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Floral biology of *Avena strigosa*

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Resumo

Biologia floral de cultivares de *Avena strigosa*. A aveia-preta é uma planta de múltiplos usos no sistema agrícola, contudo programas de melhoramento têm pouco sucesso em obter novos genótipos, não se sabe o motivo exato. Assim sendo, este trabalho teve como objetivo caracterizar e avaliar a morfologia floral e a viabilidade polínica em *Avena strigosa*. Para isso, duas cultivares de hábito e ciclo contrastantes (IAPAR 61 – Ibiporã e UPFA 21 – Moreninha) foram estabelecidas no campo e em vaso no município de Passo Fundo, no norte do estado do Rio Grande do Sul. Foram realizadas avaliações morfológicas na panícula, espiguetas e grãos de pólen das cultivares. Na cv. IAPAR 61 – Ibiporã foram encontrados os maiores comprimentos de espigueta, ráquila, gluma I, gluma II, pálea, arista e pistilo. Na cv. UPFA 21 – Moreninha encontrou-se maior comprimento da ráquis, lema e antera apicais e maior densidade de espiguetas na panícula. Em relação à viabilidade polínica, as cultivares não diferem entre si, evidenciando-se que apenas as medidas dos grãos de pólen foram contrastantes.

Palavras-chave: Aveia-preta; Morfologia; Viabilidade polínica

Abstract

Black oat is a plant of multiple uses in the agricultural system, however, without knowing exactly why, breeding programs have had little success in obtaining new genotypes. The goal of this study was to characterize floral morphology and evaluate if there is variability in *Avena strigosa* in its pollen viability. Thus, two cultivars of contrasting habits and cycles (IAPAR 61 – Ibiporã and UPFA 21 – Moreninha) were cultivated in a field and in a vase in the city of Passo Fundo, in the north of the state of Rio Grande do Sul, Brazil. Morphological evaluations were performed on the panicle, spikelets and pollen grains of the cultivars. The largest lengths of spikelet, rachilla, glumes I, glumes II, palea, ridge and pistil were found. in cv. IAPAR 61 – Ibiporã. Larger length of the rachis, lemma and floret in the apical and greater density of spikelets in the panicle were found in cv. UPFA 21 – Moreninha. As for pollen viability, the cultivars do not differ from each other, evidencing that only pollen grain measurements were contrasting.

Key words: Black oat; Morphology; Pollen viability

Introduction

The black oat (*Avena strigosa* Schreb.) is a cool season annual cycle grass and is considered a more rustic type of the genus *Avena* L. It is usually more resistant to pests and disease; however, it demands a great deal of water. It presents a high capacity for tillering and green mass production (GOELLNER; FLOSS, 2001). The species has been widely grown for Winter-Spring forage and mainly as a Winter cover crop in the southern region of Brazil. Its use in direct planting is essential since its hay aids in erosion control, produces a large quantity of dry matter and demonstrates expressive control for weed and nutrient cycling due to the allelopathic effect determined by dead crops (CARVALHO, 1998).

Though it is more important in terms of cultivated area, the black oat has not received the same amount of attention as the white oat (*A. sativa* L.) in regard to genetic improvement. This has delayed the release of cultivars with a higher yield than the "Common" variety; thus, producers have used native seeds of a population called "Common" black oat. However, after the ban on the distribution of "Common" black oat seeds (BRASIL, 2003), there was growing interest on the part of companies in improving the development of new cultivars. Despite the increase in cultivars in the Brazilian market, there is a long way ahead of us until materials with high biomass production and greater resistance to fungi-related disease are obtained (SANTOS et al., 2018).

Considering the characterization of black oat genotypes, Chini (2017) stresses the importance of choosing variables with easily identifiable differences between them and that allow the selection of materials. The use of artificial hybridizations is one of the most common techniques to obtain genetic variability in plant improvement, attempting to join in one single genotype favorable alleles in commercial cultivars, elite lineages, introduced plants or in related species (SILVEIRA, 2009). In this sense, black oat improvement programs have had very little success due to the difficulty in obtaining seeds from artificial hybridization – the reasons for this are still not very clear. It is possible that there is some morphological restriction in the species related to floral biology, in which aspects related to pollen viability and to pollen x stigma cannot be ignored, since in gametophytic incompatibility systems, the pollen/pistil interaction is genetically controlled by the haploid genome of the pollen grain and the diploid genome of the pistil. In sporophyte incompatibility systems, on the other hand, pollen reaction is determined by the genome of the tissue in which the pollen was produced (ZANETTINI, 2003).

To obtain genetic variability through crossbreeding it is essential to have genotypes of recognized agronomic value in terms of the characteristics of interest. Morphological and agronomic characterization are the stages that precede crossbreeding, the first being of paramount importance since it is the basis for any study and initial determination begins with the phenotype (LONGHI-WAGNER; CHIES, 2003).

The principal reproductive system in black oats is autogamy (KOSINA, 2015), and special attention must be given to the moment of performing emasculation and hybridization. In addition, information regarding the floral biology of this grass is described in the taxonomic keys of the genus *Avena* L., with no details concerning the possible variability between cultivars as to the morphology of floral verticils, pollen viability and stigma receptivity.

With this in mind, the present study was carried out with the purpose of verifying if there is morphological variability in *A. strigosa* concerning floral biology. Two contrasting cultivars in relation to cycles and growth habits were chosen, cv. UPFA 21 – Moreninha and cv. IAPAR 61 – Ibiporã. Their floral morphologies were described and the possibility of inter/intraspecific variability was evaluated, as well as the characterization and analysis of pollen viability.

Materials and Methods

Vegetable material

The UPFA 21 – Moreninha and IAPAR 61 – Ibiporã cultivars were released by the obtainers, the Universidade de Passo Fundo (University of Passo Fundo, Brazil) and

Instituto Agronômico do Paraná (the Agronomic Institute of the State of Parana, Brazil), respectively. The UPFA 21 – Moreninha cultivar was created for cover crop use and it can be sown earlier than the "Common" black oat, as well as handled in flowering during a period that is closer to the period for soybean cultivation (FLOSS, 2003). The IAPAR 61 – Ibiporã cultivar has a long cycle and was selected from a population of "Common" black oats. It is used for animal feeding (direct pasture, green forage, hay and silage) and for soil handling and conservation (as a cover crop and an option for culture rotation) (IAPAR, 2004).

Locality and period

The cultivars were established in different periods and localities, according to the type of evaluation that would be carried out. For the floral morphology evaluations, plants were cultivated in the fields of the experimental area of the School of Agronomic Sciences and Veterinary Medicine at the Universidade de Passo Fundo (FAMV/UPF), between May and October 2018. To evaluate pollen viability, plants were cultivated in growth chambers (PGR 36 model – Conviron – Winnipeg, Manitoba/Canada) at Embrapa Trigo, from December 2018 on, in controlled light, temperature and humidity conditions. Both these institutions are located in Passo Fundo in the northern region of the state of Rio Grande do Sul, Brazil, at 28°15' latitude South and 52°24'longitude West, altitude of approximately 700 m above sea level. The region's climate, according to the Köppen classification, is of the subtropical variety (Cfa) (KUINCHTNER; BURIOL, 2001).

Procedures

For the morphological study, two portions were established with one hundred plants of each cultivar in the experimental area at FAMV/UPF, sown in dark dystrophic Red Latosol soil (STRECK et al., 2008), with the following attributes: pH: 5.4; P: 4.2 mg/dm³; K: 13 mg/dm³; Al: 1.5 Cmol c/dm³; Ca: 3.5 Cmol c/dm³; Mg: 1.8 Cmol c/dm³; CTC: 14.0; Base saturation: 38%; S: 25.0; B: 0.1 mg/dm³; Mn: 70.3 mg/dm³; Zn: 4.57 mg/dm³ and Cu: 12.41 mg/dm³.

At Embrapa Trigo, seeding was carried out in six 8L buckets (three for each cultivar) containing a homogeneous mixture of soil:substrate:vermiculite (1:1:1 proportion), adjusted to pH 6.0. Cultivation conditions were temperatures at 18 ± 2°C (day) and 14 ± 2°C (night) and 70% relative humidity.

Floral morphology evaluations

Plants were examined in two phonologic stages (ZADOKS et al., 1974): pre-emergence of the panicle and totally emerged panicle. In both stages, ten panicles were collected from each cultivar. At stage 59 (completely exposed inflorescence), ten panicles were examined for length and number of rachilla nodes, number of ramifications and spikelets, number of spikelets per node and ramifications per node, type of ramification orientation and position. Subsequently, sexual expression of the florets of ten spikelets was examined. At stage 57 (¾ of emerged inflorescence), the morphological evaluation of the spikelets was carried out. To do so, nine spikelets per panicle were extracted, totaling ninety spikelets evaluated in each cultivar. To improve evaluative precision, and verify possible differences among flower, anther and spikelet morphology according to their position in the panicle, the longest ramification of each panicle in the 1st basal node, the 5th or 6th middle region node, and the last three rachilla apical spikelets were chosen. After the removal of the spikelets, the length of the glumes, lemma, palea, awn, anther, pistil, lodicules and rachilla were measured. In addition, the presence/absence of surface covering, color and venation of glumes, lemma and palea were recorded. Length was measured in millimeters with a pachymeter. Morphological characterization was done based on descriptors of *Avena* spp. (BRASIL, 2002).

Pollen viability evaluation

To evaluate the viability of pollen grains, ten panicles from each cultivar were collected in the emergence and inflorescence phases (Stage 52, ZADOKS et al., 1974). After collection, and the proper identification of the panicles, these 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 -20ºC.

For pollen analysis, cytological slides were made using three anthers of the same flower, taken from the basal, middle and apical regions of the same panicle, with repetition, totaling thirty slides per cultivar/one slide per panicle region. The anthers were cut into small pieces for pollen grain release, using carmine acetic acid dye (1%), and covered with the coverslips, pressing slightly for the material to spread. Subsequently, the slides were quickly run over fire to improve the concentration of the dye on the nucleus, and sealed with "luto" (a mixture of beeswax and pitch, at a 1:1 proportion). For the apical region of the panicle, DAPI fluorochrome was used (4.6-diamidino-2-fenilindol), since the large quantity of starch prevented the perfect visualization of the nuclei. The procedures for this last case were the same carried out for the basal and middle regions; the only difference being the analyses was done with an epifluorescence microscope (Axioscop FL/Zeiss).

To obtain a random sample, a cytological slide scan method was used with an optical microscope (BX 50/Olympus), 20x objective lens. 220 pollen grains per slide were counted. The pollen grains analyzed were classified into three different groups: 1) normal (uninuclear, binuclear and trinuclear, presence of one pore and adequate and uniform starch formation) and 2) unviable (empty), 3) grains with little starch. The best images were shot using the Pinnacle Studio Plus program. Additionally, 10 pollen grains per slide were measured, totaling 330 per genotype. 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).

Statistical analysis

Data was submitted to variance analysis (F test) with measurement comparison using the Tukey test at 5% significance.

Results

The vegetative period, between emergence and flowering (stages 50 – 69; ZADOKS et al., 1974) lasted 119 days for cv. UPFA 21 – Moreninha, and 143 days for cv. IAPAR 61 – Ibiporã. The flowering phenophase for cv. UPFA 21 – Moreninha began on the second week of September, while that of cv. IAPAR 61 – Ibiporã began the first week of October. The beginning of the reproductive phase was verified through panicle emergence, this being an inflorescence composed of spikelets.

Based on the descriptors for *Avena* spp. (BRASIL, 2002), the orientation of panicle ramification varied between partially unilateral to unilateral for cv. IAPAR 61 – Ibiporã, and equilateral and partially unilateral for cv. UPFA 21 – Moreninha. As for ramification position, cv. UPFA 21 – Moreninha was semi-decumbent and cv. IAPAR 61 – Ibiporã was semi-erect and erect.

In relation to panicle morphometric variables (Table 1), cv. UPFA 21 – Moreninha was 43% higher than cv. IAPAR 61 – Ibiporã regarding the number of spikelets. Higher values were also seen in rachilla length: cv. UPFA 21 – Moreninha was 12% larger in relation to cv. IAPAR 61 – Ibiporã. As for the number of ramifications and node/rachis, there was no difference between the cultivars ($p > 0,05$).

TABLE 1: Morphological characteristics of the panicle of the two black oat cultivars.

Character/Variable	IAPAR 61 – Ibiporã	UPFA 21 – Moreninha	*p**
Node per rachis	11.6	11.1	0.0962
Ramification	54.4	57.7	0.3173
Spikelet	109.5	156.4	0.0007*
Rachis size (cm)	23.59	26.7	0.0000 *

* F Test = Significance $p \leq 0,05$.

In relation to the analysis of the number of spikelets per node and ramifications per node, only the number of spikelets/nodes varied significantly between the cultivars, but only in the first five nodes counting from the base to the apex (Table 2). In this case, cv. UPFA 21 – Moreninha presented spikelet density that was 31% higher than that of cv. IAPAR 61 – Ibiporã.

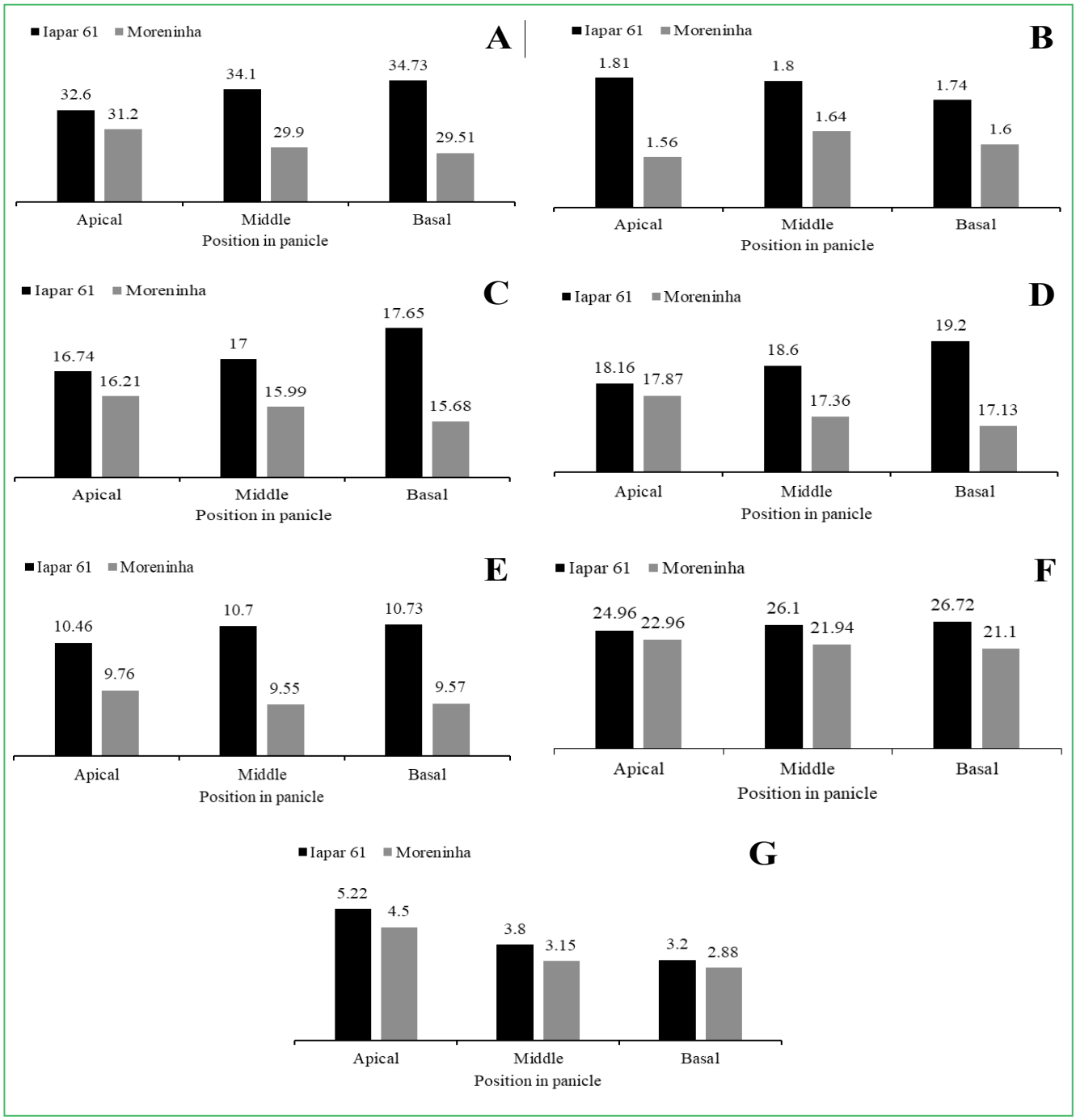
TABLE 2: Density average of spikelets in panicles of the black oat cultivars according to node position (NP).

PN	IAPAR 61 – Ibiporã	UPFA 21 – Moreninha	*p*
1º	19.6	30.1	0.0309*
2º	18.6	27	0.0205*
3º	16.3	24.2	0.0054*
4º	14.4	19.9	0.016*
5º	11.6	14.9	0.0497*
6º	8.5	10.4	0.0925
7º	7,3	7.8	0.5331
8º	5	4.3	0.2263
9º	3.5	3.1	0.4228
10º	1.7	2	0.0652
11º	1.8	1.4	0.2457

* F Test = Significance, $p \leq 0,05$; NP: sequential numbering from panicle base to apex.

In relation to the anther analyzed (180), the length of spikelets, rachilla, glumes I, glumes II, palea, awn and pistil was greater ($p < 0,05$) in cv. IAPAR 61 – Ibiporã in the three portions observed when compared to cv. UPFA 21 – Moreninha (Figure 1).

FIGURE 1: Difference in length of spikelet (A), rachilla (B), glume I (C), glume II (D), palea *E), awn (F) and pistil (G) in three panicle positions in both cultivars.



In the apical position of the lemma and anther cv. UPFA 21 – Moreninha was longer, however in the basal and middle positions, cv. IAPAR 61 – Ibiporã (Figure 2) was longer.

In this study, it was observed that the flower of the floret is achlamydeous, covered by protective bracts – the palea, lemma and glumes – and three homodynamic stigmas in a concentric line. The anthers are basifixed and present longitudinal dehiscence. The anthers of the middle portion of the panicle are purple ventrally, while the remaining are yellow (Figure 3). When found in the apical region, they are already dry. The pistil is white to colorless, has an upper pilous ovary, a bifid feathery stigma; the panicle apex is already in grain form (Figure 3). There are also lodicules frontally in the ovary (Figure 7.E); in the basal portion of the panicle these are full and, in the apex, they are already dry.

FIGURE 2: Differences in lengths of lemma (A) and anther (B) in the apical position.

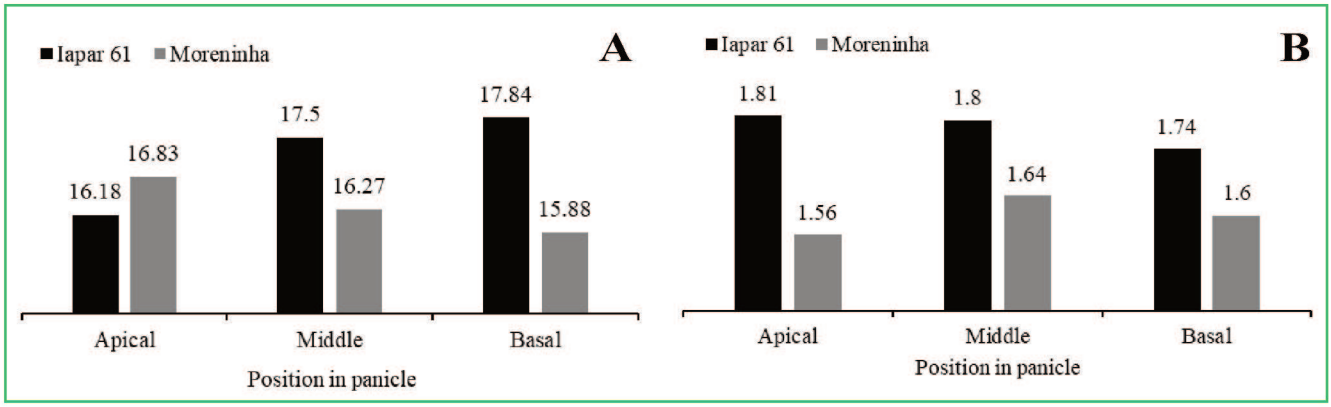
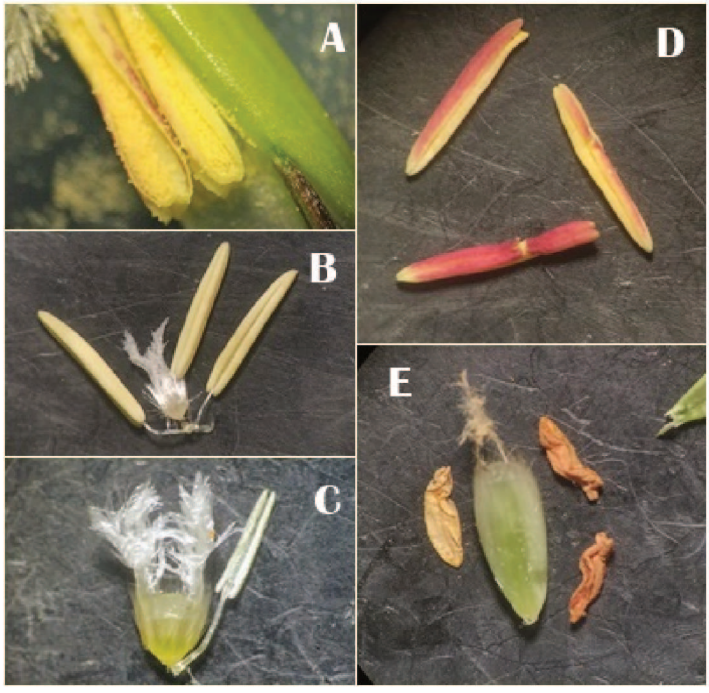


FIGURE 3: Dehiscence of black oat anthers (A). Basal region pistil and anthers of cv. UPFA 21 – Moreninha (B). Basal region pistil and anther of cv. IAPAR 61 – Ibiporã (C). Purple-colored anthers in middle region of panicle (D). Dry anthers and pistil in grain of the apical region of rachilla (E).



As for the external appearance of the protective structures of the spikelet, both cultivars present two glumes, called Glume I and Glume II; the second is larger than the first, they have a lanceolate shape, are parallel-veined and concolored; the outline is green and white, with purple stains at the apex in the middle region of the panicle. They also have small trichomes on their ridges (Figure 4). The awn is seen dorsally at the lemma, it is twisted, with trichomes; color varies from brown at the base, and green and white lengthwise. The apex of the panicle is brown and dry and there is veining (Figure 5).

FIGURE 4: Glumes (A). Purple stain on the upper portion of glume (B). Details of ridge with small trichomes (C).

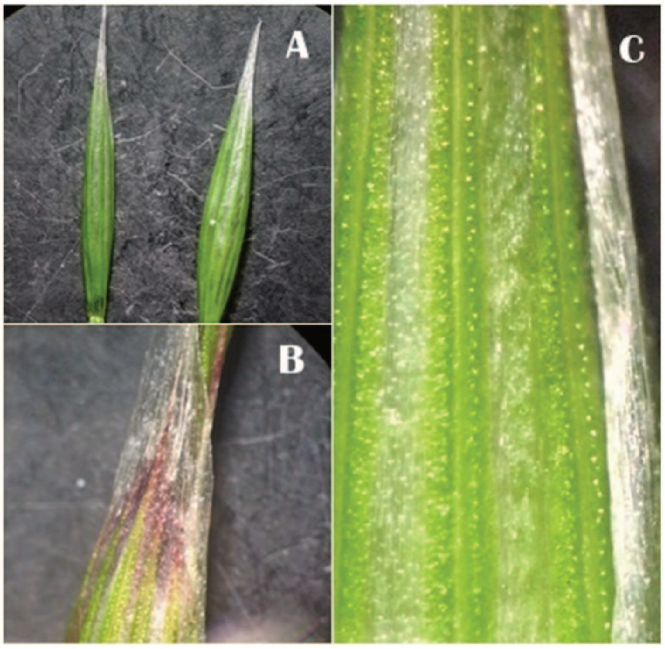
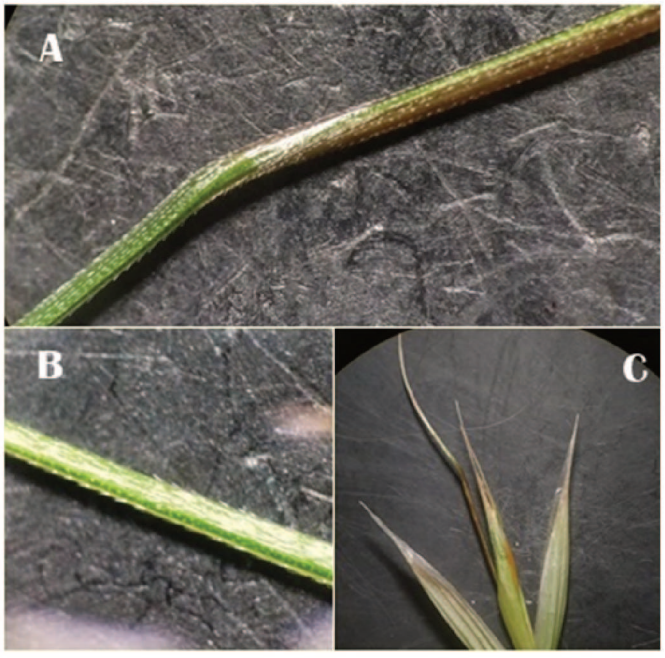


FIGURE 5: Twisted awn with trichomes (A). Details of trichomes (B). Awn drying on the apical anther (C).



The lemma is biaristulated, green on the basal and middle portion and brown on the apical portion of the panicle. It has small trichomes at its apex and a slight longitudinal ridge. Its awns can be purple in the middle region of the panicle (Figure 6). Inside the lemma, covering the flower, is the palea. This does not have ridges; it is green to colorless, with a membranous texture, and is brown in the apical region of the panicle. It has trichomes around its apex; on its dorsal side there is an anther that has not developed on the spikelet (Figure 7).

FIGURE 6: Brown lemma in the apical region of the panicle (A). Detail of the trichomes at the lemma apex (B). Detail of the bi-awned lemma (C). Lemma with purple awns in the middle region (D).

FIGURE 7: Palea covering anthers and pistil at the panicle apex (A). Palea of the apical region with undeveloped anther indicated by arrow (B). Basal palea (C). Basal lodicules (D). Lodicules facing the ovary in the basal region (E). Details of trichomes surrounding the palea (F).

The pollen grains of both cultivars are monoporous, have radial symmetry, are oval to eliptical in shape and are rarely circular at the apex. Analyses of pollen viability enabled the verification of the average of viable (Figure 8.A) and inviable (Figure 8.B) pollen grains in the cultivars. Comparison of the cultivars (interspecific) and their panicle regions for the normal/viable variables (uninuclear and bi/trinuclear, with starch and one pore), inviable (empty) and with little starch, is presented in Table 3. However, the cultivars presented differences ($p < 0,05$) only in relation to the quantity of uninuclear pollen and little starch, collected at the basal and middle position of the panicle, respectively (Table 3). In cv. UPFA 21 – Moreninha, the presence of uninuclear pollen in the basal region was 36% higher in comparison to cv. IAPAR 61 – Ibiporã (Figure 9). In relation to the presence of grains with little starch in the middle region, cv. IAPAR 61 – Ibiporã presented 41% more grains than cv. UPFA 21 – Moreninha.

FIGURE 8: Variable pollen grains in the basal region with chromosome division. Enhanced 400x (A). Empty pollen grain indicated by the arrow, enhanced 400x (B). Pollen grains with little starch indicated by arrow. Enhanced 400x (C).

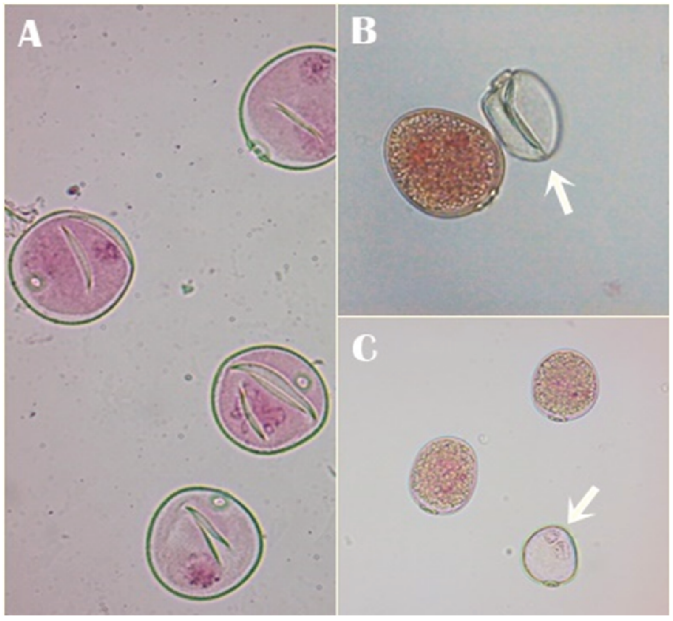
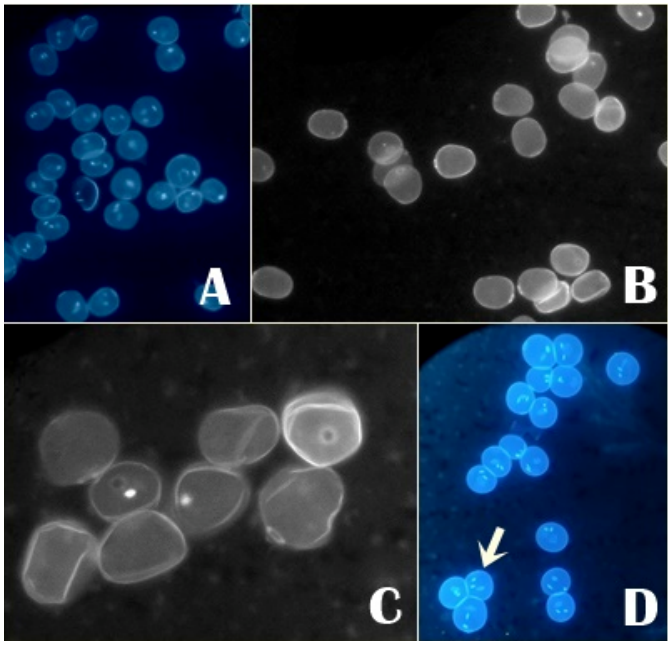


TABLE 3: Average of pollen grains in the cultivars according to variables.

Categories	IAPAR 61 – Ibiporã	UPFA 21 – Moreninha	*p*
Basal uninuclear	83.4	130.6	0.0273 *
Middle uninuclear	23.2	35.5	0.345
Apical uninuclear	14.1	9.9	0.4782
Basal bi/trinuclear	87.2	64.7	0.439
Middle bi/trinuclear	155.1	158.7	0.8406
Apical bi/trinuclear	135.1	172.8	0.2832
Basal empty	31.3	9.4	0.0801
Middle empty	20.9	12.5	0.3023
Apical empty	70.8	37.3	0.2679
Basal little starch	18.1	15.3	0.6498
Middle little starch	20.8	12.3	0.036 *

* F test = Significance, $p \leq 0,05$.

FIGURE 9: Variable pollen grain in cv. IAPAR 61 – Ibiporã, 200X (A). Empty pollen grain in cv. IAPAR 61 – Ibiporã, enhanced 200x (B). Empty pollen grain in cv. UPFA 21 – Moreninha, enhanced 400x (C). Variable pollen grains in cv. UPFA 21 – Moreninha, trinuclear, indicated by arrow, enhanced 200x (D).



When considering specifically the apical region, a large quantity of starch impaired the full visualization of the nuclei. With the DAPI fluorochrome we were only able to see the nuclei; however, we were not able to observe the little starch category with this color.

However, when comparisons are carried out interspecifically, that is, comparisons of the evaluated regions in the panicle of each cultivar, we noticed that in cv. IAPAR 61 – Ibiporã the presence of pollen grains of the uninuclear kind is 55% higher in the basal region than in the apical (Table 4). In cv. UPFA 21 – Moreninha, the presence of pollen grains in the basal region is 51% higher than in the other regions. Furthermore, the presence of bi/trinuclear types is 26% higher in the middle and apical regions than in the basal region in this cultivar (Table 5), demonstrating how the nuclei divide in the panicle basal region (Figure 8.A).

In relation to pollen grain size, at the mean of the polar and equatorial axes for the cultivars, in the respective panicle segments (apical, middle, basal), when compared interspecifically (between cultivars), only the apical polar and apical equatorial diameter presented a difference between the cultivars and were 16% and 18% larger, respectively, in UPFA 21 – Moreninha when compared to cv. IAPAR 61 – Ibiporã (Table 6).

TABLE 4: Average of pollen grain in cv. IAPAR 61 – Ibiporã according to panicle position.

Types of pollen grain	Basal	Middle	Apical	*p*
Uninuclear	4.19	2.43	1.89	0.0015*
Bi/trinuclear	3.60	5.01	4.25	0.091
Empty	4.17	4.17	6.97	0.2014
Little starch	2.50	2.91	-	0.2573

* F Test = Significance, $p \leq 0{,}05$.

TABLE 5: Average of pollen grain in cv. UPFA 21 – Moreninha according to panicle position.

Types of pollen grain	Basal	Middle	Apical	*p*
Uninuclear	4.82	3.36	2.39	0*
Bi/trinuclear	3.72	5.03	5.04	0.0014*
Empty	2.99	3.37	5.09	0.0942
Little starch	2.51	2.36	-	0.6166

* F Test = Significance, $p \leq 0,05$.

TABLE 6: Average diameter of pollen grains in black oat cultivars according to panicle position.

Diameter (µm)	IAPAR 61 – Ibiporã	UPFA 21 – Moreninha	*p*
Apical polar	18.96	22.46	0.0001 *
Medium polar	19.56	20.80	0.1379
Basal polar	18.56	18.76	0.7699
Apical equatorial	18.74	22.66	0.0000 *
Medium equatorial	19.51	20.89	0.1558
Basal equatorial	18.41	18.54	0.8331

* F Test = Significance, $p \leq 0,05$.

When the average was verified interspecifically, we could see that there were significant differences among the three regions in both cultivars (Tables 7 and 8).

TABLE 7: Measurement averages of cv. IAPAR 61 – Ibiporã in the three panicle regions.

Region	Average	*p*
Apical polar	18.96	0.0000*
Medium polar	19.56	0.0000*
Basal polar	18.56	0.0000*
Apical equatorial	18.74	0.0000*
Medium equatorial	19.51	0.0000*
Basal equatorial	18.41	0.0000*

* F Test = Significance, $p \leq 0,05$.

TABLE 8: Measurement averages of cv. UPFA 21 – Moreninha in the three panicle regions.

Region	General average	*p*
Apical polar	22.46	0.0000*
Medium polar	20.80	0.0000*
Basal polar	18.76	0.0000*
Apical equatorial	22.66	0.0000*
Medium equatorial	20.89	0.0000*
Basal equatorial	18.54	0.0000*

* Teste F = Significância, $p \leq 0,05$.

Discussion

Panicles and spikelets

According to Floss (2003), cv. UPFA 21 – Moreninha takes 155 days in average from emergence to maturation. The results obtained in the present study confirmed this, since the emergence of the panicle in this cultivar occurred within the expected number of days: 119 days before flowering. Cv. IAPAR 61 – Ibiporã, which took 143 days before flowering in this study, did not confirm the description given by the Instituto Agronômico do Paraná (IAPAR, 2004) which states it takes around 134 days from emergence to full emission of panicles. It is assumed that climate or soil conditions interfered in the process.

In this study, *A. strigosa* variability was evaluated regarding floral biology. Based on the results obtained, we confirmed that both cultivars are different in relation to their morphometrics. In relation to spikelet morphology (color, covering, veining) both are the same, though they are interspecifically different in regard to regions (basal, middle and apical). Only one floret could be observed on the spikelets of both cultivars and two florets were seldom found in some spikelets of

cv. UPFA 21 – Moreninha. However, there was always a rudimentary floret on the palea dorsal area. These characteristics, added to the twisted awn, corroborate Groth's description of *A. strigosa* (GROTH, 2009).

Spikelet flowering in the panicle occurred from the apex to the base, since when they were collected, we could see that the apex florets were already fertilized while the florets of the base were immature. This flowering orientation of the panicle is described by Borém (1999) to explain the period in which emasculation, one of the most prescribed hybridization methods in oats, should be done. According to Mundstock (1983), in the genus *Avena* L., the flowering from apex to base occurs during a period of three to four days and can take longer. He also reports that in the same plant, all the panicles flower in around ten days.

The inflorescence of the panicle type, with ramifications along the rachilla, can be equilateral, as seen in this study. This had already been described by Stanton (1955) regarding black oat morphology. In this study, it was also observed that the florets of both cultivars lean on an axis in the interior of the spikelet called rachilla. Each floret of the cultivars is formed by two glumes, a lemma, a palea, two lodicules, three stigmas and the pistil. The pistil is formed by the ovary style and bifid/feathery stigma. These structures had already been reported by Marshall and Sorrells (1992).

According to Chase (1950), the lodicules in *A. strigosa* are located at the base of the ovary, corroborating what was found in this study. Their function is to aid the opening of the floret with turgescence, thus enabling the emergence of the stamen and stigmas. Also, according to Marshall and Sorrells (1992), the feathery characteristic of the stigma helps to trap the pollen grains being dispersed by wind, which is common in Poaceae.

Pollen viability

The analysis of pollen viability and assisted selection in plants are excellent tools for genetic improvement programs since it is instantly possible to examine hundreds of pollen grains (SCARIOT, 2013). According to Brambatti et al. (2016), pollen viability in triticale helps the geneticist make decisions when crossing breeds, since it allows him/her to choose stable genotypes. In his study on Bromeliaceae, Benzing (2006) states that the use of morpho-pollen characteristics for taxonomic studies can be used instead of other characteristics such as leaf and flower coloring and structural size. The latter are more prone to alterations when compared to pollen grain characteristics, which are considered to be more preserved.

The pollen grains of the cultivars analyzed in this study are mono-porous; they have radial symmetry, an oval to elliptical shape and are rarely circular at the apex. Some of these characteristics can be seen in Radaeski et al. (2017) who described the pollen of *A. strigosa* as monads, with grains ranging from medium to large, radial symmetry, hetero-polarity, circular or spherical in shape, mono-porosity, a circular pore with annulus measuring approximately 5 µm located at the distal pole, annulus of 12 µm in diameter and 3.5 µm in thickness, tectal exine, columellae, with microechinate ornamentation. It has a sexine of 0.52 µm and a nexine of the same size.

It should be stressed that among the studies on floral biology of a species, that of the development of the pollen grain – its viability and fertility – is the basis for understanding reproductive biology as well. This contributes to the improvement and conservation of genetic resources (BRAMMER et al., 2019; SCARIOT, 2013), which is the case of results obtained with *A. strigosa* that will offer information for the development of new cultivars of the species.

In this study, the quantity of starch was a variable observed. However, the large quantity found in the apical region of the panicle prevented the observation of the nuclei. A repetition with DAPI fluorochrome was needed in order to exclusively mark the DNA (nuclei), since the carmine acetic dye stains not only the nuclei, but all the components of the cell cytoplasm (BRAMMER et al., 2015). With the DAPI we observed that binuclear and trinuclear pollen is found in the apex in larger amounts. In anthers with a dark coloring (as seen in Figure 1.E), and already deteriorating, there were many stained pollen grains, though without nuclei, while others had a different coloring (Figure 9) but were

uninuclear. This indicates that, after a while (though it is not certain how long), the mature grain supposedly begins to degenerate at the panicle apex. In the basal region of the panicle there was a larger number of cells with little starch – the pollen grains were in an earlier stage of development in this region. This suggests that most likely they would not present any problems during maturation, nor of viability, when fertilized. In the middle region of the panicle, the quantity of pollen grains with little starch decreased and a larger number of binuclear grains appeared. According to Coleman et al. (1981), when the DAPI is connected to the nuclear DNA, its fluorescence increases 20 times. This enables a more detailed observation of the apical region, though further study must be carried out regarding the absence or "deterioration" of the nuclei observed in this region.

With the results obtained here, we can affirm that there is a variability in *A. strigosa* concerning floral morphology, both in macrostructures and in pollen grains, which includes aspects of pollen viability. The interspecific differences observed in the comparison of the cultivars IAPAR 61 – Ibiporã e UPFA 21 – Moreninha indicate the need to extend this type of study to other genotypes, whether they be cultivars, lineages or plants selected from the native population. On the other hand, the high pollen viability seen in this study excludes this attribute as a possible cause for the failure in artificial hybridization of the black oat. For crossbreeding purposes, geneticists can obtain a larger amount of viable pollen in the middle region of the panicle, since maturation is basipetal.

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