	2.2 c	10.1 a	31.8 a	1635.3 c
G17	57.0 a	41.6 b	2.3 c	8.3 b	27.4 a	2565.4 b
G18	48.9 a	24.5 a	2.1 d	9.2 a	22.7 a	1753.8 c
G19	57.9 a	36.8 b	2.3 c	10.3 a	28.7 a	2819.3 b
G20	51.7 a	35.0 b	2.6 a	11.0 a	8.2 a	2665.0 b
G21	44.4 a	29.4 a	2.2 c	12.0 a	27.0 a	2378.1 c
G22	71.7 a	49.5 c	2.3 c	9.5 a	25.7 a	1881.2 c
G23	72.1 a	59.2 c	2.4 b	8.3 b	20.2 a	1451.3 c
G24	38.9 a	24.0 a	2.2 c	10.78 a	18.1 a	3550.8 a
G25	85.6 a	47.1 c	2.0 d	10.0 a	19.0 a	2766.5 b
Msoy 8527	50.5 a	39.2 b	2.2 c	10.8 a	25.0 a	3695.9 a
G27	62.1 a	41.4 b	2.4 b	9.7 a	30.8 a	2289.7 c
UFUS Xavante	61.2 a	52.5 c	2.3 c	7.1 c	18.5 a	1650.2 c
Msoy 8787	66.6 a	48.8 c	2.5 b	7.9 b	23.0 a	1173.8 c
UFUS Milionária	57.9 a	38.0 b	2.4 b	6.1 c	17.1 a	1329.4 c

L' Means followed by the same lowercase letter (column) belong to the same group at 5% level of significance by Scott and Knott test. TNP: Total number of pods; NEP: Number of empty pods; NSP: Number of seeds per pod; HSW: One hundred seed weight (g); SEV: Asian soybean rust severity (%); Y: Grain yield (kg ha^{-1}).

Along with that, the one hundred seed weight can vary from 2 to 30 g (Sediyama et al., 2013). As an expected symptomatology of the attack of stink bugs, the weight of seeds has been negatively affected. They oscillated from 6.1 (UFUS MILIONÁRIA) to 12 g (G21) (Table 4), which shows the impact of stink bugs on reducing soybean seeds weight. A pseudo-resistance mechanism, defined as damage dilution type, may reduce the proportional number of damaged seeds due to insect attack by increasing the number of seeds per plant (Rocha et al., 2015).

The Asian soybean rust severity did not statistically differ among the evaluated genotypes, showing that it has not interfered in differentiating the resistance of soybean genotypes to stink bugs. Overall, those genotypes have shown a lower disease severity compared to other studies that have identified potential sources of genetic resistance to this disease (Ribeiro et al., 2009; Glasenappet al., 2015). Still, the soybean lines' resistance to soybean rust severity may vary over time and it is associated with the genotype, the type of resistance mechanism according to the environmental factors or physiological effects (Martins and Juliatti, 2014).

The soybean grain yield fluctuated from 1.062 kg.ha^{-1} to 4.427 kg.ha^{-1} (lines G6 and G2, respectively) (Table 4). The observed variations, beyond genetic variability, may be attributed to the differential levels of resistance of genotypes to attack of stink bugs, mainly *Euschistus heros*, which were found in the highest densities in the experiment. The lines G1, G2, G24, and the cultivar Msoy 8527 achieved the highest yielding standards, staying above the national average of 3.037 kg.ha^{-1}during the same growing season of 2015/2016 (Conab, 2016). In addition, the grain yield is considered to be a good indicator parameter towards the selection

of genotypes resistant to the attack of stink bugs (Lourenção et al., 2010; Rocha et al., 2014). Another indicator of soybean stink bug resistance can be approached through the visual analyses of seeds. In the current research, most seeds were affected by the stink bug attack indicating a high damage potential to soybean seeds in nearly all genotypes (Table 5).

Table 5. Damage rates of stink bug infestation in percentages (± standard error) on soybean seeds according to visual classification into four groups over the growing season of 2015/2016 in Uberlândia.

Genotypes	Seeds (%)			
	Healthy	Slightly damaged	Damaged	Highly damaged
G1	25 ± 6.1 a	43 ± 1.4 b	23 ± 3.3 a	9 ± 4.2 a
G2	14 ± 2.8 c	51 ± 0.5 b	30 ± 1.9 a	5 ± 0.5 a
G3	9 ± 4.2 c	34 ±1 3.2 a	50 ± 15.1 b	7 ± 2.4 a
G4	22 ± 1 b	52 ± 1 b	29 ± 1.4 b	0 ± 1 a
G5	23 ± 2.4 b	57 ± 2.4 b	20 ± 4.7 a	0 ± 1 a
G6	3 ± 1.4 d	24 ± 2.8 a	36 ± 1.9 b	37 ± 6.1 c
G7	2 ± 1 d	18 ± 1 a	58 ± 1 a	22 ± 1 b
G8	26 ± 1.9 a	66 ± 1 b	8 ± 1.9 a	0 ± 1 a
G10	13 ± 3.3 c	45 ± 9.9 b	27 ± 8.0 a	15 ± 5.2 a
G11	10 ± 0.9 c	50 ± 1.9 b	33 ± 0.5 a	7 ± 3.3 a
G12	17 ± 5.2 b	47 ± 9.0 b	29 ± 10.8 a	7 ± 3.3 a
G13	27 ± 2.4 a	56 ± 3.8 b	16 ± 0.9 a	1 ± 0.5 a
G15	8 ± 1.9 c	33 ± 6.1 a	40 ± 2.8 b	19 ± 5.2 b
G17	5 ± 1.4 d	32 ± 9.4 a	32 ± 1.9 a	31 ± 9.0 c
G18	8 ± 3.8 c	38 ± 11.3 a	37 ± 9.9 b	17 ± 5.2 a
G19	35 ± 2.4 a	50 ± 0.9 b	15 ± 1.4 a	0 ± 1 a
G20	15 ± 3.3 c	60 ± 5.7 b	25 ± 2.7 a	1 ± 0.5 a
G21	16 ± 1.9 b	35 ± 1.4 a	43 ± 0.5 b	6 ± 2.8 a
G22	1 ± 0.5 d	19 ± 2.4 a	37 ± 1.4 b	33 ± 0.5 c
G23	2 ± 0.9 d	24 ± 2.8 a	27 ± 1.4 a	47 ± 3.3 c
G24	10 ± 3.8 c	34 ± 8.5 a	55 ± 12.7 b	1 ± 0.5 a
G25	31 ± 0.5 a	54 ± 0.9 b	15 ± 1.4 a	0 ± 1 a
Msoy 8527	21 ± 0.5 b	61 ± 6.1 b	18 ± 6.6 a	0 ± 1 a
G27	11 ± 0.5 c	48 ± 0.9 b	32 ± 1.9 a	9 ± 1.4 a
UFUS Xavante	3 ± 1.4 d	27 ± 12.7 a	44 ± 3.8 b	26 ± 10.4 b
Msoy 8787	3 ± 0.5 d	36 ± 11.3 a	30 ± 4.7 a	31 ± 6.1 c
UFUS Milionária	1 ± 0.5 d	26 ± 6.6 a	39 ± 7.1 a	34 ± 1 c

L' Means followed by the same lowercase letter (column) belong to the same group at 5% level of significance by Scott and Knott test.

Feeding activity of *E. heros* during seed filling stage can considerably cause direct damage to soybean seeds resulting in more often wrinkled or cracked seed coats (Cantone et al., 2011) and reduce soybean yield (Corrêa-Ferreira, 2005). The genotypes G6, G17, G22, G23, and the cultivars Msoy 8787 and UFUS Milionária have presented the largest percentage of highly damaged soybean seeds. Also, the effect of *E. heros* population on escalating the number of damaged seeds corroborates with the results found by Bridi (2012). Seeds attacked by stink bugs, especially *Euschistus heros*, show higher physical damage as well as an inferior seed weight, suggesting that the damage caused by *E. heros* is more severe than other species such as *Edessa meditabunda* (Cantone et al., 2011). On the other hand, the genotypes G1, G8, G13, G19, and G25 have shown the highest percentage of healthy seeds, which reinforce a superior behavior of those lines in respect to avoiding damage occasioned by the vast presence of brown stink bugs.

An important parameter to assess the relationship between agronomic traits and identify beneficial characteristics for soybean resistance to stink bug is the establishment of correlations. To correctly interpret correlations, beyond significance, the magnitude and direction has also to be considered (Cruz et al., 2012). Regarding direction, the correlations can be positive, which indicates a trend of increasing a variable while another also increases; on the other hand, when negative, it suggests a possibility of increasing a variable

value while reducing another (Nogueira et al., 2012). It is important to know the association among agronomic traits for selection of genotypes as they can infer a better understanding of the correlated traits and help to avoid selecting one trait that leads to an undesirable selection of another (Ramalho et al., 2012).

For most agronomic traits, the genotypic correlations were higher than the phenotypic ones, with the same direction. This aspect demonstrates that the genotypic factors have contributed more to the correlations than the environmental factors (Almeida et al., 2010). Correlation coefficients whose magnitudes are above 0.7 indicate variables that can be considered highly correlated (Cruz et al., 2012). Still, researchers tend to value mostly the signal and magnitude estimates on interpreting correlation values when they are above 0.5 in module (Lopes et al., 2002).

The increase in the number of days to maturity led to a rise in the number of empty pods (genotypic correlation of 0.57), as well as a reduction on the weight of a hundred seed and grain yield (Table 6). The association between the number of days to maturity and both grain yield and weight of one hundred seeds were highly negatively correlated, -0.65 and -0.63, phenotypically, and -0.74 and -0.69, genotypically, respectively. Similar results were observed by Rocha et al. (2015), as the longer plant cycles are, the exposure of the plant to the attack of pests increases.

Table 6 Coefficient of phenotypic (r_p) and genotypic (r_g) correlations between fifteen traits from 27 soybean genotypes over the growing season of 2015/2016 in Uberlândia.

Traits		NDM	PHF	PHM	NNB	NNM	TNP	NEP	NSP	HSW	Y	HS	SDS	DS	HDS
NDB	r_p	0.21^{ns}	0.44^*	0.98^{ns}	0.55^{**}	0.13^{ns}	-0.08^{ns}	-0.06^{ns}	0.51^{**}	-0.08^{ns}	-0.17^{ns}	-0.01^{ns}	0.01^{ns}	0.03^{ns}	-0.02^{ns}
	r_g	0.21^{ns}	0.45^+	0.10^{ns}	0.60^{++}	0.11^{ns}	-0.12^{ns}	-0.07^{ns}	0.55^{++}	-0.07^{ns}	-0.21^{ns}	0.01^{ns}	0.03^{ns}	0.02^{ns}	-0.05^{ns}
NDM	r_p		0.25^{ns}	0.49^{**}	0.06^{ns}	0.35^{ns}	-0.05^{ns}	0.49^{**}	0.08^{ns}	-0.63^{**}	-0.65^{**}	-0.79^{**}	-0.79^{**}	0.52^{**}	0.83^{**}
	r_g		0.31^{ns}	0.57^{++}	0.04^{ns}	0.37^+	-0.06^{ns}	0.57^{++}	0.09^{ns}	-0.69^{++}	-0.74^{++}	-0.85^{++}	-0.88^{++}	0.56^{++}	0.87^{++}
PHF	r_p			0.76^{**}	0.59^{**}	0.12^{ns}	-0.29^{ns}	0.07^{ns}	0.36^{ns}	0.14^{ns}	-0.45^*	-0.09^{ns}	0.04^{ns}	-0.07^{ns}	0.08^{ns}
	r_g			0.77^{++}	0.62^{++}	0.12^{ns}	-0.37^{ns}	0.38^{ns}	0.41^+	0.14^{ns}	-0.53^{++}	-0.12^{ns}	0.02^{ns}	-0.06^{ns}	0.10^{ns}
PHM	r_p				0.29^{ns}	0.44^*	-0.14^{ns}	0.38^{ns}	0.19^{ns}	-0.21^{ns}	-0.59^{**}	-0.36^{ns}	-0.21^{ns}	-0.04^{ns}	0.48^*
	r_g				0.33^{ns}	0.48^+	-0.14^{ns}	0.49^{ns}	0.26^{ns}	-0.25^{ns}	-0.65^{++}	-0.42^+	-0.30^{ns}	-0.01^{ns}	0.55^{++}
NNF	r_p					0.23^{ns}	0.09^{ns}	0.07^{ns}	0.27^{ns}	0.26^{ns}	0.07^{ns}	0.17^{ns}	0.14^{ns}	-0.17^{ns}	-0.10^{ns}
	r_g					0.22^{ns}	0.09^{ns}	0.05^{ns}	0.28^{ns}	0.32^{ns}	0.09^{ns}	0.19^{ns}	0.22^{ns}	-0.24^{ns}	-0.16^{ns}
NNM	r_p						0.24^{ns}	0.50^{**}	0.09^{ns}	-0.24^{ns}	-0.23^{ns}	-0.28^{ns}	-0.23^{ns}	-0.02^{ns}	0.44^*
	r_g						0.19^{ns}	0.52^+	0.07^{ns}	-0.24^{ns}	-0.33^{ns}	-0.30^{ns}	-0.25^{ns}	-0.04^{ns}	0.45^+
TNP	r_p							0.66^{**}	-0.25^{ns}	-0.21^{ns}	0.58^{**}	0.11^{ns}	-0.10^{ns}	-0.26^{ns}	0.22^{ns}
	r_g							0.57^+	-0.37^{ns}	-0.29^{ns}	0.48^+	0.07^{ns}	-0.24^{ns}	-0.26^{ns}	0.30^{ns}
NEP	r_p								-0.06^{ns}	-0.50^{**}	-0.15^{ns}	-0.34^{ns}	-0.48^{**}	0.06^{ns}	0.61^{**}
	r_g								-0.94^{ns}	-0.58^+	-0.38^{ns}	-0.44^+	-0.71^{++}	0.15^{ns}	0.74^{++}
NSP	r_p									-0.06^{ns}	-0.13^{ns}	-0.08^{ns}	0.75^{**}	0.01^{ns}	-0.01^{ns}
	r_g									-0.06^{ns}	-0.19^{ns}	-0.07^{ns}	0.14^{ns}	-0.02^{ns}	-0.03^{ns}
HSW	r_p										0.40^*	0.54^{**}	0.55^{**}	-0.18^{ns}	-0.74^{**}
	r_g										0.45^+	0.61^{++}	0.60^{++}	-0.16^{ns}	-0.80^{++}
Y	r_p											0.60^{**}	0.59^{**}	-0.32^{ns}	-0.69^{**}
	r_g											0.69^{++}	0.77^{++}	-0.37^{ns}	-0.82^{++}
HS	r_p												0.80^{**}	-0.72^{**}	-0.80^{**}
	r_g												0.91^{++}	-0.77^{++}	-0.85^{++}
SDS	r_p													-0.76^{**}	-0.82^{**}
	r_g													-0.72^{++}	-0.87^{++}
DS	r_p														0.35^{ns}
	r_g														0.37^{ns}

**, *: significant at 1% and 5% probability level by *t test*; ++, +: significant at 1% and 5%, respectively, by *bootstrap* method with 5 thousand simulations. NDB and NDM: number of days to bloom and to maturity, respectively; PHF and PHM: plant height at flowering and at maturity, respectively; NNF and NNM: number of nodes on the main stalk at bloom and at maturity, respectively; TNP: Total number of pods; NEP: Number of empty pods; NSP: Number of seeds per pod; HSW: One hundred seed weight; Y: Grain yield; HS: Healthy seeds; SDS: Slightly damaged seeds; DS: Damaged seeds; HDS: Highly damaged seeds.

Significant positive correlations of medium magnitude were found between one hundred seed weight (HSW) and grain yield (Y) (Table 6). Rocha and collaborators (2014) emphasized the weight of a hundred seeds as a useful trait in simultaneous selection for high yield and resistance to the stink bug complex, as well as the importance of those types of resistance associations as a strategy to lower costs for farmers.

Leite and collaborators (2016) found 0.57 (phenotypic) and 0.78 (genotypic) correlations between grain yield and total number of pods, which agrees with the results found in the current study and reinforces the importance of number of pods for achieving high yielding soybean genotypes (Alcantara Neto et al., 2011; Rocha et al., 2015; Leite et al., 2016).

The correlations between damage rates of stink bug to soybean seeds according to visual classification and other agronomic traits are indeed important to assess genotype's resistance to stink bug. For instance, an increase in the number of days to maturity has significantly escalated the percentage of damaged and highly damaged seeds (Table 6). On the other hand, phenotypic and genotypic correlations towards a larger number of healthy (-0.79, -0.85, respectively) and slightly damaged seeds (-0.79, -0.88, respectively) were achieved with shorter number of days to maturity. Another study suggests the importance of a shorter pod-filling period as an indicator of soybean genotypes' resistance to stink bug (Santos et al., 2017). Also, there was a significant positive correlation between the number of empty pods and the percentage of highly damaged seeds (0.61 and 0.74, phenotypically and genotypically, respectively), which reinforces that the occurrence of empty pods were associated with stink bug damage. Furthermore, both yield and one hundred seed weight parameters similarly correlated with the percentage of healthy and slightly damaged seeds (positive correlations of medium to high magnitude). Yet, the opposite can be inferred regarding the correlation of yield and one hundred seed weight with the number of damaged and highly damaged seeds, which were negatively correlated.

The genotypes Msoy 8527, G1, G2, and G24 have indicated higher resistance to the attack of stink bugs by withstanding or recovering from damage caused by insect and probably exhibiting antixenosis resistance (usually expressed as non-preference of the insect) to *E. heros*, which can be a valuable characteristic for soybean breeding programs that aim to develop cultivars resistant to this species (Souza et al., 2017). Also, the genotypes G1 and G2 have demonstrated great tolerance concerning stink bug damage to seeds associated with a high grain yield standard, which reveals a great potential source of genetic resistance to stink bugs.

CONCLUSIONS

Regarding the selection of the main traits of assessment in soybeans, grain yield and the evaluation of stink bug damage to seeds have demonstrated to be useful traits for indirect and direct selection, respectively, of soybean genotypes with resistance to brown stink bugs. The traits HSW and TNP are useful for indirect selection of soybean lines with high yield potential under natural infestation of brown stink bug. Also, early cycle genotypes have demonstrated a positive correlation to grain yield and lower damage percentage to seeds over the same conditions. Finally, the lines G1, G2, and G24 are very promising lines as they have shown high grain yield standards, as well as valuable agronomic traits for stink bug resistance.

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