

GOPEN ACCESS

Citation: Teodoro LPR, Bhering LL, Gomes BEL, Campos CNS, Baio FHR, Gava R, et al. (2019) Understanding the combining ability for physiological traits in soybean. PLoS ONE 14(12): e0226523. https://doi.org/10.1371/journal. pone.0226523

Editor: Istvan Rajcan, University of Guelph, CANADA

Received: July 23, 2019

Accepted: November 26, 2019

Published: December 17, 2019

Copyright: © 2019 Teodoro et al. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Funding: The author(s) received no specific funding for this work.

Competing interests: The authors have declared that no competing interests exist.

RESEARCH ARTICLE

Understanding the combining ability for physiological traits in soybean

Larissa Pereira Ribeiro Teodoro¹, Leonardo Lopes Bhering², Bruno Ermelindo Lopes Gomes², Cid Naudi Silva Campos¹, Fabio Henrique Rojo Baio¹, Ricardo Gava¹, Carlos Antonio da Silva Júnior³, Paulo Eduardo Teodoro^{1*}

1 Department of Plant Science, Universidade Federal de Mato Grosso do Sul, Chapadão do Sul, Mato Grosso do Sul, Brazil, 2 Department of General Biology, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil, 3 Department of Geography, Universidade do Estado do Mato Grosso, Sinop, Mato Grosso, Brazil

* eduteodoro@hotmail.com

Abstract

Photosynthetic efficiency has become the target of several breeding programs since the positive correlation between photosynthetic rate and yield in soybean suggests that the improvement of photosynthetic efficiency may be a promising target for new yield gains. However, studies on combining ability of soybean genotypes for physiological traits are still scarce in the literature. The objective of this study was to estimate the combining ability of soybean genotypes based on F_2 generation aiming to identify superior parents and segregating populations for physiological traits. Twenty-eight F_2 populations resulting from partial diallel crossings between eleven lines were evaluated in two crop seasons for the physiological traits: photosynthesis, stomatal conductance, internal CO_2 concentration, and transpiration. General combining ability (GCA) of the parents and specific combining ability (SCA) of the F_2 populations were estimated. Our findings reveal the predominance of additive effects in controlling the traits. The genotype TMG 7062 IPRO is the most promising parent for programs aiming at photosynthetic efficiency. We have also identified other promising parents and proposed cross-breeding with higher potential for obtaining superior lines for photosynthetic efficiency.

1. Introduction

Soybean [*Glycine max* (L.) Merril] is the most economically important oilseed in the world, whose yield has grown considerably in the last three decades. Among the factors that contributed to this scenario, we highlight the genetic breeding [1-4]. In breeding programs aiming at obtaining high yielding genotypes, the evaluation of genetic diversity to identify the crosses that provide more significant heterotic effect is an essential step, since it increases the probability of obtaining transgressive segregating progenies [5]. Among the methods based on biometric models for evaluating the diversity of parents, there are diallel crosses.

Diallel crosses provide information about the genetic control of the traits evaluated, which helps in conducting and selecting segregating populations [6, 7]. The diallel also allows the breeder to know the *per se* behavior of the parents, called general combining ability (GCA), besides their hybrid combinations, called specific combining ability (SCA). GCA is attributed

to genes with additive effects, whereas SCA is related to non-additive gene effects, which characterizes the difference of the hybrid combinations concerning the average behavior of the parent [8]. Hence, this approach enables to select segregating populations with high SCA for the traits of interest, and that includes at least one of the parents having high GCA [9].

Soybean crop has a restriction regarding the use of plants in F_1 generation for diallel analysis, due to the low availability of seeds. This problem can be overcome by using the F_2 generation [10–12]. However, for each self-fertilization generation advanced from F_1 , the contribution of the dominance deviation in the population mean is halved. This condition may contribute to the SCA effect to be insignificant in subsequent generations and cause loss of information on gene complementation among the parents used [13]. In this sense, the use of partial diallel may be more appropriate, since in this diallel the magnitude of GCA, besides quantifying the frequency of favorable alleles, indicates the genetic diversity between the parent from one group and those from the opposite group [14]. The analysis in advanced generations of a partial diallel is justified because of the possibility of bias in GCA estimates due to the predominance of dominance deviations when F_1 generation is used [15].

The use of diallel analysis in the F_2 generation has been applied to the breeding of several crops, such as wheat [10, 16, 13], soybean [11, 17, 18] and common bean [19, 20]. The study of the combining ability of genotypes, besides providing valuable information to the decision making about the choice of parents, allows the understanding of the genetic action involved in the trait inheritance, contributing to greater efficiency of soybean breeding programs.

As already mentioned, obtaining high yielding genotypes is the main goal of soybean breeding. However, increased photosynthetic efficiency has become the target of several breeding programs [21–23]. The positive correlation between the photosynthetic rate and the yield in soybean suggests that the improvement of photosynthetic efficiency may be a promising target for new yield gains [22, 24, 25]. Zhu et al. [26] estimated that at least a 50% improvement in photosynthetic efficiency will also be critical to meet the doubled global yield of grain crops over this century. Morgan et al. [27] and Dermody et al. [28] provide evidence that increasing photosynthesis in a crop under standard field production conditions does result in increased yield. In these experiments, when photosynthesis was increased by artificial elevation of CO_2 , there was an increase in yield of 15%.

In addition to photosynthetic capacity, other traits such as stomatal conductance, internal CO_2 concentration, and transpiration are essential for understanding plant physiological metabolism and identifying genotypes with greater photosynthetic and water-use efficiencies. Wong et al. [29] reported that the photosynthetic capacity is correlated with gs, suggesting that genotypes with higher gs have a higher photosynthetic rate. In turn, genotypes with lower transpiration rates have greater water-use efficiency. In this sense, genotypes with higher gs and photosynthetic rate and lower transpiration can be promising in the formation of populations in breeding programs aiming at both yield increase and resistance to abiotic stress.

Although they are crucial in traditional breeding aimed at photosynthetic efficiency, there are no studies on combining ability of soybean crop for physiological traits to date. Therefore, we performed a diallel analysis in soybean lines and F_2 populations aiming to i) estimate the combining ability of genotypes based on F_2 generation, and ii) identify superior segregating parents and populations for physiological traits.

2. Material and methods

2.1. Obtaining progenies in F₁ generation

The hybrids were obtained in a greenhouse from the Soybean Program of the Department of Plant Science at Federal University of Viçosa (20°45'14"S; 42°52'53"W, 649 m of altitude), from

October 2016 to January 2017. For assembly of the crossing blocks, we selected contrasting parents for flower color, in which the males carried alleles for purple flower (dominant) and females carried alleles for white flower (recessive). The divergence regarding the relative maturity group (RMG) was also taken as the selection criteria of parents (Table 1).

Based on the mentioned characteristics, 11 transgenic parents (carrier of Intacta RR2 PRO[™] technology) were selected and divided into two groups: male (group I) and female (group II).

2.2. Obtaining progenies in F₂ generation

The 28 F_1 hybrids were carried out in a greenhouse from the Soybean Program of the Department of Plant Science at Federal University of Viçosa (20°45'14"S; 42°52'53"W, 649 m of altitude) from February 2017 to June 2017. Hybrid seeds were sown in a 3 L plastic pot, and one plant per pot was maintained after the identification of hybrid plants characterized by purple flower. The control of weeds, pests and diseases was carried out according to technical recommendations for the crop.

2.3. Conducting progenies in F₂ generation

Two experiments (2017/2018 and 2018/2019 crop seasons) evaluating the 28 F_2 populations were carried out in the experimental field at Federal University of Mato Grosso do Sul, Campus Chapadão do Sul (18°46'26"S, 52°37'28"W and an average altitude of 810 m). The design used was an extended complete block with two replicates due to the low availability of seeds to conduct the experiments in two environments (crop seasons). The experimental unit consisted of three lines for each F_2 population. The control of weeds, pests and diseases was carried out according to technical recommendations for the crop. Phytosanitary management followed the recommendations for soybean cultivation. The climatic conditions observed over each experiment are shown in Fig 1.

2.4. Traits evaluated in F₂ generation

At 60 days after emergence (DAE), physiological traits were analyzed using a portable photosynthesis analyzer (*Infrared Gas Analyzer*—IRGA) model Li-6400XT (LiCor Inc., Lincoln, Nebraska, USA). Photosynthetically active photon flux of 1044 μ mol m⁻² s⁻¹ and environment

| Table 1. Characteristics of the 11 soybean genory | ypes used as parents in each group: F | lower color and relative | | | | |
|---|---------------------------------------|--------------------------|--|--|--|--|
| maturity group (RMG). | | | | | | |
| | | | | | | |

| Genotype | Flower color | RMG | |
|---------------------------|--------------|-----|--|
| Group I (male parents) | | | |
| BMX Prisma IPRO | Purple | 7.5 | |
| M6952 IPRO | Purple | 7.2 | |
| BMX Bônus IPRO | Purple | 7.9 | |
| BMX Flecha IPRO | Purple | 6.6 | |
| M6410 IPRO | Purple | 6.4 | |
| NS 6909 IPRO | Purple | 6.9 | |
| M7739 IPRO | Purple | 7.7 | |
| Group II (female parents) | | | |
| BMX Ponta IPRO | White | 6.1 | |
| DM 6563 RSF IPRO | White | 6.3 | |
| SYN 13671 IPRO | White | 7.1 | |
| TMG 7062 IPRO | White | 6.2 | |

https://doi.org/10.1371/journal.pone.0226523.t001





 CO_2 concentrations (372 ± 10 mol m⁻² s⁻¹) were used, according to similar studies evaluating gas exchange in soybean crop [30, 31]. The physiological traits measured were: net photosynthesis (A, mmol CO_2 m⁻² s⁻¹), transpiration (E, mmol H₂O m⁻² s⁻¹), stomatal conductance (gs, mmol m⁻² s⁻¹), and internal CO_2 concentration (Ci, mmol m⁻² s⁻¹).

In both crop seasons, the measurements were carried out between 9:00 h and 10:00 h a.m. in five plants randomly sampled from each experimental unit. Measurements were taken on cloudless days with temperatures between 26.0 and 26.5°C, and relative humidity between 50 and 80%. We used the third leaf fully developed from the apex of the plant, which is considered diagnostic for soybean nutritional analysis [32], and this is where occurs most of the metabolic processes responsible for the energy acquisition by plants.

2.5. Statistical analysis

Initially, a joint analysis of variance was performed according to the statistical model described below:

$$Y_{iik} = \mu + B/E_{ik} + G_i + E_j + GxE_{ij} + e_{iik}$$
(1)

Wherein: Y_{ijk} is the observation in the k-th block, evaluated in the i-th genotype and j-th environment (crop season); μ is the overall mean of the experiments; B/E_{jk} is the effect of k block within the j environment; G_i is the effect of the i-th genotype considered as fixed; E_j is the effect of the j-th environment considered as random; GxE_{ij} is the random effect of the interaction between i genotype and j environment; e_{ijk} is the random error associated with observation Y_{ijk} .

After verifying that the interaction between genotypes x environments was not significant, the partial diallel analysis was performed according to the Griffing model [33], adapted to partial diallel by Geraldi and Miranda Filho [34]. The treatment effect, considered as fixed, was decomposed into general combining ability (GCA) and specific combining ability (SCA) according to the statistical model described in Eq.2.

$$Y_{ij} = \mu + \frac{1}{2}(d_1 + d_2) + g_i + g_j + S_{ij} + \bar{\varepsilon}$$
(2)

Wherein: Y_{ij} is mean involving the i-th parent from Group I and the j-th parent from Group II; μ is the overall mean of the diallel; d_1 and d_2 are contrasts involving means of the Groups I and II and the overall mean; g_i is the effect of the general combining ability of the i-th parent from Group I; g_j is the effect of the general combining ability of the j-th parent from Group II; S_{ij} is the effect of specific combining ability; and $\bar{\varepsilon}$ is the mean random error.

For purposes of a proper interpretation of the results, the significance of GCA and SCA estimates were assessed by t-test. Thus, we considered only the estimates that differed from zero, that is, which were significant by t-test at 5% probability level. The sum of squares of treatments was unfolded into sum of GCA squares of the groups I and II and SCA. The magnitude of additive and non-additive effects was inferred by the ratio between sums of GCA mean squares (Groups I + II) and SCA mean squares, since the mean square has no orthogonal decomposition [35]. All statistical analysis was performed with the Genes software [36], following the procedures recommended by Cruz et al. [9].

3. Results

3.1. Diallel analysis of variance

There was a significant effect (P < 0.05) of genotypes for all physiological traits (Table 2). These results indicate the existence of genetic variability among genotypes for the evaluated traits. The effects of genotypes were unfolded into GCA effects of Groups I and II and SCA. The GCA mean squares of Groups I and II were significant for all traits. Similar to the GCA effects, there was a significant effect of SCA for all the traits. It is important to highlight that the GxE interaction was not significant for all evaluated traits. It is possible to note that the means for all traits presented by the populations, although close to the parents, were slightly higher than those obtained by the parents. The coefficients of variation (CV) were less than 4%, revealing high experimental accuracy and data reliability.

As mentioned, the sum of squares between GCA (Groups I and II) and SCA was adopted as a criterion for assessing the magnitude of genic effects. Based on this criterion, the GCA mean squares were higher than the SCA mean squares for all evaluated traits. Hence, additive effects are predominant in controlling these traits.

| Sources of variation | DF | Α | gs | Ci | E | | | |
|----------------------|----|--------------------|--------------------|---------------------|--------------------|--|--|--|
| Blocks/Environment | 2 | 0.04 | 0.06 | 43.85 | 0.22 | | | |
| Genotypes (G) | 34 | 23.22* | 0.19* | 1010.42* | 0.82^{*} | | | |
| GCA-Group I | 3 | 46.38* | 0.32* | 720.16* | 0.51* | | | |
| GCA-Group II | 6 | 74.89* | 0.24* | 1218.28* | 2.04* | | | |
| SCA | 28 | 25.86* | 0.24* | 1149.90* | 1.11* | | | |
| Environment (E) | 1 | 123.11* | 5.57* | 5934.16* | 10.29* | | | |
| GxE | 34 | 0.44 ^{ns} | 0.02 ^{ns} | 25.34 ^{ns} | 0.06 ^{ns} | | | |
| GCAxE-Group I | 3 | 1.25 ^{ns} | 0.02 ^{ns} | 15.16 ^{ns} | 0.03 ^{ns} | | | |
| GCAxE-Group II | 6 | 0.98 ^{ns} | 0.01 ^{ns} | 10.90 ^{ns} | 0.02 ^{ns} | | | |
| SCAxE | 28 | 0.44 ^{ns} | 0.02 ^{ns} | 17.33 ^{ns} | 0.03 ^{ns} | | | |
| Error | 20 | 0.39 | 0.01 | 16.33 | 0.04 | | | |
| Means-Parents | | 54.88 | 1.16 | 416.34 | 6.78 | | | |
| Means-Populations | | 55.53 | 1.39 | 437.78 | 7.29 | | | |
| CV (%) | | 1.14 | 3.85 | 0.94 | 2.75 | | | |

Table 2. Mean squares from diallel analysis for net photosynthesis (A), stomatal conductance (gs), internal CO_2 concentration (Ci) and transpiration (E), evaluated in F_2 populations and their parents grown in 2017/2018 and 2018/2019 crop seasons.

 $^{\rm ns}$ and *: not significant and significant at 5% probability by F test, respectively

DF: degrees of freedom; CV: coefficient of variation.

https://doi.org/10.1371/journal.pone.0226523.t002

3.2. Combining ability of parents and F₂ populations

The GCA estimates of the parents from Groups I and II are shown in Fig 2. The GCA and SCA estimate values and their significance (if the values differ from zero) by t-test are available in <u>S1</u> and <u>S2</u> Tables, respectively. Regarding the net photosynthesis (A), the highest GCA estimates for Group I were obtained by the parents NS 6909 IPRO and M7739 IPRO, whereas for Group II the highest estimate was observed for the parent TMG 7062 IPRO. Among the highest SCA estimates for A, the populations M7739 IPRO x BMX Ponta IPRO, NS 6909 IPRO x SYN 13671 IPRO, BMX Prisma IPRO x TMG 7062 IPRO and M6952 IPRO x TMG 7062 IPRO can be highlighted by presenting at least one parent with high GCA for the trait (Fig 3).

Significant GCA estimates for gs were observed for the parents BMX Flecha IPRO and M7739 IPRO (Group I), and TMG 7062 IPRO (Group II). The populations BMX Flecha IPRO x DM 6563 IPRO, M7739 IPRO x DM 6563 IPRO, BMX Flecha IPRO x SYN 13671 IPRO, M7739 IPRO x SYN 13671 IPRO, M6952 IPRO x TMG 7062 IPRO and BMX Flecha IPRO x TMG 7062 IPRO stood out because they had the highest SCA estimates and at least one parent with higher GCA estimates.

For the internal CO_2 concentration (Ci), the parents BMX Flecha IPRO, M6410 IPRO and M7739 IPRO (Group I) and SYN 13671 IPRO and TMG 7062 IPRO (Group II) presented significant GCA estimates. The populations BMX Flecha IPRO x DM 6563 IPRO, M7739 IPRO x DM 6563 IPRO, BMX Prisma IPRO x SYN 13671 IPRO, M6952 IPRO x SYN 13671 IPRO, BMX Bônus IPRO x SYN 13671 IPRO, BMX Flecha IPRO x SYN 13671 IPRO, M7739 IPRO x SYN 13671 IPRO, M6952 IPRO x SYN 13671 IPRO, M6952 IPRO x TMG 7062 IPRO, and BMX Flecha IPRO x TMG 7062 IPRO had the highest SCA estimates associated with high GCA estimates of at least one of their parents. Finally, the parents with negative GCA estimates for transpiration (E) were M6952 IPRO, BMX Bônus IPRO and NS 6909 IPRO (Group I) and DM 6563 IPRO (Group II). The highest SCA estimates for E were obtained by the populations M6952 IPRO x BMX Ponta IPRO and M6410 IPRO x SYN 13671 IPRO, and only the population M6952 IPRO x BMX Ponta IPRO presented one of their parents with lower SCA estimates.



Group II



Fig 2. General combining ability estimates of the parents from Groups I and II for net photosynthesis (A, mmol $CO_2 m^{-2} s^{-1}$), transpiration (E, mmol $H_2O m^{-2} s^{-1}$), stomatal conductance (gs, mmol $m^{-2} s^{-1}$), and internal CO_2 concentration (Ci, mmol $m^{-2} s^{-1}$).

https://doi.org/10.1371/journal.pone.0226523.g002

Based on the results obtained for A, gs and Ci, the genotype TMG 7062 IPRO stood out with desirable GCA and SCA estimates for these traits. Other genotypes that can be highlighted are SYN 13671 IPRO and BMX Flecha IPRO for internal CO_2 concentration, and M7739 IPRO for net photosynthesis. It is worth mentioning that although the parent M6410 IPRO presented the highest GCA estimate for Ci of the Group I, none of the cross-breeding involving this parent had satisfactory SCA estimates. Hence, we do not recommend the use of M6410 IPRO as parent in crossing blocks aiming at obtaining offspring with higher Ci.



Fig 3. Specific combining ability estimates of F_2 populations for net photosynthesis (A, mmol CO₂ m⁻² s⁻¹), transpiration (E, mmol H₂O m⁻² s⁻¹), stomatal conductance (gs, mmol m⁻² s⁻¹), and internal CO₂ concentration (Ci, mmol m⁻² s⁻¹).

https://doi.org/10.1371/journal.pone.0226523.g003

4. Discussion

4.1. Understanding of gene effects controlling physiological traits

The coefficients of variation (CV) less than 4% show high experimental accuracy and are lower than the values reported in the literature [30, 31, 37, 38]. After conducting the experiments in two crop seasons, it was observed that the genotype x environment interaction was not significant for all traits evaluated. These results were expected, since the phytosanitary management performed in the crop seasons were the same. Moreover, the climatic conditions that occurred in both crop seasons were very similar (Fig 1). Total rainfall in the first crop season was 675 mm, while in the second crop season it was 643 mm. The average temperature in the first crop season was 26.6°C, while in the second crop season it was 26.4°C.

In cases where GCA is significant, it can be inferred that at least one of the parents differs from the others regarding the concentration of favorable alleles [9, 13]. Hence, the significance of GCA effects is indicative of the existence of parents who contribute to a greater number of favorable alleles for these traits to be transmitted to offspring [39].

Significant SCA effects reveal that there are deviations in the behavior of the hybrids compared to what was expected based on the parent's GCA [8]. The GCA is attributed to genes with additive effects, whereas SCA is related to non-additive gene effects [9]. Therefore, the presence of significant GCA and SCA effects show the importance of both additive and nonadditive genetic components controlling the studied traits.

Based on the diallel analysis, it is possible to evaluate the relative importance of additive gene effects (expressed by GCA effects), as well as the effects due to dominance (associated with SCA). This information is useful in establishing the best breeding strategy [5]. The GCA mean squares higher than the SCA mean squares reveal the predominance of additive effects on the control of all traits, although non-additive effects involved [6]. There are still no studies in the literature evaluating the genetic effects involved in the control of traits A, gs, Ci and E in soybean. Our study is the first to elucidate the predominance of additive effects controlling these traits, revealing that the selection of parents based on these traits is promising.

When additive effects are pronounced, gains of greater magnitude will be predicted [5]. Additive effects of genes are cumulative over generations and are the main sources of genetic variation exploited by most breeding programs [40], since it is responsible for setting the traits of interest.

In this sense, the selection based on the physiological traits evaluated here, which are useful in soybean breeding programs aiming at photosynthetic efficiency, can be carried out at initial generations due to the predominance of additive effects in F_2 . This generates time savings in the evaluation and conduction of populations, contributing to greater efficiency of breeding programs.

4.2. Combining ability estimates to identify parents and promising populations for breeding of physiological traits

The photosynthetic rate is positively correlated with soybean yield [22, 23, 37, 38]. Todeschini et al. [25], when evaluating the genetic progress of a historical cultivar set in South Brazil released between 1965 and 2011, they verified that the photosynthetic rate, transpiration rate, and chlorophyll a and b content improved significantly over the years and were positively associated with seed yield. Therefore, photosynthetic efficiency is related not only to net photosynthesis but also to the mechanisms of stomatal conductance, internal CO_2 concentration and

transpiration. For this reason, it is important to understand the combining ability for these traits in order to select parents who have alleles favorable to be transmitted to their offspring.

Stomatal conductance (gs) is a measure of the relationship between the passage of carbon dioxide (CO₂) entering and the water vapor flowing through the leaf stomata [41]. Under non-limiting conditions for water availability and environment temperatures below thermal stress levels, the maximal stomatal conductance of a genotype will maximize the photosynthetic rates [29, 42]. This is because the first response to a water deficit is the change in gs, thereby limiting photosynthesis [23, 43]. Roche [41] also report that the CO₂ uptake maintenance, which is required by high photosynthetic rates at times of the day with high irradiation, contributes to a higher final yield.

The importance of gs in the initial responses to water stress has been reported in the literature [41–44]. As gs is related to leaf turgor and this, in turn, depends on the balance between water loss through transpiration and water supply to the leaf from the soil [45], it can be inferred that gs is directly related with transpiration. In this sense, genotypes with greater CO_2 assimilation and lower transpiration rates are desirable, since they will show greater water-use efficiency.

In this sense, identifying genotypes with great combining ability for A, Ci, gs and E is crucial to guide crossbreeding step. Based on the results obtained for combining ability, we can verify a superiority of the genotype TMG 7062 IPRO for A, gs and Ci. This fact can also be confirmed when observing the performance of its progeny resulting from the cross-breeding M6952 IPRO x TMG 7062 IPRO, which presented high SCA estimates for these traits. Due to its great combining ability for the mentioned traits, the genotype TMG 7062 IPRO shows to be promising for use as parent in breeding programs for obtaining populations with greater photosynthetic and water-use efficiencies.

Traditional breeding prevails in soybean programs in Brazil, having the use of diallel crosses and phenotypic information as the main approaches used in the choice of parents and formation of base-population. Furthermore, soybean breeding programs are still very lacking in studies on physiological traits, and thus most of them do not take into account the measurement of these parameters for evaluating parents and progenies. Our findings revealed that there are differences between genotypes regarding the concentration of favorable alleles for Ci, gs, A and E. The diallel analysis also allowed to identify the predominant genetic effects controlling the evaluated traits.

Todeschini et al. [25], in a study assessing the genetic progress of several agronomic, phenological and physiological traits in South Brazil, they reported that breeding strategies which maximize the photosynthetic rate, transpiration rate and chlorophyll content may increase the genetic progress for soybean yield in the future. Therefore, the evaluation of A, Ci, gs and E, which are easy to measure and low cost, can be valuable for soybean breeding programs, since it can contribute simultaneously to greater photosynthetic efficiency and yield gains [26]. Given the findings already reported in the literature and the results found here, we recommend using the traits net photosynthesis, stomatal conductance, internal CO_2 concentration and transpiration rate in indirect selection on soybean grain yield, due to the positive association between photosynthetic capacity and yield, as well as the additive nature of the traits, which ensures greater gains from selection.

However, we also understand that further studies on population choice based on combining ability for physiological traits, especially other traits not evaluated here, are needed for a better understanding of genetic effects involved in the control of traits related to photosynthetic and water-use efficiencies. Such studies may contribute to clarify which physiological traits are critical for identifying promising lines, as well as provide information about the genetic effect of the traits, which will guide the crossing and progeny selection steps.

5. Conclusions

By analyzing combining ability of soybean genotypes for net photosynthesis, stomatal conductance, internal CO_2 concentration, and transpiration, we identified a predominance of additive effects controlling the traits. This finding is important for setting and guiding strategies to be adopted in soybean breeding programs using these traits in the choice of genotypes for basepopulation. Since additive effects are predominant on the traits evaluated here, higher-magnitude genetic gains with selection on these traits will be predicted, since they are responsible for setting the traits of interest.

The diallel analysis also allowed to identify superior parents and segregating populations for physiological traits. The genotype TMG 7062 IPRO presented high GCA estimates for A, gs and Ci, and given these results, it can be used as parent for crosses to improve photosynthetic efficiency. Lastly, populations coming from the cross-breeding between SYN 13671 IPRO and TMG 7062 IPRO, with BMX Flecha IPRO and BMX Bonus IPRO present higher potential for obtaining superior lines for photosynthetic and water-use efficiencies.

Supporting information

S1 Table. Values used for diallel analysis of photosynthesis (A), stomatal conductance (gs), internal CO_2 concentration (Ci) and transpiration (E) obtained in F_2 populations of soybean grown in 2017/2018 and 2018/2019 crop seasons. (DOCX)

S2 Table. Values used for diallel analysis of photosynthesis (A), stomatal conductance (gs), internal CO_2 concentration (Ci) and transpiration (E) obtained in parents of soybean grown in 2017/2018 and 2018/2019 crop seasons. (DOCX)

Acknowledgments

The authors thank the CAPES (Coordination for the Improvement of Higher Education Personnel) for financial assistance (Finance Code 001), and CNPq (National Council for Scientific and Technological Development) for scholarship support.

Author Contributions

Conceptualization: Larissa Pereira Ribeiro Teodoro.

Data curation: Bruno Ermelindo Lopes Gomes, Paulo Eduardo Teodoro.

Formal analysis: Paulo Eduardo Teodoro.

Investigation: Bruno Ermelindo Lopes Gomes.

Methodology: Carlos Antonio da Silva Júnior.

Supervision: Carlos Antonio da Silva Júnior, Paulo Eduardo Teodoro.

Visualization: Leonardo Lopes Bhering, Cid Naudi Silva Campos, Fabio Henrique Rojo Baio, Ricardo Gava, Paulo Eduardo Teodoro.

Writing - original draft: Larissa Pereira Ribeiro Teodoro.

Writing – review & editing: Larissa Pereira Ribeiro Teodoro, Leonardo Lopes Bhering, Bruno Ermelindo Lopes Gomes, Cid Naudi Silva Campos, Fabio Henrique Rojo Baio, Ricardo Gava, Carlos Antonio da Silva Júnior, Paulo Eduardo Teodoro.

References

- 1. Diers BW, Specht J, Rainey KM, Cregan P, Song Q, Ramasubramanian V, Shannon G. Genetic architecture of soybean yield and agronomic traits. G3-Genes Genom. Genet. 2018; 8: 3367–3375.
- Wiggins B, Wiggins S, Cunicelli M, Smallwood C, Allen F, West D, Pantalone V. Genetic Gain for Soybean Seed Protein, Oil, and Yield in a Recombinant Inbred Line Population. J. Am. Oil Chem. Soc. 2019; 96: 43–50.
- Hegstad JM, Nelson RL, Renny-Byfield S, Feng L, Chaky JM. Introgression of novel genetic diversity to improve soybean yield. Theor. Appl. Genet. 2019; 1–12. https://doi.org/10.1007/s00122-018-3219-y
- Miranda C, Culp C, Škrabišová M, Joshi T, Belzile F, Grant DM, Bilyeu K. Molecular tools for detecting Pdh1 can improve soybean breeding efficiency by reducing yield losses due to pod shatter. Mol. Breeding 2019; 39: 27.
- Bhering LL, Peixoto LA, Cruz CD. Seleção de genitores. In: Silva FL, Borém A, Sediyama T, Ludke W, editors. Melhoramento da Soja. Viçosa: UFV; 2017.
- 6. Baker RJ. Issues in diallel analysis. Crop Sci. 1978; 18: 533–536.
- Gerhardt IFS, do Amaral Junior AT, Pena GF, Guimarães LJM, de Lima VJ, Vivas M, Santos PHAD, Ferreira FRA, Freitas MSM, Kamphorst SH. Genetic effects on the efficiency and responsiveness to phosphorus use in popcorn as estimated by diallel analysis. PloS one 2019; 14: e0216980. <u>https://doi.org/10.1371/journal.pone.0216980</u> PMID: 31095632
- Cruz CD, Vencovsky R. Comparação de alguns métodos de análise dialélica. Rev. Bras. Gen. 1989; 12: 425–438.
- Cruz CD, Regazzi AJ, Carneiro PCS. Modelos biométricos aplicados ao melhoramento genético. Viçosa: UFV; 2012.
- Bhullar KS, Gil KS, Khehra AS. Combining ability analysis over F₁-F₅ generations in diallel crosses of bread wheat. Theor. Appl. Genet. 1979; 55: 77–80. https://doi.org/10.1007/BF00285194 PMID: 24306488
- 11. Cho Y, Scott RA. Combining ability of seed vigor and seed yield in soybean. Euphytica 2000; 112: 145– 150.
- Friedrichs MR, Burton JW, Brownie C. Heterosis and Genetic Variance in Soybean Recombinant Inbred Line Populations. Crop Sci. 2016; 56: 2072–2079.
- Pimentel AJB, Souza MA, Carneiro PCS, Rocha JRASC, Machado JC, Ribeiro G. Partial diallel analysis in advanced generations for selection of wheat segregating populations. Pesq. Agropec. Bras. 2013; 48: 1555–1561.
- Viana JMS. Heterosis and combining ability analyses from the partial diallel. Bragantia 2007; 66: 641– 647.
- Vencovsky R. Herança quantitativa. In Melhoramento e produção do milho no Brasil; Paterniani E., Ed. Campinas: Fundação Cargill; 1978.
- Javaid A, Masood S, Minhas NM. Analysis of combining ability in wheat (Triticum aestivum L.) using F2 generation. Pakist. J. Biol. Sci. 2001; 4: 1303–1305.
- Carvalho ADF, Geraldi IO, Santos VS. Evaluation of F_{2:4} and F_{4:6} progenies of soybeans and perspectives of using early generation testing for grain yield. Bragantia 2009; 68: 857–861.
- Rocha GAF, Pereira FAC, Vello NA. Potential of soybean crosses in early inbreeding generations for grain yield. Crop Breed. Appl. Biotechnol. 2018; 18: 267–275.
- Rosal CJS, Ramalho MAP, Gonçalves FMA, Abreu AFB. Early selection for common bean grain yield. Bragantia 2000, 59: 189–195.
- 20. Do Vale NM. Melhoramento de feijão carioca com ênfase em precocidade. D.Sc. thesis, Universidade Federal de Viçosa, Viçosa, 2015. Available from: https://www.locus.ufv.br/handle/123456789/6872
- Manavalan LP, Guttikonda SK, Tran LS, Nguyen HT. Physiological and molecular approaches to improve drought resistance in soybean. Plant Cell. Physiol. 2009; 50: 1260–1276. <u>https://doi.org/10.1093/pcp/pcp082</u> PMID: 19546148
- 22. Ainsworth EA, Yendrek CR, Skoneczka JA, Long SP. Accelerating yield potential in soybean: potential targets for biotechnological improvement. Plant Cell. Environ. 2012; 35: 38–52. https://doi.org/10.1111/j.1365-3040.2011.02378.x PMID: 21689112
- Mutava RN, Prince SJK, Syed NH, Song L, Valliyodan B, Chen W, Nguyen HT. Understanding abiotic stress tolerance mechanisms in soybean: a comparative evaluation of soybean response to drought and flooding stress. Plant Physiol. Biochem. 2015; 86: 109–120. <u>https://doi.org/10.1016/j.plaphy.2014</u>. 11.010 PMID: 25438143

- Koester RP, Nohl BM, Diers BW, Ainsworth EA. Has photosynthetic capacity increased with 80 years of soybean breeding? An examination of historical soybean cultivars. Plant Cell. Environ. 2016; 39: 1058– 1067. https://doi.org/10.1111/pce.12675 PMID: 26565891
- Todeschini MH, Milioli AS, Rosa AC, Dallacorte LV, Panho MC, Marchese JA, Benin G. Soybean genetic progress in South Brazil: physiological, phenological and agronomic traits. Euphytica 2019; 215: 124.
- Zhu XG, Long SP, Ort DR. Improving photosynthetic efficiency for greater yield. Ann. Rev. Plant Biol. 2010; 61: 235–261.
- Morgan PB; Bollero GA, Nelson RL, Dohleman FG, Long SP. Smaller than predicted increase in aboveground net primary production and yield of field-grown soybean under fully open-air [CO₂] elevation. Global Change Biol. 2005; 11: 1856–1865.
- Dermody O, Long SP, McConnaughay K, DeLucia EH. How do elevated CO₂ and O₃ affect the interception and utilization of radiation by a soybean canopy? Glob. Change Biol. 2008; 14: 556–564.
- 29. Wong S, Cowan IR, Farquhar GD. Stomatal conductance correlates with photosynthetic capacity. Nature 1979; 282: 424–426.
- de Oliveira TB, de Azevedo Peixoto L, Teodoro PE, de Alvarenga AA, Bhering LL, Campo CBH. The number of measurements needed to obtain high reliability for traits related to enzymatic activities and photosynthetic compounds in soybean plants infected with *Phakopsora pachyrhizi*. PloS one 2018a; 13: e0192189.
- de Oliveira TB, Peixoto LA, Teodoro PE, Alvarenga AA, Bhering LL, Hoffmann-Campo CB. Relationship between biochemical and photosynthetic traits with Asian soybean rust. An. Acad. Bras. Cien. 2018b; 90: 3925–3940.
- Raij BV, Cantarella H, Quaggio JA, Furlani AMC. Recomendações de adubação e calagem para o Estado de São Paulo. 2nd ed. Campinas: Instituto Agronômico; 1996.
- Griffing B. Concept of general and specific combining ability in relation to diallel crossing systems. Austr. J. Biol. Sci. 1956; 9: 463–493.
- 34. Geraldi I, Miranda Filho J. Adapted models for the analysis of combining ability of varieties in partial diallel crosses. Rev. Bras. Gen. 1988; 11: 419–430.
- 35. Bueno TV. Capacidade combinatória de genitores de soja nas gerações F1 e F2 visando melhoramento para precocidade e produtividade de grãos. M.Sc. Thesis, Universidade Federal de Viçosa. 2015. Available from: https://www.locus.ufv.br/handle/123456789/11688
- Cruz CD. Genes: a software package for analysis in experimental statistics and quantitative genetics. Acta Sci. Agron. 2013; 35: 271–276.
- Thompson JA, Schweitzer LE, Nelson RL. Association of specific leaf weight, an estimate of chlorophyll, and chlorophyll concentration with apparent photosynthesis in soybean Photosynth. Res. 1996; 49: 1– 10. https://doi.org/10.1007/BF00029422 PMID: 24271528
- Zobiole HS, Oliveira L, Morgan RS, Huber DM, Constantin J, Castro C, Oliveira FA, Oliveira A Jr. Glyphosate reduces shoot concentrations of mineral nutrients in glyphosate-resistant soybeans. Plant Soil 2010; 328: 57–69.
- **39.** Ramalho MAP, Ferreira DF Oliveira A.C. Experimentação em genética e melhoramento de plantas. UFLA: Lavras, Brazil, 2012.
- Isik F, Li B, Frampton J. Estimates of additive, dominance and epistatic genetic variances from a clonally replicated test of loblolly pine. Forest Sci. 2003; 49: 77–88.
- **41.** Roche D. Stomatal conductance is essential for higher yield potential of C3 crops. Critical Rev. Plant Sci. 2015; 34: 429–453.
- 42. Hetherington AM, Woodward FI. The role of stomata in sensing and driving environmental change. Nature 2003; 424: 901–908. https://doi.org/10.1038/nature01843 PMID: 12931178
- Flexas J, Medrano H. Drought-inhibition of photosynthesis in C3 plants: Stomatal and non-stomatal limitations revisited. Ann. Bot. 2002; 89: 183–189. https://doi.org/10.1093/aob/mcf027 PMID: 12099349
- Medrano H, Escalona JM, Bota J, Gulias J, Flexas J. Regulation of photosynthesis of C-3 plants in response to progressive drought: Stomatal conductance as a reference parameter. Ann. Bot. 2002; 89: 895–905. https://doi.org/10.1093/aob/mcf079 PMID: 12102515
- Tuzet A, Perrier A, Leuning R. A coupled model of stomatal conductance, photosynthesis and transpiration. Plant, Cell. Environ. 2003; 26: 1097–1116.