

Multivariate analysis revealed genetic divergence and promising traits for indirect selection in black oat

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ABSTRACT: This study aimed to identify important traits for indirect selection and to evaluate the variability among black oat populations through cause and effect relationships and canonical variables. Fourteen (14) black oat populations were collected in the 2013 cropping season which were evaluated in the laboratory, and then in the field in the 2014 cropping season. The seed width has a high and positive association with physiological quality of black oat seeds. The number of grains and thousand-grain weight has greater direct effects on the grain yield of black oat; thus, these traits may be considered for indirect selection in earlier phases of future black oat breeding programs. Targeted crosses between black oat genotypes coming from Salvador das Missões with genotypes coming from the other studied locations should be promising to obtain recombinant offspring in the future.

Key words: *Avena strigosa* S.; breeding; cause and effect; populations; variability

Análise multivariada revela divergência genética e características promissoras para seleção indireta em aveia preta

RESUMO: Este estudo teve como objetivo identificar características importantes para seleção indireta e avaliar a variabilidade entre populações de aveia preta através de relações de causa e efeito e variáveis canônicas. Quatorze populações de aveia preta foram coletadas na safra 2013, que foram avaliadas em laboratório e em campo, na safra de 2014. A largura das sementes apresenta alta e positiva associação com a qualidade fisiológica das sementes de aveia preta. O número de grãos e o peso de mil grãos têm maior efeito direto no rendimento de grãos de aveia preta; assim, essas características podem ser consideradas para seleção indireta em fases anteriores de futuros programas de melhoramento de aveia preta. Cruzamentos direcionados entre genótipos de aveia preta procedentes de Salvador das Missões com genótipos procedentes dos demais locais estudados devem ser promissores para obtenção de descendentes recombinantes no futuro.

Palavras-chave: *Avena strigosa* S.; reprodução; causa e efeito; populações; variabilidade

Introduction

Black oat (*Avena strigosa* S.) stands out among the most cultivated winter cereals worldwide. Among other grains of lesser importance, black oat is widely used for soil coverage, as well for forage production during the winter in the Southern region of Brazil (Caierão et al., 2001). The growing demand for superior black oat genotypes with more adequate vegetative and reproductive cycles to the current needs and greater potential for fresh and dry matter production lead genetic breeding programs to continually search for techniques which help in the process of early genotype selection. Another important factor considered by breeding programs is the association degree among traits of interest during the selection process.

Pearson's linear correlation stands out among the most used techniques for identifying association among traits, which reveals the sense and degree of linear association between two random traits (Nogueira et al., 2012). However, quantification and interpretation of the sense and magnitude of linear relationships may result in misunderstandings in selection strategy, because a third or a group of traits may be acting on the trait response in evidence (Cruz et al., 2012).

Path analysis has been widely used aiming at mitigating possible bias caused by the effect of other traits in correlation. This analysis enables understanding associations of cause and effect, meaning the direct and indirect effects of explanatory traits on the main trait. The indirect selection by traits with easy measurement and high heritability, which are associated with the dependent trait, has enabled faster genetic gains in relation to using direct selection (Vesohoski, 2011; Cruz et al., 2012). Santos et al. (2014) highlight that multiple character selection may be used to identify a superior genotype when a given character shows low to moderate possibility of gain from indirect selection.

As breeding programs work with a high number of genotypes and/or populations, it is often necessary to determine the dissimilarity degree among these individuals in order to identify possible parents with higher recombination ability. Thus, cluster analysis brings together parents or populations in groups which have similarity within the group and dissimilarity among groups.

The use of canonical variables allows to simplify the dataset, reducing the number of traits into a few components which are independent, thus aiming at retaining most of the observed variation (Cruz et al., 2012). Classifications of these groups are obtained through genetic divergence by quantifying the (dis)similarity among the tested genotypes using data from phenotypic traits. Group distinction may contribute to choosing parents (Cruz et al., 2012). It is crucial for breeders to identify traits which most contribute to (dis)similarity, thus enabling to plan the trait selection. Therefore, Singh's method (1981) is one of the most used methodologies to determine relative trait contribution to the total variation (Marchioro et al., 2003).

In view of the fact that few studies have been conducted in this field on black oat, the aims of this study were to: (i) assess

the cause and effect relationships among quantitative traits aiming to identify promising traits for indirect selection; and (ii) to use canonical variables to evaluate the variability among black oat populations.

Materials and Methods

Source of plant material

First, 3-kg samples of seeds of black oat populations were collected from farms in 14 municipalities of Rio Grande do Sul State in the 2013 cropping season. A total of 14 populations were collected and named according to the municipality of origin, namely: Taquaruçu do Sul, Palmeira das Missões 1, Palmeira das Missões 2, Condor, Alto Alegre, Campos Borges, Chapada, Tenente Portela, Boa Vista das Missões, Salvador das Missões, Santa Rosa, Espumoso, Planalto, and Ajuricaba. These municipalities faithfully represent the greater producing oat region of the state of Rio Grande do Sul, and are geographically represented in Figure 1.

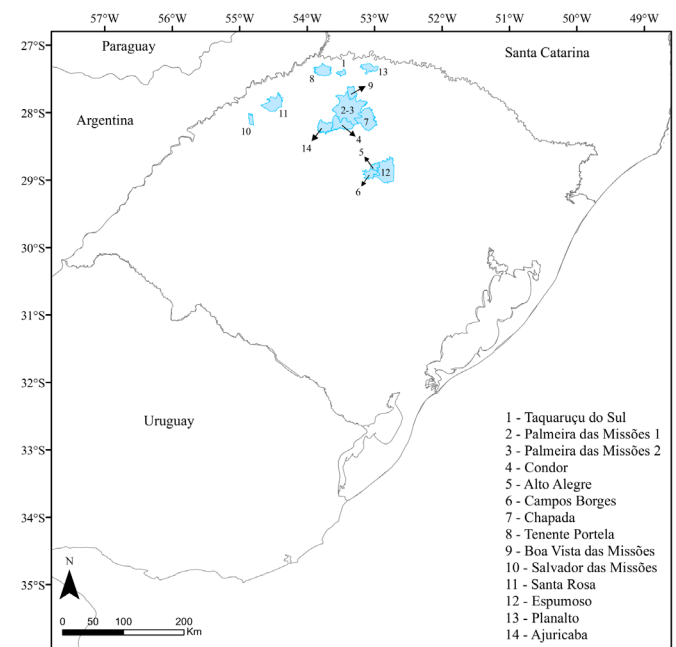


Figure 1. Collection site map of the 14 populations of black oat in Rio Grande do Sul (RS).

Preliminary laboratory analysis of the samples

After the samples were collected, some initial evaluations were performed with the seed samples of each black oat population in the Genetic Breeding and Plant Production Laboratory (GBPPL) of the Federal University of Santa Maria, Campus Frederico Westphalen, RS. The following traits were assessed: seed length (SL, mm) and seed width (SW, mm) measured with a digital caliper in four replicates by the mean of 10 seeds (subsamples) each replicate. The thousand-seed weight (TSW, g) was evaluated by counting eight replicates of 100 seeds of each black oat population was weighed on an analytic scale and extrapolated to the weight of one thousand seeds.

Physiological characters were evaluated in an experiment conducted in a germination chamber in a randomized complete block design with four replicates. To do so, 400 seeds of each population (four subsamples of 100 seeds of each replicate) were used. The seeds were placed on a germitest-type paper, and then moistened with distilled water in a proportion of 2.5 times their dry weight. Next, the samples were kept in a B.O.D germination chamber at a constant temperature of 20 °C (MAPA, 2009).

The traits evaluated in this preliminary experiment were: first germination count (FGC), obtained by percentage of normal seedlings at five days after test installation; and germination percentage (GP), evaluated at ten days by counting the percentage of normal seedlings. Ten seedlings were then randomly selected from each replicate at the end of the germination test, and the following characters were assessed in each seedling: shoot length (SHL, mm) and root length of seedling (RL, mm), as measured with a digital caliper. The results were then averaged for each replicate. Seedling dry mass (SDM, g) was assessed with the same previously randomly selected seedlings. To do so, the seedlings were conditioned in an oven with forced air circulation at 60 °C until reaching a constant mass. Afterward, the dried seedlings of each replicated were weighed on an analytical scale.

Field experiment

The field experiment was carried out in the experimental area of the Genetic Breeding and Plant Production Laboratory of the Federal University of Santa Maria, Campus Frederico Westphalen, RS, located at the geographic coordinates of 27°23'26"LS; 53°25'43"LW, at 461 m asl. The region's climate is classified as subtropical humid with an average annual temperature of 19.1 °C and average annual precipitation of 1880 mm, and the soil is classified as Hapludox (Alvares, 2013).

The 14 collected populations were conducted in the 2014 cropping season in a randomized complete block design, with three replications. Experimental units were composed of twelve 3-m long sowing rows, spaced at 0.17 m. Seeding was carried out on June 17th, 2014, with a seed density to ensure 300 suitable plants m⁻². Nutritional management was according to soil analysis.

The harvest was performed at the physiological maturation stage. At that time, 10 plants per experimental unit were randomly collected and the following traits were analyzed: plant height (PH, cm), measured from the ground up to the plant apex; panicle length (PL, cm), measured from the insertion of first branch of panicle to the panicle apex; number of grains per main panicle (NG); number of branches in the panicle (NB); weight of one thousand grains in the panicle (TGW, g) and grain weight/panicle weight ratio (GPR), also known as harvest index.

We used the harvested grains of each plot to assess the thousand-grain weight (TGW, g). To do so, eight replicates of 100 grains of each plot were weighted on an analytical scale, and the results were averaged (MAPA, 2009); grain yield (GY, kg ha⁻¹) was obtained by averaging the total grain mass of

the ten plants collected per experimental unit after moisture correction to 13%, and subsequently extrapolated to the plant density per experimental unit.

Statistical analysis

The data from the 15 evaluated traits was submitted to ANOVA by F-test, at $p \leq 0.05$ after observing the assumptions of variance homogeneity and residual normality. A Pearson correlation matrix was calculated, and its magnitude classified according to Carvalho et al. (2004). The Pearson's correlation analysis was performed for the original data of all evaluated traits using the *agricolae* package (Mendiburu, 2017), and it was graphically represented using the *corrplot* package (Wei & Simko, 2017) in R software (<http://www.R-project.org>).

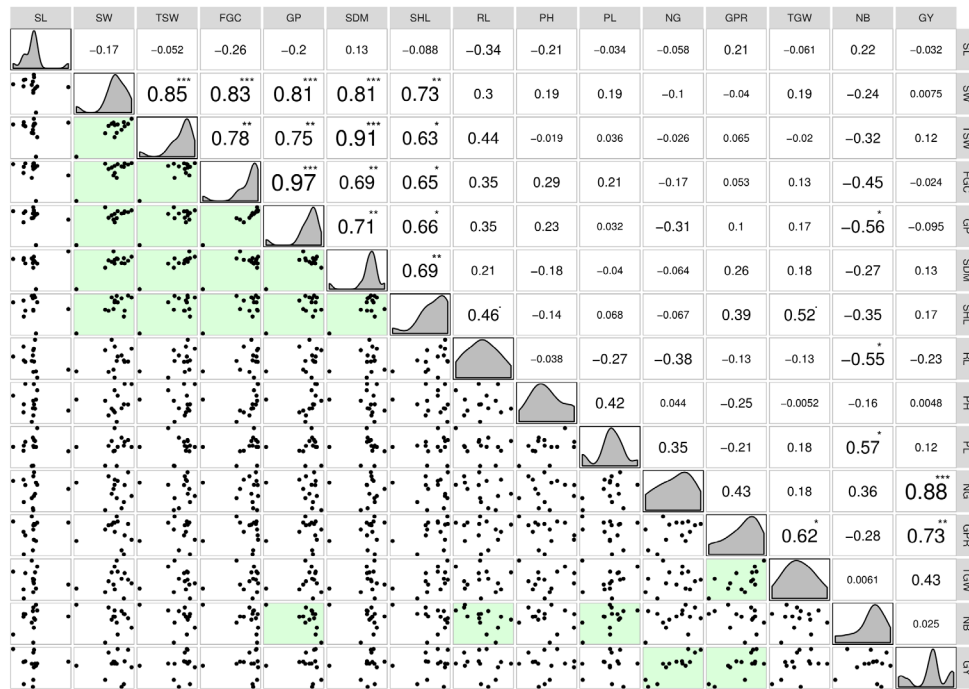
To proceed with the path analysis, the multicollinearity of the correlation matrix among the 14-explanatory traits (put here the explanatory traits) was initially diagnosed by the condition number (CN), given by the ratio between the largest and smallest eigenvalues of explanatory traits correlation matrix. We decided to exclude the variables causing severe multicollinearity problems from the analysis, as suggested by Olivoto et al. (2017). After solving possible problems with multicollinearity, a phenotypic path analysis was performed considering GY as the dependent trait and those variables that remained after multicollinearity adjustment as explanatory traits. Path analysis was realized using *cursoR* (Olivoto, 2019) package in R software (<http://www.R-project.org>).

Canonical variate analysis was computed to assess the (dis)similarity among the studied populations based on the 14-phenotypic variables evaluated (Albrecht, 1980). Canonical variate is in probing the multivariate data space for a relatively few dimensions, which efficiently summarize the majority of the originally widely distributed information among the descriptor traits, in addition to maximizing the variation in a reduced number of axes which are constructed so as to be uncorrelated with respect to both. Unlike principal component analysis, the residual variance-covariance matrix obtained in the ANOVA procedure can be and was used to consider the covariation and within-population dispersion in the multivariate analysis (Gower, 1996). The relative contribution of each trait in the (dis)similarity was estimated by the method proposed by Singh (1981). Statistical procedures were performed with Genes statistical software (Cruz, 2016) and graphically represented using the *ggplot2* package (Wickham, 2016) in R software (<http://www.R-project.org>).

Results and Discussion

Linear relationships among evaluated traits

The SW, TSW, FGC, GP, SDM, and SHL showed strongly and positive associations between them (Figure 2). Seeds with higher SW tend to increase TSW; in addition, they may contain higher energy reserves, improving the germination percentage and vigor. Ahmad et al. (2013) showed that selection for seed length and width associated to the number of leaves per plant, number of tillers, plant height, stem diameter, and thousand-



*, **, *** Pearson linear correlation coefficients (n=42) significant levels at $p < 0.05$, 0.01 and 0.001 , respectively. SL: seed length; SW: seed width; TSW: thousand-seed weight; FGC: first germination count; GP: germination percentage; SDM: seedling dry mass; SHL: shoot length of seedlings; RL: root length of seedlings; PH: plant height; PL: panicle length; NG: number of grains; GPR: weight of panicle grains and weight of panicle ratio; TGW: thousand-grain weight; NB: number of branches per panicle; GY: grain yield.

Figure 2. Pearson linear correlation of 14 black oat populations relative to 2014 cropping season.

seed weight may improve plant breeding, aiming at a higher forage and better quality seed production.

NB presented moderate and negative association with GP and RL; in other words, the increase in NB reduces seed size and physiological quality. Alves & Kist (2011) verified that the best germination and vigor rates were obtained from seeds with higher weight, regardless of which oat genotype was evaluated. A positive and moderate association between NB and PL was also observed; this can be practically explained because a larger panicle length can support a greater number of branches.

The NG and GPR traits showed strongly positive correlations with GY, revealing that there is an increase in grain yield when the number of grains per plant and its weight are increased. In studies with white oat, Benin et al. (2003) observed that panicle weight, number of panicles per plant and average grain weight might be used in the indirect selection for grain yield. The same was observed by Caierão et al. (2001) regarding traits, number of grains per panicle and grain weight.

The GPR showed a moderate and positive correlation with TGW, meaning that the increment in TGW has a positive effect on GPR since heavier grains tend to increase the GY. The identification of genotypes that show association among NG, GPR, and TSW with GY must be prioritized, but care must be taken because the increase in NG may lead to a reduction in grain weight due to competition by photoassimilates. In wheat research, Valério et al. (2008) have concluded that the increase in number of spikes results in lower grain filling, and a compensatory effect occurs when there is less tillering, thereby improving the efficiency in using photoassimilates.

In this sense, the selection of plants with a high capacity of producing photoassimilates with a greater number of grains per panicle and maintenance of grain weight must be prioritized. Studies conducted by Valério et al. (2009) corroborate our findings, showing that there is an increase in grain yield when there is a greater number and grain weight per panicle.

Cause and effect relationships

When evaluating the multicollinearity of the phenotypic correlation matrix among the explanatory traits, we observed severe multicollinearity effects caused by the traits SL, SW, TGW, FGC, SHL, RL. Thus, these traits were removed from path analysis to circumvent the harmful effects of multicollinearity (Olivoto et al., 2017).

The phenotypic estimates of cause and effect in the path analysis, showed to be trustworthy due to the high value of the determination coefficient (0.97) and low residual effects (0.17). Thus, it is important to identify the direct and indirect effects between explanatory traits and the main trait in black oat populations (Table 1).

GP expressed a negative direct effect of weak magnitude on the dependent trait (GY). The indirect effects of GP revealed a negative and low magnitude with NG. It is worth pointing out that population seeds were collected from small farms, meaning they were saved seeds so the generation and quality of storage were not known. SDM showed a positive direct effect of low magnitude to GY, while null values were verified for indirect effects of other traits.

No direct and indirect effects were verified for PH. In contrast, Hartwig et al. (2006) pointed out that high plants

Table 1. Phenotypic estimate of direct and indirect effects in eight traits on dependent trait (grain yield) of 14 black oat populations.

Effects	Explanatory traits							
	GP	SDM	PH	PL	NG	GPR	TGW	NB
Direct on GY ¹	-0.01	0.13	0.03	-0.08	0.89	0.10	0.22	-0.25
Indirect via GP	-	-0.07	-0.02	0.00	0.03	-0.01	-0.02	0.05
Indirect via SDM	0.09	-	-0.02	-0.01	-0.01	0.03	0.02	-0.03
Indirect via PH	0.01	0.00	-	0.01	0.00	-0.01	0.00	0.00
Indirect via PL	0.00	0.00	-0.03	-	-0.03	0.02	-0.01	-0.04
Indirect via NG	-0.28	-0.06	0.04	0.32	-	0.39	0.16	0.33
Indirect via GPR	0.01	0.03	-0.03	-0.02	0.05	-	0.06	-0.03
Indirect via TGW	0.04	0.04	0.00	0.04	0.04	0.13	-	0.00
Indirect via NB	0.14	0.07	0.04	-0.14	-0.09	0.07	0.00	-
Linear (r)	-0.10	0.13	0.01	0.12	0.88**	0.73**	0.43	0.02
Determination coefficient								0.97
Variable residual effects								0.17
Condition number								32.61

** , * Pearson linear correlation coefficients (n=42) significant at 1 and 5% error probability, respectively. ¹GY: grain yield; GP: germination percentage; SDM: dry matter of seedlings; PH: plant height; PL: panicle length; NG: number of grains; GPR: weight of panicle grains and weight of panicle ratio; TGW: thousand-grain weight; NB: number of branches of panicle.

in white oat crosses tend to be more productive because they present physiological advantages over competitors, and they also pointed out identification of genetic constitutions, presenting associations of desirable quantitative traits with qualitative traits which require complex studies. In studying traits for dry and fresh matter production, Cargnelutti Filho et al. (2015) showed that plant height has a positive linear relation and can be used for indirect selection.

The direct effect of PL for GY was null, but it expressed a moderate indirect positive effect via NG, noting that a panicle increase may result in a higher number of grains. The NG has a direct positive influence and high magnitude in GY, and indirect effects are null in the other traits, with Pearson correlation significant to 1% probability of error, evidencing a high magnitude of a positive relationship ($r = 0.86$).

The GPR revealed direct positive effects of low magnitude for GY and expressed a positive and moderate indirect effect for NG and TGW. In studying generations of crosses, Hartwig et al. (2006) obtained high phenotypic correlations between number of grains per plant and grain weight per plant. On the other hand, Benin et al. (2003) point out that the traits with greatest direct and indirect effects such as panicle weight, number of panicles per plant and average grain weight to grain yield as the main traits for superior genotype selection. Hartwig et al. (2006) evidenced that there is a positive correlation between grain mass and grain yield per plant in generations of crosses.

The TGW expressed a positive direct effect of weak magnitude for GY, and indirect effects via NG. Lorencetti et al. (2006) found that a combination between panicle weight and thousand-grain weight traits; while Benin et al. (2003) and Hartwig et al. (2006) found that a combination between number of panicles per plant and grain yield per plant potentiate indirect selection, aiming for higher grain yield. In wheat, Vesohoski et al. (2011) verified that indirect selection for grain yield from number of grains per spike considering thousand-grain weight is the best strategy.

NB showed a negative direct effect of weak magnitude for

GY, and indirect positive of moderate magnitude for NG. Thus, the higher NB indicates a greater amount of NG, but it cannot be claimed that an increase in GY occurs.

Multivariate-based genetic divergence among black oat populations

In the present study, it was verified that the first two canonical variables (CV) explained 85.32% of the total variation observed (Figure 3). Thus, the accumulated variance in the first two canonical variables may be considered reliable to represent the divergence among the populations in a two-dimensional graphic representation (Cruz et al., 2012).

By examining the graphical dispersion of the two firsts scores it was possible to identify two groups of black oat populations. Group 1 was composed of 13 populations, except for the population from Salvador das Missões (Figure 3). Aiming at increasing the probability of obtaining superior segregant genotypes, the crosses between superior genotypes coming from different groups should be prioritized. Thus, targeted crosses between superior genotypes from Salvador das Missões with superior genotypes from the other locations should be promising to generate segregant populations in future black oat breeding programs.

Four assessed variables (TSW, GP, SW and SL) were responsible for 82.33% of the differentiation among the studied populations, namely, 51.54%, 15.73%, 7.84% and 7.22%, respectively (Table 2). Scheffer-Basso et al. (2012) verified that base pilosity of grain and cycle are traits that contribute to a greater percentage for dissimilarity in wild oat. The GY, NB, TGW, PH, and PL contributed less than 2% for differentiation of the studied oat populations. Thus, these traits can be disregarded from evaluation, reducing cost and phenotyping time in future genetic divergence studies of black oat.

The seed width has a high and positive association with physiological quality of black oat seeds. The number of grains and thousand-grain weight has the greater direct effects on grain yield of black oat; thus, these traits may be considered for indirect selection in earlier phases of future black oat

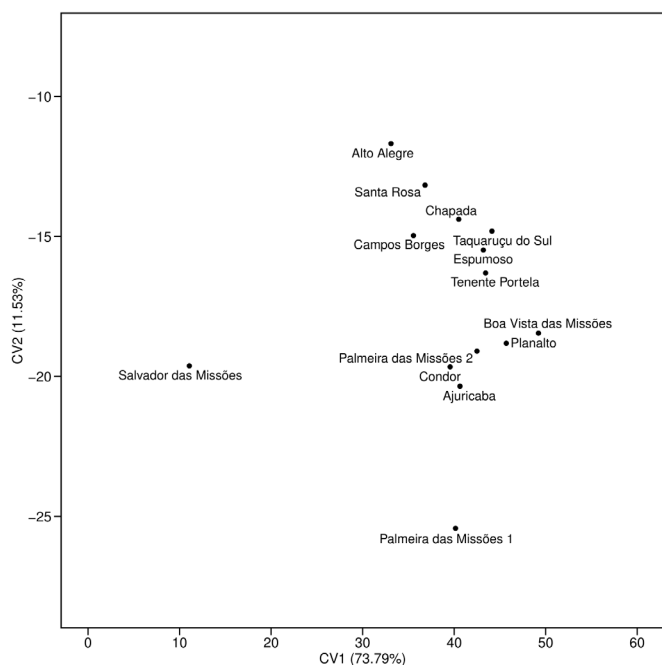


Figure 3. Graphical dispersion of scores obtained by canonical variables analysis of 14 black oat populations obtained in the 2014 crop season.

Table 2. Relative contribution of traits to divergence - Singh (1981) in 14 black oat populations.

Trait	S _j	Value (%)
SL ⁽¹⁾	1455.205	7.22
SW	1581.658	7.84
TSW	10396.62	51.54
FGC	1190.337	5.90
GP	3172.249	15.73
SDM	486.0609	2.41
SHL	458.9793	2.28
RL	360.8592	1.79
PH	85.03659	0.42
PL	89.20996	0.44
NG	215.9593	1.07
GPR	333.969	1.66
TGW	125.8303	0.62
NB	37.80056	0.19
GY	180.6303	0.90

¹SL: seed length; SW: seed width; TSW: thousand-seed weight; FGC: first germination count; GP: germination percentage; SDM: seedling dry mass; SHL: shoot length of seedlings; RL: root length of seedlings; PH: plant height; PL: panicle length; NG: number of grains; GPR: weight of panicle grains and weight of panicle ratio; TGW: thousand-grain weight; NB: number of branches of panicle; GY: grain yield.

breeding programs. Targeted crosses between black oat genotypes coming from Salvador das Missões with genotypes coming from the other studied locations should be promising to obtain recombinant offspring in the future.

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