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Agronomic, pollen and molecular aspects of *Avena strigosa* and *Avena brevis* and their respective hybrids

Aspectos agronômicos, polínicos e moleculares de *Avena strigosa* e *Avena brevis* e respectivos híbridos

Bianca Oliveira Machado

Passo Fundo, 25 de setembro de 2024.

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“Crê em ti mesmo, age e verás os resultados. Quando te esforças, a vida também se esforça para te ajudar”.

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RESUMO

MACHADO, Bianca Oliveira. Aspectos agronômicos, polínicos e moleculares de Avena strigosa e Avena brevis e respectivos híbridos. 83 f. Tese (Doutorado em Agronomia) – Universidade de Passo Fundo, Passo Fundo, 2024.

Reconhecida entre os principais cereais de inverno a aveia é mundialmente cultivada e uma das principais culturas de inverno no sul do Brasil. O gênero *Avena* é representado por várias espécies, incluindo espécies silvestres e cultivadas, com diferentes níveis de ploidia: diploides, tetraploides e hexaploides. A espécie diploide *Avena strigosa* Schreb. é amplamente utilizada como forrageira anual de inverno para o pastejo e empregada no sistema plantio direto como cobertura do solo. Devido a sua importância, o melhoramento genético busca selecionar genótipos superiores, além de incorporar variabilidade genética nos programas, o que inclui o interesse por parentes silvestres. O cruzamento entre *A. strigosa* e *A. brevis* Roth. (ambas diploides) geram descendentes férteis. Entretanto, poucas informações estão disponíveis sobre a *A. brevis* e seu uso na hibridação interespecífica com a *A. strigosa*. O capítulo I visa inferir quanto à fertilidade dos grãos de pólen em genótipos de *A. strigosa* e *A. brevis*, assim como nos genótipos resultantes do cruzamento entre essas espécies nas gerações F_1 e F_2. O capítulo II tem como objetivo avaliar o desempenho genotípico de cultivares e linhagens de aveia, cultivadas no Rio Grande do Sul, de 2016/2017 a 2021/2022, utilizando a metodologia de modelo misto. O capítulo III busca determinar a dissimilaridade genética das populações oriundas dos cruzamentos entre *A. strigosa* e *A. brevis* na geração F_3.

Palavras-chave: 1. Aveia. 2. Hibridação interespecífica. 3. Dissimilariddae genética. 4. Melhoramento genético. 5. Interação genótipo x ambiente.

ABSTRACT

MACHADO, Bianca Oliveira. Agronomic, pollen and molecular aspects of Avena strigosa and Avena brevis and their respective hybrids. 83 f. Thesis (Doctorate in Agronomy) – University of Passo Fundo, Passo Fundo, 2024.

Recognized as one of the main winter cereals, oats are cultivated worldwide and represent one of the leading winter crops in southern Brazil. The genus *Avena* is represented by several species, including wild and cultivated species, with different levels of ploidy: diploids, tetraploids, and hexaploids. The diploid species *Avena strigosa* Schreb. is widely used as a winter annual forage for grazing and employed in no-till systems as soil cover. Due to its importance, genetic breeding aims to select superior genotypes and incorporate genetic variability into programs, including an interest in wild relatives. Crossbreeding between *A. strigosa* and *A. brevis* Roth. (both diploids) produces fertile offspring. However, limited information is available about *A. brevis* and its use in interspecific hybridization with *A. strigosa*. Chapter I aims to infer pollen grain fertility in genotypes of *A. strigosa* and *A. brevis*, as well as in genotypes resulting from the interspecific crossing of these species in F_1 and F_2 generations. Chapter II aims to evaluate the genotypic performance of oat cultivars and lines cultivated in Rio Grande do Sul from 2016/2017 to 2021/2022, using the mixed model methodology. Chapter III aims to determine the genetic dissimilarity of populations derived from crosses between *A. strigosa* and *A. brevis* in the F_3 generation.

Key words: 1. Oat. 2. Interspecific Hybridization. 3. Genetic breeding. 4. Genetic dissimilarity. 5. Genotype x environment interaction.

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1 INTRODUCTION

Oats hold a prominent position among cereals and are one of the main winter crops in southern Brazil. The genus *Avena* comprises various species, including both wild and cultivated forms, with different ploidy levels: diploid, tetraploid, and hexaploid. Among them, the hexaploid species *Avena sativa* (2n = 6x = 42) is primarily used for human consumption, though it is also employed as forage under certain conditions. However, the diploid species *Avena strigosa* Schreb. (2n = 2x = 14) is the most widely used annual winter forage for grazing and is also utilized as cover crop residue in no-till farming systems. Additionally, the diploid species *Avena brevis* (2n = 2x = 14) presents an excellent alternative for gene introgression, as it exhibits desirable traits such as leaf area, leaf-to-stem ratio, and regrowth capacity, all of which have significant potential to enhance forage production.

Forage oat breeding programs primarily aim to develop genotypes that demonstrate stability under varying climatic conditions and animal grazing. These programs are focused on identifying cultivars that can enhance animal performance through high productivity, excellent nutritional quality, and resistance to pests and diseases. However, a major challenge in this context is the genotype-by-environment interaction, where a genotype that performs best in one environment may not do so in another. This interaction can affect selection gains and complicate the recommendation of cultivars with broad adaptability.

As a strategy, genetic breeding programs aim to incorporate genetic variability, including a focus on wild relatives. Wild oat species possess both qualitative and quantitative traits that can be advantageous when incorporated into interspecific hybridizations. However, using wild species for the transfer of advantageous genes in crop improvement programs may result in the transfer of deleterious genes carried along by linkage drag. There is limited information available on *A. brevis* and its use in

interspecific hybridization, highlighting the need for further studies and strategies for its utilization.

In this regard, biotechnological tools such as pollen viability and DNA molecular markers can aid in inferences about the fertility of crosses between specific genotypes and in analyzing the genetic dissimilarity among the resulting hybrids. Additionally, precise statistical analysis methods are essential for selecting the best materials for the release of a new cultivar.

Therefore, this work aims to select and characterize potential genotypes with the capability to develop new oat cultivars with high dry matter productivity. This will be achieved by using a mixed model approach to test the adaptability, stability, and fertility of these genotypes. Additionally, the study seeks to elucidate the genetic proximity and potential use of *A. brevis* in the breeding of *A. strigosa*.

2 LITERATURE REVIEW

This literature review presents information on the genus Avena, with a focus on the species *A. strigosa* and *A. brevis*. Additionally, it discusses the potential for gene transfer between species, the genetic breeding of forage oats, and finally, cytogenetics and microsatellite molecular markers for detecting genetic viability and diversity.

2.1 Genus *Avena* and the species *Avena strigosa* and *Avena brevis*

The species of the genus *Avena* L. belong to the family Poaceae, subfamily Poideae, and tribe Aveneae. This genus consists of annual plants that are self-pollinating, with anthesis occurring before the flower opens (BARBIERI, 2008, p. 213).

The probable center of origin for the genus *Avena* L. is located in the western part of the Mediterranean region, including the Atlas Mountains, the northwest coast of Africa, and the western Pyrenees, where the highest diversity of diploid species is concentrated. From this region, the species spread eastward (LOSKUTOV, 2008). The Russian geneticist Nikolai Vavilov concluded that the migration of oats into Europe was primarily driven by the spread of wheat cultivation (BARBIERI, 2008, p. 214). The advancement into colder and wetter environments led to a decline in the adaptability of previously used crops, such as wheat and barley, thereby facilitating the development of oats (TAVARES et al., 1993).

According to Baum (1977), there are 16 diploid taxa within the genus *Avena*, which are divided into sections and karyotypic groups. *Avena sativa* L. is classified in the section *Avena*, while the four diploid species are classified in the section (Baum), namely: *A. brevis* Roth., *A. hispanica* Ard., *A. nuda* L., *A. strigosa* Schreb. The section Tenuicarpa (Baum) includes eight more diploid species, and finally, three taxa of the C genome are designated to the section Ventricosa (Baum) (JELLEN; LEGGETT, 2006, p. 202).

The A genome of diploid species consists of five different karyotypes, As, Al, Ad, Ap, and Ac, which are associated with the following species: *A. strigosa*, *A. longiglumis*, *A. damascena*, *A. prostata* e *A. canariensis* (BARBIERI, 2008, p. 214). The species in the section Agraria (Baum) are part of the cytogenetic group with the AsAs genome, which is interfertile. Specifically, *A. brevis*, *A. hispanica*, *A. nuda*, and *A. strigosa* are included in this group, characterized by their AsAs genome (JELLEN; LEGGETT, 2006, p. 203).

According to Fu (2018), the phylogenetic tree of 25 oat species is divided into three main clades (I, II, III). Calibration with the wheat-oat divergence, dated at 25 million years ago, indicates that the C genome oat species diverged from other oat species around 20 million years ago. The separation between clade III (with As and AB genomes) and clade II (AC and ACD genomes) is estimated to have occurred 11 million years ago. Within clade III, divergence times for the nine species ranged from 4 to 8.9 million years ago, with *A. atlantica* being the oldest in the clade. The species *A. strigosa*, *A. nuda*, *A. brevis*, and *A. hispanica* shared a common ancestor approximately 6.3 million years ago. The separation of *A. strigosa* from *A. nuda* was estimated at 4.2 million years ago, and the separation between *A. brevis* and *A. hispanica* occurred around 4 million years ago.

A. strigosa was one of the first diploid species described. It is a tufted plant that can reach up to 120 cm in height during flowering. The inflorescence is a panicle, and the grains are caryopses that are semi-cylindrical and pointed at the ends, covered by the lemma and the palea (FONTANELI et al., 2012).

A. strigosa produces grains with low mass, which is why it is not widely used for human consumption. However, it is extensively employed in pasture production during periods of significant forage deficit in southern Brazil. It is also used in direct planting systems, where it helps control erosion, produces a large amount of dry matter, and significantly controls weeds due to the allelopathic effects of its mulch cover (CARVALHO, 1998).

The improvement of *A. strigosa* faces challenges in artificial hybridizations and the restriction of parental lines for crosses. Given that crosses in this species are still limited, it is crucial to identify new genetic constitutions to develop a genotype with the desired characteristics (HARTWIG et al., 2007).

For genetic progress through artificial selection in any species, it is essential to have genetic variability within the population's constitution (TAVARES et al., 1993). Wild species are a valuable source of new, useful alleles for genetic breeding programs. Consequently, there is interest in *A. brevis*. This species naturally occurs in various parts of the world, primarily as an invasive plant and currently as a cultivated crop (LEGGETT, 1992, p. 39). Additionally, some researchers recognize it as a subspecies of *A. strigosa*, naming it *A. strigosa* subsp. *brevis* Husn. due to its morphological similarity to *A. strigosa* (LOSKUTOV, 2008).

Baum (1977) noted that *A. strigosa* and *A. brevis* differ by only a few characteristics, such as the morphology of the spikelet tips, the height of the awn insertion on the lemma, and the type of lodicule. Ladizinsky (2012, p. 8) suggested that these differences may be merely intraspecific variation. Research being conducted at Embrapa Trigo indicates a close relationship between these species, as hybrids from crosses between *A. strigosa* and *A. brevis* were found to be fertile (NASCIMENTO et al., 2015).

However, there is limited information available on *A. brevis*, highlighting the need for further research to understand its potential for gene transfer of agronomic interest between species.

2.2 Genetic Breeding of Forage Oats

The integration of crop and livestock systems, particularly for fattening beef cattle during the off-season in traditional grain-producing areas such as the Planalto, Missões, and Alto Uruguai regions in Rio Grande do Sul, can offer a consistent and profitable economic activity (FONTANELI et al., 2000).

The preference for using oats as a cover crop is primarily due to their ease of establishment and excellent tillering capacity, which results in rapid ground cover formation (GFELLERA et al., 2018). From an economic perspective, it is recommended to produce this cereal for dual purposes, as this alternative production method can provide a competitive advantage for cattle farming, improving the economic return of the activity (LIZOT et al., 2017).

With the evolution of the crop-livestock integration system, there is a concern to enhance the forage production potential to make the system viable. This involves both increasing pasture production and maintaining soil cover after the grazing period (BALBINOT JUNIOR et al., 2009). Considering the above, there is a search for new genotypes that are better adapted to grazing and cutting.

Kichel and Miranda (2000) note that forage oats have excellent nutritional value, potentially reaching up to 26% crude protein at the start of grazing, with good palatability and digestibility (60 to 80%). They are non-toxic to animals at any vegetative stage, and productivity ranges from 10 to 30 tons of green mass per hectare, with 2 to 6 tons per hectare of dry matter. Therefore, genetic breeding programs for forage oats aim to develop genotypes with stability under climatic conditions and animal grazing, improved regrowth capacity after grazing, and resistance to diseases and environmental challenges.

Due to the complexity of genetic breeding programs, which optimize the selection of new genotypes to enhance forage quality and productivity, with the goal of improving animal production efficiency, such as meat and milk, it is essential to have a detailed characterization and accurate understanding of the germplasm used (JANK et al., 2011). Forage cultivars should exhibit rapid growth in autumn and spring, produce a high number of tillers, and remain in the vegetative stage for a longer period compared to cultivars intended for grain production (KIM et al., 2014). A genotype with a late cycle is of greater interest because, in the vegetative stage, the forage quality is significantly better compared to the reproductive stage (COBLENTZ; WALGENBACH, 2010).

Due to the differential behavior of a genotype when cultivated in different environments, cultivar recommendations are challenging, primarily because of genotype × environment interactions (ZAKIR, 2018). Estimating the G (genotype), E (environment), and G × E (genotype-environment interaction) ensures valid recommendations of genotypes that can cope with varying conditions, which helps breeding programs direct the selection of materials for broad or specific environments (EGEA-GILABERT et al., 2021).

The application of more refined genetic-statistical procedures to estimate genetic parameters and variance components, and to predict mean components, constitutes a trend in plant breeding (MAIA et al., 2011). Several methods for quantifying genotype × environment interactions are available in the literature and are used for various crops. Among these, the Best Linear Unbiased Prediction (BLUP) method via Restricted Maximum Likelihood (REML) stands out.

2.3 Cytogenetics in Genotype Characterization

Cytogenetics, the study of genetics through cytology, encompasses all research related to chromosomes, whether isolated or in conjunction, condensed or dispersed. This includes the study of their morphology, organization, function, replication, as well as their variation and evolution (BRAMMER; ZANOTTO; CAVERZAN, 2007).

In genetic breeding programs, cytogenetic evaluation is useful for selecting parental lines and other crosses, aiming to assess and infer genetic stability quickly and efficiently (TONIAZZO et al., 2018). Additionally, this technique can be used to determine phylogenetic and evolutionary relationships between plant groups, helping to identify the potential for successful crosses between plants (DOBIGNY et al., 2004).

The knowledge of ploidy levels, through chromosome number counting, is crucial for breeders as it helps in selecting parents with compatible ploidy levels, thus avoiding the production of sterile hybrids. Additionally, studying the meiotic index determines

chromosome behavior during meiosis by analyzing pollen or microspores, including the presence of one or more micronuclei (ZANOTTO et al., 2009).

Damasceno et al. (2010) inferred that higher rates of abnormal cells are expected during meiosis and post-meiosis in wild plants or those with minimal domestication. These irregularities directly impact pollen viability and, consequently, affect fruit and seed formation in crosses and self-pollinations (POZZOBON et al., 2015). In this context, the meiotic index (MI), which analyzes the percentage of normal tetrads, and pollen viability emerge as useful tools for these evaluations. They assist breeders in making decisions regarding the elimination of genotypes that are either undesirable or unstable (BRAMBATTI et al., 2016).

By definition, two individuals share the same genome if their hybrid develops normally, exhibits normal chromosomal pairing during meiosis, is fertile, and no breakages occur in the segregating generations (LADIZINSKY, 2012, p. 8). Therefore, genetically similar species have similar karyotypes, while distantly related species exhibit more divergent karyotypes.

Karyotype structure analyses in diploid oat species have revealed two main genomes: A and C. Species carrying the A genome have a symmetrical karyotype and a lower content of heterochromatin. In contrast, species with the C genome are characterized by asymmetrical, heterochromatic chromosomes (FOMINAYA; VEGA; FERRER, 1988).

The karyotypes of *A. strigosa* subsp. *brevis* and *A. strigosa* subsp. *strigosa*, studied by Badaeva et al. (2005), are structurally similar. They each include two metacentric chromosomes, two submetacentric chromosomes, one subtelocentric chromosome, and two morphologically distinct satellite chromosomes. All species have a low content of heterochromatin.

The genomes of *Avena* L. species form a distinct series of polyploids, occurring at three ploidy levels: diploids (2n=2x=14), tetraploids (2n=4x=28), and hexaploids

(2n=6x=42) (TAVARES et al., 1993). Diploid species have AA or CC genomes, while tetraploids may have AABB or AACC genomes, and hexaploids possess the AACCDD genome (LOSKUTOV; RINES, 2011, p. 118).

2.4 Molecular Markers: Microsatellites

For the efficient use of plant genetic resources, DNA-based techniques are widely employed in crop improvement programs. Unlike morphological and biochemical markers, molecular markers are not influenced by environmental changes or plant developmental stages (CAO et al., 2022).

Molecular markers, such as RFLP (Restriction Fragment Length Polymorphism), RAPD (Random Amplified Polymorphic DNA), AFLP (Amplified Fragment Length Polymorphism), ISSR (Inter Simple Sequence Repeat), DArT (Diversity Arrays Technology), SSR or microsatellite (Simple Sequence Repeats), and SNP (Single Nucleotide Polymorphism), characterize genetic diversity within and between plant populations. Among these various molecular markers, SSR markers are frequently used to characterize genotypes (BEGNA, 2021; SALGOTRA; STEWART JR, 2020).

Microsatellites are typically composed of repetitions of 1 to 6 nucleotides. These markers are abundant, widely distributed throughout the genome, and highly polymorphic compared to other genetic markers. Additionally, they are species-specific and co-dominant (MIAH et al., 2013).

The development of microsatellite markers can be very useful for cultivar identification, evaluating genetic diversity of germplasm, and assisting in breeding to enhance crop characteristics (WU et al., 2012). In oats, although there have been reports of SSR markers since the early 2000s (LI et al., 2000), they are challenging to develop, and there are fewer markers available compared to other crops.

MONTILLA-BASCÓN et al. (2013) used microsatellite markers to estimate genetic diversity in an oat collection and concluded that these markers are useful for

examining changes in allelic diversity. They also have the potential to be linked to genomic regions that control different traits. This allows for the efficient and precise transfer of useful alleles from local varieties to modern cultivars.

KUMAR et al. (2023), in studying genetic diversity using SSR markers among 177 oat accessions (*A. sativa* L.), including 3 landraces and 36 commercial cultivars, reported that the markers revealed a high level of polymorphism, detecting a total of 454 alleles. The cultivars showed less diversity compared to the landraces, indicating a reduction in genetic diversity during improvement. This suggests considerable genetic variation exists in landraces that is not present in cultivars, providing opportunities for breeding new cultivars by exploring the genetic diversity found in landraces.

DA-SILVA et al. (2001) analyzed the transferability and utility of microsatellite markers from *A. sativa* (genome AACCDD), for genetic studies of *A. strigosa* (genome AA). The microsatellites from *A. sativa* showed a high transferability rate and are a valuable tool for genetic studies and the characterization of *A. strigosa* genotypes.

3 CHAPTER I

Genotypic performance of forage oat genotypes using mixed models

Bianca Oliveira Machado; Jane Rodrigues de Assis Machado; Nadia Canali Langaro; Sandra Patussi Brammer.

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3.1 Abstract

The presence of environmental effects and genotype x environment interaction (GxE) in the phenotypic constitution of a trait makes the selection of superior genetic constitutions challenging for breeders. The use of mixed models enhances the efficiency of selecting self-pollinated plant progenies or lines. The aim of this study was to apply mixed model methodology to assess the agronomic performance of oat lines and cultivars from Embrapa and partners. Data from Value for Cultivation and Use (VCU) trials of oat cultivars belonging to the oat breeding program were utilized. The experimental design was randomized blocks with three replications. Leaf dry matter was the evaluated variable. Variance components and genetic parameters were estimated using mixed models, and adaptability and stability were determined through REML/BLUP methodology. There were no statistically significant differences for genotypes, genotype x locations, and genotype x years, indicating the absence of genetic variability among the tested genotypes and the interaction of locations and years with each individual genotype.

Keywords: 1. *Avena strigosa*. 2. Plant breeding. 3.BLUP. 4. Stability. 5. Adaptability.

3.2 Introduction

Characterizing and selecting genotypes with the potential to become new oat cultivars is a significant challenge pursued by breeders. The integration between agriculture and livestock is a way of improving the livestock production indices and

reducing the risks of agriculture, maximizing the use of crop areas during the year. Among the various species to be cultivated in this system, the black oat (*Avena strigosa* Schereb.) is presented as alternatives for use within this context.

Oat forage can be grown for hay, pasture, or silage, which can be incorporated in dairy rations for high-producing cows, where it can replace corn silage in 10% of the diet (Harper et al., 2017) or fed in large quantities to beef cattle (Stevens et al., 2004). The popularity of black oats as a winter crop is attributable to a combination of characteristics, amongst them: high carbon : nitrogen ratio, ensuring high biomass (over 6 $t.ha^{-1}$) (Stevens et al., 2004). The oat dry matter production can be high but varies, among other factors, according to the region and the cultivar used. For the recommendation of its use, it is necessary to first identify which ones adapt better to a specific region (Alvim & Coser, 2000).

In more advanced stages of breeding, as it occurs in Value for Cultivation and Use (VCU) trials, it is common to test developed genotypes in multiple environments and years, where, in this way, in addition to the main effects of environment (E) and genotype (G), the genotype x environment interaction effect arises (Pour-Aboughadareh et al., 2022). The interaction between genotype and environment effect is important for breeders, and reflects yield variation unexplained by individual genotype and environment effects (Yan and Hunt, 2001).

One of the methods for quantifying genotype x environment interaction, estimating gains, genetic progress, and assessing efficiency within the breeding program is the mixed model. For the use of mixed model equations and the prediction of Best Linear Unbiased Prediction (BLUP), it is necessary to know the variance and covariance components.

The REML method, Restricted Maximum Likelihood (Patterson & Thompson, 1971), stands out as a methodology to be used in unbalanced data since it provides non-negative estimates of variance components and considers the loss of degrees of freedom for fixed effects estimates. The yield stability is one of the most desirable properties of a

genotype to be released as a variety for cultivation. Stability is a complex product of genetic yield potential to stress conditions (Dewi et al., 2014).

Therefore, the aim of this study was to assess the genotypic performance of oat cultivars and lines, evaluated in Rio Grande do Sul, from 2016/2017 to 2021/2022, using a mixed model.

3.3 Material and Methods

The results obtained from the Value for Cultivation and Use (VCU) trials of the cooperative oat breeding program, developed by the Brazilian Agricultural Research Corporation (EMBRAPA), in the 2016/2017, 2017/2018, 2018/2019, 2019/2020, 2020/2021, and 2021/2022 cropping seasons, conducted in the following locations in the state of Rio Grande do Sul: Bagé, Passo Fundo, and Eldorado do Sul (Table 1).

Table 1. Environments where oat genotypes were assessed in the growing seasons of 2016/2017 to 2021/2022.

Growing season	Environments
2016/2017	Bagé; Passo Fundo; Eldorado do Sul
2017/2018	Bagé; Passo Fundo; Eldorado do Sul
2018/2019	Bagé; Passo Fundo; Eldorado do Sul
2019/2020	Passo Fundo
2020/2021	Passo Fundo
2021/2022	Passo Fundo

The data pertains to the production of leaf dry mass production (DM) in kg/ha. The fresh mass was collected and the samples were submitted to a drying temperature of 65ºC until reaching a constant mass. The trials were set up in a randomized complete block design with three replications. In the 2016/2017 season, 7 genotypes were evaluated, and in the 2017/2018, 2019/2020, 2020/2021, and 2021/2022 seasons, 9

genotypes were evaluated, while in the 2018/2019 season, 10 genotypes were evaluated (Table 2).

Table 2. Treatments to test oat genotypes in 2016/2017 to 2021/2022.

Growing season	Genotypes
2016/2017	Agro Planalto; Embrapa 139 Neblina*; BRS Pampeana; PFA201602; PFA201603; PFA201604; BRS Tropeira
2017/2018	Agro Planalto; Agro Zebu; Embrapa 29 Garoa; Embrapa 139 Neblina*; BRS Pampeana; PFA201604; BRS Tropeira; PFA201701, PFA201702
2018/2019	IPR Cabocla*; Agro Planalto; BRS Pampeana; PFA201604; BRS Tropeira; PFA201701; PFA201702; PFA201801; PFA201802; PFA201803
2019/2020	IPR Cabocla*; Agro Planalto; BRS Pampeana; BRS Tropeira; PFA201701; PFA201702; PFA201801; PFA201802; PFA201803
2020/2021	IPR Cabocla*; Agro Planalto; BRS Pampeana; BRS Tropeira; PFA201701; PFA201702; PFA201801; PFA201802; PFA201803
2021/2022	IPR Cabocla*; Agro Planalto; BRS Pampeana; BRS Tropeira; PFA201701; PFA201702; PFA201801; PFA201802; PFA201803

* Control treatment.

In each trial, the experimental plots consisted of six rows of five meters, spaced seventeen meters apart. The crop management was performed as recommended for each region and technology level.

Statistical analyses were performed using model 115 of software SELEGEN-REML/BLUP, assuming that $y = xf + Zg + Qa + Ti + e$, where y is the data vector, f is the vector of effects of the repetition-location-year combinations (assumed as fixed) added to the overall mean, g is the vector of genotypic effects (assumed as random), a is the vector of genotype-by-year interaction effects (random), i is the vector of genotype-by-location interaction effects, and e is the vector of errors or residuals (random). The vector f encompasses the effects of replications within locations within years, locations, years, and the interaction of locations by years.

The values of the harmonic mean of genotypic values (MHGV) for stability assessment are given as per Resende (2002; 2004). The relative performance of genotypic values (RPVG) for adaptability is based on Annicchiarico's method (1992). The harmonic mean of relative performance of genotypic values (MHPRVG) for stability, adaptability, and productivity is similar to Linn and Binns' method (1988).

The significance of the model effects was estimated by deviance analysis, following the recommendations of Resende (2007). The deviances were obtained through analyses with and without the effects of g, ga, gl, and gla. Then, each deviance from the full model was subtracted from the deviance without the respective effect and compared to the chi-square value with one degree of freedom at 1% and 5% probability. Mathematically: $LRT = -2ln\left(\frac{MV\ of\ the\ reduced\ model}{MV\ of\ the\ full\ model}\right)$, where ln is the natural logarithm, and LR is the maximum likelihood ratio.

3.4 Results and Discussion

The result of the deviance analysis for genotype effects and the effects of genotype x location and genotype x year interactions, their respective variance components, and coefficients of determination in the joint analysis of the 3 locations and 6 years are presented in Table 3. It was determined through the deviance analysis that the genotype effects, genotype x location interactions, and genotype x year interactions, as well as their respective variance components (Vg, Vgl, and Vga) and coefficients of determination (h^2, c^2gl e c^2ga), were not statistically significant. The *deviance* analysis, therefore, indicated the absence of genetic variability among the tested genotypes and the interaction of locations and years with each individual genotype.

The coefficients of determination indicate how much each component contributes to the total phenotypic variance. Thus, the interactions between genotypes x locations and genotypes x years contributed with 4% and 0.54%, respectively.

The heritability for this trait was low (0.05), which indicates a strong environmental influence. The accuracy obtained, approximately 90%, reveals excellent experimental quality and, consequently, confidence in the selection.

Table 3. *Deviance*, variance components and determination coefficients related to the overall joint analysis involving oat cultivars and lines across different locations and 6 years.

Effect	*Deviance*	LRT (X^2)	Variance Components	Determination Coefficients
Genotypes	3711.41	1.90^{ns}	Vg = 16620.5109	$h^2g = 0.0535$
Genotypes x locations	3709.54	0.03^{ns}	Vgl = 15404.6045	$c^2gl = 0.0496$
Genotypes x years	3710.87	1.36^{ns}	Vgy= 2267.6899	$c^2gy = 0.0073$
Residue		-	Ve = 276105.6730	
			CVg (%) = 3,58	
			CVe (%) = 14,05	
Complete model	3709.51	-	-	

LRT: (Likelihood-ratio test); Vg: genotypic variance; Vgl: variance of genotype x locations interaction; Vgy: variance of genotype x years interaction; Ve: variance of error and random factors; CVg: genetic variation coefficient; CVe: environmental variation coefficient; h^2g: broad-sense heritability; c^2gl: coefficient of determination of the effects of the interaction genotype x environment; c^2gly: coefficient of determination of the effects of the interaction genotype x years.

In the study by Gadisa et al. (2023), the genotype-environment interaction (GxE) also did not show a statistically significant difference, leading them to conclude that the genotypes tested in the trial have low genetic distances.

The genetic structure of a plant population can be well partitioned through predictions of mean components (Table 4).

Table 4. Individual mean components (individual BLUP) for leaf dry matter production in oat genotypes.

Genotypes	Individual mean components (individual BLUP)				
	g	u+g	Gain ($kg.ha^{-1}$)	New mean	u+g+gem
Agro Planalto	160.0557	3910.9938	160.0557	3910.9938	3960.4426
BRS Pampeana	140.4182	3891.3563	150.2370	3901.1751	3934.7382
PFA201603	58.8496	3809.7877	119.7745	3870.7126	3827.9692
PFA201801	47.1966	3798.1347	101.6301	3852.5682	3812.7160
PFA201702	33.1378	3784.0759	87.9316	3838.8697	3794.3138
PFA201701	15.0960	3766.0341	75.7923	3826.7304	3770.6980
IPR Cabocla	12.7867	3763.7248	66.7915	3817.7296	3767.6752
Embrapa 29 Garoa	-18.4565	3732.4816	56.1355	3807.0736	3726.7795
BRS Tropeira	-19.1460	3731.7921	47.7709	3798.7090	3725.8770
PFA201802	-23.0745	3727.8636	40.6864	3791.6245	3720.7348
PFA201602	-27.5009	3723.4372	34.4875	3785.4256	3714.9409
PFA201803	-64.2924	3686.6457	26.2559	3777.1940	3666.7827
Agro Zebu	-78.4765	3672.4616	18.1995	3769.1376	3648.2164
Embrapa 139 Neblina	-117.0530	3633.8851	8.5386	3759.4767	3597.7218
PFA201604	-119.5410	3631.3971	0.0000	3750.9381	3594.4652

g: genetic value; u+g: predicted genotypic values; u+g+gem: average genotypic value

The negative values of u+g indicate that the genotype is below the overall mean (3746,23). This means that if only the dry matter production were considered for

selection, these genotypes would be discarded. In this case, the genotypes BRS Tropeira, Embrapa 29 Garoa, PFA201602, PFA201802, PFA201803, Agro Zebu, Embrapa 139 Neblina, PFA201604 exhibited the worst performance although not differing statistically. (Table 4).

The genotypic value (u + g + gem) for the mean across years, taking advantage of the average effect of the interaction, generates results similar to the methods in which both adaptability (PRVG) and stability (MHPRVG) are capitalized simultaneously (Tables 5 and 6).

Genotypes that consistently appear ranked highest in all environments for leaf dry matter production are not significantly influenced by the environment and, consequently, exhibit minimal variation in the genotype x environment interaction. The stability of genetic values can be evaluated through the Harmonic Mean of Genetic Values (MHVG). This measure indicates predictability, that is, the maintenance of production across diverse environments. According to Table 5, the most stable genotypes across environments are, in the following order, BRS Pampeana; Agro Planalto; PFA201603; PFA201801 and PFA201702.

The adaptability of genetic values can be predicted through the method of Relative Performance of Genotypic Values (PRVG), across environments, in relation to the yearly average. Adaptability assesses the level of response to environmental stimuli, meaning it is the capacity of lines to be responsively and advantageously reactive to environmental improvement (Mariotti et al., 1976). By using the product of PRVG and the overall mean (3746.23 $kg.ha^{-1}$), the column PRVGMG was obtained for the genotypes, the genotypic value average capitalized by the interaction. Thus, when observing the values of PRVGMG, it was found that the genetic materials that exhibited the highest adaptive synergy were the following: Agro Planalto; BRS Pampeana; PFA201603; PFA201801; PFA201702.

Table 5. Stability of genotypic values (MHVG), adaptability of genotypic values (PRVG), genotypic values capitalized by the interaction (PRVG*MG) for leaf dry matter production of oat cultivars and lines evaluated in Rio Grande do Sul, from 2016 to 2021.

Genotype	MHVG	Genotype	PRVG	PRVG*MG[a]
PFA201801	4213.0250	Agro Planalto	1.0547	3956.0064
IPR Cabocla	4161.9169	BRS Pampeana	1.0527	3948.6610
PFA201802	4107.9413	PFA201603	1.0186	3820.8436
PFA201803	4048.7428	PFA201801	1.0169	3814.1572
BRS Pampeana	3722.4646	PFA201702	1.0127	3798.4314
Agro Planalto	3708.2027	PFA201701	1.0065	3775.2529
PFA201603	3582.6320	IPR Cabocla	1.0046	3768.2131
PFA201702	3574.7221	BRS Tropeira	0.9966	3738.2208
PFA201701	3553.7335	Embrapa 29 Garoa	0.9926	3723.3543
BRS Tropeira	3527.0892	PFA201802	0.9918	3720.0515
Embrapa 29 Garoa	3497.6729	PFA201602	0.9878	3705.3290
PFA201602	3473.9277	PFA201803	0.9773	3665.9709
Agro Zebu	3425.4359	Agro Zebu	0.9718	3645.0050
PFA201604	3388.7703	PFA201604	0.9591	3597.4031
Embrapa 139 Neblina	3364.1038	Embrapa 139 Neblina	0.9566	3587.9802

[a]: overall experiment mean.

The method of the Harmonic Mean of Relative Performance of Genotypic Values (MHPRVG) which is based on predicted genotypic values via mixed models, combines stability, adaptability, and production into a single statistic, facilitating the selection of superior genotypes (Borges et al., 2010). The MHPRVG, as well as the product of

MHPRVG by the overall mean, are presented in Table 6. MHPRVG*MG provides the genotypic values of each cultivar and/or line penalized for instability and capitalized for adaptability.

Table 6. Stability and adaptability of genotypic values (MHPRVG) and genotypic values averaged across locations (MHPRVG*MG) for leaf dry matter production of oat cultivars and lines evaluated in Rio Grande do Sul, from 2016 to 2021.

Genotype	MHPRVG	MHPRVG*MG
Agro Planalto	1.0544	3954.9060
BRS Pampeana	1.0525	3947.9004
PFA201603	1.0184	3819.8375
PFA201801	1.0168	3814.0532
PFA201702	1.0126	3798.1285
PFA201701	1.0064	3775.1231
IPR Cabocla	1.0046	3768.1906
BRS Tropeira	0.9963	3736.9612
Embrapa 29 Garoa	0.9926	3723.2025
PFA201802	0.9918	3720.0288
PFA201602	0.9876	3704.5696
PFA201803	0.9773	3665.9690
Agro Zebu	0.9717	3644.8155
PFA201604	0.9589	3596.8996
Embrapa 139 Neblina	0.9561	3586.3038

Simultaneous selection, considering the variable leaf dry matter production and the genetic stability and adaptability parameters, highlights the genotypes: Agro Planalto; BRS Pampeana; PFA201603; PFA201801; PFA201702, with the best performances for these parameters.

Finally, although there is no statistical difference, the cultivars Agro Planalto and BRS Pampeana evaluated from 2016 to 2021 were the ones that showed the best performance in terms of MHPRVG, meaning higher stability, adaptability, and leaf dry matter production. Among all the lines, PFA201603 stood out based on this criterion.

The mixed model methodology is highly useful in the evaluation of Value for Cultivation and Use (VCU) trials, especially in the selection of lineages. The obtained results indicate little variability among the tested genotypes. This outcome may be attributed to the high degree of genetic proximity among the parents, therefore, it is suggested to broaden the genetic base of the breeding program.

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4 CHAPTER II

Pollen viability of interspecific hybrids of *Avena strigosa* Schreb. and *Avena brevis* Roth.

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4.1 Abstract

Crossing of *Avena brevis* with *A. strigosa* can be used in genetic breeding programs to introduce genes related to animal foraging traits, however, pollen viability has not been elucidated for these species. This work, therefore, aimed to evaluate pollen viability between these two species. The parental genotypes of *A. brevis* (BRS Centauro) and *A. strigosa* (BRS Pampeana, IAPAR 61, PFA201702 and UPFA 21 Moreninha), and the genotypes resulting from F_1 and F_2 generation crosses, were analyzed. The percentage of normal pollen grains for F1 generation crosses ranged 77.20 – 98.4%, while for F_2 generation crosses it ranged 92.47 – 95.27%. Pollen grains with little starch reached maximum percentages of 15.93% and 5.30% for F_1 and F_2 crosses, respectively. The overall pollen grain length averaged 41.40 µm. The evaluated genotypes have stable pollen viability in the F_1 and F_2 generation.

Keywords: hybridization, oat, oat forage, plant generation, pollen grains.

4.2 Introduction

Oats are an annual grass from the *Poaceae* family, *Aveneae* tribe, and *Avena* genus. The genus *Avena* L. is one of the most ancient cereal genera, consisting of diploid (2n = 2x = 14; A and C genomes), tetraploid (2n = 4x = 28; AB and AC genomes) and

hexaploid (2n = 6x = 42; ACD genome) species (Nikoloudakis *et al*. 2008). The genus contains about 29 species exhibiting considerable morphological and ecological diversity in the Mediterranean Basin, Eastern Africa, Europe, Asia, and the Americas (Loskutov 2001, Lin *et al*. 2015). Powo (2024) recognizes 27 species of the genus *Avena*, including *A. strigosa* and *A. brevis*.

Oat is one of the main winter crops in southern Brazil. *Avena strigosa* Schreb. is the most commonly planted diploid oat species usually utilized as a cover crop, as well as in crop-livestock systems, where the pasture is used to meat or milk production (Moraes *et al*. 2014, Nascimento Junior *et al*. 2015).

Genetic breeding seeks to incorporate qualitative and quantitative characteristics present in different genotypes, which includes crosses between closely related species. *Avena brevis*, as well as *A. strigosa*, is part of a particular group with 14 (2n) chromosomes (Tavares *et al*. 1993), classified in the Agrarian section (Baum) and composes the subdivision of the interfertile AsAs genome cytogenetic group (strigosa) (Jellen & Leggett 2006). As *A. strigosa* and *A. brevis* differ by few characteristics, *A. brevis* is sometimes recognized as a subspecies of *A. strigosa* and is referred to as *A. strigosa* subsp. *brevis* Husn (Loskutov 2008).

The viable pollen grains pool available during flowering is crucial to reproductive success (Tomaszewska & Kosina 2022). The normal and harmonious course of meiosis in pollen mother cells including regular bivalent formation and normal cytokinesis ensures 100% pollen viability (Pagliarini 2000). Any abnormality in course of meiosis causes the formation of sterile gametes and low percentage of pollen viability (Jiang *et al.* 2011). Pollen viability plays a significant role in plant diversity and distribution in

various habitats, as it is a valuable tool for determining the degree of stability of plant species growing under favorable or unfavorable conditions (Nazish & Althobaiti 2022).

In germplasm characterization and genetic breeding programs, cytogenetic evaluation is useful for choosing parents and other crosses, aiming to quickly and efficiently assess and infer genetic stability (Toniazzo *et al*. 2018). Furthermore, this technique can be used to determine the phylogenetic and evolutionary relationships between plants groups, allowing to indicate the cross potential between plants (Dobigny *et al*. 2004). Factors influencing the pollen grains viability include flower morphology, environmental conditions (temperature, humidity, soil conditions, nitrogen availability, seasonality) and indirect factors (pollen metabolism, number of nuclei, genetic conditions, breeding methods) (Dafni & Firmage 2000).

Viable pollen is important for species dispersal, fitness, and survival of the next plant generation (Impe *et al*. 2020). It is also essential for directed plant breeding and, consequently, crop improvement. Therefore, the aim of this study was to evaluate pollen viability and morphology in *A. strigosa* and *A. brevis* genotypes, as well of the resulting genotypes from the cross between these species in the F_1 and F_2 generation.

4.3 Material and Methods

Plant material

In generation F_1 the crosses between the five parental genotypes of *A. brevis* Roth (BRS Centauro) and *A. strigosa* L. (IAPAR 61, BRS Pampeana, PFA 201702 and UPFA 21 Moreninha) species were analyzed: BRS Centauro x IAPAR 61, BRS Pampeana x BRS Centauro, PFA 201702 x BRS Centauro and UPFA 21 Moreninha x BRS Centauro.

Pollen viability and grain length were assessed in three repetitions, where each repetition is represented by a panicle.

In generation F_2 the five parental genotypes and the plants derived from crosses between these two species (Table I) were analyzed. The parental genotypes analyses were performed with four repetitions and, for each cross, from 15 to 18 repetitions, where each repetition is represented by a panicle, totaling 192 plants.

Table I. Artificial hybridizations in generation F_2 between *A. brevis* and *A. strigosa*

Population	Hybridization	Line	Genealogy
1	BRS Centauro x IAPAR 61	L1	A2x1802/1
	BRS Centauro x IAPAR 61	L2	A2x1802/2
	BRS Centauro x IAPAR 61	L3	A2x1802/3
2	BRS Pampeana x BRS Centauro	L1	A2x1803/1
	BRS Pampeana x BRS Centauro	L2	A2x1803/2
	BRS Pampeana x BRS Centauro	L3	A2x1803/3
3	PFA 201702 x BRS Centauro	L1	A2x1902/1
	PFA 201702 x BRS Centauro	L2	A2x1902/2
4	UPFA 21 Moreninha x BRS Centauro	L1	A2x1906/1
	UPFA 21 Moreninha x BRS Centauro	L2	A2x1906/2
	UPFA 21 Moreninha x BRS Centauro	L3	A2x1906/3

Locality and period

F_1 and F_2 generations plants were cultivated in a greenhouse at Embrapa Trigo (Brazilian National Wheat Research Center) in controlled humidity conditions during the recommended season for cultivating these species. This institution is located in Passo Fundo in the northern region of Rio Grande do Sul state, Brazil, at 28° 15’ S, 52° 24’ W and an altitude of 700 m above sea level.

Pollen viability evaluation

To evaluate pollen grains viability, panicles from each hybridization were collected when a quarter of the inflorescence emerged (Stage 52, Zadoks et al. 1974). After panicle collection and proper identification, they were placed immediately in flasks containing Carnoy fixative (ethyl alcohol: glacial acetic acid, in a 3:1 proportion) and kept at room temperature for 24 h. After fixation, the material was transferred to 70% alcohol and stored in a freezer at -20 ^{o}C.

For pollen analysis, cytological slides were made using three anthers of the same flower, based on previous studies, taken from the middle region. The anthers were cut into small pieces for pollen grain release, using carmine acetic acid dye (1%), and covered with the coverslips, slightly pressed for better material spread. Subsequently, the slides were quickly run over fire to improve dye concentration on the nucleus, and sealed with "luto" (a mixture of beeswax and pitch, at a 1:1 proportion).

To obtain a random sample, a cytological slide scan method was used with an optical microscope (BX 50/Olympus), 20x objective lens. On each slide, 500 pollen grains were counted. The pollen grains analyzed were classified into three different groups (Figure 1): 1) normal (uninuclear, binuclear and trinuclear, presence of one pore and adequate and uniform starch formation) and 2) unviable (empty) and 3) grains with little starch. Additionally, 10 pollen grains per slide were measured. The polar axis and equatorial axis were measured with the aid of the AxioVision User's Guide Release 4.6.3 – Axioscop FL 40 microscope program (Zeiss).

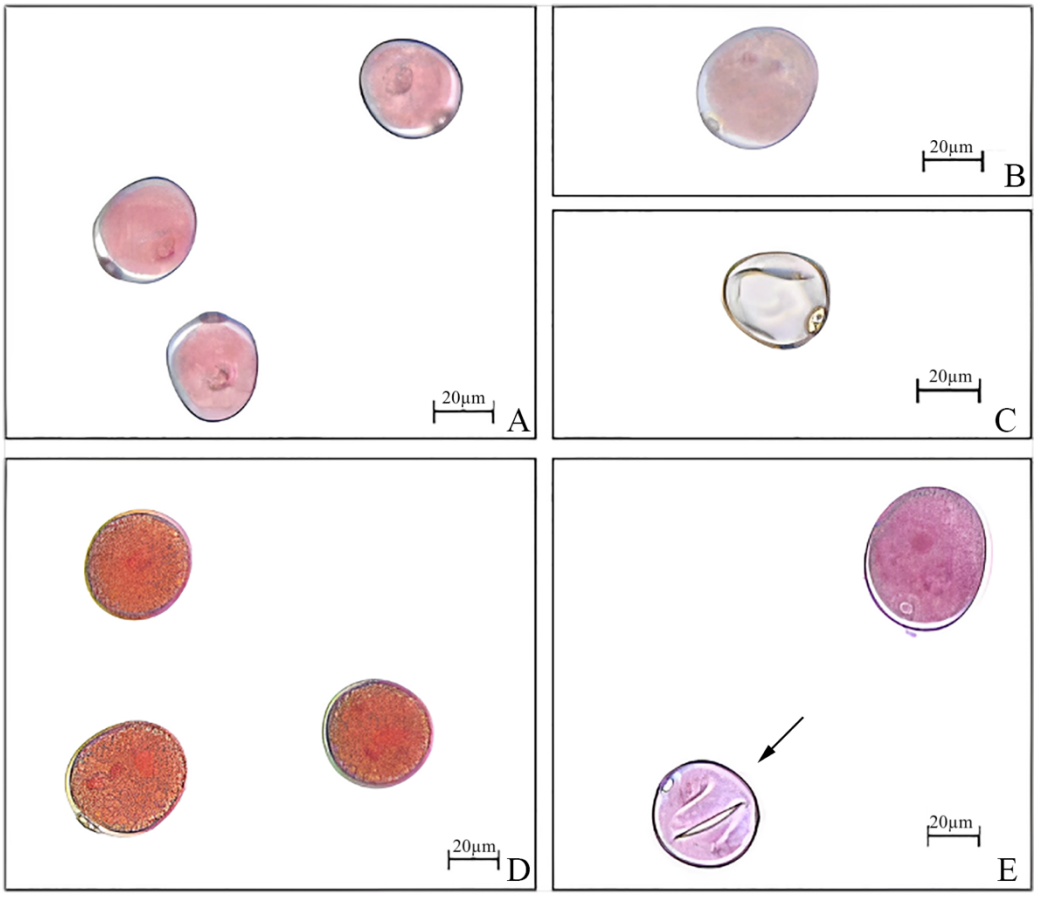


Figure 1. Classification of pollen grains. A – Uninuclear; B – Binuclear; C – Empty; D – Trinuclear; E - grains with little starch indicated by arrow. Bar = 20µm.

Statistical analysis

Data were analyzed using the R software (R Core Team 2022). Normality test was performed and when the data follow a normal distribution, analysis of variance (ANOVA) followed by comparison of means using Tukey's test at 5% error probability was carried out. When data did not follow normal distribution, it was submitted to Nonparametric Kruskal–Wallis Test. A value of $p < 0.05$ was considered significant.

Heatmap and cluster analysis was executed for visual separation of oat genotypes employing function “pheatmap” in R software package. This hierarchical clustering was based on the association of Euclidean distances of different genotypes in rows and characteristics of pollen grains in columns. Principal component analysis (PCA) was performed using the correlation matrix of trait values with the built-in R function "prcomp" and the setting "scale = TRUE". PCA transforms a dataset of continuous variables into a new set of uncorrelated variables called “principal components”. The first principal component derived explains the largest amount of the variability in the underlying dataset. The second principal component derived explains the second largest amount of variability in the dataset and so on.

4.4 Results

Results revealed a high level of pollen grain viability for plants derived from crosses and their parental species, in the F_1 and F_2 generation.

In the parental genotypes, the highest percentage of viable pollens was 99,53% in the IAPAR 61 genotype, however it did not differ from the other parents. The IAPAR 61 cultivar also stood out for presenting no pollen grains with little starch. Despite not statistically differing from other genotypes, the largest pollen was found in PFA201702 (49.30 µm).

In the F_1 generation, the highest percentage of normal pollen grains was in the cross between PFA 201702 x BRS Centauro, but it did not differ statistically from UPFA 21 Moreninha x BRS Centauro and BRS Pampeana x BRS Centauro. BRS Centauro x IAPAR 61 showed the lowest percentage of viable pollen grains and, consequently, the

highest percentage of unviable pollen grains, despite not differing from other crosses. The highest percentage of pollen grains with little starch was in BRS Centauro x IAPAR 61 and did not differ from the cross BRS Pampeana x BRS Centauro (Table II).

The average length of pollen grains ranged from 37.70 (BRS Centauro x IAPAR 61) to 42.72 µm (PFA 201702 x BRS Centauro) in the F_1 generation. There was no statistical difference between the crosses in terms of average length (Table II).

The average percentage of viable pollen was above 90% in the F_2 generation. Plants derived from crosses showed slightly lower viability of pollen grains compared with their parental species, between 92,47% and 95,27%. The plants resulting from the cross between UPFA 21 Moreninha x BRS Centauro (L1) resulted in the highest percentage of pollen grains with little starch (5.30%), even though they did not differ from other materials (Table II).

The average pollen grain length, in the F_2 generation, ranged from 39.72 to 49.30 µm. The smallest grains were observed in UPFA 21 Moreninha x BRS Centauro (L3), even though they did not differ from other materials (Table II).

Table II. Parental and crosses performed in the F_1 and F_2 generation and normal, unviable and pollen grains with little starch percentages.

Genotype/Cross	Normal (%)	Unviable (%)	Grains with little starch (%)	Average diameter (µm)
Parental				
Iapar 61	99.53 a	0.47 c	0.00 e	40.70 c
PFA201702	99.20 ab	0.47 c	0.33 de	49.30 a
BRS Pampeana	98.90 abc	0.50 c	0.60 bcde	45.17 abc
UPFA 21 Moreninha	98.66 abc	0.87 abc	0.47 cde	46.72 ab
BRS Centauro	98.20 abc	0.93 abc	0.87 bcde	44.44 abc
F_1 generation				
PFA 201702 x BRS Centauro	98.40 a	1.06 b	0.53 b	42.72 a
UPFA 21 Moreninha x BRS Centauro	97.20 ab	1.60 ab	1.20 b	39.03 a
BRS Pampeana x BRS Centauro	90.80 ab	7.53 ab	1.66 ab	40.83 a
BRS Centauro x Iapar 61	77.20 b	6.86 a	15.93 a	37.70 a
F_2 generation				
BRS Centauro x Iapar 61 (L1)	95.27 bc	0.72 c	3.83 bcd	42.24 c
BRS Pampeana x BRS Centauro (L3)	95.89 c	0.75 bc	3.26 bc	41.62 c
PFA 201702 x BRS Centauro (L1)	96.82 cd	1.38 a	1.79 bcd	44.24 abc
BRS Pampeana x BRS Centauro (L2)	95.18 cd	2.67 ab	2.03 bcd	42.38 bc
BRS Centauro x Iapar 61 (L2)	96.80 cd	0.93 abc	2.27 ab	43.02 bc
UPFA 21 Moreninha x BRS Centauro (L2)	95.68 cd	1.67 abc	2.52 ab	44.74 abc
UPFA 21 Moreninha x BRS Centauro (L3)	96.59 cd	1.49 a	1.88 bc	39.72 c
BRS Centauro x Iapar 61(L3)	96.64 cd	1.39 a	1.93 b	41.50 c
PFA 201702 x BRS Centauro (L2)	96.05 cd	1.34 a	2.61 ab	43.75 abc
BRS Pampeana x BRS Centauro (L1)	94.15 cd	3.04 a	2.80 ab	42.57 bc
UPFA 21 Moreninha x BRS Centauro (L1)	92.47 d	2.23 a	5.30 a	43.43 abc

CV= 5,11%

In the F_1 generation, pollen grains characteristics developed two main clusters (Figure 2). First cluster consisted of unviable and grains with little starch and the second cluster was composed by normal and average pollen grain diameter. The four crosses evaluated formed four groups based on the pollen grain characteristics. The crossings BRS Centauro x IAPAR 61 and BRS Pampeana x BRS Centauro proved to be more genetic distant from the others, as it resulted in the lowest percentage of normal pollen grains and average diameter, in addition to high percentages of grains with little starch and unviable. The other group is formed by parents, except IAPAR 61. Also, proximity between PFA 201702 x BRS Centauro, UPFA 21 Moreninha x BRS Centauro and IAPAR 61 was observed.

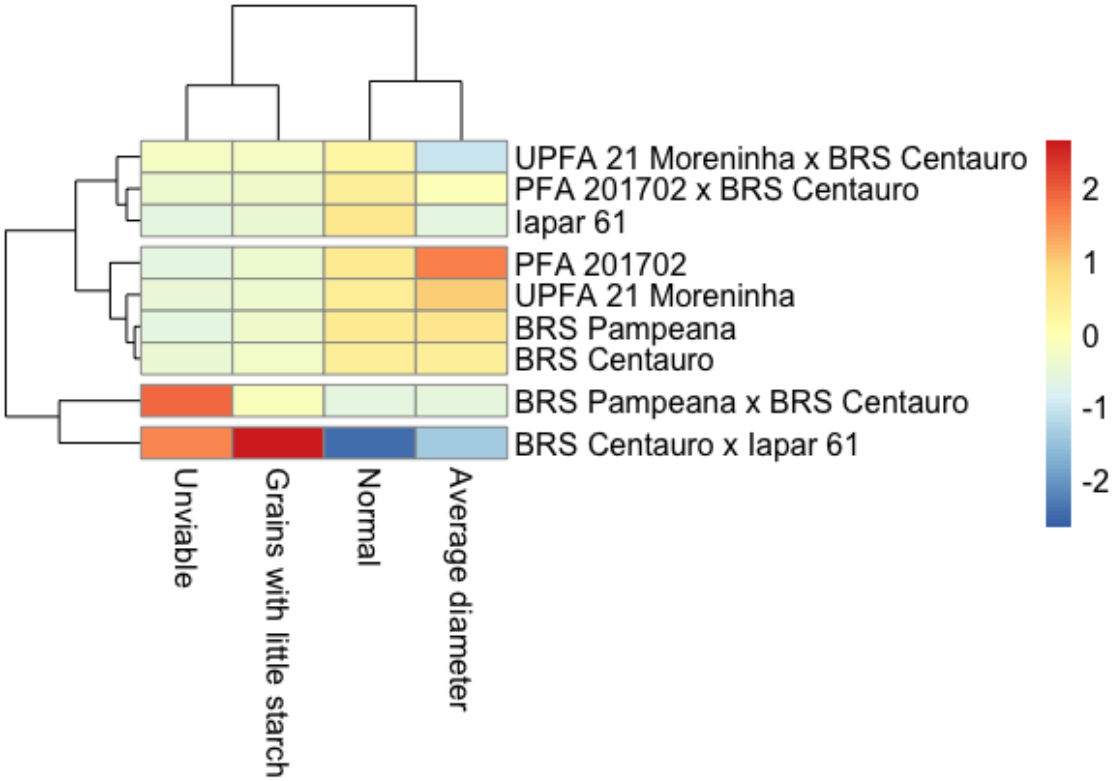


Figure 2. Heat-map and two-dimensional dendrogram of F_1 generation between genotypes of *A. brevis* and *A. strigosa* for pollen grain characteristics (normal, unviable, grains with little starch and average diameter). Dendrograms illustrate the relation between crosses (rows) and pollen grain characteristics (columns) using different color shades based on the average z-scores.

In the F_2 generation, pollen grain characteristics developed two main clusters, in the same way as in the F_1 generation (Figure 3). Parents and plants derived from the F_2 generation were divided into four groups based on pollen grain characteristics. The first group, formed by a line 1 of the population 4 UPFA 21 Moreninha x BRS Centauro and two lines (L1 and L2) of the population 2 BRS Pampeana x BRS Centauro, was the one that presented the highest number of unviable pollen grains, intermediate to high values of grains with little starch, lower values of normal pollen grains and intermediate values

of average grain diameter. Parents formed the second group, except for IAPAR 61 (group 3), as it had a lower mean diameter value compared to the others. The fourth group is composed of the other plants derived from the crosses, presenting intermediate values for the vast majority of pollen grain characteristics.

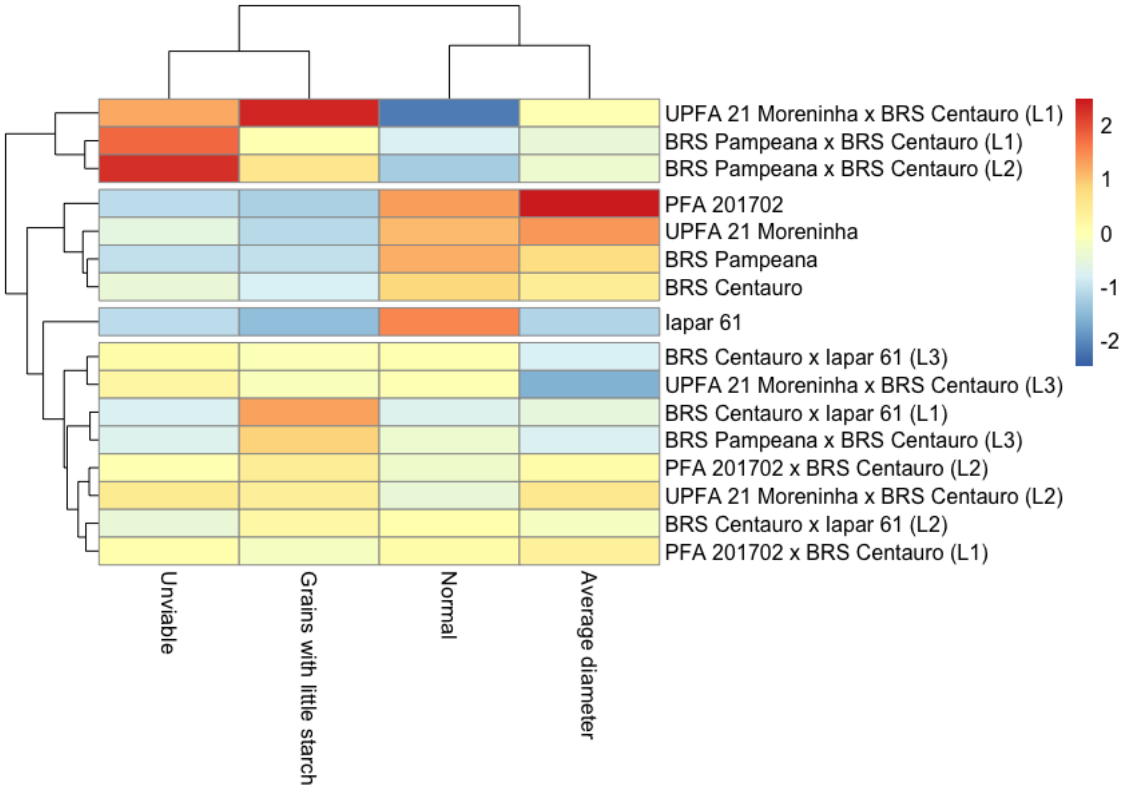


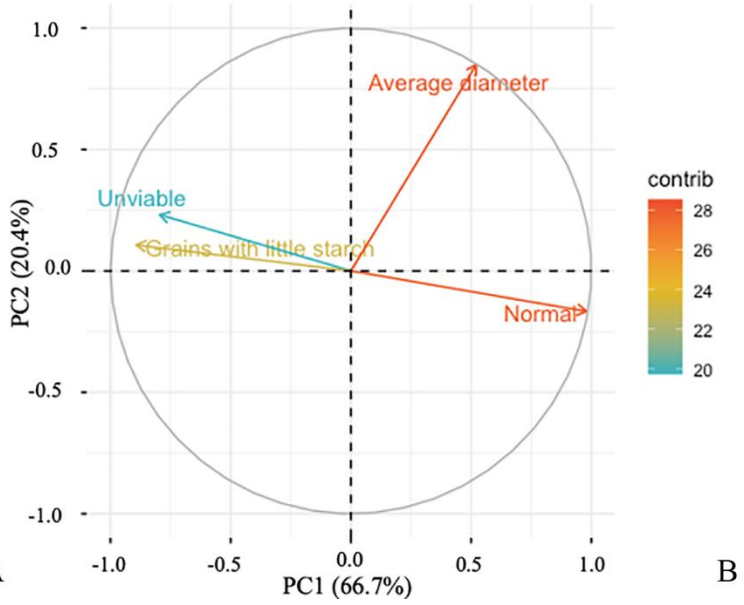
Figure 3. Heat-map and two-dimensional dendrogram of F_2 generation for pollen grain characteristics (normal, unviable, grains with little starch and average diameter). Dendrograms illustrate the relation between crosses (rows) and pollen grain characteristics (columns) using different color shades based on the average z-scores.

To investigate the relationships among trait variables and the factors underlying trait variation, PCA was performed for all four traits. In F_2 generation PC1 explained 66.7% of the trait variance (Figure 4A). The trait normal showed high positive loading (0.98), while unviable and grains with little starch showed negative loading (Figure 4A-

B). This result suggested that plants with high PC1 scores exhibited high number of normal pollen grains and small number of unviable and grains with little starch. PC2 explained 20.4% of the total variance, and the loading on PC2 was high for average diameter (0.85) (Figure 4A), suggesting that PC2 is representative of average diameter. The PCA results for traits measured in F_1 generation were consistent with F_2 generation.

Traits	PC1	PC2
Normal	0.98	-0.17
Unviable	-0.80	0.23
Grains with little starch	-0.89	0.11
Average diameter	0.52	0.85
Eigenvalue	2.66	0.81
Proportion of Variance	0.66	0.20
Cumulative Proportion	0.66	0.87

A



B

Figure 4. PCA for pollen grain characteristics in the F_2 generation. A – Summary of the two PCs (PC1 and PC2) for four traits in the dataset of five parents and four populations. B – Loading plot of PC1 and PC2. Proportion of variances for PC1 and PC2 are shown in parentheses.

4.5 Discussion

The quality of pollen grain is an important characteristic which provides information about the viability and vigor of the pollen grain. For pollen feasibility studies, values above 70% can be considered high for pollen viability, and this percentage would be enough for wheat genetic

breeding studies (Souza *et al*. 2002). Our results regarding the pollen viability percentages revealed a high level of them.

If pollen viability of genotypes is high, they can be considered good pollinators and assessment of pollen fertility and germination potential are important criterions for pollen evaluation (Gaaliche *et al.* 2013). According to Giacomin *et al.* (2015) several studies have reported improvements in the number of normal cells during meiosis when segregating genotypes are crossed with their parents. The authors emphasize that cytogenetic study is an excellent tool for measuring heritable male reproductive capacity and, it is possible to infer that plant breeding associated with cytogenetics will be successful in decreasing the rate of meiotic abnormalities through selection.

Studies like this have already been conducted for other species. Mirzaghaderi *et al.* (2020) made different cross combinations between diverse genotypes of wheat and *Aegilops* species including emmer wheat × *Ae. tauschii* (2n = DD or DDDD), durum wheat × *Ae. tauschii*, *T. timopheevii* × *Ae. tauschii*, *Ae. crassa* × durum wheat, *Ae. cylindrica* × durum wheat and *Ae. ventricosa* × durum wheat in the field over three successive years. However, contrary to what we described in our results, the pollen grain viability, calculated as the percentage of pollen stained with Alexander's solution, in the analyzed *Ae. crassa* × *T. turgidum* hybrids were low, varying from 0.39% (for *Ae. crassa* 'Bookan' × *T. durum* '6268' hybrid) to 17.33% (for *Ae. crassa* 'Sanandaj' × *T. durum* '6268' hybrid). No viable pollen was observed for *T. durum* '17' × *Ae. cylindrica* '236'. Mean of unreduced gamete in *Ae. ventricosa* 'AE 1522' × *T. durum* '11' hybrid was 1.02%. These hybrids also involve evolutionarily more distant species, and genetic stability did not occur even in the early generations, which is a complicating factor for breeding programs. Since aiming for genetic variability is essential, stability with high pollen viability is crucial as well.

The results presented by Tomaszewska & Kosina (2022) were similar to those described in this study, since in all examined different oat accessions, including accessions of *A. strigosa*,

the average viable pollen percentage, using the acetocarmine staining technique, for three years was above 90%. Oat amphiploids showed slightly lower viability of pollen grains compared with their parental species and the average length of grains ranged from 37.6 to 53.9 µm.

In our study, the average pollen grain length was 41.40 µm. Oats pollen grains are usually slightly elongated and elliptical in shape. In comparison to other plant phenotypic traits, pollen size varies somewhat less, because there is a strong selection favoring small pollen size, and likewise, selection pressures against extremely large pollen (Knight *et al*. 2010). Small pollen may have a higher probability of transport to a receptive stigma both by wind and insect vectors. Gornall (1977) found variation in pollen grain size from 36.30 to 40.70 µm amongst *Avena* diploid species. When analyzing pollen grains from 39 species of grasses, Joly *et al.* (2007) found pollen grains with an average diameter of 48.22 µm for *Avena fatua* and 46.02 µm for *Avena barbata*.

Starch is the main storage reserve in mature pollen grains and used for supplying energy and a carbon skeleton to support pollen germination and pollen tube growth for proper fertilization (Lee *et al*. 2016). Thus, insufficient starch synthesis in pollen grains is related to male sterility. The percentage of pollen grains with low starch in the study was low, with the highest value found in the F_1 generation, which was 15.93% in the cross between BRS Centauro and IAPAR 61. The other percentages in the F_1 generation ranged from 0.53% to 1.66%, and in the F_2 generation, from 0% to 5.30%.

Starch accumulation in cereal pollen occurs in amyloplasts that differentiate from proplastids (Clément & Pacini 2001, Pacini *et al.* 1992). Starch synthesis genes that are both specific and essential to cereal pollen have been identified, but how maturing pollen controls starch production remains unknown (Lee *et al*. 2016). Amanda *et al*. (2022) hypothesize that once pollen receives enough sucrose from the mother plant, auxin ensures the completion of pollen development into a dehydrated structure that stores energy in the form of starch, ready for its terminal functions, germination, tube growth, and fertilization.

4.6 Conclusion

The results indicate that genotypes resulting from crosses between *A. brevis* and *A. strigosa* generate viable offspring. Thus, the combinations between parents of both species proved to be reproductively efficient. Therefore, it is possible to infer that there is proximity between the species, which makes it possible to use *A. brevis* in the genetic improvement of *A. strigosa*.

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5 CHAPTER III

Genetic dissimilarity in populations of *Avena strigosa* and *Avena brevis* revealed by microsatellite markers

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Bragantia

5.1 Abstract:

The use of genetic dissimilarity indicators can help in the selection of genetic breeding strategies. The objectives of this work were to estimate the genetic dissimilarity among populations derived from the crossing of the species *A. strigosa* and *A. brevis*. The genetic characterization was carried out using the microsatellite marker technique. Considering the Polymorphic Information Content (PIC) coefficient, the average value was 0.34. The average genetic similarity among the parents was 0.81, while it was 0.70 across the four studied populations. A coefficient of genetic differentiation showed a low genetic differentiation among the genotypes. Therefore, efforts should be made to increase the genotypic variability. This would enhance the possibility of selecting new accessions having high potential for genetic breeding and the sustainable use of the species.

Key words: molecular markers, SSR marker, genetic similarity, genetic breeding.

5.2 Introduction

Oat is a minor cereal cultivated worldwide with a high range of applications from animal feed, human food, and industry purposes (Boczkowska et al. 2016). The black oat (*Avena strigosa*) is considered an excellent feed for cattle due to its high nutritional value and good digestibility, and it is also widely used in sustainable agricultural production systems as a cover crop (Restelatto et al. 2014). It has been observed that the species *Avena brevis* has high potential for black oat variety improvement.

Studying genetic variability is of great relevance in plant genetic resource management programs. Through information it provides, it is possible to identify genotypes of interest and use them in the establishment of effective conservation strategies (Abdelaziz et al. 2020). The assessment of genetic diversity plays an important role in the characterization of breeding lines, cultivars, or species and is the basis for the selection of appropriate parental forms in the development of crossing (Cieplak et al. 2021). Genetic dissimilarity can be estimated using morphological and molecular markers.

Molecular markers are by far more suitable to analyze the genetic diversity than morphological and biochemical traits because they segregate as a single gene and they are not affected by the environment (Kumar et al. 2009). Simple Sequence Repeats (SSR) marker has been preferred to DNA based marker system due to high levels of polymorphism and information content, selective neutrality, high reproducibility, and wide dispersion in diverse genomes (Hussain and Nisar 2020). SSRs are sequence blocks containing 1 to 6 nucleotide units repeated in tandem and flanked by sequences that are generally unique in the genome, but conserved in organisms (YU et al. 2017). SSR

markers have been effectively utilized to study the variability and genotypic characterization in oat (Fu et al. 2003; Nersting et al. 2006; Montilla-Bascon et al. 2013; He and Bjornstad 2012; Cieplak et al. 2021).

Molecular markers are indispensable tools for characterizing genetic resources by detecting variation of the DNA sequences among genotypes. The objectives of this study were to use microsatellites DNAs and to assess the genetic dissimilarity among four oat populations formed by the cross between the species *A. brevis* and *A. strigosa*.

5.3 Material and Methods

The F_3 generation resulting from crosses between the five parental genotypes of *A. brevis* (BRS Centauro (5)) and *A. strigosa* L. (IAPAR 61 (3), BRS Pampeana (1), PFA 201702 (4) and UPFA 21 Moreninha (2)) species were analyzed: BRS Centauro x IAPAR 61, BRS Pampeana x BRS Centauro, PFA 201702 x BRS Centauro and UPFA 21 Moreninha x BRS Centauro (Table 1). The populations were previously established in 2020 by the oat breeding program team.

Table 1. Artificial hybridizations in generation F_3 between *A. brevis* and *A. strigosa*.

Population	Hybridization	Line	Genealogy	Identification
1	BRS Centauro x IAPAR 61	L1	A2x1802/1	6-23
	BRS Centauro x IAPAR 61	L2	A2x1802/2	24-38
	BRS Centauro x IAPAR 61	L3	A2x1802/3	39-56
2	BRS Pampeana x BRS Centauro	L1	A2x1803/1	57-74
	BRS Pampeana x BRS Centauro	L2	A2x1803/2	75-91
	BRS Pampeana x BRS Centauro	L3	A2x1803/3	92-109
3	PFA 201702 x BRS Centauro	L1	A2x1902/1	110-126
	PFA 201702 x BRS Centauro	L2	A2x1902/2	127-144
4	UPFA 21 Moreninha x BRS Centauro	L1	A2x1906/1	145-159
	UPFA 21 Moreninha x BRS Centauro	L2	A2x1906/2	160-173
	UPFA 21 Moreninha x BRS Centauro	L3	A2x1906/3	174-192

Plants were cultivated in a greenhouse at Embrapa Trigo (Brazilian National Wheat Research Center) in controlled humidity conditions during the recommended season for cultivating these species. This institution is located in Passo Fundo in the northern region of Rio Grande do Sul state, Brazil, at 28° 15’ S, 52° 24’ W and an altitude of 700 m above sea level.

DNA extraction was performed at the Embrapa Trigo Biotechnology Laboratory according to the methodology described by Doyle and Doyle (1990). Total genomic DNA was extracted from leaf tissue per each genotype, totaling 192 plants, including the parentals that formed the four populations.

Sixteen oat microsatellite markers were selected for genotyping, following the protocol by Li et al. (2000) (Table 2).

Table 2. Description of the sixteen microsatellite primers tested and specific for *Avena*, sequence, repetition motif, and annealing temperature (AT) in ºC.

Primer	**Primer sequence**	**Repetition motif**	**AT* (ºC)**
AM1	5’GGA TCC TCC ACG CTG TTG A 5′CTC ATC CGT ATG GGC TTT A	$(AG)_{21}$ $(CAGAG)_6$	46
AM2	5′TGA ATT CGT GGC ATA GTC ACA AGA 5′AAG GAG GGC ATA GGG AGG TAT TT	$(AG)_{24}$	49
AM5	5′TTG TCA GCG AAA TAA GCA GAG A	$(AG)_{27}$	46

	5′GAA TTC GTG ACC AGC AAC AG		
AM7	5′GTG AGC GCC GAA TAC ATA 5′TTG GCT AGC TGC TTG AAA CT	$(AG)_{21}$	48
AM14	5′GTG GTG GGC ACG GTA TCA 5′TGG GTG GCG AAG CGA ATC	$(AC)_{21}$	48
AM15	5′GTG ACC GTA AAC GAT AAC AAC 5′AAG CAA GAC GCG AGA GTA GG	$(AC)_{14}$	47
AM20	5′TGT CGA TTT CTT TAG GGC AGC ACT 5′TCG CGA GAA AGA TGG AAA GGA GA	$(TG)_{10}(CG)_5$	50
AM22	5′ATT GTA TTT GTA GCC CCA GTT C 5′AAG AGC GAC CCA GTT GTA TG	$(AC)_{22}$	46
AM25	5′AGC CTG GAC ATG TAA TCT GGT 5′AGC CCT GGT CTT CTT CAA CA	$(AC)_{8(}AC)_4$ $(CT)_4$	47
AM30	5′TGA AGA TAG CCA TGA GGA AC 5′GTG CAA ATT GAG TTT CAC G	$(GAA)_{14}$	43
AM31	5′GCA AAG GCC ATA TGG TGA GAA 5′CAT AGG TTT GCC ATT CGT GGT	$(GAA)_{23}$	47
AM38	5′TGA TGA CCT CTT GAG TAA GCA 5′TGC CTT TCG TGG ACT TAC TA	$(GAA)_9$	45
AM43	5′AGC CCC TAC AAA GCC ATC A	$(GAA)_{17}$	46

	5′CAA GCA AAG GAC GAA CAA TAG		
AM47	5′GCA CCG GTT AAA AAG GAG TCA G 5′TTT CTT CTT ACC CAC CCA CCA C	$(AC)_{14}$	50
AM50	5′CTT GAG CGC TAG ATG GTT CC 5′CTC TGT TAC TCA AGT GTT TCA ATA	$(AT)_6(AC)_5$	47
AM61	5′TCG GAG CCG GTA TGG AAG C 5′GGT GGC AAG GGG TGT ATG AG	$(TTTC)_4$ $(CCT)_6$	51

*AT – annealing temperature. Fonte: LI et al. (2000)

For the PCR (Polymerase Chain Reaction) reaction were carried out in a GeneAmp Thermal Cycler 9700 thermocycler (Applied Biosystems - ABI) using the following basic programming: one cycle at 94ºC for 3 minutes; 5 cycles of 94ºC for 1 minute, 60ºC for 1 minute (decreasing 1ºC per cycle up to 55ºC), 72ºC for 1 minute; 30 cycles of 94ºC for 1 minute, 55ºC for 1 minute, 72ºC for 1 minute; and a cycle of 72ºC for 10 minutes.

The amplification products visualized on the gel, produced by each primer, were used to create a genetic similarity matrix by recording the presence (1) and absence (0) of bands in the electrophoretic profile of each genotype, as exemplified in Figure 1.

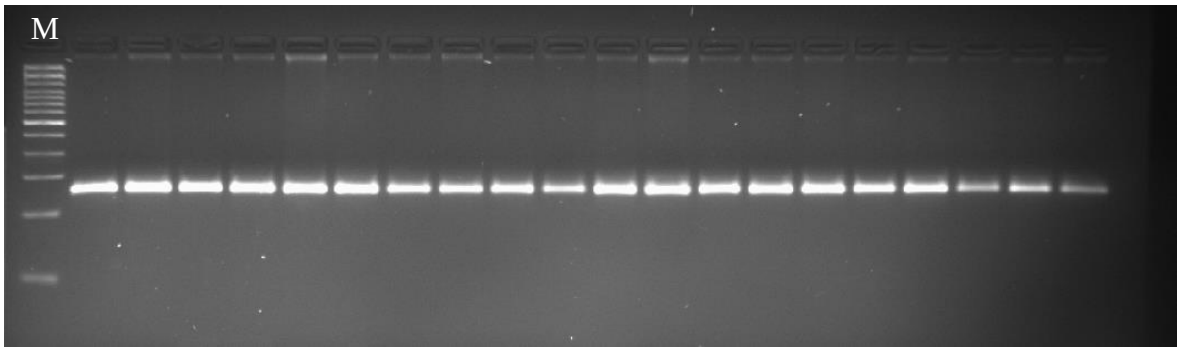

Figure 1. PCR amplification in the electrophoretic profile. M= 100 bp DNA ladder.

The estimate of genetic dissimilarity (Sgij) between each pair of genotypes was calculated using the Jaccard coefficient (Jaccard 1991), represented by the following expression:

$$Sgij = \frac{a}{a + b + c}$$

Where:

a is the number of bands present in both genotypes,

b is the number of bands present in genotype i but absent in genotype j,

c is the number of bands present in genotype j but absent in genotype i.

The value of the polymorphism information content (PIC) was determined using the following formula: PIC = 1 - Σpij^2, where pij^2 is the squared frequency of allele j for locus i, covering all alleles by locus (Nei 1973).

A phylogenetic tree was constructed based on Jaccard matrix using R statistical package‘vegan’ implemented in open software program Rstudio (R Studio Team 2020).

5.4 Results and Discussion

The numerous analyses of genetic similarity between different species within the genus *Avena* can help elucidate the evolutionary process within this genus and support genetic breeding programs for developing new varieties (Okoń & Kowalczyk, 2012).

Considering only the results obtained from polymorphic primers, the Polymorphism Information Content (PIC) was determined in the present study, which is an indicator of the informative capacity of a marker in genetic studies. According to the classification by Botstein et al. (1980), markers with PIC values greater than 0.50 are considered very informative, values between 0.25 and 0.50 are moderately informative, and values below 0.25 are not very informative. Therefore, markers with PIC values above 0.5 are more recommended to genetic studies while those below 0.25 are not recommended (Serrote et al. 2020).

In the present study, PIC varied from 0.11 (AM5) to 0.50 (AM50) with a mean of 0.34 (Table 3). The results showed that among the polymorphic primers, 71.4% are moderately informative and 28.6% are not very informative.

Table 3. Variation in fragment size and PIC (Polymorphism Information Content) for microsatellite markers evaluated in oat genotypes.

Marker	Variation in the size of fragments (pb)	PIC
AM1	180-190	0,46
AM5	130-150	0,11

AM22	110-210	0,38
AM30	210-230	0,26
AM31	150-160	0,48
AM50	290-300	0,50
AM61	200-205	0,21

Considering the species *A. strigosa*, interesting results were evidenced by Podyma et al. (2019) who evaluated different accessions kept in the Polish Germplasm Bank (National Center for Plant Genetic Resources/Radzików), but coming from different parts of the world. For this analysis, a total of 589 fragments were amplified, ranging from 50 bp to 828 bp, with a PIC ranging from 0.26 to 0.41. The authors emphasize that the combined analyses were crucial for providing a more reliable and comprehensive description of these genetic resources, which should lead to their optimal use in a genetic breeding program.

The dendrogram shows that IAPAR 61 and PFA201702 have a high genetic similarity, as do BRS Pampeana and BRS Centauro. UPFA 21 Moreninha, while related to the BRS Pampeana and BRS Centauro group, maintains a certain genetic distance that differentiates it from them (Figure 2).

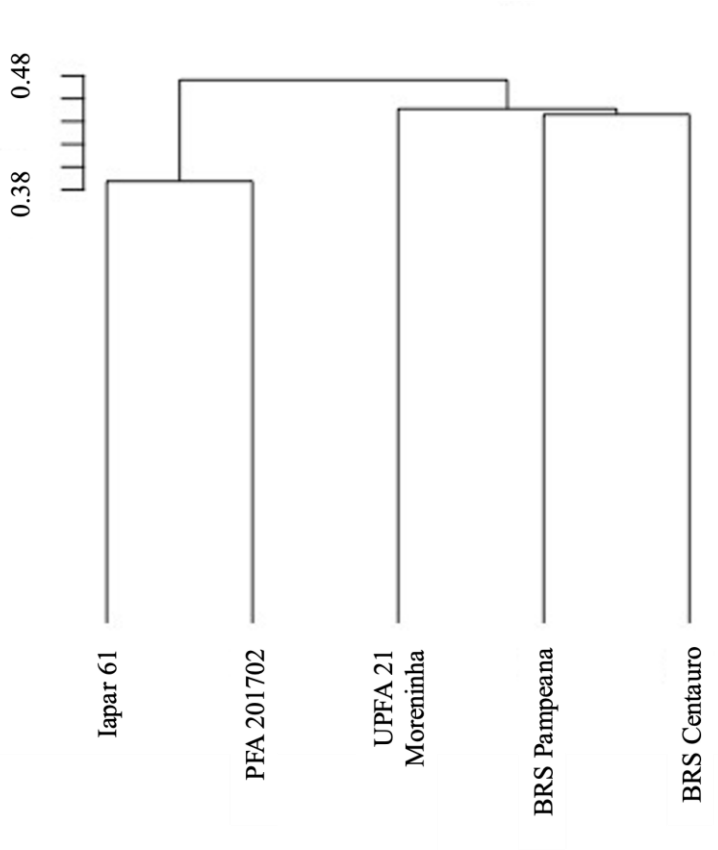

Figure 2. Genetic relationship dendrogram among parental *Avena* genotypes.

The analysis of the Jaccard matrix revealed that the average similarity between the evaluated genotypes was 0.81 (Table 4). This value indicates that, on average, 81% of the genetic characteristics compared among the genotypes are shared. Such a high similarity index suggests a significant genetic homogeneity among the studied genotypes, indicating a large number of common characteristics.

Table 4. Jaccard Similarity Matrix for genetic comparison among oat genotypes.

	BRS Pampeana	UPFA21 Moreninha	IAPAR 61	PFA201702
UPFA 21 Moreninha	1.00			
IAPAR 61	0.82	0.82		
PFA201702	0.83	0.83	0.68	
BRS Centauro	0.77	0.77	0.72	0.83

Analysis of population 1 using the Jaccard matrix revealed an average similarity of 0.69 between the evaluated genotypes. Genetic dissimilarity estimates among the populations ranged from 0.0 to 0.30 (Figure 3).

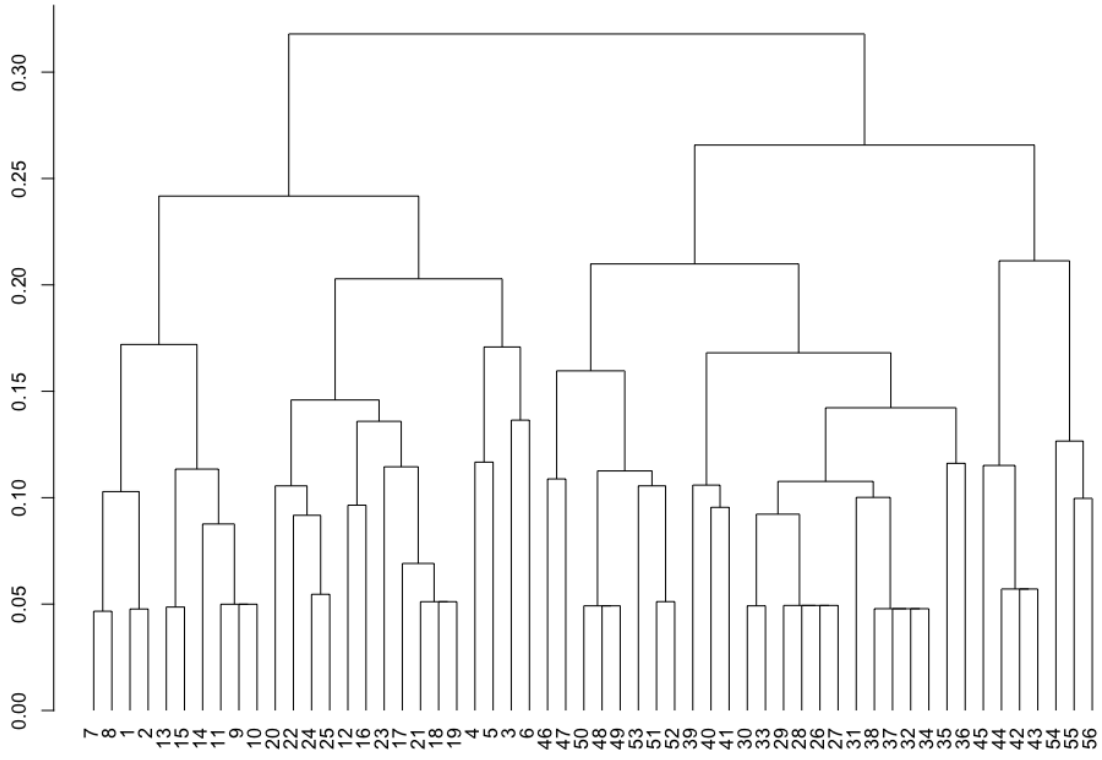

Figura 3. Dendogram of genetic distance obtained from the Jaccard coefficient for population 1.

Analysis of population 2 using the Jaccard matrix revealed an average similarity of 0.70 between the evaluated genotypes. Genetic dissimilarity estimates among the populations ranged from 0.0 to 0.35 (Figure 4).

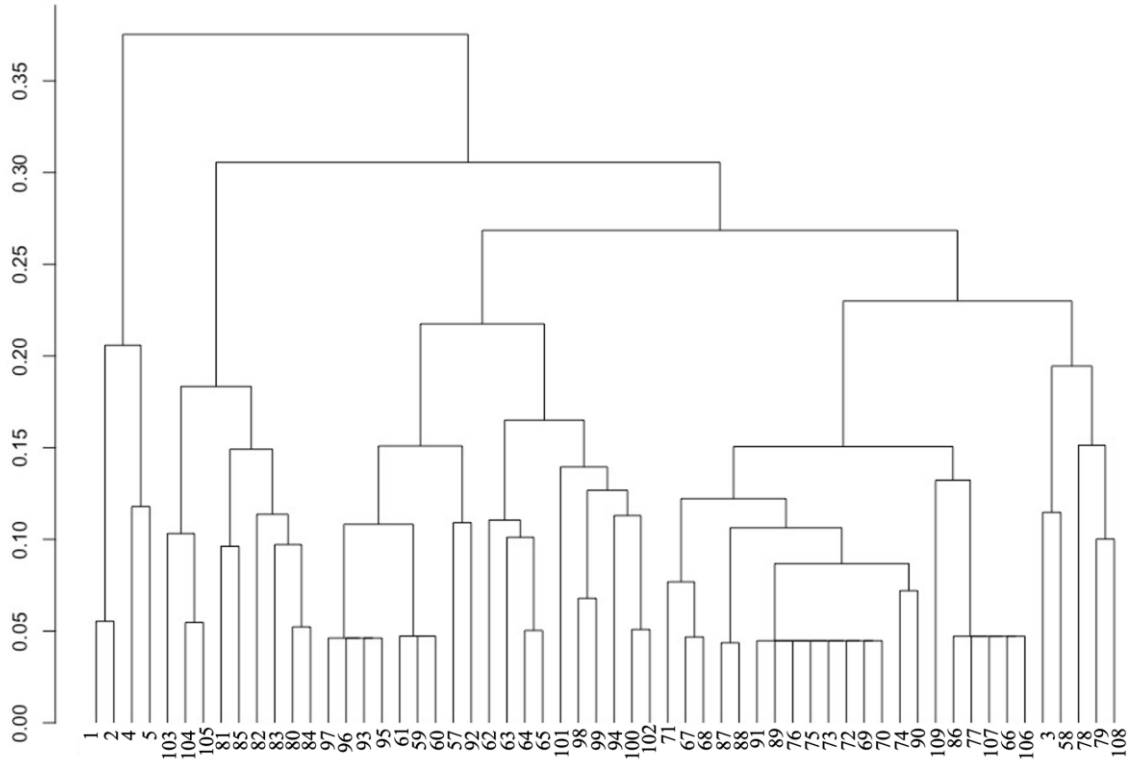

Figure 4. Dendogram of genetic distance obtained from the Jaccard coefficient for population 2.

Analysis of population 3 using the Jaccard matrix revealed an average similarity of 0.75 between the evaluated genotypes. Genetic dissimilarity estimates among the populations ranged from 0.05 to 0.40 (Figure 5).

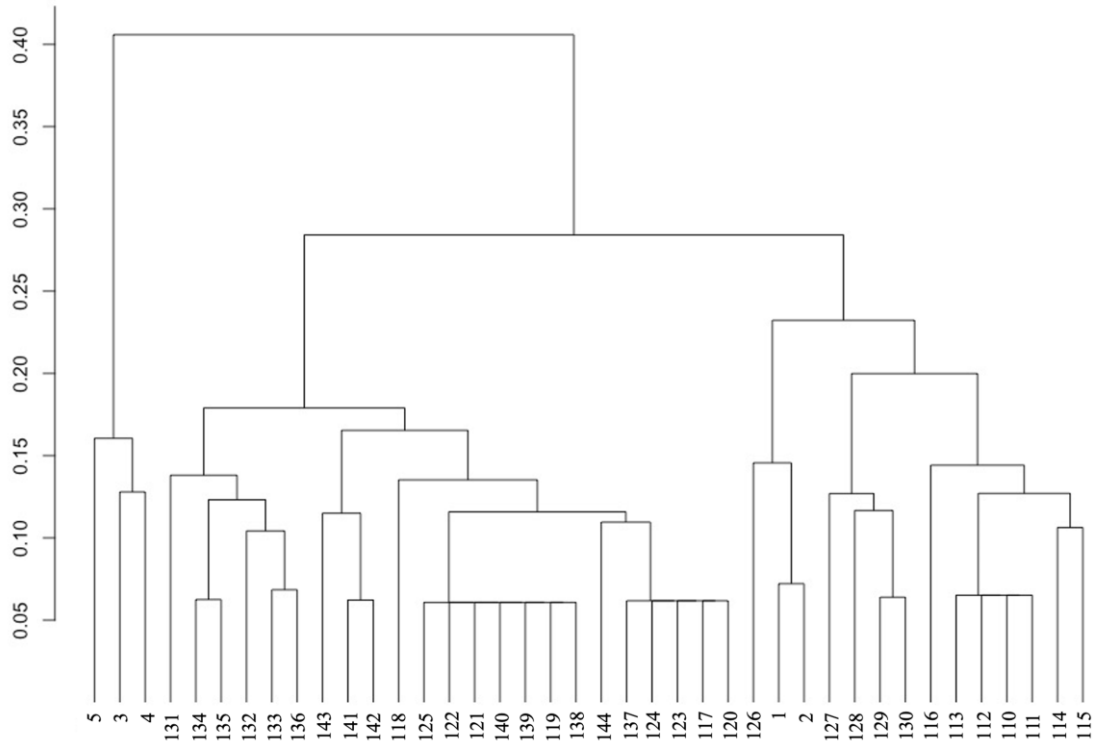

Figure 5. Dendogram of genetic distance obtained from the Jaccard coefficient for population 3.

Analysis of population 4 using the Jaccard matrix revealed an average similarity of 0.74 between the evaluated genotypes. Genetic dissimilarity estimates among the populations ranged from 0.00 to 0.35 (Figure 6).

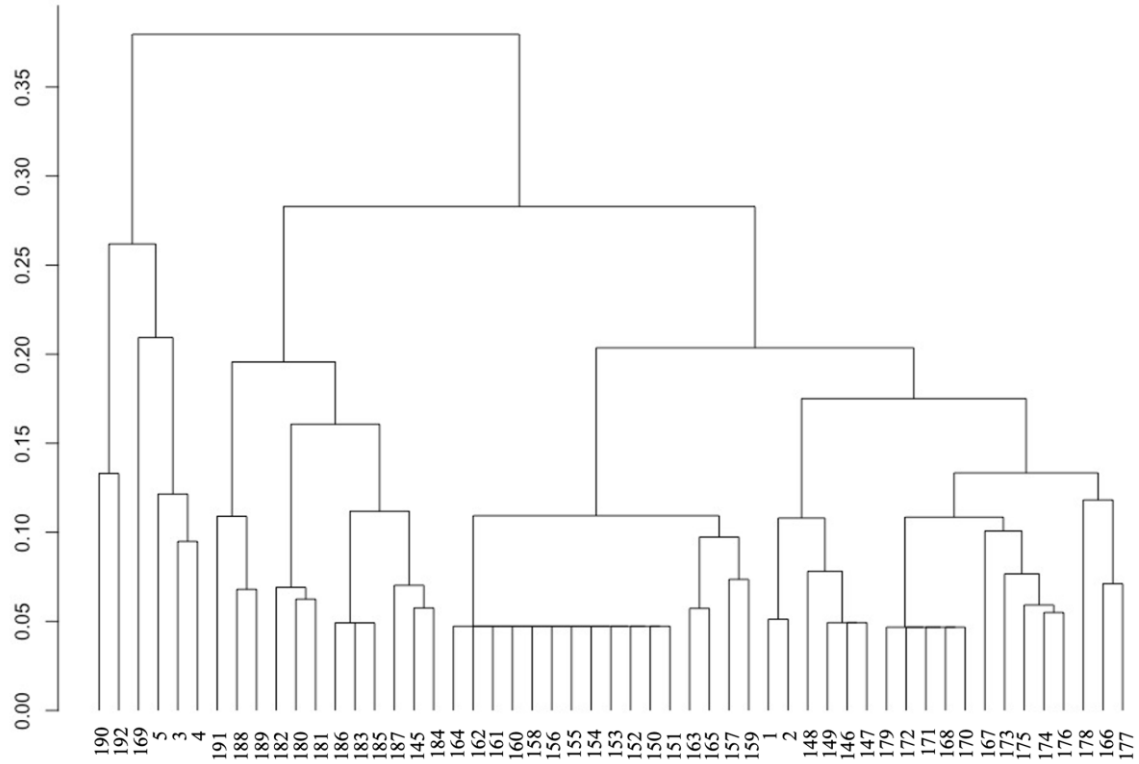

Figure 6. Dendogram of genetic distance obtained from the Jaccard coefficient for population 4.

When all populations were analyzed together, the generated dendrogram indicated that the genotypes could not be grouped into distinct clusters. The Jaccard matrix revealed an average similarity of 0.69 among the evaluated genotypes, with genetic dissimilarity estimates ranging from 0.0 to 0.35 across the populations (Figure 7).

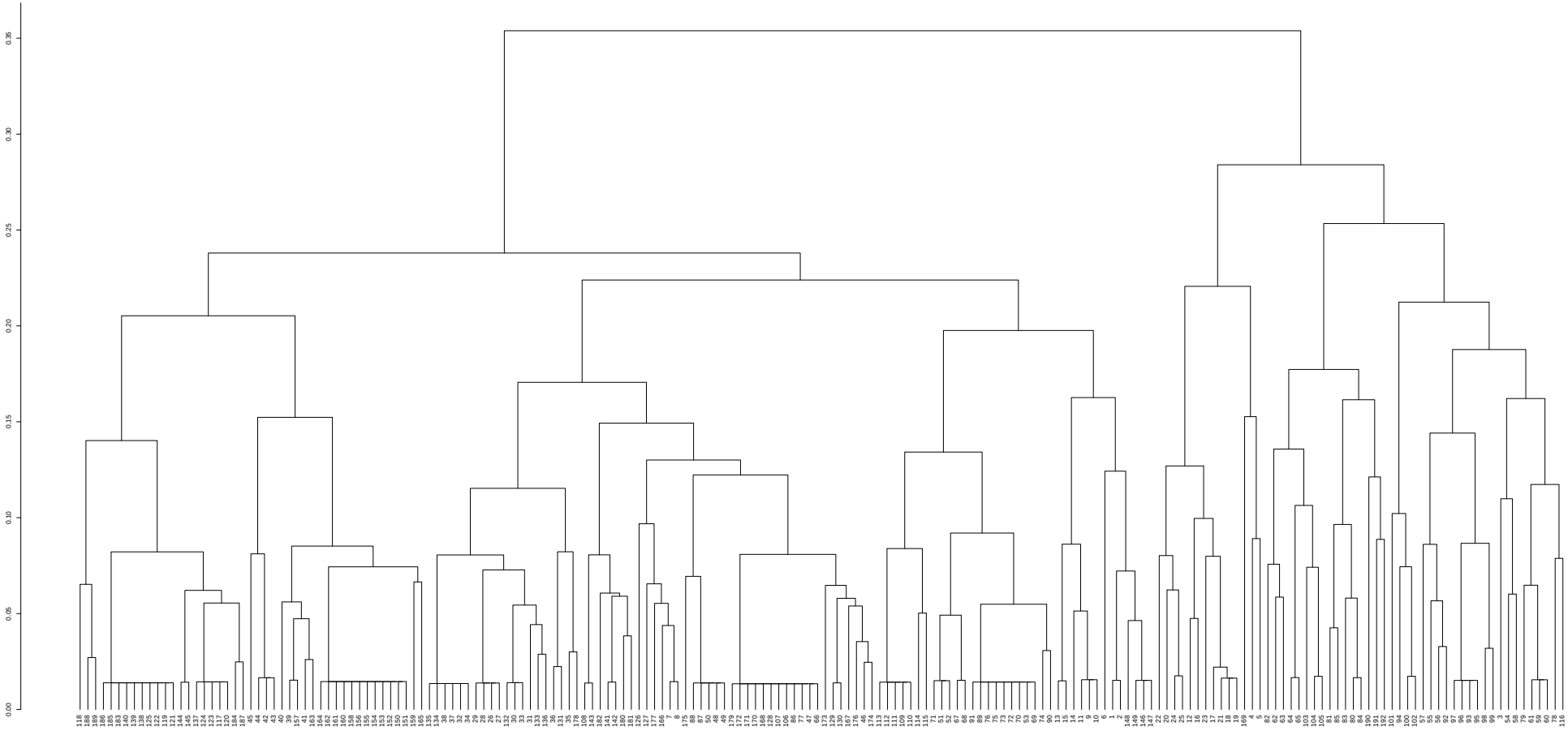
0.00
0.05
0.10
0.15
0.20
0.25
0.30
0.35

Figure 7. Dendogram of genetic distance obtained from the Jaccard coefficient

The narrow genetic variation observed in this study among oat genotypes suggests that only a small portion of the available genetic diversity is currently utilized for oat breeding. According to Fu et al. (2003), the reduction in genetic diversity may increase the vulnerability of crops to new pests, pathogens, and climate changes.

Despite the relatively large representation of this species is in various gene banks, it is highly probable that the vast majority of stored worldwide accessions are duplicates, and the protected gene pool is relatively narrow (Podyma et al. 2019).

Throughout the twentieth century, landraces have increasingly been replaced by modern cultivars, leading to a significant loss of genetic diversity (Warburton et al., 2008; Reif et al., 2005; Roussel et al., 2004). This loss has become a critical issue in both natural plant populations and important crop species (Montilla-Bascón et al., 2013). Similar lack of diversity has been observed in Canadian and Chinese oat varieties, further underscoring the potential consequences for crop vulnerability and adaptability (Baohong et al., 2003; Fu et al., 2004).

5.5 Conclusions

Thus, our results suggest that microsatellite markers can play an important role as a source of additional information in oat. Genetic diversity studies, assessed by various tools including DNA markers, provide important information both for genetic conservation and for use in efficiently breeding new commercial varieties. The results demonstrated a relatively low level of genetic variation, but it is still possible to exploit

it. The introduction of new germplasm sources is recommended to provide the necessary genetic variability.

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6 FINAL CONSIDERATIONS

The search for oat genotypes with forage potential that are both well-adapted and stable requires the application of quantitative genetic methods and advanced statistical techniques to achieve more accurate predictions of genotypic value. Understanding the interaction between genotype and environmental effects is essential for breeders, as it accounts for yield variation that cannot be explained by genotype or environmental effects alone.

Additionally, there is a push to increase genetic variability by broadening the genetic base. To assess the viability resulting from crosses between different species/individuals, cytogenetic analysis is employed, which allows for monitoring interspecies transfer and assists in selecting stable plants for breeding programs. Pollen viability contributes to taxonomic and evolutionary studies and is a crucial factor influencing fertilization success. Crosses made between plants with non-viable pollen will result in sterile plants and reduced grain production.

DNA markers are indispensable tools in breeding and genetic resource management. They are employed to estimate genetic similarity and distance, select and identify desirable traits, evaluate parental genotypes and crossing efficiency, assess seed purity, identify genes responsible for important functional traits, and enhance the density of genetic maps.

7 GENERAL CONCLUSION

The absence of genotype × environment interaction, both across locations and years, indicates that genotypes with good performance in one environment tend to maintain their performance in different environments. Nonetheless, the cultivars Agro Planalto and BRS Pampeana, evaluated from 2016 to 2021, stood out in terms of stability, adaptability, and adequate leaf dry matter production. Among all the lines, PFA201603 appears to be promising based on this criterion.

The combinations between the parents of *A. brevis* and *A. strigosa* demonstrated reproductive efficiency, suggesting that there is a close relationship between the species. This proximity facilitates the use of *A. brevis* in the genetic improvement of *A. strigosa*.

In the study, the coefficient of genetic differentiation indicated low genetic variation between the genotypes of the two different species. Therefore, it is crucial to enhance the genotypic diversity of the assessed introductions.

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