http://dx.doi.org/10.18512/1980-6477/rbms.v16n2p287-296

INDIRECT SELECTION FOR DROUGHT TOLERANCE IN MAIZE THROUGH AGRONOMIC AND SEEDS TRAITS

VIVIANE MARIA DE ABREU[1], ÉDILA VILELA DE RESENDE VON PINHO[1], MARCELA REZENDE DE CARVALHO[1], GLÓRIA MARIA DE FREITAS NAVES[1], RENZO GARCIA VON PINHO[1] and HELOÍSA OLIVEIRA DOS SANTOS[1]

[1]*Universidade Federal de Lavras, Caixa Postal 3037, CEP 37200-000 Lavras, MG. E-mail: vivianeabreu_ufla@yahoo.com.br, edila@dag.ufla.br, cecelarc@hotmail.com, glorinha.fn@gmail.com , renzo@dag.ufla.br, heloisasantos@dag.ufla.br*

Revista Brasileira de Milho e Sorgo, v.16, n.2, p. 287-296, 2017

ABSTRACT - High prolificacy, stay green, and a reduced anthesis-silking interval are among the traits most used for indirect selection for drought tolerance. The objective of this research was to evaluate five maize lines from the maize breeding program of the company Geneseeds Recursos Genéticos Ltda, to drought tolerance, grown at four plant population sizes, 40, 60, 80 and 100 thousand plants ha^{-1}. In these conditions, the following traits were evaluated: prolificacy, stay green and interval between female and male flowering, which are indirectly related to drought tolerance. The physiological quality of seeds and expression of the enzymes alpha amylase, esterase, catalase, superoxide dismutase, alcohol dehydrogenase, malate dehydrogenase, and heat resistant proteins were also evaluated. The experiment was conducted in a randomized block design in a split plot layout. Differences were observed between the lines for all traits analyzed. Lines 63, 64 and 91 showed the greatest yields and prolificacy, and lines 63, 64, 54 and 91 had the lowest intervals between male and female flowering. No effetc of plant population on the physiological quality of maize seeds was detected, and the highest values of germination and seed vigor were observed in lines 64, 44 and 91. Lines 63, 64 and 91 are considered as promising for drought tolerance.
Keywords: *Zea mays*, Abiotic stress, Flowering, Plant population, indirect selection.

SELEÇÃO INDIRETA PARA TOLERÂNCIA À SECA EM MILHO POR MEIO DE CARACTERÍSTICAS AGRONÔMICAS E DE SEMENTES

RESUMO - Alta prolificidade, *stay green*, o reduzido intervalo entre florescimento masculino e feminino estão entre os caracteres mais utilizados para seleção indireta para a tolerância à seca. O objetivo nesta pesquisa foi avaliar cinco linhagens de milho do programa de melhoramento da empresa Geneseeds Recursos Genéticos Ltda., quanto à tolerância à seca, cultivadas em quatro populações de plantio, 40, 60, 80 e 100 mil plantas ha^{-1}. Nessas condições foram avaliados os seguintes caracteres indiretos relacionados à tolerância à seca: *stay green*, prolificidade, intervalo entre os florescimentos feminino e masculino. Foi avaliada também a qualidade fisiológica das sementes e a expressão das enzimas alfa amilase, esterase, catalase, superóxido desmutase, álcool desidrogenase, malato desidrogenase e proteínas resistentes ao calor. O experimento foi conduzido em delineamento em blocos casualizados, no esquema de parcelas subdividas. Houve diferenças entre as linhagens para todos os caracteres analisados. As linhagens 63, 64 e 91 foram as que apresentaram maiores valores de produtividade e prolificidade e nas 63, 64, 54 e 91 foram observados os menores valores de intervalo entre os florescimentos masculino e feminino. Não houve a influência das populações de plantas sobre a qualidade fisiológica de sementes de milho, sendo que os maiores valores de germinação e vigor foram observados em sementes das linhagens 64, 44 e 91. As linhagens 63, 64 e 91 são consideradas promissoras quanto à tolerância à seca.
Palavras-chave*:* *Zea mays*, Estresse abiótico, Florescimento, População de plantas, Seleção indireta.

Stress by low water availability is one of the factors that limit the agricultural crops development. Thus, plant breeding programs have been selecting varieties with high productivity and water stress tolerance at the same time. Thereby, the identification and comprehension of drought tolerance mechanisms are crucial in the development of these new cultivars tolerant to drought stress conditions (Shao et al., 2008). In this sense, using phenotypic traits and visible factors, which are indirectly related to water stress tolerance, can improve cultivar selection efficiency to drought stress tolerance.

There are several indirect traits related to water stress tolerance, such as high prolificacy, stay green, reduced interval between male and female flowering (Bänzinger et al., 2000; Kamara et al., 2003). The synchronism of male and female flowering in maize under water stress can ensure the seed production (Xie et al., 2010) and is one of the most important traits to select genotypes to drought stress tolerance (Spitkó et al., 2014). According to Mugo et al. (2003), increased drought tolerance occurs simultaneously with increased tolerance to high densities, without incurring losses in productivity.

The characterization of genotypes through the traits associated with abiotic stress tolerance and evaluation of seed physiological quality can reduce costs and make the selection easier, in addition to providing parameters that will assist the selection of new genotypes.

Thus, the objectives of this study were to evaluate the performance of maize lines, grown in four plant population sizes, through indirect traits related to drought tolerance; evaluating the physiological quality of seeds of five maize lines.

Material and Methods

This study was conducted on an experimental area and the Central Seed Laboratory of the Agriculture Department, Federal University of Lavras (UFLA), in Lavras, Minas Gerais, Brazil.

The field was installed in the 2011/2012 crop season, for evaluation and seeds multiplication of the maize lines 63, 54, 64, 44 and 91, which were genotypes from the maize breeding program of the Geneseeds Genetic Resources Ltda. Four population sizes were used: 40, 60, 80 and 100 thousand plants ha^{-1}. All cultural and phytosanitary treatments were carried out according to the crops needs.

During the flowering, the plants were manually self-pollinated. Ears were harvested and husked manually, and dried at 35°C until the seeds reached 13% water content. Threshing was performed manually. Later, the seeds were stored in a cold chamber at 10°C until the physiological and enzymatic analysis.

The following characters were assessed: Male and Female Flowering, determined by the number of days between sowing until 50% of the plants in each plot had, respectively, visible blooming and silks. Male flowering was defined as the date of pollen release and the female flowering when the silks reached 1 cm long; Interval between female and male flowering in days (IFMF), determined in days by the sowing difference between male and female blooms. To remove negative values, it was added to all IFMF values a 100 constant value; Stay green, evaluated from the physiological maturity of grain at 125 days after sowing, on a scale varying from 1 to 5, according to the methodology suggested by Costa et al. (2008). Grades have been assigned to all plants of the plot in general, 7 in 7 days to reach the highest score. For analysis, it was used the grade average corrected by the date of the female flowering of each strain; Prolificacy, evaluating the average number of ears per plant on each plot; Grain yield, obtained by transforming the grain yield obtained in the floor area of the plot, expressed in kg ha^{-1} and corrected by a moisture content of 13%; and Weight of 100 grains, determined by the weight of six samples of 100 grains from the final production of each plot, corrected by a moisture content of 13%.

To evaluate the physiological quality, seeds produced in three replicates on the field (60 plots) were used, separately. In the laboratory, each repetition turned into two sub-samples, totaling six repetitions. After conducting the tests, it was considered the average of the two subsamples. The seeds were treated with the Vitavax-Thiram 200 SC fungicide at a dose of 3 ml kg^{-1} of seeds, prior to testing.

The evaluation of the seed quality was held by germination on paper towels, Germitest type, according to the Rules for Seed Analysis (Brasil, 2009); accelerated aging, according to the methodology cited by Andrade et al. (2013), with assessments at the fourth and seventh day after seeding, accounting for the percentage of normal seedlings; soilless cold test, according to the methodology proposed by Dias and Barros (1995) with assessments at the fourth and seventh day after seeding, accounting for the percentage of normal seedlings; germination speed index (ESI), germination speed index in aged seeds and germination speed index of seeds subjected to the cold test according to the methodology proposed by Maguire (1962), which was used as a parameter to evaluate the number of seedlings that have reached at least 3 cm of shoot and 4 cm of root, until stabilization.

For esterase isozyme (EST), catalase (CAT), superoxide dismutase (SOD), alcohol dehydrogenase (ADH), malate dehydrogenase (MDH) analysis in seeds produced under different populations of plants, two samples were used with 50 seeds of each line. The samples were macerated manually in a mortar in the presence of polyvinylpyrrolidone (PVP) antioxidant and in liquid nitrogen and stored in a deep freezer at -86°C. For the extraction of α-amylase enzyme, it was used the methodology proposed by Oliveira et al. (2013). The extraction of heat resistant proteins was performed according to the methodology described by Andrade et al. (2013). After electrophoresis, the gels were revealed and stained for enzymatic systems according to Alfenas (2006).

The experimental design was a randomized block design with three replicates, with plots subdivided in space, and the maize lines arranged in main plots and subplots were allocated four plant populations ha^{-1}. The results of evaluations of indirect traits in the field and the physiological quality analyzes were submitted to analysis of variances and comparison of means of each treatment for all tests was taken by Scott & Knott test, at 5% probability. Statistical analyzes were performed on Sisvar® software (Ferreira, 2011). The evaluation of enzymatic patterns was made according to the intensity of the bands.

Results and Discussion

The number of days observed from sowing to female flowering was 76.0 days for the earliest line (54) and 86.2 days for the latest line (44) (Table 1). About the male flowering, were observed 76.0 days and 84.4 days, respectively, for earliest and latest lines. By analyzing the male flowering data, there were no differences between these different plant population sizes (Table 2). However, to female flowering the number of days for projection of the silks was increased by the increase in plant population size. The occurrence of stress during flowering may delay the release of style-stigma, however the release of pollen is little influenced by this process (Xie et al., 2010).

Regarding the interval between female and male flowering, lower values were found for lines 63, 54 and 91, followed by 44 and 64. In those at 40,000 and 60,000 plants ha^{-1} the lowest values were observed for this trait, unlike the population sizes of 80 to 100 thousand plants ha^{-1}, which also produced the highest rates of IFMF. The viability of most of the silks of corn genotypes is around 14 days and 2 or 3 days after the release of pollen grains lasting between five to eight days after anthesis (Viana et al., 1999). Thus, negative values of IFMF indicate that the silk emission occurred before the onset of pollination, ensuring that the fertilization process is optimized.

The larger flowering interval between the male and female parent is negatively correlated with grain yield (Spitkó et al., 2014). So, it can derail the production of hybrid seeds once the flowering synchronization between parent plants is required to secure crossing (Xie et al., 2010). Also, according to Spitkó et al. (2014), protandry is one of the most important traits to select genotypes to drought tolerance. It was observed in the lines with lower IFMF values, 63 and 91, larger prolificacy values. According to Bänzinger et al. (2002), reduced IFMF decreases cob abortion and has close correlation with the number of ears per plant (prolificacy). Highest prolificacy values were observed for lines 63, 64 and 91, followed by lines 54 and 44 (Table 2). Among the analyzed populations, the 40,000 plants ha^{-1} resulted in higher prolificacy values. Populations of 80,000 to 100,000 plants ha^{-1} provided lower prolificacy values. According to Bolaños and Edmeades (1993), maize cultivars that have high prolificacy are better able to adapt in an environment with stress, such

Table 1. Averages for grain yield, weight of 100 grains (P100), prolificacy (PROL), stay green (SG), days to female flowering (FF), days to male flowering (MF) and interval between female and male flowering (IFMF).

Line	Grain yield (kg ha^{-1})	P100 (g)	PROL	SG	FF (days)	MF (days)	IFMF
63	5212 a	30.66 a	1.05 a	4.44 c	78.0 b	78.7 b	-0.7 a
54	2849 b	25.90 b	0.89 b	5.00 e	76.0 a	76.4 a	-0.4 a
64	4683 a	29.50 a	1.07 a	3.90 b	84.5 d	83.2 c	1.3 b
44	3312 b	23.98 b	0.88 b	2.95 a	86.2 e	83.2 c	3.0 c
91	4547 a	22.29 c	1.12 a	4.81 d	83.4 c	84.4d	-1.0 a
Plant Population size	Grain yield	P100	PROL	SG	FF	MF	IFMF
40,000	3376 b	27.11 a	1.19 a	4.19 a	80.9 a	81.1 a	-0.1 a
60,000	3808 b	26.46 a	1.00 b	4.17 a	81.3 a	81.1 a	0.2 a
80,000	4495 a	26.23 a	0.92 c	4.23 a	81.9 b	81.1 a	0.7 b
100,000	4804 a	26.07 a	0.89 c	4.30 a	82.5 b	81.5 a	1.0 b

* Means followed by the same letter in the column do not differ statistically by Scott and Knott test at 5% probability.

as high density sowing. However, selection for this singly trait could result in undesirable effects on other characteristics (Jampatong et al., 2000), making it necessary to select for stress tolerance based on a set of traits, enabling application of the prolificacy benefits.

Lower stay green was found for line 44 followed by 64. Among the different plant population sizes there were no significant differences. According to Zaidi et al. (2004), the stay green trait is directly related with tolerance to water stress after flowering. By comparing the data of grain yield and stay green it was not observed connection among traits, since higher productivity was observed for lines 63, 64 and 91 and the respective notes to stay green were 4.44; 3.90 and 4.81, respectively. According to Zaidi et al. (2004), higher presence of stay green would be associated with a greater number of seeds developed by ears, a higher weight average and a better performance, results which were not observed in this study.

Regarding grain yield (kg ha^{-1}), it is possible to observe the superiority of lines 63, 64 and 91 in comparison to others. In populations of 100,000 and 80,000 plants ha^{-1} were observed higher grain yield, a fact that was already expected due to the higher number of plants.

To the weight of 100 grains, lines 63 and 64 there had the highest average, followed by lines 54 and 44. The lowest weight of 100 grains average was observed in line 91. There were no differences between plant population sizes.

The average of the data obtained in the tests for physiological quality evaluation is shown in Table 2. There was no significant effect for the interaction between lines and plant population size, demonstrating that there was no influence of plant population on seed quality. The average degree of humidity of seeds at the time of testing was 12.2% with a maximum variation of 1%. The conditions during the seed production process may affect the behavior of the inbred line, which requires assessment of quality of seeds produced under different climatic conditions. The performance of maize lines can be affected by the conditions during the seed production. So, the quality of seeds produced under different conditions is an important parameter to be assessed, since the physiological quality is controlled by several genes (Silva et al., 2008).

The vigor in the early stage of germination process is critical for the establishment of plants in the field, especially under stress conditions, after sowing (Veiga et al., 2010). By the results of the germination test at four and seven days it was possible to differentiate the lines in two classes, with the lines 64, 44 and 91 higher compared to lines 63 and 54. Further ESI values were observed in seeds from lines 54 and 91. In the accelerated aging test, there was a greater vigor at day four in seeds from lines 91, 44, 64 and 54 and at day four for the seeds of lines 91, 44 and 64. As for the ESI assessed in aged seeds it was not possible to differentiate lines. With the cold test results, it appears the superiority of lines 91, 44 and 64, at four and seven days of assessment. Further ESI values under these conditions were observed for line 91 (Table 2).

Overall, higher values were observed for seed germination and vigor in the evaluated lines. It is noteworthy that these seeds were harvested, dried and processed using technologies that provide high seed quality. However, even in these conditions, considering all the tests, it was observed a higher germination and vigor values in seeds from lines 64, 44 and 91 in six of the nine tests used and lower

Table 2. Average results (%) of germination, accelerated aging and cold test, in seeds of maize lines 63, 54, 64, 44 and 91, produced under different plant population sizes.

Line	Germination			Accelerated aging			Cold test		
	4 days	7 days	ESI	4 days	7 days	ESI	4 days	7 days	ESI
63	95 b	96 b	23.9 b	94 b	96 c	24.8 a	96 b	96 b	40.6 c
54	96 b	97 b	29.1 a	97 a	97 b	29.5 a	96 b	98 a	45.5 b
64	98 a	99 a	27.5 b	98 a	99 a	29.0 a	97 a	98 a	45.7 b
44	97 a	99 a	24.5 b	99 a	100 a	28.2 a	98 a	99 a	39.1 c
91	99 a	99 a	28.2 a	99 a	99 a	29.9 a	99 a	100 a	48.4 a

* Means followed by the same letter in the column do not differ statistically by Scott and Knott test at 5% probability.

germination and vigor values were observed in seeds of the line 63.

Figure 1 shows α-amylase isozyme patterns in seeds of maize lines produced in different populations of plants. In general, higher expressions were observed for seeds in lines 63, 54 and 64. In seeds of line 64 it was observed an additional isoform (marked in Figure 1A with the number 1) with the patterns observed for the other lines. Also in seeds from lines 63 and 54 were observed increased enzyme expression when produced in the population of 100,000 plants ha^{-1}.

In maize, the alpha-amylase enzyme, when promoting the starch hydrolysis, provides carbohydrates necessary for the embryo development, allowing the germination process. However, Oliveira et al. (2013) points out that in addition to the amylase genes, several other genes may be involved in controlling the physiological quality of seeds trait, as an example, the genes directly related to respiration.

The esterase enzyme expression patterns are shown in Figure 1B. It was not possible to observe significant differences in the expression of this enzyme in seeds produced under different plant populations, result also observed when evaluating the physiological quality of seeds. Higher expression of the esterase enzyme was observed in seeds from line 64 and lower expression in line 91.

In Figure 1C, there is shown the expression profile of heat-resistant proteins. The differences in expression of these proteins refer to genotypes. According to Andrade et al. (2013), the difference in heat resistant proteins expression is dependent on genotypes and at different stages of development. Also, these proteins were stable for maize cultivar identification (Menezes et al., 2008). Similar patterns were observed in seeds of lines 63, 54 and 91, independently of the plant populations in which they were produced. In seeds of line 44 was observed lower expression of heat-resistant proteins.

For the MDH enzyme was not possible to see significant differences in expression in seeds produced under different populations of plants (Figure 1D). This same trend was observed by Veiga et al. (2010), where they do not found alterations in MDH expression in seeds submitted at different levels of potassium fertilization and liming. Lower expression of this enzyme was observed in seeds of line 54 and higher expression in seeds of line 63. This enzyme, related to aerobic respiration, is encoded by five loci and is found in great abundance in different cell organelles, in the mitochondria and cytoplasm

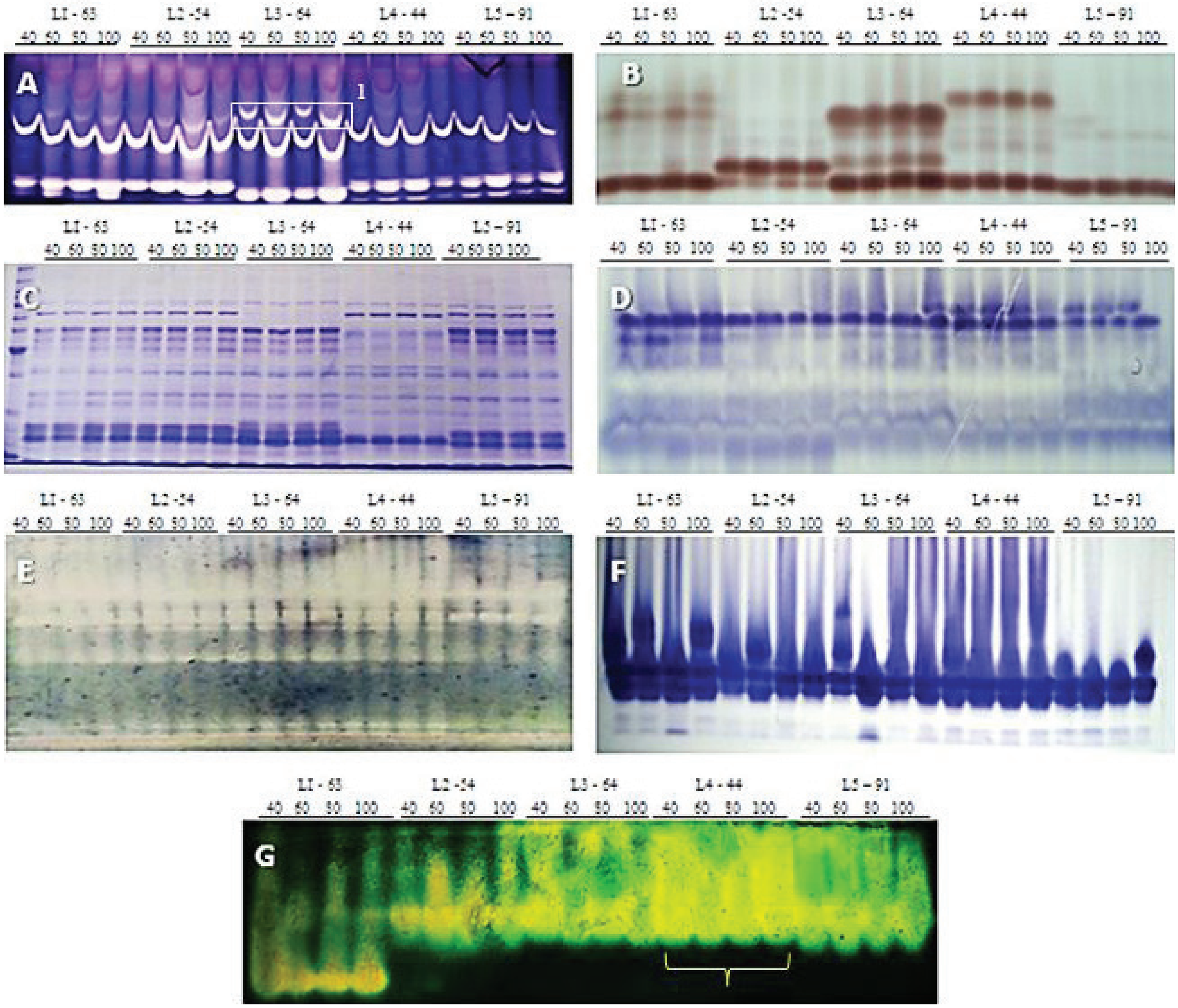

Figure 1. Protein standards for alpha-amylase (A), esterase (B), heat resistant protein (C), malate dehydrogenase (D), superoxide dismutase (E), alcohol dehydrogenase (F) and catalase (G), seeds of maize lines 63, 54, 64, 44 and 91 produced in different plant populations (40: 40,000 plants ha $^{-1}$; 60: 60,000 plants ha $^{-1}$; 80: 80,000 plants ha $^{-1}$ and 100: 100,000 plants ha $^{-1}$).

(Goodman & Stuber, 1987). Due to this, the change in its expression is observed only in more advanced deteriorating process in seeds and therefore, considered an inefficient marker of physiological quality.

At the SOD zymogram, no differences were observed in the expression in seeds produced under different populations of plants (Figure 1E). Lower expression of the SOD enzyme was observed, in general, in seeds of line 91, which were considered of higher physiological quality in most of the tests used to evaluate the physiological quality. The SOD is considered an antioxidant enzyme that acts in detoxification mechanisms acting as the first line of defense against reactive oxygen species, transforming the superoxide into hydrogen peroxide (Noctor & Foyer, 1998).

For the ADH enzyme, in general, there was a higher expression in seeds produced in the population of 100,000 plants ha^{-1} (lines 64, 44 and 91) (Figure 1F). The ADH enzyme acts in anaerobic metabolism, reducing acetaldehyde to ethanol oxidizing NADH to NAD+ (Bray et al., 2000). However, it were not observed differences in ADH expression in maize seeds submitted or not in controlled deterioration (Menezes et al., 2008).

Higher expression of the catalase enzyme was observed in seeds of line 44 (Figure 1G). The activity of this enzyme is related to the hydrogen peroxide decomposition formed by SOD in cells, acting as a second line of defense (Mallick & Mohn, 2000). The reduction in CAT activity may make the seed more sensitive to the effects of oxigen, free radicals, membrane unsaturated fatty acids and peroxidation production of secondary lipids (Gomes et al., 2000).

Even while working with six enzyme systems related to antioxidant systems, respiration and heat resistant proteins, it was not possible to associate the expression of proteins with the physiological quality results observed in this study. As already mentioned there are several genes that control this physiological quality of seeds trait.

Conclusions

For the grain yield, prolificacy and intervals between female and male flowering traits, the lines 63, 64 and 91 were the most promising for drought tolerance.

Increases in plant population sizes did not affect the physiological quality of seeds.

Best physiological quality was observed in seeds from lines 64, 44 and 91.

Acknowledgments

The authors acknowledge Fundação de Amparo à Pesquisa do Estado de Minas Gerais, Conselho Nacional de Desenvolvimento Cientifico e Tecnológico e Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, for supporting this research.

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