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PHENOTYPIC SELECTION OF GRAIN SORGHUM RESTORER LINES

Abstract – This study aimed to select grain sorghum R lines based on grain yield and disease traits. The experiment consisted of 360 experimental R lines and two checks cultivars CMSXS180R and 9503062R. The experimental design used was of augmented blocks. The evaluated characteristics were plant height, days to flowering, thousand-grain mass, grain yield, and evaluation of leaf blight (*Exserohilum turcicum*) and anthracnose (*Colletotrichum graminicola*). In the analysis of variance, the interaction between Lines and checks was significant for all characteristics evaluated. All traits showed heritability above 70%, except a thousand-grain mass below 60%. In this work, the lines that obtained the best means were L57 and L74, considered earlier, with plant height ideal for grain and a high grain yield and resistance to foliar diseases.

Keywords: *Sorghum bicolor*, Line development, Sorghum breeding.

SELEÇÃO FENOTÍPICA DE LINHAGENS RESTAURADORAS DE SORGO GRANÍFERO

Resumo - O objetivo desse estudo foi realizar a seleção *per se* de linhagens restauradoras (R) de sorgo granífero, que se mostraram mais promissoras para atributos de rendimento de grãos e doenças em nível de campo. O experimento foi constituído de 360 linhagens R experimentais, e duas testemunhas elites CMSXS180R e 9503062R. O delineamento experimental utilizado foi de blocos aumentados. As características avaliadas foram altura de plantas, florescimento, massa de mil grãos, produtividade de grãos e avaliação de helmintosporiose (*Exserohilum turcicum*) e antracnose (*Colletotrichum graminicola*). Na análise de variância a interação entre as linhagens e testemunhas foi significativa para todas as características avaliadas. Todas as características apresentaram herdabilidade superior a 70%, exceto para massa de mil grãos que foi abaixo de 60%. Nesse trabalho as linhagens que obtiveram as melhores médias foram as L57 e L74, sendo consideradas precoces, com altura de plantas ideal para granífero, juntamente com alta produtividade de grãos e resistentes a doenças foliares.

Palavras-chave: *Sorghum bicolor*, Desenvolvimento de linhagens, Melhoramento de sorgo.

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Sorghum bicolor (L.) Moench is the fifth most important cereal globally, after wheat, corn, rice, and barley (Menezes et al., 2015). Furthermore, it has been used for animal feeding in Brazil due to its good digestibility and low production cost (Liu et al., 2013). Thus, grain sorghum is an excellent option as a supplement in feed formulations for animals (Rodrigues et al., 2014).

Grain sorghum has been grown in Brazil as the second crop in succession to soybean cultivation (Cruz et al., 2012; Tardin et al., 2013). As a result, Brazil reached about 2.6 million tons in approximately 849,000 ha in 2020 (Acompanhamento da Safra Brasileira [de] Grãos, 2020). Much of the production gain over time is due to improved agricultural practices and plant breeding (Pfeiffer et al., 2019).

Sorghum is a plant from a tropical climate; it presents its maximum production potential between 20 to 33 °C (Menezes et al., 2015). In addition, the African continent is the center of both origin and diversity, which has given it adaptive mechanisms and a certain rusticity, such as higher tolerance to water deficit than other plants, such as corn (Landau et al., 2015). Therefore, sorghum-breeding programs have sought new hybrids combinations with earliness, high grain yield, and ideal plant height for harvest, aiming to have cultivars adapted to these conditions (Kumar et al., 2011; Oliveira et al., 2019).

The development of lines is essential in the breeding program to develop more resistant, tolerant, and productive parents (Santos et al., 2005). Before discovering the sources of male sterility in sorghum, crosses were performed by manual emasculation, which demanded more time and money (Schertz, 1973; Secrist & Atkins, 1989). However, with the discovery of the male genetic sterility (GMS) with maternal inheritance in sorghum, the cultivars development made significant advances enabling the development of hybrids more efficiently. Even today, this system allows the use of A (sterile male), B (maintainer), and R (restorer) lines.

The steps of obtaining and selecting R lines are essential to the grain sorghum breeding program for male fertility restoration of line A of sorghum. The R lines are pollen sources in seed production fields, so both the elite lines already developed and the new lines in test crosses must-have traits of the high agronomic performance. Besides that, R lines have more significant genetic variability than the A and B lines (Menezes et al., 2017; Oliveira et al., 2019), requiring more extensive experiments and better performance evaluation methods. Given the above, the present study aimed to identify and select the most promising grain sorghum R lines for hybrid development based on traits of agronomic interest.

Material and Methods

The experiment was conducted at the Embrapa Maize and Sorghum Experimental Station, located in Sete Lagoas-MG, 19° 27’ 57” S and 44° 14’ 49” W, at 732 meters altitude. Three hundred and sixty R lines were evaluated, together with two check controls (CMSXS180R and 9503062R) already used as male parents in the grain sorghum breeding program. The 360 lines were distributed in 30 experimental blocks, in which the two controls were repeated, totaling

420 plots. The experimental design used was augmented blocks with one replication. The plot consisted of two rows of 5 m, with a spacing of 0.5 m inter-rows.

Sowing was carried out in a mechanized manner, with ten plants per meter and a final stand of 180,000 plants/ha. Before sowing, 400 $kg.ha^{-1}$ of 8-28-16 fertilizer (NPK) was applied with FPT, and 25 days after emergence, 160 $kg.ha^{-1}$ of urea was applied as a top-dressing. After sowing, a post-emergence herbicide (Atrazine) was applied at a dosage of 3.0 $L.ha^{-1}$, and the sorghum management was carried out according to the crop's need. In the experiment, sprinkler irrigation was used to supplement the crop's water requirement, according to the technical recommendation of approximately 450 mm.

The following characteristics were evaluated: plant height, measuring the length between the collar of the plant and panicle apex at the time of physiological maturation; flowering, counting the days between sowing and flowering, when 50% of the plants of the plot were flowered. The evaluations of the diseases leaf blight (*Exserohilum turcicum)* and anthracnose (*Colletotrichum graminicola*) diseases were performed using scores from 1 to 5 (1 = resistant and 5 = susceptible) and transformed by using $\sqrt{x}$. Grain yield was obtained by measuring the mass of grains, correcting the mass value to 13% moisture. The mass of a thousand-grains mass was determined by weighing 1,000 grains.

The statistical model of augmented block analysis was:

$$Y_{iyk} = \mu + E_i + B_{(i)j} + G_k + \varepsilon_{ijk}$$

Y_{iyk} is the observed value of treatment k, in block j, of experiment i; E_i is the effect of experiment i; $B_{(i)j}$ is the effect of block j, j =1,2, ...,30, within experiment i; G_k *is* $T_{k'} + T_{(j)k}$, G_k is the effect of the treatment. $T_{k'}$ is the fixed effect of the common treatment, $_{k'} = 1, 2$; $T_{(j)k}$ of the treatment is the random effect of the regular treatment *k* within block j, being k = 1, 2, ..., 420; $T_{(j)k} \sim NID(0, \sigma^2)$. ε_{ijk} is the effect of the experimental error. The statistical analyses were performed using the R program version 3.1.1, *augmented RCBD* package (R Core Team, 2014).

Results and Discussion

The effect of Lines (L) was significant ($p < 0.01$) for all traits (Table 1), meaning variability among the lines to be explored by genetic selection. The effects of the interaction Lines x Controls (L x C) and Checks (C) were significant too for all characteristics, indicating a difference between the evaluated lines, except for Thousand-grain mass. The evaluation of the blocks was not significant. According to the classification proposed by Pimentel-Gomes (2009), a coefficient of variation (CV) below 20% is a satisfactory thousand grains standard for traits evaluated in the field. Thus, considering that parameter, it can be inferred that plant height (3.64%), anthracnose (5.39%), flowering (6.67%), Leaf blight (15.56%), thousand-grain mass (15.53%), and grain yield (16.52%) showed CVs that demonstrate experimental precision and reliability.

The characteristics of resistance to Leaf blight (95%), plant height (84%), flowering (80%) and grain yield (73%), and anthracnose (73%) showed a high heritability, indicating

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Table 1. Analysis of variance for Plant height (cm), Anthracnose, Flowering (days), Leaf blight, Thousand-grain mass (g), and Grain yield (t ha^{-1}) for 362 grain-sorghum R lines.

Source of variation	DF	Plant height	Anthracnose	Flowering	Leaf blight	Thousand-grain mass	Grain yield
Lines (L)	359	0.44^{**}	0.11^{**}	165.97^{**}	36.24^{**}	31.51^{**}	2.61^{**}
Checks (C)	1	1.65^{**}	2.76^{**}	7370.42^{**}	147.27^{**}	1.61^{ns}	13.18^{*}
C vs L	1	0.8^{*}	1.36^{**}	3925.01^{**}	1713.53^{**}	7.49^{ns}	139.8^{**}
Block	29	0.15^{ns}	0.01^{ns}	35.76^{ns}	2.83^{ns}	15.62^{ns}	2.35^{ns}
Residue	29	0.13	0.01	28.18	7.47	12.93	2.32
h^2		0.73	0.95	0.84	0.80	0.59	0.73
CV (%)		5.39	15.56	3.64|	6.67	15.53	16.52

^{ns} non-significant, * and **Significant at 5% and 1% probability, respectively.

the possibility of high genetic progress (Table 1). Therefore, it is feasible to obtain a more significant genetic gain by selecting these R lines as male parents. These heritability values are similar to those attained by Silva (2016) in a study on grain sorghum, where they found results for heritability of 87% for flowering, 86% for plant height, 76% for grain yield, and 80% for anthracnose. Furthermore, a study carried out on sorghum by Cruz and Regazzi (1997) showed higher values than 70% for all the traits indicating a better opportunity to be successful in selecting those genotypes for these traits.

In Figure 1, all the 362 lines are represented, divided into groups according to the Scott & Knott test. The lines were divided into nine groups according to flowering. They flowered before the 75 days. The check cultivars flowered between 78 and 85 days, which means selection gain in earliness (Figure 1a). Flowering and grain filling are the most sensitive phases regarding drought tolerance, and earlier lines can escape the stress and have higher yields. In the second season in Brazil, sowing is carried out in February and March, and flowering occurs approximately in May and June, coinciding with a period of low rainfall. Therefore, the earlier the sorghum, the shorter the exposure time to water stress (Menezes et al., 2015). The existence of differences between flowering means between the lines contributes to the occurrence of the G x A interaction, as observed in Table 1. Possibly, the earlier lines had a better adaptation to the edaphoclimatic conditions at the time.

According to the grain production criterion, the lines were grouped into ten distinct groups (Figure 1b). Thus, it can be found that the two check cultivars are in groups 5 and 6, presenting the same yield statistically, with a mean of 7 t ha^{-1}. These values were close to those found by Tardin et al. (2013) studying sorghum hybrids, which indicates that the lines have high yield potential. Thus, the lines that are in the group 8 (5 lines), 9 (4 lines), and 10 (2

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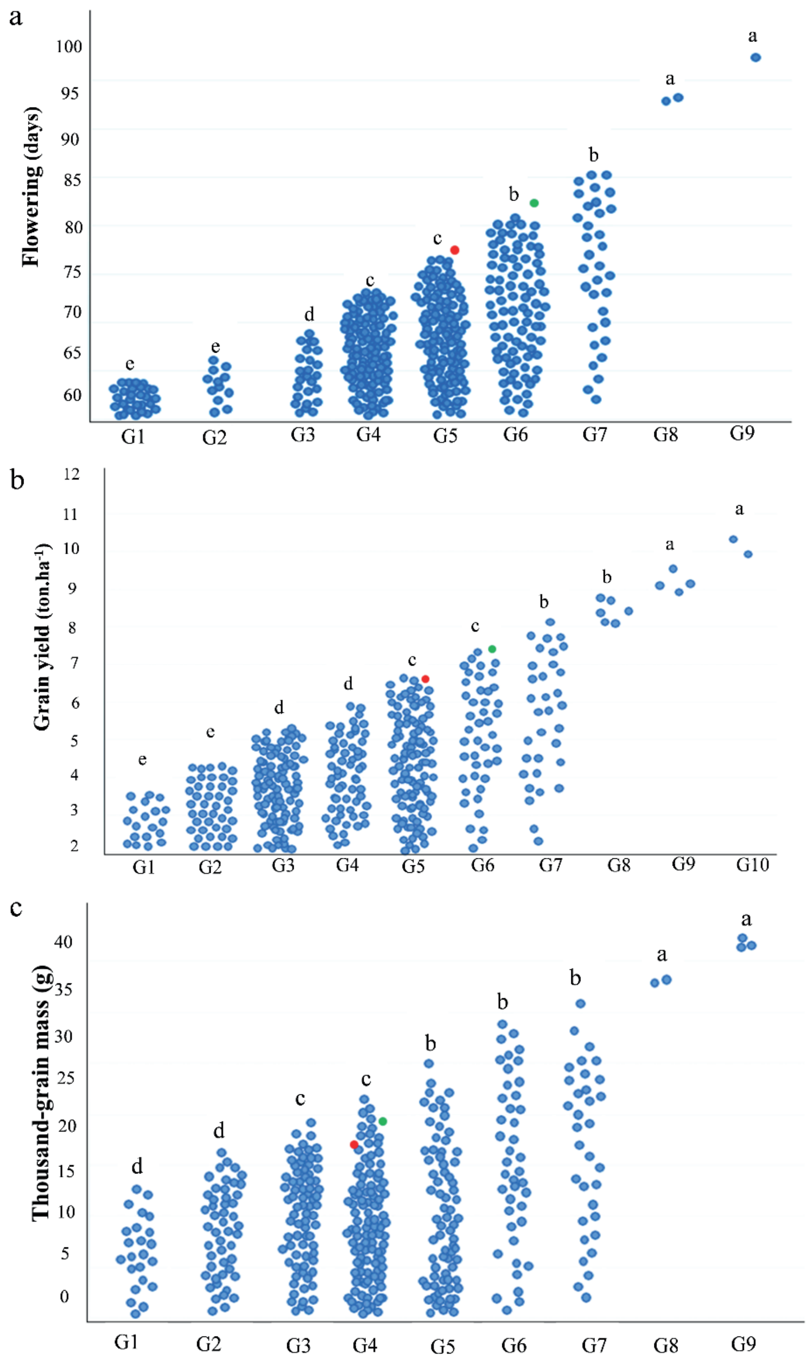

Figure 1. (a) Flowering, (b) Grain Yield, (c) Thousand-grain mass of the 362 lines classified in nine groups according to Scott & Knott. Checks: CMSXS 180R (L361) (red) and 9503062R (L362) (green).

lines) were the ones that outperformed the two controls: L361 (6.49 t ha^{-1}) and L362 (6.91 t ha^{-1}), with a yield above 7 t ha^{-1}, which presented yield higher than the national production. In the study carried out by Guedes et al. (2007), the mean productivity was five t ha^{-1}. Therefore, lines with grain yield below 4 t ha^{-1} are allocated in group 1. That grouping of genotypes was also carried out by Perazzo (2012) in sorghum cultivars in the semiarid region, forming five distinct groups, being possible to observe the existence of variability among the genotypes.

The means for thousand-grain mass are grouped into nine classes (Figure 1c). The two check cultivars are in group 4 with an average of 15 grams. The lines grouped from 5 to 9 showed means higher than 25g and had a thousand grains mass statistically higher than the controls. In work carried out by Heckler (2002) on grain sorghum hybrids, he obtained similar results, which ranged from 23.4 to 32.3 g, showing that the lines evaluated in the present study demonstrated a mass of a thousand grains compatible with the hybrids, which in theory tends to be superior to that of parental lines. Thousand-grain mass is a significant component of grain yield, and selecting lines with larger grains will result in more yielding hybrids.

Twenty-one lines were selected, through a selection index of 5% under the R lines evaluated. The two checks (L361 and L362) and lines L74 (36.52 g) and L50 (37.31 g) had the highest means for Thousand-grain mass. It was observed that the selected lines have a higher Thousand-grain mass than those obtained by Batista et al. (2017), which were 25 g, that evaluated the grain cultivation in a semiarid environment.

Table 2 presents the best 21 lines selected according to the best performance of each trait. However, the main parameters were plant height (between 90 and 130 cm) and high grain yield. Observing the flowering, the lines that proved to be the earliest concerning the check cultivars were lines L57, L74, and L88, with flowering days of 66, 68, and 68, respectively.

The plant height ranged from 90 to 130 cm in the selected lines and 78 to 98 cm for the controls. These results demonstrated that the trait plant height for the lines evaluated is within the range recommended by Menezes et al. (2015).

For resistance to leaf blight among the 21 selected lines, all had a score of 1, demonstrating resistance to this disease. Moreover, for resistance to anthracnose, we can see that the lines that showed better resistance than the controls were lines L18, L28, L44, and L99, which scored 1. Therefore, according to Cota et al. (2018) and Miedaner (2016), genetic resistance is the most efficient way to control these diseases.

There are currently eight systemic fungicides for controlling leaf blight and anthracnose in sorghum. However, most hybrids on the market already show high resistance to these diseases. Therefore, genetic resistance is the most effective method for diseases control. Furthermore, there is evidence of vertical and horizontal resistance in sorghum (Cota et al., 2012; Costa et al., 2008).

In plant breeding, the breeder works with many lines and progenies, with the need to reduce the number of replications in competition trials. The augmented block design proved easy to use and showed sufficient accuracy to select the best lines safely. The selection per se of lines

Table 2. Selection index of 5% under the R lines evaluated for the traits; plant height, anthracnose, flowering, leaf blight, Thousand-grain mass, grain yield, standing out the controls CMSXS 180R (L361) and 9503062R (L362).

Lines	Flowering (Days)	Plant height (cm)	Leaf blight (1-5)	Anthracnose (1-5)	Grain yield (t ha^{-1})	Thousand-grain mass (g)
L18	72	95	1.0	1.0	6.30	29.26
L28	72	120	1.0	1.0	5.22	27.92
L38	72	110	1.0	2.0	5.40	29.40
L44	77	120	1.0	1.0	5.94	26.90
L50	77	110	1.0	2.0	5.63	31.32
L57	66	95	1.0	2.0	7.65	30.88
L73	72	110	1.0	2.0	5.10	30.78
L74	68	90	1.0	2.0	8.78	31.52
L86	72	110	1.0	2.0	9.23	27.12
L87	72	90	1.0	2.0	5.18	27.52
L88	68	110	1.0	2.0	6.12	25.96
L91	77	100	1.0	2.0	6.00	26.68
L92	72	105	1.0	2.0	8.10	27.32
L99	77	105	1.0	1.0	5.63	28.88
L100	72	95	1.0	2.0	5.70	25.46
L109	72	110	1.0	2.0	7.20	27.76
L114	72	105	1.0	2.0	6.12	28.76
L217	77	100	1.0	2.0	7.20	28.98
L220	77	115	1.0	2.0	5.40	25.04
L300	72	130	1.0	2.0	7.65	29.72
L352	72	105	1.0	2.0	7.80	25.68
L361	75	98	1.0	2.0	6.49	20.62
L362	78	78	1.0	2.5	6.91	23.33

is crucial because these traits have a high general combining ability.

The high variability of the population allowed the selection of more yielding lines with good earliness and tolerance to leaf blight and anthracnose. These lines should be test crossed to female sterile “A” lines to check their combining ability, and those with the best performance will be registered as parental lines.

Conclusions

It was possible to select restorer lines with performance superior to those of the check cultivars already used as parentals in the grain sorghum program. In addition, the study showed high variability among the lines, resulting in high heritability for all the traits. Thus, genetic progress is feasible by selecting those lines and crossing them to female lines to produce new hybrids.

Lines L57 (7.65 t ha^{-1}) and L74 (8.78 t ha^{-1}) stood out as they presented earliness, ideal plant height, and high grain yield. In addition to being resistant to leaf blight and anthracnose. L86, L92, L300, and L352 lines also proved promising concerning the evaluated traits, surpassing the controls.

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