ABRATES

Journal of Seed Science
ISSN 2317-1545
www.abrates.org.br/revista

ARTICLE

Journal of Seed Science, v.46, e202446016, 2024

Viability of simultaneously selecting for grain yield and seed physiological quality in maize

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http://dx.doi.org/10.1590/2317-1545v46278696

ABSTRACT: Currently, the demand is not only for more productive corn hybrids, but also for those with high physiological seed quality. Seed quality is considered the sum of genetic, physical, physiological and sanitary attributes that directly interfere in plant vigor. The objective of this study was to evaluate the physiological parameters of the seeds of parents and maize inter varietal hybrids obtained in a reciprocal recurrent selection program through physiological and image analysis techniques and either to study the feasibility of simultaneously selecting for seed physiological quality and grain yield. Two experiments were performed, one in the field and the other in the laboratory. The evaluated traits in the field were days of female flowering, days of male flowering, insertion of the 1st ear, plant height and grain yield. For the physiological quality, the attributes of germination at four days, germination at seven days, vigor by the cold test, emergence speed index, and the ratio of the root length to shoot length were obtained using GroundEye®. Heterosis was measured for the agronomic and physiological traits. Through the contrast between the inter varietal crosse hybrids and reciprocals we measured the maternal effect. The magnitudes of heterosis allowed us to infer that as greater as the number of selection and recombination cycles, on average, greater heterosis for the traits grain yield and seed germination. The correlated response indicated that, high yield intervarietal hybrids have better seed physiological quality.

Index terms: heterosis, maternal effect, reciprocal recurrent selection, *Zea mays* L.

RESUMO: Atualmente a demanda não é somente para híbridos de milho mais produtivos, mas também que apresentem alta qualidade fisiológica de sementes. A qualidade de sementes é considerada o somatório dos atributos genéticos, físicos, fisiológicos e sanitários que interferem diretamente no vigor das plantas. Objetivou-se avaliar os parâmetros fisiológicos de sementes dos parentais e híbridos de milho obtidos, por meio de análises fisiológicas e técnicas de análise de imagens, bem como estudar a viabilidade da seleção simultânea para a qualidade fisiológica de sementes e produtividade de grãos. Foram realizados dois experimentos, um à campo e o outro em laboratório. Os parâmetros avaliados em campo foram dias para o florescimento feminino, dias para o florescimento masculino, inserção da 1ª espiga, altura de plantas e produtividade de grãos. Para a qualidade fisiológica foram obtidos os atributos de germinação aos quatro dias, germinação aos sete dias, vigor pelo teste de frio, índice de velocidade de emergência e a razão do comprimento da raiz pelo comprimento da parte aérea através do GroundEye®. Obteve-se a heterose para os caracteres agronômicos e fisiológicos. O desdobramento dos efeitos por meio do contraste entre híbridos e recíprocos permitiu testar o efeito materno. As magnitudes da heterose permitem inferir que quanto maior o número de ciclos seletivos e de recombinação, em média maior será a heterose para os caracteres produtividade de grãos e germinação das sementes. A resposta correlacionada indica que híbridos intervarietais mais produtivos apresentam melhor qualidade fisiológica de sementes.

Termos para indexação: heterose, efeito materno, seleção recorrente recíproca, *Zea mays* L.

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Received: 09/19/2023.
Accepted: 05/05/2024.

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INTRODUCTION

The use of high-quality seeds result in the production of vigorous and uniform plants in the field, a low diseases incidence, and consequently a yield increase. These can lead also to better competition with weeds and more efficient use of nutrients and water. This might exhibit better tolerance to environmental stresses such as drought, heat, or salinity, and also more consistent yields under adverse conditions (Rodrigues et al., 2018).

Although the selection process in maize breeding programs traditionally focuses on agronomic traits, there is growing recognition of the importance of including seed physiological quality parameters in the selection criteria. These parameters, such as germination rate, vigor, can significantly impact crop performance and stand establishment. By incorporating seed quality parameters into selection strategies, breeders can develop maize varieties with not only superior agronomic traits but also improved seed performance, contributing to overall crop productivity and resilience. Besides the great majority of efforts in plant breeding programs are focused on selecting for agronomic traits, seed physiological quality parameters have been also considered essential for breeders to take into consideration in their selection strategies (Monteiro et al., 2021; Batista et al., 2022; Bianchi et al., 2022).

There are some reports in the literature regarding the genetic parameters associated with seed quality in the scenario of plant breeding programs (Cervantes-Ortiz et al., 2016; Nerling et al., 2018; Monteiro et al., 2021; Batista et al., 2022; Bianchi et al., 2022). To evaluate the physiological quality of seeds, several tests are considered, especially vigor and germination tests (Rodríguez et al., 2014). On other hand, one of the techniques used to complement the structural analyses of plants and seeds is image analysis. Through this analysis it has been possible to optimize the time to obtain vigor data and reduce the bias in the evaluation, resulting in more accurate decision-making (Medeiros et al., 2019).

Thus, we aim to evaluate the physiological parameters of parental and intervarietal maize hybrids seeds, as well as to study the feasibility of simultaneous selection for physiological quality of seeds and grain yield in maize.

MATERIAL AND METHODS

Sites

The study was conducted in the laboratory and in the field. To assess the physiological quality of the seeds, the tests were performed in the of the Department of Agriculture (DAG) Seed Analysis Laboratory (LAS) at *Universidade Federal de Lavras* (UFLA). In the field, the experiment was conducted in the experimental area of the Center for Scientific and Technological Development in Agriculture (*Fazenda Muquém* - UFLA) in the municipality of Lavras during the 2020/2021 crop season.

Field Experimental Data

For the study, four Hardy-Weinberg equilibrium populations were used (UFLA A, UFLA B, UFLA C, and UFLA D) originating from simple commercial hybrids. The UFLA A and UFLA B populations were conventional, and the UFLA C and UFLA D populations were Roundup Ready (RR) and *Bacillus thuringiensis* (BT). The UFLA A and UFLA B populations involved in this study were in the eighth cycle of RRS, whereas the populations UFLA C and UFLA D were in the first cycle (C_0).

A total of 48 treatments were implemented: i) six intervarietal hybrids of the UFLA AB population; ii) six intervarietal hybrids of the UFLA CD population; iii) six intervarietal reciprocals hybrids of the UFLA AB population; iv) six intervarietal reciprocals of the UFLA CD population; v) six parents of the UFLA A population; vi) six parents of the UFLA B population; vii) six parents of the UFLA C population; and viii) six parents of the UFLA D population.

A randomized complete block design (RCBD) was adopted in four contiguous areas, with i) the UFLA A x UFLA B hybrids and their reciprocals; ii) the UFLA A and UFLA B parents; iii) the UFLA C and UFLA D hybrids and their reciprocals; and iv) the UFLA C and UFLA D populations.

A no-tillage system was implemented, with plots consisting of two rows of three meters, with a spacing of 0.60 m between rows and 0.25 m between plants (4 plants per linear meter). Fertilization at sowing was performed in the furrow with the formulation 08-28-16 (N, P_2O_5, and K_2O, respectively). Side dressing nitrogen fertilization was performed between phenological stages V2 to V4. A total of 400 kg of urea per hectare. The other crop treatments were performed according to the need and recommendation for the corn crop in the region (Sousa and Lobato, 2004).

The following variables were evaluated in the field:

Days to male flowering (DMF): 50% of the plants in the plot exhibiting exposed tassels with pollen release by the anthers;

Days to female flowering (DFF): 50% of the plants in the plot with ear shoots showing visible style and stigma;

Plant height (PH): height, in m, from the soil to the insertion of the flag leaf;

Height of first ear insertion (HFEI): height, in m, from the ground to the insertion of the first ear;

Grain yield (GY): determined during the harvest of the plots. After standardizing the grain moisture to 13%, the yield was defined in $kg.ha^{-1}$ from the conversion of each plot area.

Data from the same treatment were evaluated against each other for the same response variable. The outliers analysis was performed by the quantiles of data distribution (Mchugh, 2003). The normality of the residues was checked by Shapiro-Wilk test (Shapiro and Wilk, 1965) and the detection of homogeneity of variance, according to the maximum F test (Hartley, 1950). The data obtained were subjected to statistical analysis using the R environment and the Scott-Knott test (1974) at $p<0.05$ for means comparison (R Core Team, 2023).

For the agronomic traits attributes, the genetic and phenotypic parameters were measured, and heterosis (h) was determined using the estimator proposed by Hallauer et al. (2010). The maternal effect (ME) was measured by analyzing the contrast between the hybrid x reciprocal, using analysis of variance and the F test at a 5% probability.

Laboratory Experimental Data

The harvest was performed at the R6 stage when the crop reached physiological maturity. We used the Nogueira® mechanical thresher, model SDMN5/10C, and then, the seeds were separated from the other material with the aid of a mechanical shaker and stored in a paper bag.

The laboratory experiment was conducted in a complete randomized design (CRD) with 48 treatments, corresponding to the parental and hybrid treatments. To perform the tests in the laboratory, a set of circular sieves was used to standardize the seed sizes, and the seeds were retained in the sieve with a 20-inch diameter.

The following tests were performed to evaluate the physiological quality of the seeds:

Germination test (G): The germination test was performed with three replications of 25 seeds per sample, according to the criteria established in the Rules for Seed Testing (Brasil, 2009).

Image analysis in GroundEye® (R/A.): To obtain images of the seedlings, the G was performed with two replications of 10 seeds per treatment. The G was performed according to the criteria established in the Rules for Seed Testing (Brasil, 2009), and seedling images were captured four days after sowing. To obtain the images, the GroundEye® system, version S800, was used according to the criteria recommended by Pinto et al. (2015), and subsequently, the mean values of the root length to shoot length ratios (R/S) were extracted.

Cold test with soil (CT): Four replications of 25 seeds were performed for each treatment according to the method proposed by Krzyzanowski et al. (2020).

Emergence speed index (ESI): This index was performed with four replications of 25 seeds for each treatment and calculated according to the estimator proposed by Maguire (1962).

The tests performed to evaluate the physiological quality of the seeds were analyzed according to a CRD. Data from the same treatment were evaluated against each other for the same response variable. The outliers analysis was performed by the quantiles of data distribution (Mchugh, 2003). The normality of the residues was checked by Shapiro-Wilk test (Shapiro and Wilk, 1965) and the detection of homogeneity of variance, according to the maximum F test

(Hartley, 1950). The data obtained were subjected to statistical analysis using the R environment and the Scott-Knott test (1974) at p<0.05 for means comparison (R Core Team, 2023).

For the physiological traits, the genetic and phenotypic parameters were measured, and heterosis (h) was determined using the estimator proposed by Hallauer et al. (2010). The maternal effect (ME) was measured by analyzing the contrast between the hybrid x reciprocal, using analysis of variance and the F test at a 5% probability.

RESULTS AND DISCUSSION

Experimental precision is essential for obtaining accurate inferences, thus making it possible to obtain estimates close to the real value of a trait (Cargnelutti-Filho et al., 2020). The CV(%) ranged from 4.38 to 28.05%. This results show us high to average experimental precision observed for most of the evaluated traits. The higher CV% was observed for root length to shoot length ratio (Table 1).

The maternal effect (ME) was measured by contrasting the hybrids and reciprocals (Table 1). For the traits measured on the field no ME was observed. On the other hand for the physiological seed quality the ME was significative for G4 (AB e CB) and for R/A (AB). When the inheritance of a trait is controlled by nuclear genes, no significant differences are observed between a hybrid and its reciprocal. However, if there is a cytoplasmic effect on the inheritance of a trait, then the *offspring* will have the female parent phenotype (Hallauer et al., 2010). So by the achieved results we can assume that the agronomic traits are controlled by nuclear genes and the physiological seed quality are effected by both.

In maize breeding, heterosis can be exploited using RRS, a method in which the additive and nonadditive effects are maximized to improve hybridization between two populations of different heterotic groups (Vieira et al., 2021). Crosses with high heterosis show a high potential for use in breeding programs. Baretta et al. (2019) measured the heterosis of intervarietal hybrids, which presented mean values of 88.35% for yield values similar to those obtained in this study (AB 71.29% Table 1). Khamphasan et al. (2020) obtained high and positive values of heterosis in all hybrids for the grain yield trait. For germination after four days, it was observed that the average heterosis of the AB population was 51.04% and for CD it was 2.67% (Table 1). Thus, the magnitudes of heterosis allowed us to infer that the greater the number of selection and recombination cycles, on average, the greater the heterosis for the seed germination characteristic.

The means for the AB and CD populations are described in Figure 1. There was a wide variation in the magnitude for the grain yield. In the AB population, the lowest value observed was 3278,76 kg.ha^{-1} for the hybrid 2AB, and the highest value observed was 6104,04 kg.ha^{-1} for the hybrid 1BA, which indicates the presence of genetic variability among the progenies of the eighth cycle of the RRS and reveals the existence of differences in the complementarity of the crosses between the two populations. In the CD population, there was a range of variation of 2202,61 kg.ha^{-1} (Figure 2). When evaluating the potential of sixth cycle maize progenies of the RRS, Vieira et al. (2021) found superior progenies in relation to the control, which enabled them to infer about the potential of the progenies for generating commercial hybrids.

Similar to grain yield, other agronomic characteristics, such as plant height and first ear insertion height, are important and desirable in maize crops. Regarding the average plant height, the highest estimates were observed for parents 4B, 5B, and 6B, with averages of 1.87, 1.86, and 1.81 m, respectively, and the lowest plant height was observed in parent 3B, with a mean of 1.46 m (Figure 1). For the first ear insertion height, within the AB population, a higher value was found for hybrid 1AB and for parent 5B (1.14 m and 1.03 m, respectively). The lowest values observed were 0.92 m for hybrid 3AB and 0.73 m for parent 3B. For the DC population, an amplitude of variation equivalent to 0.24 m was observed (Figure 1).

Historically, maize has been improved by selecting for produce smaller, compact corn plants with lower ear height to decrease the lodging percentage and enable planting density. Taller plants tend to be susceptible to lodging and/or breaking, drastically reducing their productivity (Xue et al., 2017). This scenario was evident in the AB population, where parent 2B had a high plant breakage percentage (47.17%) and low yield (data not shown). The other treatments resulted in mean plant breakage percentages ranging from 39.97% (5B) to 9.37% (3A) (data not shown). Lodging can reduce productivity by up to 28% during the 12-leaf stage (V12) and by up to 48% during the grain filling stage. In addition, bedded or broken plants are difficult to harvest and usually have malformed and burned grains (Li et al., 2015).

Table 1. Heterosis (%); mean maternal effect (ME); coefficient of variation for the traits grain yield (GY), days of female flowering (DFF), days of male flowering (DMF), height insertion of the 1st ear (HIFE), and plant height (PH); germination at four days (G4); germination at seven days (G7); vigor by the cold test (CT); emergence speed index (ESI); and root length to shoot length ratio (R/S) using GroundEye®. Data on the parent, hybrid, and reciprocal A, B, C, and D maize populations.

Population	Parameters	GY ($kg.ha^{-1}$)	DFF (days)	DMF (days)	HIFE (m)	PH (m)
AB	Heterosis AB	71.29	-7.98	-10.12	12.61	12.11
	Parents Mean	2689.49	67.33	68.19	0.91	1.66
	AB Hybrid Mean	4488.18	61.94	61.27	1.02	1.86
	General Mean	3661.19	64.65	64.85	0.97	1.77
	CV (%)	26.94	4.38	5.12	9.92	7.96
CD	Heterosis CD	2.39	1.54	0.97	-0.12	0.04
	Parents Mean	3269.77	60.66	60.11	0.96	1.69
	CD Hybrid Mean	3279.58	61.61	60.44	0.96	1.69
	General Mean	3336.21	60.97	60.45	0.96	1.70
	CV (%)	23.24	4.00	5.13	10.81	8.13
Population	Parameters	G4	G7	CT	ESI	R/A.
AB	Heterosis AB	51.04	2.19	2.21	-3.33	11.01
	Heterosis BA	70.47	-2.89	0.14	-3.67	-6.37
	Parents Mean	30.36	87.75	94.75	17.31	3.36
	Hybrid AB Mean	44.13	89.54	95.83	16.71	3.59
	Hybrid BA Mean	51.24	84.91	94.83	16.65	3.12
	General Mean	39.02	87.49	95.04	17.00	3.36
	p-value (ME)	0.042	0.080	0.354	0.905	0.013
	CV (%)	26.23	8.88	8.14	9.82	12.69
CD	Heterosis CD	2.67	1.09	5.36	1.8	12.19
	Heterosis DC	21.96	1.36	6.09	1.3	7.22
	Parents Mean	37.17	83.39	92.67	17.42	3.46
	Hybrid Mean CD	37.33	84.00	95.17	17.71	3.10
	Hybrid Mean DC	44.16	84.33	95.5	17.6	3.76
	General Mean	38.96	83.78	94.00	17.54	3.45
	p-value (ME)	0.047	0.913	0.840	0.841	0.502
	CV (%)	25.85	10.9	10.09	10.44	28.05

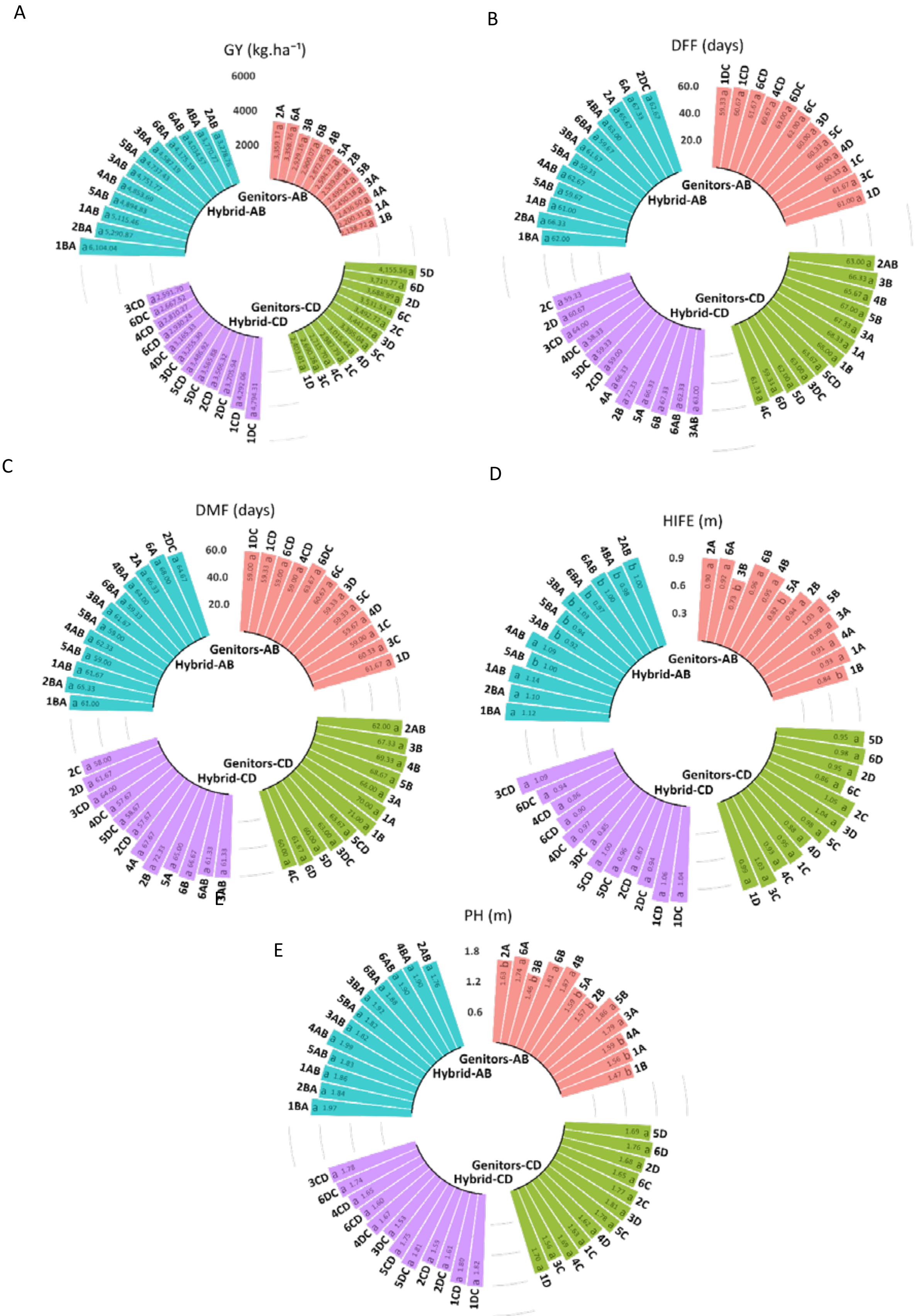

Figure 1. (A) grain yield (GY), (B) days of female flowering (DFF), (C) days of male flowering (DMF), (D) , height insertion of the 1st ear (HIFE) and (E) plant height (PH).

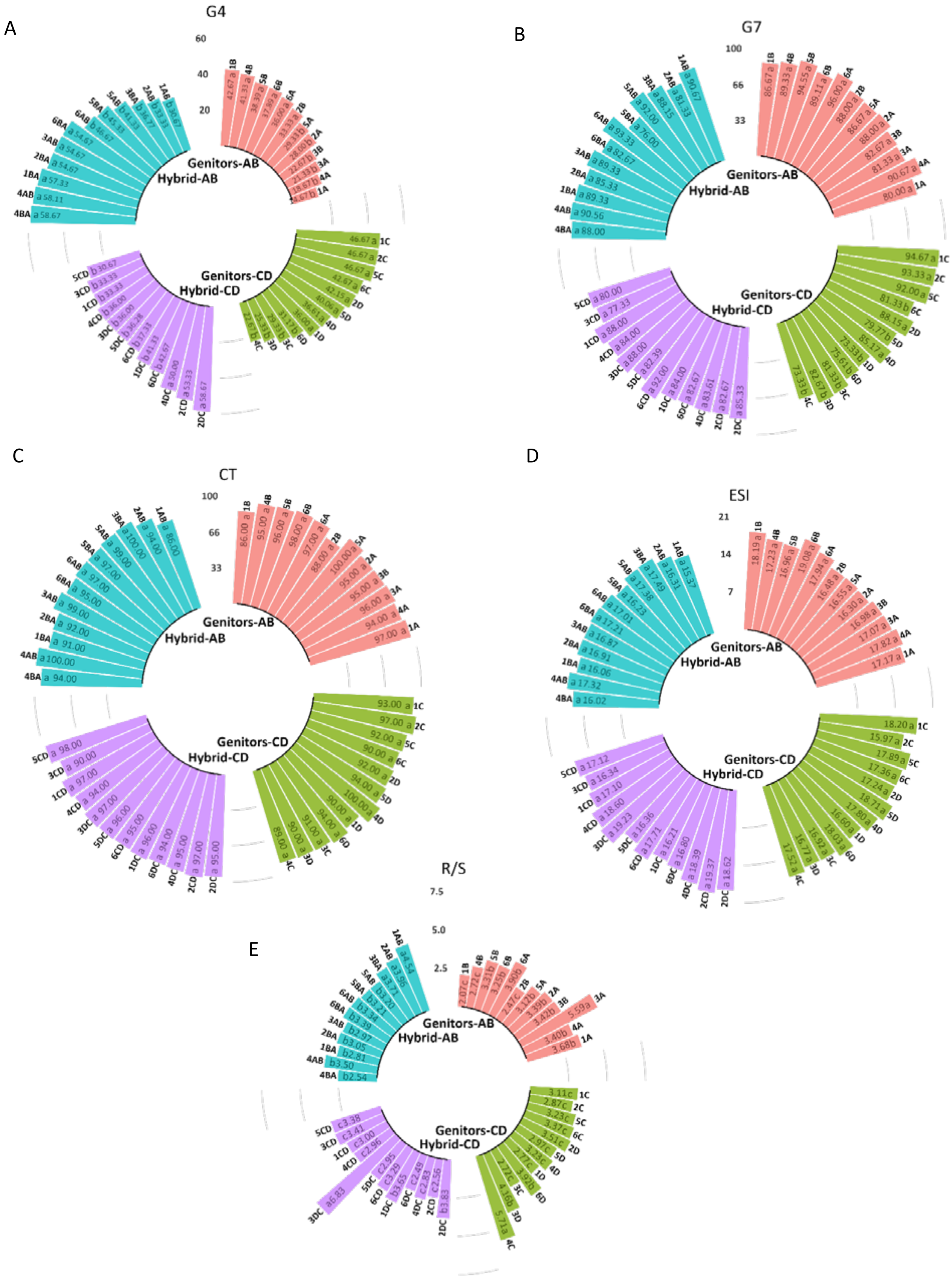

Figure 2. (A) Germination at four days (G4), (B) germination at seven days (G7), (C) vigor by the cold test (CT), (D) emergence speed index (ESI), and (E) root length to shoot length ratio (R/S) using GroundEye®.

In maize breeding programs, thousands of crosses are performed annually and tested, especially with a primary focus on the grain yield trait. In addition to this attribute, it is feasible to measure the potential physiological quality of the seed. Thus, in this study, an experiment was conducted in the laboratory to evaluate the physiological potential of maize seeds. Considering the coefficient of variation obtained, the characteristics evaluated in the laboratory obtained good experimental precision (Table 1).

Regarding the heterosis for germination at 4 days, it was found that on average, both the AB and DC populations had a greater heterosis values for the reciprocal. The contrast observed shows that there was a ME on the germination trait at 4 days for both the AB and CD populations (Table 1). Abreu et al. (2019) studied genetic trait control associated with tolerance to water deficit and observed a significance for the reciprocal effect on the physiological quality traits of maize seeds, highlighting the importance of correctly selecting the female parent for obtaining hybrids.

The means for all seed quality tests are shown in Figure 2. In the AB population, hybrids 4BA, 4AB, 1BA, 2BA, 3AB, and 6BA and parents 1B, 4B, 5B, 6B, 6A, and 2B showed superior performance over the others for the germination trait at four days. In the CD population, the superior hybrids for this trait were 2DC, 2CD, and 4DC, and the parents were 1C, 2C, 5C, 6C, 2D, 5D, 4D, and 1D. For the percentage of germination at seven days, a higher percentage was observed for hybrid 6AB (93.33%) and parental 6A (96.00%). In the CD population, there was a higher percentage of germination for the hybrid 6CD (92.00%) and for the parental 1C with 94.67% (Figure 2).

The results for the vigor test for the AB population, through the CT, indicated excellent performance of the treatments, and the test was performed with the soil moistened at 60% of its water retention capacity (Figure 2). Machado et al. (2020) evaluated the physiological quality of maize seeds under conditions of water deficit in the soil and found that irrigation depths lower than 70% of the field capacity decreased the water content in the soil and negatively affected the physiological quality of the maize seeds produced.

There was greater R/S values for the hybrids 1AB, 2 AB, and 3 BA and for the parent 3A using images in GroundEye®. Although parent 3A stood out, there was a low germination rate (81.33) at 7 days (Figure 2). Thus, the use of parameters that allow the identification of reduced seed quality is extremely important in seed analysis, as it will assist in the removal of genotypes with low plant emergence potential (Silva and Cicero, 2014). For the DC population, a higher value was observed for the hybrid 3DC (6.83) and the parent 4C (5.71).

For the four-day germination trait in the AB population, it was found that in all hybrids, there was heterosis (data not shown). Silva-Neta et al. (2020) evaluated the germination of corn seeds and obtained heterosis values ranging from -0.25 to 81.25%, revealing that hybrid vigor can and should be exploited for the production of hybrids tolerant to low temperatures during the germination process.

The correlated response estimates allowed an evaluation of hybrid behaviors for the different traits, considering the selection performed for grain yield. Once the most productive treatments were selected, there was also an increase in germination values for both the AB and CD populations (data not shown).

Thus, the importance of the selection cycles in the RRS program is evident, as the population that was in the 8th cycle of the RRS (AB) showed a higher mean heterosis value than the population that was in the 1st cycle (CD). This observation reinforces the importance of repeated selection and recombination cycles.

CONCLUSIONS

There is genetic variability between the parents and the hybrids for the attributes of grain yield and physiological quality, and it is possible to select superior genotypes.

The correlated response indicated that in general, more productive hybrids have better seed physiological quality.

The magnitudes of heterosis allowed us to infer that as greater as the number of selection and recombination cycles, on average, greater will be the heterosis for the seed germination.

ACKNOWLEDGMENTS

The authors thank the National Council for Scientific and Technological Development (*Conselho Nacional de Desenvolvimento Científico e Tecnológico* - CNPq) for the productivity fellowship for the author Dr. Adriano Teodoro Bruzi and the Minas Gerais Research Foundation (*Fundação de Amparo à Pesquisa do Estado de Minas Gerais* - FAPEMIG) for the financial support, as well as the Support and Evaluation Agency of Graduate Program (*Fundação Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* - CAPES) for the master's scholarship for the author Júlia Carvalho Costa.

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