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Genetic stability in interspecific hybridizations of wheat populations determined by meiotic index and pollen viability

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Resumo

Estabilidade genética em hibridizações interespecíficas de populações de trigo estimada por índice meiótico e viabilidade polínica. O objetivo do estudo foi verificar a estabilidade genética, determinando-se o Índice Meiótico (IM) e a viabilidade polínica em populações segregantes F_1RC_2, oriundas do cruzamento entre trigos sintéticos e cultivares comerciais de trigo, que visam introgressão/resistência genética. Para o IM, três espigas foram coletadas por genótipo, antes da antese, e fixadas em Carnoy. As lâminas citológicas foram preparadas pelo método de maceração com corante carmim acético 1%, observadas em microscópio ótico e analisadas tétrades normais e com micronúcleos. Para a viabilidade polínica, as espigas foram coletadas no estágio de grãos de pólen maduro. O procedimento metodológico foi semelhante ao das tétrades, avaliando-se: grãos de pólen viáveis, com pouco amido, com presença de dois poros, tamanhos diferentes e inviáveis. O tamanho dos grãos de pólen foi medido pelo programa Axion Vision. Todas as populações segregantes apresentaram IM acima de 90% e viabilidade polínica elevada (acima de 85%), refletindo comportamento meiótico estável. Quanto à variação no tamanho, o cruzamento CIGM90.909/BRS 179 apresentou grãos de pólen com 58,43 µm e CIGM93.298/BRS Guamirim, 47,15 µm. Com base nos resultados, as populações segregantes de trigo apresentam-se meioticamente estáveis e adequadas para seguirem em programas de melhoramento de trigo e incorporação de novos genes importantes.

Palavras-chave: Comportamento meiótico; Retrocruzamentos; Trigo sintético; *Triticum estivum*

Abstract

The objective of this study was to evaluate genetic stability by determining the meiotic index (MI) and pollen viability in F_1RC_2 segregating populations, obtained by the crosses between wheat commercial cultivars and synthetic wheat, aimed at introgression/genetic resistance. For MI, three ears were collected per genotype, before anthesis, and they were fixed in Carnoy's solution. The cytological slides were prepared by crushing the anthers and staining with 1% acetocarmine, and the normal tetrads and the ones with micronuclei were observed under a light microscope. For pollen viability, the ears were collected at the stage of mature pollen grain. The methodological procedure was similar to that of the tetrads, evaluating the pollen grains as viable, with little starch, with two pores, different sizes and non-viable. The pollen grain size was measured by the Axion Vision software. All the segregating populations with MI above 90% and high pollen viability (above 85%) reflected a stable meiotic behavior. About the variation in size, the CIGM90.909/BRS 179 cross showed pollen grains with 58.43 µm and CIGM93.298/BRS Guamirim, 47.15 µm. According to the results, wheat segregating populations were considered meiotically stable and suitable for use in wheat breeding programs and for incorporation of new important genes.

Key words: Backcrosses; Meiotic behavior; Synthetic wheat; *Triticum estivum*

Introduction

The hexaploid wheat (*Triticum aestivum* L.) is one of the most important cereals in the world, and there have been several cytogenetic, molecular and evolutionary studies on the genetic and genomic structure of the species of the group (*Aegilops*, *Amblyopyrum* and *Triticum*) and about the relationships between various wild relatives and between them and domesticated species as well (MIRZAGHADERI; MASON, 2019).

Among the species that contributed to the evolution of wheat, the species *Aegilops tauschii* stands out, being considered a valuable genetic resource for the improvement of cultivated wheat (ARORA et al., 2018), mainly because its genome has important commercial features such as tolerance/resistance to biotic and abiotic stresses. The development of a synthetic hexaploid wheat is a way to obtain potential resistance genes from *Ae. tauschii* for wheat breeding programs (OGBONNAYA et al., 2013).

Such synthetic wheats are obtained from artificial crosses between the tetraploid species *Triticum turgidum* or *T. durum* (genome AABB, 2n = 28) and the wild relative *Ae. tauschii* (genome DD, 2n = 14), followed by in vitro immature embryo culture and colchicine application, aimed at chromosome duplication and fertility in regenerated seedlings to restore the hexaploid condition (OGBONNAYA et al., 2013).

Wild species are considered gene repositories, and through interspecific hybridization, it has been possible to improve many agronomically important crop varieties. Since the 1940s, more than 1,500 synthetic lines have been developed, and a large number have been identified as resistant to major wheat diseases (leaf rust, septoria, crown rot, leaf spots, nematodes, powdery mildew and fusariosis, among others) and insects and tolerant to abiotic stresses (drought, heat, salinity and waterlogging), as well as having new grain yield and quality characteristics (JAFARZADEH et al., 2016). It is noteworthy that some of the germplasms derived from synthetic wheat have been used successfully to develop commercial varieties of wheat, such as the high-producing variety Chuanmai 42' (YANG et al., 2009) and insect resistant varieties "TAM 110" (LAZAR et al., 2004) and "TAM 112" (RUDD et al., 2014).

However, it is common to observe some chromosomal irregularities (meiotic instability) due to the hybridization practice or because of self-fertilization (FELDMAN; LEVY, 2012). Plant breeding programs stand on obtaining superior cultivars, based on the reproductive potential of the gametes and on the manipulation of the genetic variability in the species germplasm. The meiotic index (MI) sheds light on the percentage of normal tetrads (without the presence of micronuclei) and infers about the chromosomal behavior during meiosis I and II, and it has been considered an important criterion in studies of genetic/chromosomal stability in different species (TONIAZZO et al., 2018).

To guarantee success in the use of selected superior individuals and, especially, for the production of new cultivars through the recombination of characters by controlled hybridization, it is important that the pollen grain be viable (POZZOBON et al., 2011), since that directly influences fertilization success. In this way, testing the viability of the pollen grain is indispensable.

Irregularities are related to pollen viability, which influences the rate of fruit survival at crosses and in self-fertilizations and, consequently, the production of hybrid or self-fertilized seeds. Information about pollen viability, in other words, the ability of the pollen grain to germinate on flower stigma and to fertilize the ovule, is essential for germplasm conservation and characterization as well as for breeding. The study on the reproductive biology of plant species had been allowed the confirmation and understanding of the reported results of certain crosses (DA SILVA et al., 2017).

Pollen grain examination by cytogenetic analysis is a rapid method to determine abnormalities of chromosomal behavior during meiosis. In addition, the quartet studies (meiosis final stage) serve as an additional criterion for breeding programs, where plants that are cytologically abnormal can be discarded or reserved for further studies (ZANOTTO et al., 2009).

The objective of this study was to determine the MI and to analyze pollen grain viability and size of wheat segregating populations in F_1RC_2 originated by the cross between synthetic and commercial wheat cultivars.

Material and Methods

Six crosses were assessed in the F_1RC_2 generation, which were originated from the combination of synthetic and commercial wheat cultivars obtained in 2016 and 2017: CIGM90.909/BRS Guamirim; CIGM90909/BRS 179; CIGM 92.1666/BRS Guamirim; CIGM 92.1666/BRS 179; CIGM93.298/BRS Guamirim and CIGM93.298/BRS 179. The material selection was based on the phytopathological and cytogenetic characterization of male (BRS Guamirim and BRS 179) and female (CIGM 90.909, CIGM92.1666 and CIGM93.298) parentals in 2014 and 2015, by Frizon et al. (2017), emphasizing that these genotypes showed an MI above 90% in the cytogenetic characterization. The maternal parents and sources of exotic resistance, coming from CYMMIT (International Center for Improvement of Maize and Wheat - Mexico), are the result of the cross *T. durum* x *Ae. squarrosa* and were previously characterized as moderately resistant to *gibberella*. Male parents were also characterized as moderately resistant to gibberella (Table 1).

The trial was conducted in the experimental field at Embrapa Trigo (28°15'46"S and 52°24'24"W and altitude of 684 m), in Passo Fundo/RS, in 2018, and the cytogenetic analysis was performed at the Biotechnology Laboratory, in the cytogenetic area, also located at Embrapa Trigo.

TABLE 1: Genealogy of the genotypes used in the crosses aimed at resistance to gibberella. Embrapa Trigo, Passo Fundo, RS, 2019.

Accession	Genealogy	Resistance
CIGM90.909	GAN/ *Ae. Squarrosa*	MR
CIGM92.1666	RASCON// *Ae. Squarrosa*	MR
CIGM93.298	RASCON// *Ae. Squarrosa*	MR
BRS Guamirim	Embrapa 27/Buck Nandu// PF93159	MR
BRS 179	BR 35/PF 8596/3/PF 772003*2/PF 813//PF 83899	MR

The experimental design was in randomized blocks with six treatments, in addition to the male parentals (BRS Guamirim and BRS 179), and with four replications. Each cross was sown in a plot of 5-m rows, using 50 suitable seeds spaced 10 cm apart. The experiment was carried out following the technical indications for wheat crops.

To determine the MI, tetrads were examined to identify the presence of micronuclei (MN). Initially, three wheat ears were randomly collected from each treatment at phenological stage 10. The ears were fixed in Carnoy 3:1 (absolute ethyl alcohol; glacial acetic acid) for 24 h at room temperature and stored in 70% alcohol at -20°C. Slides were prepared using three anthers of the same flower from the middle region of the ear. The ears were crushed and stained with 1% acetocarmine. Normal tetrads (without the presence of MN) and the ones with the presence of MN were analyzed. Each treatment was composed of three replicates and each replicate represented one ear. Each slide was prepared with one ear, and the first 200 tetrads were then evaluated per slide. The MI was calculated according to Love (1949), in which MI = number of normal tetrads/total number of analyzed tetrads x 100. The material is considered meiotically stable when the MI is greater than 90%.

For pollen viability analysis, the inflorescences were collected in the mature pollen stage (prior to anthesis) and fixed in Carnoy's solution by the same method described for tetrad assessment, using here 200 pollen grains. The analyzed variables were: I) viable pollen grains; II) pollen grains with little starch; III) pollen grains with more than one pore; IV) pollen grains of different sizes and V) non-viable or empty pollen grains.

The pollen grain measurement was performed by the Axion Vision Release 4.8.2 (Zeiss) software, and the images were captured with a Zeiss microscope – Axioscop FL40 model. Ten pollen grains were analyzed on each slide, and three slides were evaluated per block.

For tetrads assessment and determination of pollen viability, the slides were examined under a light microscope with magnification of 200 to 400x. The best images were captured by the Honestech TVR 2.5 software using an Olympus BX5 light microscope. Analysis of variance and the Tukey test at 5% level of significance were used for all variables.

Results

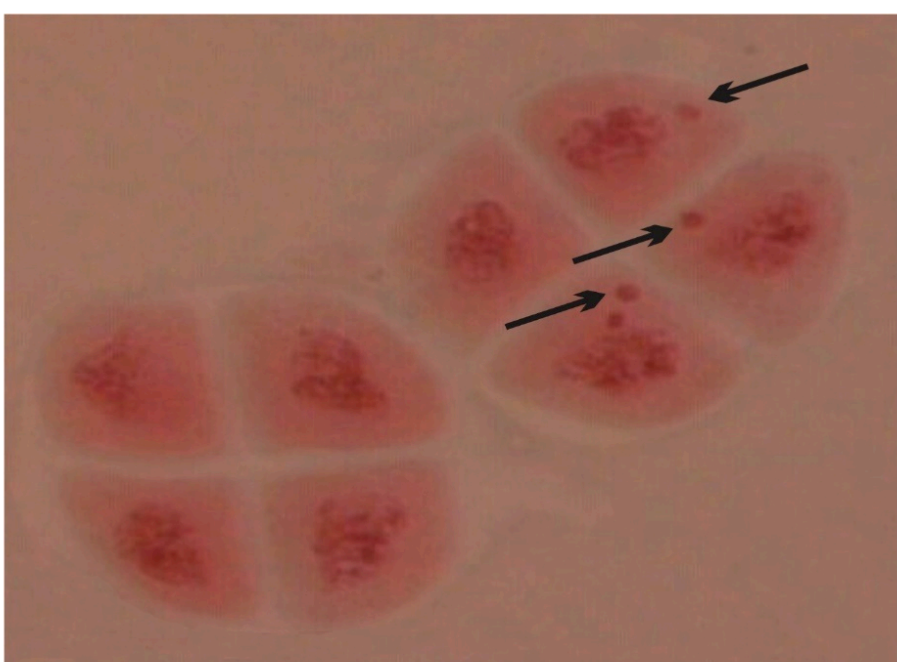
Meiotic Index

The presence of MN in the tetrad stage, therefore, will result in the formation of pollen grains with different genetic material from the mother cell (Figure 1).

For MI determination, all crosses showed values higher than 90% (Table 2). This is considered ideal, since in cytogenetic studies, MI is the parameter that allows the inference of genetic stability.

In the present study, MN percentage was low (less than 10%). The cultivar BRS 179, used as a male parental, displayed the highest MI (99.37%), and BRS Guamirim also had a high MI percentage (98.66%). Frizon et al. (2017) also found high MI values for wheat commercial cultivars. For example, the cultivar BRS 179, in both 2014 and 2015, had the highest MI (98.98 and 99.54%, respectively) and lowest MN percentage

FIGURE 1: Wheat cells in normal tetrad and micronucleus tetrad stage (arrow) in F_1RC_2 population resulting from the combination of CIGM93.298/BRS 179. Magnification: 400x. Embrapa Trigo, Passo Fundo, RS, 2019.



(1.02% and 0.46%), and the mean MI of the same cultivars was 95.91 and 95.26% in the same years. Urio (2013) also found a high MI in wheat cultivars, with exception of BRS 220 and BR 25 cultivars, which showed MI values below the recommended rate (90%).

TABLE 2: Meiotic index (MI) and micronuclei (MN) percentage of male parentals and of wheat segregating populations in F_1RC_2, obtained in the cytological analysis of wheat tetrads. Embrapa Trigo, Passo Fundo, RS, 2019.

Cross	MI (%)	MCN (%)
BRS 179	99.37 A	0.63 C
BRS Guamirim	98.66 Ab	1.34 Bc
CIGM90.909/BRS Guamirim	98.04 Ab	1.96 Bc
CIGM90.909/BRS 179	97.85 Ab	2.15 Bc
CIGM93.298/BRS 179	97.79 Ab	2.21 Bc
CIGM92.1666/BRS Guamirim	97.25 Ab	2.75 Bc
CIGM92.1666/BRS 179	96.70 B	3.3 B
CIGM93.298/BRS Guamirim	93.62 C	6.38 A

Means followed by the same letters do not differ statistically by the Tukey test at 5% level of significance.

Therefore, according to the results of the tetrad analysis (Table 1), it can be inferred that there was regular meiosis in wheat segregating populations and male parentals.

Pollen viability

For pollen viability (Table 3), all crosses and male parentals did not differ statistically, considering the following variables: viable pollen grains (bi/trinucleate and with presence of starch); little starch; more than one pore; difference in size and non-viable (empty) (Figure 2).

However, the cross CIGM90.909/BRS 179 had the lowest percentage of normal pollen grains (92%). The analysis of variance indicated a significant difference for little starch and different pollen grain sizes.

For the pollen grain with little starch variable, the crossings plus the male parentals resulted in three statistically distinct groups, but they showed low values that did not exceed 6%, which is considered ideal.

The variable of pollen grain with more than one pore was the least found, both in crossbreeding combinations and in the male parental. The only combinations that showed an unremarkable number were the ones with the CIGM93.298 synthetic wheat. This may mean a characteristic of the genotype itself, but it did not affect the parameters analyzed in this work (MI and pollen viability).

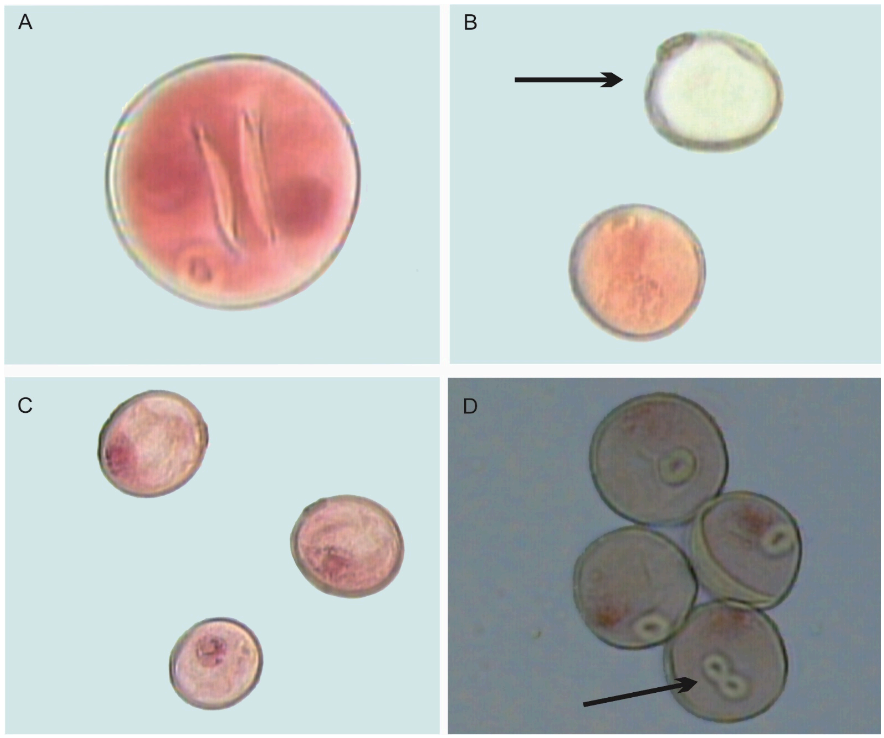
Pollen grains with different sizes formed three statistically different groups. This is expected since crosses were performed using more than one genotype.

TABLE 3: Pollen viability of wheat segregating populations in F_1RC_2 and male parentals, obtained in the cytological analysis of pollen grains. Embrapa Trigo, Passo Fundo, RS, 2019.

Cross	Feasible	%	Little starch	%	More than one pore	%	Different sizes	%	Unfeasible	%
CIGM90.909/BRS Guamirim	189.425 a	94.72	6.725 ab	3.37	0.000 a	0.00	0.917 b	0.46	2.917 a	1.46
CIGM90.909/BRS 179	184.400 a	92.20	3.325 ab	1.66	0.000 a	0.00	2.417 ab	1.21	1.500 a	0.75
CIGM92.1666/BRS Guamirim	194.825 a	97.41	3.500 ab	1.75	0.000 a	0.00	1.333 b	0.67	0.333 a	0.17
CIGM92.1666/BRS 179	190.525 a	95.27	2.075 b	1.04	0.000 a	0.00	5.750 a	2.88	1.667 a	0.85
CIGM93.298/BRS Guamirim	186.275 a	93.13	11.500 a	5.75	0.250 a	0.12	1.250 b	0.63	0.750 a	0.38
CIGM93.298/BRS 179	192.500 a	96.25	6.600 ab	3.30	0.417 a	0.21	0.500 b	0.25	0.000 a	0.00
BRS Guamirim	191.750 a	95.87	8.250 ab	4.13	0.000 a	0.00	0.00 b	0.00	0.000 a	0.00
BRS	192.600 a	96.30	4.350 ab	2.18	0.000 a	0.00	2.000 ab	1.00	1.083 a	0.55

Means followed by the same lowercase letter do not differ statistically by the Tukey test at 5% level of significance.

FIGURE 2: Pollen viability analyzed in the segregating populations (F_1RC_2): (A) normal pollen grain; (B) empty pollen grain (arrow); (C) pollen grain of different size; (D) pollen grain with two pores (arrow). Magnification: 400 x. Embrapa Trigo, Passo Fundo, RS, 2019.



Pollen grains with different sizes are commonly found in species belonging to the Triticeae tribe (ROSA et al., 2006).

Another variable that did not statistically differ was the empty or non-viable pollen grains. This variable showed low values, which is also desired. The empty pollen grain was poorly formed, elliptical and poorly stained by carmine dye (SOARES et al., 2011).

Pollen grain size

Five distinct groups were formed when evaluating the pollen grain sizes. Group I was composed of the CIGM90.909/BRS 179 cross, containing the greatest pollen sizes. Group II was composed of the CIGM90.909/BRS Guamirim cross. Group III had the male parental BRS 179 and two crosses (CIGM92.1666/BRS 179 and CIGM93.298/BRS Guamirim). Group IV also had a male parental, BRS Guamirim, and the CIGM92.1666/BRS Guamirim cross. Group V was composed of the CIGM93.298/BRS179 cross, in which the smaller pollen grain sizes were found (Table 4).

TABLE 4: Pollen grain sizes of wheat segregating populations in F_1RC_2 and male parentals, obtained in the cytological analysis. Embrapa Trigo, Passo Fundo, RS, 2019.

Cross	Average size (µm)
CIGM90.909/BRS179	58.430 A
CIGM90.909/BRS Guamirim	55.713 Ab
CIGM92.1666/BRS179	53.985 Abc
BRS 179	52.988 Abc
CIGM93.298/BRS Guamirim	51.778 Abc
CIGM92.1666/BRS Guamirim	50.503 Bc
BRS Guamirim	49.205 Bc
CIGM93.298/BRS 179	47.155 C

Means followed by the same letters do not differ statistically by the Tukey test at 5% level of significance.

In this study, it was observed that wheat segregating populations and male parentals showed pollen grain sizes within the pattern observed by other authors and also that the pollen grain size did not influence pollen viability.

Discussion

Meiotic index

When meiosis occurs regularly, it must result in four daughter cells with half of the chromosomes number of the original cell at the end of the cell division process. However, these chromosomes must be intact and containing all the same genes that were present in the original cell (URIO, 2013).

The parameter MI, previously analyzed, reflects meiosis occurrence with regular chromosomal behavior, which is of fundamental importance during hybridizations and genetic exchanges in crosses between different species (SOUZA et al., 2008). Plants that have an MI greater than 90% can be considered cytologically stable, because the higher the index, the more regular is the meiotic process (LOVE, 1949).

There are many studies in this area, and some of them are briefly described below. Arabbeigi et al. (2010) evaluated wheat germplasm and determined the MI in different combinations of synthetic hexaploid wheat (99%: Altar84/*Ae. squarrosa*; Sca/*Ae. squarrosa* and Doy1/*Ae. squarrosa*). In the case of *T. dicoccum*, *T. compactum* and *T. durum* the MI was 99.8%, while 99.5% for *T. dicoccoide*. An MI of 100% was observed for the diploid species *Ae. umbellulata*, *Ae. cylindrica*, *Ae. crassa* and *Ae. trivially*. Ghorbani et al. (2015) analyzed the meiotic behavior of eight *T. monococcum* subsp. *aegilopoids* genotypes and eight genotypes of *Ae. cylindrica*, which naturally grow in four different regions in western Iran.

The results indicated an MI of 82.6% for *Ae. cylindrical*, and it was characterized as having relatively unstable meiotic behavior. However, *T. monococcum* subsp. *aegylopoids* was considered as having a stable meiotic behavior, with MI of 97.1%. Similarly, Frizon et al. (2017) studied synthetic wheats and found MI values above 90% for CIGM90.909 (GAN/*Ae. squarrosa*), CIGM92.1666 (RASCON/*Ae. squarrosa* and CIGM93.298 (RASCON/Ae. *squarrosa*) in two years of study (2014 and 2015), wherein tetrads were analyzed by crushing anthers and staining them with 2% acetocarmine. Toniazzo et al. (2018), evaluating 67 accessions of synthetic wheat from CIMMYT stored at Embrapa Trigo Germplasm Active Bank, found that only 15 of them were considered stable, with MI above 90%, and that 52 of the accessions showed MI between 46 and 89%, being considered cytogenetically non-viable and unstable.

Another parameter that can compromise genetic stability is the presence of MN in tetrads. This is undesirable since high meiotic instabilities, associated with chromosomal abnormalities, can result in the formation of atypical, male-sterile or non-pollen-forming plants, which may prejudice the achievement of the minimum standards required for seed production as well as affecting pollination (POZZOBON et al., 2011).

Pollen viability

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). In general, a viable pollen grain was more developed, more circular and better stained by acetocarmine, whereas the non-viable grain was poorly formed, elliptical and less stained by carmine dye (SOARES et al., 2011). Brambatti et al. (2016) demonstrated that in triticale genotypes, pollen viability was higher than 90% in more than 66% of the tested materials.

A high percentage of viable pollen grains is normally expected as a result of a high percentage of normal tetrads, which would directly reflect a regular meiotic process (CORRÊA et al., 2005). Moreover, viable cells indicate high male fertility, since the effectiveness of the cross depends directly on pollen viability (TECHIO et al., 2006). Several species produce a high percentage of viable pollen grains, although many of these are not used in fertilization because they are lost in transport through the winds or serve as insect feed (SANTOS et al., 2015).

The presence of starch as a reserve substance is considered important in maintaining pollen grain viability, because the starch is totally or partially converted to glucose, fructose, sucrose and pectin which increase pollen grain resistance in hostile environments, as well as aiding in pollen tube germination at the time of fertilization (PACINI et al., 2006).

Information on pollen viability is essential for germplasm conservation and characterization as well as genetic breeding, since pollen viability rates are associated with meiotic behavior (OLIVEIRA; PIERRE, 2018). The study of pollen viability is commonly used in plant genetic breeding of several species, due to the ease, speed, low cost and reliability of the technique (CORRÊA et al., 2005).

Since the experiments and the development of the plants occurred in the field and because biotic and abiotic factors and the genotype/environment interaction (location/year) also influence pollen grain formation, pollen viability should be determined routinely in a plant breeding program to increase knowledge about the material under study, to advance selection and to prioritize the best crosses, excluding unstable materials or leaving them for later studies (BRAMBATTI et al., 2016).

Pollen grain size

The pollen grain of the family Poaceae are described as spherical, with operculum surrounded by exine thickening near the pore, forming a ring and with dimensions ranging from 20 to 70 µm (POÇAS, 2004). Cardoso (2007), studying different species of wheat, found that an *Ae. tauschii* accession showed pollen grains with the smallest diameter (39.14 µm) when compared to four Brazilian cultivars and four accessions of synthetic wheats that had diameters varying from 55.82 to 59.87 µm. In addition, diameters intermediate to those found by Cardoso 2007 in synthetic wheat were found in 4 commercial varieties of *T. durum* with values from 46.57 to 47.64 µm. Similarly, Urio (2013) analyzed 17 wheat cultivars and observed that the pollen grain measurements varied between 48.3 and 61.8 µm. These values were similar to those obtained from two cultivars used in this work: BRS 179 (52.98 µm) and BRS Guamirim (49.29 µm).

In view of the evaluated variables, it was evidenced that the understanding of meiosis is essential and has implications in the reproduction, fertility and, consequently, economic viability of commercial seed production of a new cultivar (POZZOBON et al., 2015).

Considering the analysis performed on MI, presence/absence of MN and pollen viability of wheat segregating populations in F_1RC_2 and male parentals, all parameters were found to be normal in meiosis, allowing them to be used in crossing blocks of breeding programs, as a consequence of their genetic stability.

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