ORIGINAL RESEARCH

Selection of soybean lines exhibiting resistance to stink bug complex in distinct environments

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Abstract

In soybean, stink bugs are considered the most important pest insect as they feed directly from the grain, causing significant losses in seed yield and quality. The use of resistant genotypes is a promising strategy to control these insects. Focusing on selection of soybean lines with resistance and high yield potential, 251 recombinant inbred lines (RILs), derived from a cross between IAC-100 (resistant) and CD-215 (susceptible), were evaluated in two experiments, designed as alpha-lattice, with three replicates in Piracicaba, during the growing seasons of 2012/13 and 2013/14. The evaluated traits were as follows: number of days to maturity (NDM), plant height at maturity (PHM), grain filling period (GFP), lodging (L), agronomic value (AV), grain yield (GY), weight of a hundred seeds (WHS), leaf retention (LR), and healthy seeds weight (HSW). Variance components were estimated by the Restricted Maximum Likelihood method (REML). Heritability and selection gain (SG) parameters were also calculated. Selection was carried out based on 2012/13 season, considering the genotypes that exhibited a minimum HSW of 2908.26 kg ha^{-1} (acceptable losses of 20% from the average GY). Insect population was monitored by cloth beating. An increase in stink bug population was observed during the grain filling period, with the highest population density occurring in the season 2012/13. Estimates of the variance components demonstrated the elevate influence of the interaction genotype x environment on GY and HSW, which exhibited the lowest estimates of heritability (23 and 34%, respectively). The estimate selection gain, calculated from the predicted means of GY and HSW, was of 665.4 and 482.4 kg ha^{-1} season 2012/13. Therefore, the applied selection allowed the identification of the genotypes exhibiting higher yields and resistance to the stink bug complex. From the RIL population, lines or genotypes potentially useful to generate novel cultivars were identified.

Introduction

Will the available agricultural production be enough to supply the demands of the world growing population? Currently, it is a debatable topic in sustainability discussions (Odegard and Voet 2014). Estimates have demonstrated that the required increase in agricultural production has to be from 100 to 110% to prevent the failure of food supply (Tilman et al. 2011). According to Ray et al. (2013), the production increase must come from yield improvement, instead of extending the cultivated area.

Soybean is among the four crops responsible for providing 2/3 of calories derived from agriculture worldwide (Ray et al. 2013). Therefore, a consistent production of this legume crop is necessary to guarantee food security. Plant breeding is an important tool to these goals, as it generates superior, high yielding, and adapted genotypes to a wide range of adverse conditions.

Several challenges need to be overcome for soybean production to sustain the predicted global population growth rate. The current yield increase rate is of 1.3%, however, it is required an increase around 2.4% to supply

the food demands by 2050 (Ray et al. 2013). An important challenge is the increasing number of pests attacking the crop and causing yield losses (Belorte et al. 2003). Stink bugs are considered the most important pest insect to soybean. In Brazil, three species, consisting in the stink bug complex, are predominant: small green stink bug (*Piezodorus guildinii*), green stink bug (*Nezara viridula*), and neotropical brown stink bug (*Euschistus heros*) (Guedes et al. 2012). The brown stink bug is the predominant specie in soybean growing areas (Corrêa-Ferreira et al. 2009). *Piezodorus guildinii* is a neotropical specie, found from the South of the United States down to Argentina. This specie has secondary importance, as it occurs at low densities, although it is responsible for more severe damages to soybean due to the larger area of the insect feeding apparatus (Depieri and Panizzi 2010). *Nezara virudula* occurs mainly in the states of Santa Catarina and Rio Grande do Sul (Hoffmann-Campo et al. 2000), but has also expanded toward the Central-West regions of the country (Wiest and Barreto 2012).

Larger nymphs, from the 3rd to 5th instars, and adults cause direct and indirect damages, irreversible to seed development (Panizzi and Slansky 1985; Prado et al. 2010). The insects feed directly from the pods, by inserting its sucking mouth apparatus, reaching the grains (Corrêa-Ferreira 2000). The most important injuries are associated to the injection of digestive enzymes, leading to deformation, abortion, loss in germination, and seed vigor (Oliveira 2010). Moreover, the attack also allows the transmission of pathogens, such as the yeast *Eremothecium coryli*, delay in the physiological maturity or leaf retention, impairing mechanical harvesting of the crop (Gazzoni and Moscardi 1998; Silva et al. 2013). The losses caused by the stink bug complex may reach up to 125 kg ha^{-1}, considering the presence of a single stink bug per square meter (Guedes et al. 2012).

Chemical control has been widely employed to control the insects in soybean (Musser and Catchot 2008). However, besides the costs, the excessive use of insecticides has induced the appearance of insect populations exhibiting resistance to certain molecules (Corrêa-Ferreira et al. 2013); thus, requiring higher numbers of applications and the use of broad spectrum chemicals (Temple et al. 2009). The current scenario of chemical control consists in the prohibition of some chemicals and shortage of innovation (Guedes et al. 2012). Moreover, environmental pollution consequences are drastic. According to Hart and Pimentel (2002), only 0.1% of the pesticides applied reach the target insect and 99.9% impact the surrounding environment. Therefore, the use of resistant genotypes is an interesting alternative or substitute to insect chemical control (Smith 2005), offering a series of benefits from the environmental and economical standpoints.

Thus, the current work aimed to identify high yielding soybean lines exhibiting resistance to the stink bug complex.

Materials and Methods

Development of the population

The F_8 population (recombinant inbred lines, RIL) used in this study was developed from the cross between IAC-100 (resistant to the stink bug complex) and CD-215 (susceptible). Seeds of the F_1 generation were harvested in 2007/2008, sown in a greenhouse and gave rise to a total of 251 progenies that were advanced up to F_8 under these conditions using the single-pod descendent (SPD) method.

Experiments

The experiments were carried out in the field, at the Experimental Station Anhumas, in Piracicaba, from the Genetics Department at Luiz de Queiroz College of Agriculture (ESALQ/USP). In 2012/2013, the used experimental design was alpha-lattice 10 × 25 with three replicates, totalizing 248 RILs and the respective parents. The experimental plot consisted of two lines of 4 m in length. In 2013/2014, 251 RILs and the respective parents were analyzed, along with three checks (BMX Potência, Vmax, and BMX Apolo). The 256 treatments were arranged as 32 × 8 alpha-lattice and each experimental plot consisted of four lines of 5 m in length.

Line spacing for the experiments was of 0.5 m, with 18 seeds per linear meter. Natural stink bug infestation was evaluated and the insect population density was recorded weekly according to the cloth beating method (Stürmer et al. 2012).

Plant phenotyping

The agronomical performance of the lines was evaluated for the following traits:

1. NDM: number of days to maturity, counted from seeding up to the date when 95% of the pods were ripe;
2. PHM: plant height (cm) at maturity, measured from the base of the plant (on the ground) up to the apex of the main stem;
3. L: lodging evaluated at maturity by a scale of visual grades ranging from 1 to 5, where 1 corresponds to the erect plant and 5, to lodged plants;
4. AV: agronomic value, evaluated at maturity according to visual grading system ranging from 1 to 5, where 1 corresponds to plants with no agronomic value and

5, to plants with excellent agronomic features (large number of pods, height superior to 60 cm, vigorous, erect plants, absence of green stems and leaf retention, absence of pod shattering, and absence of disease symptoms);

5. GY: grain yield, presented as kg ha^{-1}.

Moreover, four traits associated with insect resistance were evaluated:

1. GP: grain filling period (days) was obtained by the difference between the reproductive stages R7 and R5;
2. WHS: weight of a hundred seeds (g), obtained from a randomly selected sample after standardization of the moist contents;
3. LR: leaf retention, determined according a grading system ranging from 0 for plants with normal senescence to 5 for plants with several green stems and leaves (mechanical harvest impracticable);
4. HSW: healthy seeds weight (kg ha^{-1}), weight of seeds with no damage from stink bug attack, evaluated after grain harvest and processing. Seeds were processed by spiraling in order to remove empty, green, and ill-formed seeds by centrifugal and gravitational forces.

Data analyses

The data analyses considered all parameters of the model as random. The variance components (by the restricted maximum likelihood method - REML), minimum significant difference, coefficient of variation and heritability were estimated by combining the two environments (seasons). Predicted genetic means were estimated by the sum of the grand mean and the random estimate of the effect of each genotype (Best Linear Unbiased Prediction – BLUP).

The data were analyzed using the PROC MIXED, via META suite (Vargas et al. 2013) at SAS, according to the following statistical model (eq. 1):

$$Y_{ijkl} = \mu + G_i + A_j + R_k(A_j) + B_l(A_jR_k) + G_i * A_j + \varepsilon_{ijkl} \quad (1)$$

where:

Y is the value of the observation corresponding to the genotype i at replicate k in environment j;

μ is the mean of the principal effect;

G_i is the effect of genotype i;

A_j is the effect of the environment (season) j;

R_k is the effect of the replicate k within environment j;

B_l is the effect of the block l within environment j within replicate k;

$G_i * A_j$ is the effect of the interaction genotype x environment; and

ε_{ijkl} is the error or random residue.

Heritability was estimated based on the mean of the genotypes (eq. 2):

$$h^2 = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_{ga}^2}{n\text{Env}} + \frac{\sigma_e^2}{n\text{Env} \times n\text{Rep}}} \quad (2)$$

Where:

σ_g^2, σ_{ga}^2 and σ_e^2 are variances: genotypic, from the interaction Genotype × Environment and error, respectively;

nEnv is the number of environments where the experiments were conducted; and

nRep is the number of replicates.

The verification of genetic progress was performed from the selection based on the target environment – presence of stink bugs. Then, the verification of the progress for the season 2013/2014 was carried out based on the selection of individuals from the previous season. This approach was chosen based on the higher population density of stink bugs in 2012/13, in comparison to 2013/14. Moreover, the climate conditions of the later growing season were atypical, consisting of high temperatures and low precipitation.

The selection target trait was HSW, using a classification based on the ideotype: assuming 2908.26 kg ha^{-1} as minimum HSW value. The value was estimate considering the maximum acceptable loss of 20% in comparison to the general yield average of the genotypes used in the experiment. The consideration of a single trait for selection, namely HSW, was decided after the demonstration by Rocha et al. (2014) that it is a useful trait for the simultaneous selection of genotypes exhibiting resistance and high yield.

Selection gain (SG) was estimated based on the formula (eq. 3):

$$GS = \overline{X}_S - \overline{X}_O \quad (3)$$

Where:

$\overline{X}_S$ represents the means predicted by BLUP for the selected lines; and $\overline{X}_O$ represents the means predicted by BLUP for the original population.

Results and Discussion

Stink bug population

The expressive increase in the stink bug population at the end of the crop cycle was observed during both seasons (Figs. 1, 2), which is due to the presence of pods in the plants that are directly correlated to the presence of stink bugs migrating from harvest neighboring areas (Panizzi et al. 2000).

In 2012/13, the maximum number of stink bugs was as high as six (Fig. 1), whereas in 2013/14, it reached 3.5 (Fig. 2). In the later season, the infestation was below

Figure 1. Fluctuation of stink bug population in the experimental area in the growing season 2012/2013 along soybean phenological stages R3–R8, evaluated by the cloth beating method (2 m line), for soybean genotypes.
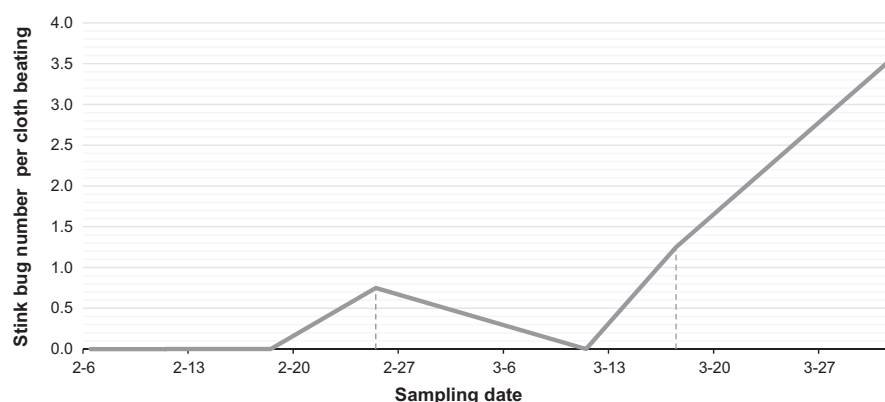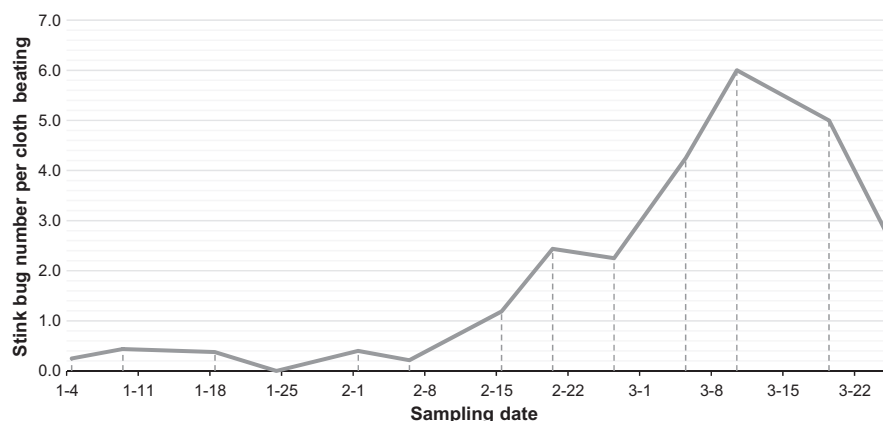

Figure 2. Fluctuation of stink bugpopulation in the experimental area in the growingseason 2013/2014 along soybean phenological stages R3–R8, evaluated by the cloth beating method (2 m per line), for soybean genotypes.

the economic injury level. The action threshold for all stink bug species is 4 stink bugs per 2 m row on a drop cloth, considering grain production and 2 if we consider seed production (Corrêa-Ferreira and Panizzi 1999).

In 2012/13, an elevate increase in the number of insects per cloth beating was observed when the majority of the genotypes was entering stage R5, when soybean is most susceptible to attack, the called critical period (Corrêa-Ferreira and Panizzi 1999). Although stink bugs can be detected at vegetative growth, their negative effects on grain filling and seed quality are noteworthy during pod formation (Corrêa-Ferreira et al., 2013). The population of stink bugs reached its maximum growth in the beginning of March, when the majority of the genotypes were mature. At harvest, the stink bugs are dispersed to alternative host plants, and, considering the brown stink bug, to diapause niches (Corrêa-Ferreira and Panizzi 1999).

During the harvesting season 2013/14, insects were absent from the first two evaluations (Fig. 2). The experiment was installed in the last days of the seeding window, with the expectations that the number of insects per cloth beating would be high. However, the inexistence of soybean crops in the neighboring areas, which could favor stink bug migration, the occurrence of drought and high temperatures during the period may have interfered with the growth of the insect population.

Variance components and Heritability

Variance components, determined by the restricted maximum likelihood (REML), minimum significant difference, variation coefficient, and heritability combined for two environments (seasons) for the traits PHM, NDM, GFP, LR, AV, L, GY, WHS, and HSW are shown in Table 1. For the majority of the investigated traits, variation coefficients were below 20%, indicating experimental precision (Pimentel Gomes 2000).

The use of the random model focus the results on the estimation of the variance components (Table 1), from which it is possible to estimate the genetic parameters, such as heritability and gains obtained from selection. The estimate components of the phenotypic variance demonstrate the strong influence of the environment on the expression of the majority of the traits.

Table 1. Variance components, mean, LSD (least significant difference), coefficient of variation (CV), and heritability (h^2) for the traits plant height at maturity (PHM in cm), number of days to maturity (NDM), grain filling period (GFP in days), leaf retention (LR in grading scale from 1 to 5), agronomic value (AV in grading scale ranging from 1 to 5), lodging (L in grading scale ranging from 1 to 5), grain yield (GY in kg ha^{-1}), healthy seeds weight (HSW in kg ha^{-1}), and weight of a hundred seeds (WHS in g), evaluated in soybean recombinant inbred lines with resistance alleles to stink bug complex in two environments.

Statistics	PHM	NDM	GFP	LR	AV	L	GY	HSW	WHS
σ_a^2	10.187	43.759	19.798	0.092	0.009	0.564	311513.111	227615.949	2.336
σ_g^2	41.132	20.440	8.613	0.273	0.045	0.069	30662.445	39439.256	2.117
σ_{ga}^2	30.875	8.700	3.203	0.103	0.077	0.043	111196.233	65697.742	0.126
σ_e^2	57.358	15.062	27.754	0.489	0.381	0.337	262668.872	249105.875	0.975
Mean	61.685	117.290	35.899	1.826	3.364	1.765	2771.313	2516.049	12.913
LSD	14.347	7.439	7.080	1.046	0.918	0.807	903.984	788.923	1.380
CV	11.809	3.220	10.014	29.104	13.859	23.215	16.562	15.920	5.425
h^2	0.622	0.749	0.580	0.672	0.306	0.471	0.236	0.347	0.904

σ_a^2: environmental variance; σ_g^2: genotypic variance; σ_{ga}^2: genotype × environment interaction variance; and σ_e^2: residual variance.

PHM and LR were the only traits displaying higher genetic variance (30 and 28% of the total variation, respectively) than environmental and genotype × environment interaction effect (G × E). These results indicate that for these traits the variability existent among the genotypes was more expressive than the remaining variances present in the model. While for the trait AV, genetic variance had higher values than the environment, but was the component of G × E interaction that had the most determinant influence on the mean of the genotypes.

Genetic variation for the trait leaf retention is highly important, as it is associated to soybean resistance to the stink bug complex. Genotypes exhibiting lower scores for the trait are desirable. The evaluation of leaf retention takes into account the amount of green stems and leaves in the plant. Green stem corresponds to the maintenance of green primary and secondary stems, and leaf retention is the absence of leaf loss, even after physiological seed maturity (Silva et al. 2013). Stink bug attacked soybean plants at the reproductive phase may lose grains and pods and the stress causes the retention of green leaves and stems. Leaf retention impairs mechanical harvesting and seed storage and processing, thus reducing the quality. Fernandes et al. (1994) have demonstrated the lower leaf retention indices of IAC-100, in comparison to the genotypes IAC-8, IAC-12, IAC-17, Dourados, Emgopa 304, and Emgopa 309, suggesting that the cultivar is less prone to leaf retention even under stink bug attack.

For the traits NDM, GFP, L, GY, HSW, and WHS, environmental variance was of higher magnitude in comparison to genotypic and G × E interaction variances. For the trait AV, the component of G × E interaction had the most determinant influence on the mean of the genotypes. Moreover, for GFP and HSW, the effect of G × E interaction displayed higher estimates than that of genotypic variance.

Heritability is one of the genetic parameters underlying the success and delimitation of selection strategies (Laviola et al. 2010). Higher values for the estimates of the parameter correspond to higher possible gains by selection (Gravois and Bernhardt 2000). Considering the investigated genotypes, the estimates of heritability coefficients, based on the means for the genotypes, ranged from 0.23 to 0.90. Values at the lower end correspond to the traits HSW and GY. In general, grain yield exhibits medium to low values due to the quantitative inheritance of the trait (Bueno et al. 2013). Traits controlled by several genes are highly influence by the environment. The complex nature of the inheritance prevents the selection of genetically superior genotypes (Li et al. 2003), as the correlation between the phenotype and the genotype is reduced (Coimbra et al. 2009).

Heritability values for GY and HSW were of 0.24 and 0.35, respectively. The HSW is directly correlated to GY, as it is measured by the weight of seeds from a given plot, after the removal of the grains injured by stink bug attack. Bueno et al. (2013) have found a heritability value superior to 83.50% for grain yield, when four investigated environments were considered as a group. According to the authors, the high heritability values are explained by the elevated genetic variance. In contrast, for Lopes et al. (1997), the heritability was of 57.92% in an experiment with natural stink bug infestation. The heritability is a parameter that could help the breeder choosing the breeding strategies for the interested traits. When the trait has low heritability or high dominance effect, it is not recommended to make selections in initial generations of selfing. Thus, according the hereditability values found in this work for GY and HSW, the selection should be made after F_5 generations, when it is possible to consider that the individuals have enough level of homozigose. GY and HSW are related. So, according Rocha et at. (2014) if

the selections are based on HSW in an environment with stink bug stress, it is possible identify that genotypes with resistance and high yield potential.

For GFP, the estimated heritability value was of 0.58, higher than the number found by Godoi and Pinheiro (2009). The heritability for GFP in the previous work was of 36.06%, in narrow sense. However, the estimates were based on plots. Estimates based on genotypes mean tend to result in higher values of heritability, as the effect of the residual variance is divided by the number of blocks and by the number of locations. For LR, heritability value was of 67%, also superior to 20% found by Godoi and Pinheiro (2009) and 20.6%, by Santos (2012).

The heritability values for PHM, NDM, and AV were 0.622, 0.749 and 0.306, respectively. These values were inferior to 90, 81, and 63%, respectively, found by Santos (2012). For L, Lopes et al. (1997) have found heritability estimates of 82.97%, whereas in our current work, the value was 0,471 or 47,1%.

Selection gain

The reaction of the genotypes to stink bug infestation could only be effectively considered during the 2012/13 season, thus allowing their classification according to resistance to the insect attack. This work focus on the identification of resistance and high yield lines, we have selected the genotypes exhibiting high performance for the traits associated with resistance and elevated yield for the 2012/13 season and estimate their gain for the 2013/14 season.

Based on HSW, genotypes producing more than 2908.26 kg ha^{-1} were selected. In order to reduce the number of lines and increase selection gain, a second criterion was adopted: minimum GY of 3635.32 kg ha^{-1} (20% superior to the mean).

Selection gains estimated based on the season 2012/13 (Table 2) were of (1) PHM: +8.8 cm – the mean height for the selected genotypes was of 72.6 cm, thus, the gain is considered satisfactory. According to Garcia et al. (2007), values higher than 60 cm are ideal to minimize grain losses during harvest; (2) NDM: +5.1 days – the increase in the number of days to maturity is not positive for insect resistance, as it causes higher exposition periods of the plants to the attacking insects; (3) GFP: +1.5 – the period between R5 and R7 is the most susceptible phase of the crop. Thus, genotypes exhibiting shorter grain filling periods tend to suffer less damage by stink bugs, it is a pseudo resistance mechanism of the host evasion type, when the time during the most susceptible phase is shortened. The use of the mechanism has been suggested to reduce damages by stink bug attack; (4) LR: +0.2 – mechanical harvest, seed storage, and processing are difficult by leaf retention. The highest

note in the grading system is 5 and the mean of the selected lines correspond to 2.1, a reasonable performance and a nonexpressive increment; (5) AV: +0.0 - the selection gain was null for the trait. The parameter considers a general plot pattern for the number of pods, plant height, vigor, lodging, green stems and leaf retention, pod shedding, and disease symptoms. Although no selection gain was detected for the isolated parameter, it was positive for its components; (6) L: +0.0 – the selection gain was null for lodging, which is a positive aspect as higher grades represent lodge of plants in the plot. Moreover, the parents are cultivars, which means that they have undergone selection for the trait; (7) GY: +665.4 kg ha^{-1} – an excellent gain, corresponding to more than 11 sacs per ha; (8) HSW: +482.4 kg ha^{-1} – elevated and simultaneous with yield gain. Our results demonstrate that it is possible to concomitantly select for stink bug resistance and grain yield; (9) WHS: +0.2 g – the weight of a hundred seeds is another pseudo resistance mechanism, defined as damage dilution type. It can be used to reduce losses due to insect attack. Lower WHS correspond to a higher number of seeds per plant and smaller proportional number of damaged seeds. Therefore, the increase in the mean for the trait is not considered positive for stink bug resistance.

The selection of superior progenies based only on traits that are strongly influenced by the environment and often correlated could provoke effects in other. As the results showed, the selection based on GY and HSW increased all the other traits evaluated, expect for AV. No changes in AV could be justified because both parents involved in the cross were commercial cultivars, i.e. have good agronomic performance. PHM and GY have near QTLs reported in the literature, and shared the same direction of additive effects (Liu et al. 2013), so when the selection focus the increase of one between the two traits cited, it is expect higher values for both. In the same way, Lee et al. (1996) identified same QTLs for plant height, lodging, and maturity. But, correlations between L and GY (Panthee et al. 2007) and LR and GY (Lopes et al., 2007) are negative. Therefore, we did not expect increase in these traits making selection based in GY. However, the changes were just 0.1 and 0.2, values that we believe that did not have affect the harvest processes, as well GY. The increase in the GFP is supported by it positive correlation with GY, in order of 0.42 (Panthee et al. 2007). Yield-component traits, as WHS, are responsible in determining GY, thus the increase in WHS was expect, even it is not a positive aspect when the breeding program is focusing in the reduction of stink bug damage.

During the season 2013/14 (Table 3), selection gains were estimated to be in the same direction, however,

Table 2. Performance of 29 soybean lines selected based on the genetic mean estimated by BLUP (Best linear unbiased prediction) for plant height at maturity (PHM in cm), number of days to maturity (NDM), grain filling period (GFP in days), leaf retention (LR in grading scale from 1 to 5), agronomic value (AV in grading scale ranging from 1 to 5), Lodging (L in grading scale ranging from 1 to 5), grain yield (GY in kg ha^{-1}), healthy seeds weight (HSW in kg ha^{-1}), and weight of a hundred seeds (WHS in g) during the growing season 2012/13.

Genotype	PHM	NDM	GFP	LR	AV	L	GY	HSW	WHS
17	74.6	124.5	39.4	2.4	3.2	1.4	3703.5	3271.0	13.6
18	77.2	128.7	37.4	2.3	3.3	1.6	3669.4	3227.0	13.1
22	71.9	127.6	41.6	2.5	3.1	1.3	4021.6	3540.9	14.3
32	82.8	124.2	38.0	1.3	3.7	1.3	3842.7	3432.8	11.7
40	77.1	124.2	40.8	2.0	3.3	1.2	3747.8	3316.1	15.7
62	82.2	115.8	34.9	2.0	3.5	1.2	3648.4	3235.8	16.8
69	76.1	141.0	43.0	4.1	3.2	1.1	3648.6	3181.9	14.9
71	71.8	139.1	42.6	1.8	3.2	1.3	3752.6	3104.3	15.0
85	69.9	129.4	39.4	2.7	3.2	1.6	3890.5	3081.7	15.8
89	63.3	125.3	40.8	2.5	3.1	1.4	3798.8	3297.1	16.6
93	72.9	131.5	43.8	1.8	3.4	1.2	3936.7	3456.0	14.3
101	65.3	124.1	40.8	2.2	3.3	1.2	3669.0	3137.2	14.0
108	63.8	118.3	40.2	2.4	3.3	1.3	3693.4	3295.8	14.8
117	78.8	124.8	40.8	2.2	3.3	1.6	4089.5	3416.5	16.5
126	68.3	123.2	41.8	1.8	3.4	1.1	3858.4	3528.2	13.3
127	72.3	124.9	40.8	3.2	2.9	1.2	4137.3	3700.1	12.8
132	79.4	134.6	39.6	2.3	3.0	1.3	3833.2	3246.9	12.7
142	71.0	137.1	43.0	3.6	3.2	1.2	3758.7	3129.6	16.2
155	66.0	129.8	42.8	2.4	3.3	1.2	3812.4	3329.8	12.8
167	78.5	132.2	36.9	2.0	3.4	1.3	4044.9	3443.2	14.1
178	71.5	132.6	42.2	2.0	3.2	1.3	3656.2	2974.9	13.4
195	68.2	120.3	39.0	1.8	3.6	1.1	3783.6	3453.8	13.5
198	73.0	119.5	43.5	1.5	3.6	1.3	3796.7	3440.5	13.0
202	65.5	131.0	42.8	3.7	3.1	1.1	3978.8	3277.1	16.4
215	71.7	127.3	38.0	1.8	3.3	1.2	3950.5	3450.2	15.0
219	71.5	125.1	40.8	1.8	3.3	1.6	3776.6	3329.3	13.2
224	75.0	124.5	41.0	2.5	3.4	1.1	3916.4	3603.9	13.9
245	62.6	123.4	40.6	1.8	3.3	1.2	3707.1	3313.1	12.9
251	83.8	120.6	39.0	1.6	3.1	1.9	3925.1	3455.0	12.1
CD-215	71.3	115.7	40.2	1.3	3.8	1.1	2973.7	2777.7	15.6
IAC-200	64.8	135.9	44.1	1.4	3.1	1.1	2676.2	2372.3	11.4
μ_L	63.8	121.9	39.0	2.1	3.3	1.2	3163.9	2851.0	14.0
μ_S	72.6	127.1	40.5	2.3	3.3	1.3	3829.3	3333.4	14.2
SG	8.8	5.1	1.5	0.2	0.0	0.1	665.4	482.4	0.2

μ_L: general mean from the lines included in the experiment; μ_S: mean from the selected lines; and SG: selection gain.

at smaller proportion. Selection based on data from the season 2013/14 would have the minimum selectable parameters at 2723.32 kg ha^{-1} for GY (15% superior to the mean) and 2178.65 kg ha^{-1} for HSW (acceptable loss of 20% of the mean GY). The selectable values are inferior to those considered for the previous harvesting season and only three genotypes would have been selected, without correspondence to those selected in 2012/13. In the light of the conditions during both growing seasons and the problems that occurred in the later one, the most plausible decision was to perform indirect selection, based on the results from 2012/13.

The lack of consistency between the selected genotypes for each growing season, along with the variance components, shows the magnitude of the G × E interaction component for GY and HSW. The G × E interaction may occur in two forms: simple, when there are differences in the genotypes variation for distinct environments, but their relative ranking is not altered; or complex, when the genotypes responses are different depending on the environment (Cruz and Carneiro 2006). In the later situation, breeders' decision making is difficult (Coelho et al. 2010).

The effects and the genotypic values for the 29 selected soybean lines based on yield and resistance is presented in Table 4. In order to facilitate data interpretation, the general mean was added to each deviation. Positive values indicate that the given genotype has contributed to increase the general mean of the assay, whereas, negative

Table 3. Performance of 29 soybean lines selected based on the genetic mean estimated by BLUP (Best linear unbiased prediction) for plant height at maturity (PHM in cm), number of days to maturity (NDM), grain filling period (GFP in days), leaf retention (LR in grading scale from 1 to 5), agronomic value (AV in grading scale ranging from 1 to 5), Lodging (L in grading scale ranging from 1 to 5), grain yield (GY in kg ha⁻¹), healthy seeds weight (HSW in kg ha⁻¹), and weight of a hundred seeds (WHS in g) during the growing season 2013/14.

Genotype	PHM	NDM	GP	LR	AV	L	GY	HSW	WHS
17	67.4	113.2	33.5	2.3	3.1	2.5	2505.7	2284.8	11.0
18	67.2	117.1	37.9	1.5	3.5	2.5	2590.7	2382.5	10.9
22	64.6	114.6	37.1	1.7	3.4	2.6	2431.3	2233.5	13.0
32	80.6	115.0	32.6	1.3	3.4	2.5	2588.4	2423.0	9.8
40	68.2	112.8	33.4	1.6	3.2	2.5	2334.8	2166.7	13.9
62	71.0	112.0	32.3	1.4	3.5	2.4	2415.3	2232.3	13.0
69	68.9	118.1	34.5	1.9	3.7	2.3	2472.8	2180.0	12.1
71	60.9	116.6	35.0	1.8	3.4	2.4	2577.7	2323.4	12.5
85	53.8	115.5	35.8	1.2	3.6	2.4	2189.3	2019.6	12.4
89	57.8	114.5	36.2	1.2	3.6	2.3	2472.2	2303.5	12.4
93	58.9	117.3	35.1	1.2	3.7	2.1	2464.3	2310.1	12.5
101	48.2	113.1	34.2	1.5	3.0	2.6	2362.8	2178.4	11.5
108	58.2	113.6	34.0	1.4	3.6	2.3	2478.0	2296.3	12.9
117	56.5	114.1	34.1	2.0	3.6	2.5	2424.8	2212.2	13.3
126	66.8	111.0	34.7	1.5	3.4	2.4	2640.6	2472.4	11.4
127	56.4	114.2	35.1	2.0	3.3	2.4	2282.0	2107.9	10.4
132	78.2	116.8	33.9	1.2	3.4	2.6	2354.5	2159.9	10.3
142	61.3	115.3	35.4	1.9	3.5	2.3	2432.2	2084.7	15.3
155	68.3	117.0	35.5	2.1	3.0	2.5	2360.6	2187.3	11.1
167	66.2	114.7	35.9	1.8	3.2	2.3	2304.3	2108.8	12.3
178	60.9	115.4	32.6	1.1	3.6	2.4	2243.5	2022.6	10.7
195	63.4	109.5	30.2	1.1	3.8	2.3	2725.9	2516.3	10.9
198	66.6	110.5	42.9	1.4	3.2	2.4	2304.4	2170.3	11.4
202	49.5	118.0	35.7	2.1	3.2	2.3	2076.9	1878.4	15.4
215	62.5	116.3	34.9	1.5	3.4	2.5	2399.0	2225.0	12.2
219	55.2	116.0	31.0	1.5	3.2	2.4	2044.3	1863.2	12.6
224	57.2	115.8	35.2	2.4	3.4	2.3	2468.9	2267.1	11.9
245	59.9	104.8	31.5	1.4	3.0	2.6	2497.8	2291.5	11.3
251	66.2	114.1	29.2	1.9	3.3	2.3	2408.1	2220.8	10.6
CD-215	68.4	110.9	30.6	1.2	3.7	2.4	2633.4	2450.3	13.0
IAC-100	55.2	116.3	36.1	1.7	3.3	2.4	2252.2	2076.5	9.6
BMX Potência	71.4	115.6	37.3	2.3	3.7	2.3	2697.2	2457.8	13.7
Vmax	60.8	118.7	37.1	2.1	3.2	2.3	2453.8	2108.2	14.0
BMX Apolo	53.8	104.7	28.1	1.3	3.9	2.1	2214.6	1963.4	12.1
μ_L	59.3	112.6	32.7	1.6	3.4	2.3	2366.5	2173.1	11.8
μ_S	62.8	114.4	34.5	1.6	3.4	2.4	2408.7	2211.1	12.0
GS	3.5	1.8	1.7	0.0	−0.1	0.1	42.2	38.1	0.2

μ_L: general mean from the lines included in the experiment; μ_S: mean from the selected lines; and SG: selection gain.

values indicate the opposite effect. Thus, RIL 62 contributed to reduce the general mean in 2.36 days for GFP, whereas RIL 198 contributes to increase this trait in 7.52 days. For LR, RIL 32 reduced the general mean up to 0.43 points, whereas RIL 69 increased the parameter up to 1.10 points of the grading system. For GY, the totality of the investigated genotypes contribute to increase the general mean, as expected, as they were selected based on the desired ideotype. For HSW, only RIL 178 contributes to reduction of the general mean in 38 kg, whereas, the effect of the remaining lines was positive. For WHS, RIL 32 contributed to a 2.20 g reduction, whereas RIL 202 promoted an increase of 3.10 g.

Although the predicted selection gains for GFP, LR, and WHS, traits associated with resistance to stink bug complex, were positive; the results indicate that among the selected 29 lines those contributing negatively to the mean of the traits can still be identified. The most noteworthy RILs contributing to those traits are 32, 195, 219, 245, and 251, which contribute to the reduction of GP, LR, and WHS means and to the increase in GY and HSW, among the selected lines.

Table 4. Genotypic effect (GE) and genotypic value (GV) for 29 selected lines, considering the traits involved in soybean resistance to stink bug complex: leaf retention (LR grading scale from 1 to 5), grain filling period (GFP in days), grain yield (GY in kg ha^{-1}), healthy seeds weight (HSW in kg ha^{-1}), and weight of a hundred seeds (WHS in g), for two assays conducted in the growing seasons 2012/13 and 2013/14.

Genotype	GFP		LR		GY		HSW		WHS	
	GE	GV	GE	GV	GE	GV	GE	GV	GE	GV
17	0.71	36.60	0.55	2.37	109.14	2875.62	154.03	2667.11	−0.55	12.36
18	2.11	38.00	0.12	1.94	120.08	2886.56	165.38	2678.46	−0.91	12.00
22	3.60	39.49	0.23	2.05	140.43	2906.91	206.99	2720.07	0.76	13.67
32	−0.41	35.48	−0.43	1.39	155.60	2922.08	249.76	2762.84	−2.20	10.71
40	1.18	37.07	0.01	1.83	62.10	2828.58	97.23	2610.31	1.98	14.89
62	−2.36	33.53	−0.10	1.72	66.22	2832.70	100.42	2613.50	2.04	14.95
69	2.95	38.84	1.10	2.92	86.26	2852.74	81.30	2594.38	0.50	13.41
71	2.76	38.65	0.01	1.83	108.37	2874.85	86.82	2599.90	0.85	13.76
85	1.64	37.53	0.12	1.94	68.79	2835.27	16.97	2530.05	1.32	14.23
89	2.67	38.56	0.01	1.83	94.31	2860.79	127.83	2640.91	1.70	14.61
93	3.32	39.21	−0.32	1.50	121.70	2888.18	182.02	2695.10	0.52	13.43
101	1.55	37.44	0.12	1.94	76.84	2843.32	82.08	2595.16	−0.15	12.76
108	1.46	37.35	0.12	1.94	118.69	2885.17	185.36	2698.44	0.86	13.77
117	1.36	37.25	0.23	2.05	139.19	2905.67	154.46	2667.54	1.93	14.84
126	2.30	38.19	−0.21	1.61	152.93	2919.41	266.03	2779.11	−0.69	12.22
127	2.11	38.00	0.66	2.48	128.83	2895.31	211.11	2724.19	−1.38	11.53
132	1.01	36.90	−0.10	1.72	101.69	2868.17	110.86	2623.94	−1.45	11.46
142	3.60	39.49	0.99	2.81	102.36	2868.84	42.75	2555.83	2.85	15.76
155	3.14	39.03	0.44	2.26	92.12	2858.60	123.81	2636.89	−0.97	11.94
167	0.81	36.70	0.12	1.94	117.62	2884.10	146.28	2659.36	0.20	13.11
178	1.36	37.25	−0.21	1.61	29.28	2795.76	−38.55	2474.53	−0.90	12.01
195	−1.34	34.55	−0.32	1.50	150.30	2916.78	248.53	2761.61	−0.77	12.14
198	7.52	43.41	−0.32	1.50	78.28	2844.76	146.93	2660.01	−0.74	12.17
202	3.23	39.12	0.99	2.81	62.05	2828.53	23.32	2536.40	3.10	16.01
215	0.71	36.60	−0.21	1.61	120.30	2886.78	170.83	2683.91	0.58	13.49
219	−0.03	35.86	−0.10	1.72	20.29	2786.77	32.23	2545.31	−0.03	12.88
224	2.30	38.19	0.66	2.48	134.78	2901.26	234.38	2747.46	0.03	12.94
245	0.34	36.23	−0.21	1.61	111.84	2878.32	169.39	2682.47	−0.88	12.03
251	−1.71	34.18	−0.10	1.72	136.29	2902.77	201.82	2714.90	−1.58	11.33
CD-215	−0.50	35.39	−0.54	1.28	31.13	2797.61	67.54	2580.62	1.38	14.29
IAC-100	4.25	40.14	−0.27	1.55	−112.82	2653.66	−188.34	2324.74	−2.53	10.38
BMX Potência	3.46	39.35	0.52	2.34	76.98	2843.46	112.25	2625.33	1.92	14.83
Vmax	3.34	39.23	0.38	2.20	13.79	2780.27	−34.55	2478.53	1.93	14.84
BMX APolo	−3.52	32.37	−0.29	1.53	−50.81	2715.67	−103.10	2409.98	0.27	13.18

Conclusions

The generated population exhibits variation for the traits of interest, thus, allowing the identification of lines exhibiting adequate agronomical performance and resistance to the stink bug complex.

Heritability estimates were higher for the traits: plant height at maturity (PHM), number of days to maturity (NDM), grain filling period (GFP), leaf retention (LR), lodging (L), and weight of a hundred seeds (WHS). For agronomical value (AV), grain yield (GY), and healthy seeds weight (HSW), the values were intermediate to low.

From the investigated 251 genotypes, 29 were selected with a minimum GY of 3635.32 kg ha^{-1} (15% higher than the general mean) and a minimum HSW of 2908.26 kg ha^{-1} (acceptable loss of 20% of the mean GY), thus allowing the identification of lines that simultaneously exhibit high yield and resistance to the stink bug complex.

The predicted selection gain for GY and HSW was of 665.4 and 482.4 kg ha^{-1} for the growing season of 2013/13, and for 2013/14, it had the same direction, but distinct magnitude.

It is possible to select cultivars from the current RIL (recombinant inbred line) population.

RIL 32 stands out due to its negative genotypic effects on GFP, LR, and WHS HSW and positive effects on GY and HSW.

Acknowledgments

Conflict of Interest

None declared.

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