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Phenotypic, cytogenetic and spike fertility characterization of a population of male-sterile triticale

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ABSTRACT: Triticale (*X Triticosecale* Wittmack) is a good cereal for production of flour and feed. A segregating population of triticale was developed from a male-sterile (MS) plant. To determine whether this new source of male sterility in triticale is appropriate for use in breeding programs the expression of the male sterility phenotype was characterized through spike fertility, meiotic behavior, and pollen. Controlled crosses between male-sterile plants and control varieties male-fertile (MF) of triticale were also conducted, and cytological analyses were performed in the F_2 and backcross plants. Plants with male-sterile phenotypes displayed reduced spike fertility when compared to plants with male-fertile phenotypes. Compared to male-fertile plants, male-sterile plants exhibited a lower percentage of normal meiotic cells, a reduced meiotic index and reduced pollen viability. The F_2 plants had improved pollen fertility when compared to the male-sterile population; however there were no corresponding improvements in the percentage of normal meiotic cells or in the meiotic index. A single generation of backcrosses resulted in an improved meiotic index and increased pollen viability. However, no changes in the percentage of normal meiotic cells were observed. Meiotic instability, which was shown to be inheritable, was the likely cause of male sterility. Therefore, the use of this population in triticale breeding was considered to be inappropriate because it could promote or contribute to the maintenance of meiotic instability, which is commonly observed in this species.
Keywords: male sterility, meiotic behavior, pollen fertility, productivity

Introduction

Triticale (*X Triticosecale* Wittmack), the first man-made cereal, is derived from the hybridization of two species, wheat (*Triticum* spp.) and rye (*Secale cereale*) (Lelley, 1992; Oettler, 2005), which are classified into different genera and feature genetic barriers of incompatibility and crossbreeding sterility (Oettler, 2005). Triticale is considered a self-pollinating plant, although it has varying levels of outcrossing and its allogamy rates appear to be directly associated with environmental, such as temperature and water deficiency, and genetic factors (Romano and Antunes, 2002).

Low fertility and the presence of hollow grains are considered serious defects in triticale. Potential causes of these defects include meiotic irregularities, differences in the duration of meiosis in parent plants (Scoles and Kaltsikes, 1974), and the presence of heterochromatin (Thomas and Kaltsikes, 1971). Male sterility in triticale and other crops can be defined as a condition in which the pollen is not viable and cannot germinate or fertilize normally to produce seeds (Kerstin and Kempken, 2006). This may be due to either genetic or environmental factors, such as water deficits or thermal stress. Male sterility usually results from the interaction between nuclear and cytoplasmic genes, where mutations in mitochondrial DNA is the main cause of the sterility (Carlsson et al., 2008). Only nuclear genetic control of male sterility has also been reported, as in wheat (Klindworth et al., 2002). Meiotic abnormalities are also associated with male sterility as observed in soybean mutants *ms1* and *ms4* (Bione et al., 2003). Male sterility is used in agriculture for hybrid seed production (Duvick, 1959) and development of segregating populations in breeding programs (Fujimski, 1979).

In the field, a source of male sterility was found because it presented wide-open flowers during anthesis (Singh, 2001). This plant was selected; however, it has been difficult to detect the mode of inheritance of male sterility from generation to generation. Therefore, the present study aims to: i) assess and characterize the triticale population with respect to the expression of the male fertility or male sterility phenotype; ii) determine the degree of sterility in terms of seed production; iii) investigate the meiotic behavior and pollen grain viability of the populations with male sterility and male fertility phenotypes; iv) assess the meiotic behavior and pollen grain viability of the F_2 and backcross populations.

Materials and Methods

The present study was conducted between 2005 and 2007 under field conditions. In 2001, a source of male sterility was found and selected, giving rise to a breeding population of triticale in which male sterility segregated, allowing one to select male-sterile (MS) and male-fertile (MF) plants. This source of male sterility was a single plant belonging to the Brazilian triticale cultivar IAPAR 54–OCEPAR 4. It was identified as male-sterile, evaluated by the Brazilian Cultivar Triticale Trial and it was conducted in Eldorado do Sul, state of Rio Grande do Sul, Brazil (30°05′ S and 51°39′ W, 46 m

altitude). For unknown reasons, this genotype exhibited an extremely low germination rate, and only two plants have developed from one of the experimental units. One of these two plant exhibited a male-sterile phenotype identified because its wide-open flowers during anthesis. This plant was marked and harvest when ripe. Its seeds were sown in the field the following year and the resulting plants displayed segregation of the male sterility phenotype. This segregation was observed throughout the whole period that the population was assessed (2002 to 2005), and each year several male-fertile and male-sterile plants were selected, thus giving rise to a breeding population of triticale.

In 2005, a subset of male-sterile plants was protected from cross-fertilization using wax paper packages, while other male-sterile plants were identified and marked with tape at the time of anthesis. All plants were individually harvested when mature. Other plants with good agronomic characteristics, but with undetermined male sterility phenotypes, were also selected at the end of the cycle. Spikelets were counted in all harvested spikes, which were threshed individually and had their grains counted. The fertility of each individual spike was estimated by dividing the number of seeds by the number of spikelets multiplied by two. From this evaluation, a sample of 117 plants representing the different classes of spike fertility was selected and planted in the field in 2006 due to the purposes of this study.

The 2006 experiment was implemented in 3-m-long, double-row plots containing less than 20 seeds, depending on their availability, with a distance of 0.3 m between plants and rows. Individual plant classification records were maintained as adopted in 2005: P (spike protected from cross-fertilization with an undefined male sterility phenotype); NP Me (unprotected spike marked as having the male-sterile phenotype); NP Und (unprotected spike not marked as having the male-sterile phenotype and thus undefined for this phenotype). Seeds from each individual plant were planted in the field. In addition, the Brazilian triticale cultivars IAPAR 54–OCEPAR 4, BR 4, Embrapa 53, and Fundacep 48 were planted as controls. The implementation of field plots was performed based on an order determined by lottery.

The characterization of male sterility in 2006 was performed using the phenotypic evaluation of flowers during anthesis (i.e., plants exhibiting wide-open flowers during anthesis were classified as having the male-sterile phenotype, and plants exhibiting flower buds were classified as having the male-fertile phenotype). Evaluations were conducted in the field by characterizing all of the flowers on each plant twice a week for three weeks, and all plants in each row were re-evaluated at each evaluation time point. The characterization of male sterility in the present study is called the "spike" phenotype.

To evaluate spike fertility in 2006, plants in pre-anthesis had the main stem of one spike protected against cross-fertilization using wax paper packages and had the number of spikelets and the number of seeds counted after physiological maturity to determine the degree of fertility. A fertility index was created by considering an average number of three flowers per spikelet. Spike fertility was determined using the following equation: Spike fertility = (Number of seeds / number of spikelets $\times$ 3) $\times$ 100. Differences in the average spike fertility of various groups of plants were compared using Student's t-tests.

In 2006, crosses were performed in the field between plants characterized as having either male sterility or male fertility and the control cultivars. Plants selected as female parents were emasculated to perform the crosses, and manual hybridization was performed approximately 24 h later using pollen grains obtained from the control cultivars. Spikes were identified and protected by wax paper packages following pollination. Consequently, 44 plants were selected as female parents for crosses with the control cultivars. A total of 1,006 seeds were obtained from the crosses (with an average of 23 seeds per cross).

During the summer and fall of 2007, seeds obtained from the crosses and from the control cultivars were planted in a greenhouse in 3-m-long, double-row plots with a distance of 0.3 m between plants and rows. Individual plant characterizations were performed during the flowering stage based on the phenotype presented. To obtain F_2 populations, backcrosses for the control cultivars were conducted in the greenhouse and spikes were protected against cross-fertilization using wax paper. Sixteen backcrosses were performed between the plants obtained from crosses (female parents) and control cultivars (pollen donors), which resulted in 224 seeds (an average of 14 seeds per cross). The other spikes gave rise to the F_2 populations.

Seeds from plants belonging to the male-sterile population that had been protected against cross-fertilization in 2006 were selected based on the percentage of seed production per spike in an effort to obtain plants with variable fertility (i.e., from low to high). In the winter of 2007, plants selected from the male-sterile population were sown in the field along with seeds from the control cultivar IAPAR 54-OCEPAR 4 and seeds from plants of the F_2 population. In 2007, at a second sowing date, the backcrosses and control cultivars obtained in the greenhouse were planted in the field for the phenotypic characterization of male sterility and the analysis of meiotic behavior in these populations. The phenotypic characterization of male sterility in 2007 was performed using the same methodology that was adopted in 2006.

For meiotic analyses, young inflorescences of plants from the F_2 population and the control IAPAR 54–OCEPAR 4 were collected at various stages of development and fixed, from early booting stage up to pre-anthesis, while still in the field, keeping the spikes in a solution containing alcohol and acetic acid (3:1), for fixation. The material was stored at room temperature,

and the fixative solution was exchanged 6 h after collection. The material was transferred to 70 % ethanol 24 h after collection and stored at approximately 4 °C. For cytogenetic analyses, anthers were cut and macerated on a slide and stained with 2 % propionic carmine.

Studies of meiotic behavior were performed by assessing all possible pollen mother cells (PMCs) on each slide, and two slides per plant were evaluated during the following phases of meiosis: diakinesis, metaphase I, anaphase I, telophase I, metaphase II, anaphase II, and telophase II. Cells in which chromosomes were displayed in bivalent associations were considered to be normal, and cells exhibiting univalent, trivalent, or tetravalent chromosomes, or multiple unidentified associations, were considered to be irregular. Moreover, the presence of chromosome bridges was also recorded. The meiotic index was calculated as an alternative to meiotic behavior by dividing the number of normal tetrads by the total number of tetrads identified and multiplying by 100. Tetrads were considered to be normal when they exhibited four equally sized microspores and no micronuclei. Tetrads were considered abnormal when they exhibited microspores of different sizes or had micronuclei. All cells on each slide were assessed, and two slides were evaluated per plant. Pollen grain viability was estimated by the ability to stain with 2 % propionic carmine. Three slides per plant were assessed, and 200 pollen grains were randomly counted per slide, for a total of 600 grains assessed. Well-stained grains were considered viable, whereas empty or colorless grains were considered non-viable.

Flowers from the F_2 plant populations, backcrosses, and control cultivars were collected and fixed, and their meiotic behavior, meiotic index, and pollen grain viability were estimated according to the methodology described above.

Results and Discussion

Phenotypic analyses of plants with male sterility allowed one to observe segregation of this phenotype between 2006 and 2007, with some plants exhibiting male fertility phenotypes and others exhibiting male sterility phenotypes. In 2006, 64 % of the evaluated plants displayed the male fertility phenotype and 36 % exhibited the male sterility phenotype (Table 1). In 2007, proportions were 71 % for male fertility and 29 % for male sterility (Table 1), demonstrating a slight increase in the average percentage of plants exhibiting male fertility in the second year of this study. Probably because there was selection for plants with higher levels of male fertility, since pollination of male sterile plants depends on the pollen coming from totally or partially fertile plants.

Plants were evaluated according to the classification adopted, which was based both on the spike that gave rise to the plant and whether the plant had been protected from cross-pollination in 2005. Notably, plants from all groups segregated to the male sterility phenotype (Table 1).

In 2006, plants derived from spikes that had been protected against cross-fertilization and NP Me plants, from 2005, displayed a similar proportion of male-sterile descendants (i.e., approximately 45 %). However, among descendants of NP Und plants, 19 % had the male sterility phenotype in 2006 (Table 1). This smaller proportion of male-sterile descendants reflects the selection of more fertile panicles and higher grain filling, performed in 2005. Plants exhibiting male-sterile phenotypes tended to produce not only a low number of seeds per spike, but also small and hollow grains. These results may be associated with the lack of viable pollen grains for self-fertilization, depending on cross-fertilization. Variation in the autopollination percentage in triticale were reported by Gregory (1976) determined an outcrossing rate of 21 % in triticale, while Romano and Antunes (2002) observed variations in the rate of allogamy between 3 % and 31 % depending on the cultivar of triticale of hibernal, facultative, and springtime habits. Yeung and Larter (1972) reported the influence of environmental factors on outcrossing rates in triticale, such as temperature and soil humidity.

Based on the analyses performed in 2007, a segregation of the male sterility phenotype was again observed (Table 1). Plants classified as male-fertile or male-sterile in 2006 both generated progenies with male-fertile and male-sterile phenotypes in 2007. Interestingly, the lowest proportion of male-sterile progeny in 2007 (14 %) was observed in plants that were classified as male-fertile in 2006 and were descendants of spikes that had been protected in 2005. This result reveals an increase in the male-fertile phenotype of plants that necessarily performed self-fertilization in 2005 because they had protected spikes and displayed the male-fertile phenotype in 2006. This result indicates that male sterility is likely a genetic trait that is

Table 1 – Percentage of plants with male-fertile and male-sterile phenotype in a male-sterile population led the field in the years 2006 and 2007, according to the classification of the plant selected in 2005.

Year	Male-Sterile Population																Mean 2007	
	Protected (2005)				NP Me (2005)				NP und. (2005)				General Mean					
2006	MF		MS		MF		MS		MF		MS		MF		MS			
	54.94		45.06		55.17		44.83		81.40		18.60		63.84		36.16			
2007	MF	MS	MF	MS	MF	MS	MF	MS	MF	MS	MF	MS	MF	MS	MF	MS	MF	MS
	86.45	13.58	60.72	39.28	72.08	27.92	68.94	31.06	76.96	23.02	61.04	38.96	78.49	21.51	63.57	36.43	71.03	28.97

NP Me: Unprotected with male-sterile phenotype; NP Und: Unprotected with undefined phenotype; MF: Male-fertile; MS: Male-sterile.

partially heritable because the male sterility phenotype was observed in all evaluations.

Characterization of male sterility in this study was performed by observing flower phenotypes (i.e., wide-open flowers during anthesis), which allowed us to classify individual plants within the population. Similarly, Singh (2001) performed phenotypic characterizations and identifications of male-sterile wheat plants (*Triticum aestivum*) that were subsequently confirmed by changes in the color and size of pollen grains observed through the microscopic analysis of their progeny. Changes in the floral morphology of plants with male sterility have been described in soybean, and changes in the color of anthers have been described in *Sesamum* sp., a typical African plant where male-sterile plants exhibit green anthers and male-fertile plants have yellow anthers (Rao et al., 1990).

Fertility assessments of plants in 2006 revealed an average of 40 % seed production (Table 2). Plants were evaluated based on their adopted classifications, which were based on the spike from which the plant was derived, whether the plant had been protected against cross-pollination in 2005 and phenotypic analysis. Notably, plants characterized as male-fertile in 2006 had an average of 54 % seed production, and plants characterized as male-sterile displayed an average of 24 % seed production (Table 2). These were lower values than those observed for plants with the male-fertile phenotype.

Differences in the percentage of seed production in plants classified as male-sterile (from different sources) were also noted in this evaluation. The 2006 progeny that originated from plants that had been protected in 2005 displayed the best production results (41 %) when compared to the progeny of NP Me and NP Und plants, which generated production rates of 21 % and 25 %, respectively (Table 2). This increase in seed production per flower in plants that were protected in 2005 may be associated with a possible selection for increased pollen fertility that year, which could thus increase the ability of protected spikes to self-fertilize.

Fertility comparisons performed using Student's t-test revealed an increase ($p < 0.05$) in spike fertility in plants with the male-fertile phenotype compared to plants with the male-sterile phenotype in 2006 (Table 2). Comparisons of spike fertility in the male-sterile population (an average of 40 %) and the control cultivar IAPAR 54-OCEPAR 4 (70 %), which was the cultivar that gave rise to this new source of male sterility, revealed differences in the percentage of seed production per flower. However, a greater difference was observed when comparing the average of plants with the male-sterile phenotype (24 %) to the average of control plants (70 %) than was observed in plants with the male-fertile phenotype, whose average (54 %) did not differ ($p < 0.05$) from the average of the control cultivar IAPAR 54-OCEPAR 4. Therefore, it is evident that the male-sterile phenotype (open flower) observed in plants in the field is associated with alterations in spike fertility (i.e., plants with the male-sterile phenotype exhibit reduced seed production per flower when compared to male-fertile plants). Nevertheless, a reduction in seed production per flower was noted in plants characterized as male-fertile (54 %) when compared to controls (70 %); however, there was no difference, an effect that may be due to the small sample size. The observed reduction in spike fertility in plants with the male-fertile phenotype indicates that the population that originates from a plant with male sterility preserves this characteristic, even though individual plants within this population do not always express the open flower phenotype.

Table 2 – Percentage of seeds/flowers of the male-sterile population in 2006, classified according to the male sterility phenotype and type of ear protection against cross-fertilization, and mean comparison between genotypes of the male-sterile population and with the control cultivar IAPAR 54–OCEPAR 4.

		Phenotype MF 2006				Phenotype MS 2006				General Mean
		Mother plant 2005				Mother plant 2005				
	IAPAR 54	Prot.	NP Me	NP ind.	Mean MF	Prot.	NP Me	NP ind.	Mean MS	(MF+MS)
Mean	69.71	56.39	53.07	54.34	53.77	40.88	21.43	24.52	24.38	40.44
S.D.	5.78	20.05	16.97	8.40	15.98	16.79	18.03	28.59	20.23	17.37
N. Obs.	4	12	60	18	90	8	54	11	73	163
	Mother plant 2005	Comparison of the Medium for Student's t-tests								
Phenotype MF 2006 – Prot.		–	Ns	Ns	Ns	Ns	**	**	**	*
Phenotype MF 2006 – NPMe			–	Ns	Ns	Ns	**	**	**	**
Phenotype MF 2006 – NP ind.				–	Ns	*	**	**	**	**
Phenotype MF 2006 – Mean MF					–	*	**	**	**	**
Phenotype MS 2006 – Prot.						–	*	Ns	Ns	Ns
Phenotype MS 2006 – NPMe							–	Ns	Ns	Ns
Phenotype MS 2006 – NP ind.								–	Ns	Ns
Phenotype MS 2006 – Mean MS									–	Ns
Witness	IAPAR 54	Ns	Ns	**	Ns	*	**	*	**	**

Prot: Protected; NP Me: Unprotected with male-sterile phenotype; NP Und: Unprotected with undefined phenotype; MF: Male-fertile; MS: Male-sterile; S.D: Standard deviation; N. Obs: Number of observations; **significant at 1 %; *significant at 5 %; Ns: not significant.

Reduced spike fertility of plants with the male-fertile phenotype when compared to the cultivar that gave rise to the population suggests that male fertility is generally low in the segregating male-sterile population. These results also point to a possible cytogenetic component affecting male fertility, based on the investigation conducted in 2007, which appointed to a possible association between irregular meiotic behavior and male sterility. The occurrence of male sterility in maize (Defani-Scoarize et al., 1995) and soybean (Bione et al., 2003) was associated with abnormalities in meiotic behavior. According to Lukaszewski et al. (1987) and Oettler (2005), high levels of meiotic instability may reduce fertility, especially when the instability affects pollen grain viability. Moreover, according to Stebbins (1951), cytological disorders represent one of the primary conditioning factors for sterility in plants.

Cytogenetic analyses of the plants from the male-sterile population were performed using the classifications that had been adopted for the mother plant in 2005. Moreover, plants were classified according to the male sterility or fertility phenotypes observed in the field in 2007. Cytogenetic evaluations performed on plants from the male-sterile population confirmed the number of chromosomes to be 21, and this population was characterized as hexaploid. Furthermore, many irregularities were observed in the meiotic process, such as the presence of univalent chromosomes, lagging chromosomes, chromosomal bridges, micronuclei in the tetrad stage and unviable pollen grains.

When analyzing the meiotic behavior of plants from the male-sterile population using the classifications that were adopted based on their origin and protection from cross-fertilization in 2005, reduced regularity values were observed in each of the three characteristics examined in all three groups (Table 3). The lowest percentage of normal cells (28 %) was observed in the group of male-sterile plants that were not protected against cross-fertilization in 2005. The lowest meiotic index value (36 %) was observed in the group of protected plants, and the lowest percentage of pollen grain viability was observed in plants from the NP Und group (20 %). The lowest rates of regularity for the three characteristics under assessment were observed in plants characterized as exhibiting the male sterility phenotype in 2007. The male sterility phenotype is associated not only with reduced spike fertility (Table 2), but also with reduced meiotic regularity and pollen grain viability. It is possible that the high meiotic instability compromised pollen grain viability and served as the limiting factor in the process of self-fertilization or even cross-fertilization. Meiotic abnormalities was also reported as responsible for reduced pollen and grain fertility in maize (Defani-Scoarize et al., 1995), soybean (Bione et al., 2003), green pepper (Luo et al., 2006) and *Arabidopsis thaliana* (Ku et al., 2003; Coimbra et al., 2004).

In general, male-fertile plants had higher regularity values than male-sterile plants of the same group. An exception was found for plants belonging to the protected male-fertile group, which displayed a lower percentage of normal meiotic cells (41 %) when compared to plants classified as male-sterile (45 %). Similarly, plants from the NP Und group with the male-fertile phenotype had a slightly lower meiotic index (58 %) when compared to plants with the male-sterile phenotype (59 %) (Table 3). Nevertheless, lower pollen grain viability was observed in plants with the male sterility phenotype in groups where comparisons between male-sterile and male-fertile phenotypes were possible. Therefore, the male-sterile phenotype appears to be associated with irregularities in general meiotic behavior because plants classified as male-sterile in 2007 had at least two of the three parameters greatly reduced when compared to

Table 3 – Meiotic analysis, meiotic index and pollen fertility of the progeny of the male-sterile population and control cultivar IAPAR 54–OCEPAR 4 conducted in the field in 2007. The genealogy was maintained according to the type of protection against cross-fertilization of the spike or the plant that originated in 2006 and according to the phenotype observed in 2007.

Phenotype		Phases of Meiosis				Normal Cells				Meiotic Index				Pollen Fertility			
2005	2007	Diakinesis/ Metaphase I[a]	Anaphase/ Telophase I[b]	Metaphase II[b]	Anaphase/ Telophase II[b]	No. Eval.	Min.	Max.	Mean	No. Eval.	Min.	Max.	Mean	No. Eval.	Min.	Max.	Mean
							——— % ———				——— % ———				——— % ———		
P	F	12(27)	79 (232)1*	66(47)	198(206)	4	14.54	55.42	40.89	7	18.40	71.99	47.01	4	11.13	82.19	56.08
P	S	36(52)	147(150)	17(28)	74(107)	3	29.06	47.29	44.84	2	30.10	42.41	36.25	4	0.0	74.57	48.62
NP Me	F	44(103)	383(637)8*	86(126)	298(461)	12	11.82	49.46	37.79	12	20.95	71.13	43.77	10	5.24	96.21	60.23
NP Me	S	22(46)	44(107)4*	6(34)	27(57)	2	26.60	31.78	28.11	2	31.70	42.75	37.22	6	0.66	81.58	22.30
NP Und	F	26(91)	207(312)	52(94)	105(117)	5	27.89	45.92	38.84	2	57.28	59.11	58.20	-	-	-	-
NP Und	S	6(17)	78(199)12*	16(25)	153(226)	3	23.71	43.43	34.56	3	44.44	90.0	59.21	3	9.09	30.50	19.75
IAPAR 54		15(25)	201(171)1*	27(20)	44(35)	2	53.17	53.29	53.24	2	45.45	50.68	48.06	2	90.69	91.90	91.29

P: Protected; NP Me: Unprotected with male-sterile phenotype; NP Und: Unprotected with undefined phenotype; F: Fertile; S: Sterile; No. Eval.: Number of evaluated plants; [a]Number of normal cells (outside the parentheses); number of cells with chromosomes I, III, IV or multiple unidentified associations (between the parentheses); [b]Number of normal cells (outside the parentheses); number of cells containing irregularities (between the parentheses); *Number of recorded bridges.

plants classified as male-fertile in that same year (Table 3). Conversely, the male-fertile phenotype of spikes in the studied plants was not synonymous with high pollen fertility, even though plants with male-fertile phenotypes in 2007 displayed higher pollen viability (56 % to 60 %) than plants with the male-sterile phenotype (20 % to 49 %). Plants with male-fertile phenotypes also had lower pollen fertility when compared to the IAPAR 54–OCEPAR 4 control plants (91 %) and exhibited a lower percentage of normal cells undergoing meiosis (Table 3). Once again, the presence of the male sterility trait in plants from this population has compromised their development in relation to meiotic behavior, with a direct relationship to spike fertility (Table 2).

Many meiotic irregularities were observed in the triticale population investigated, both in male-fertile and male-sterile plants, but mainly in the later ones. These irregularities included presence of univalents and lagging chromosomes in the meiosis. Several irregularities in the meiotic process and in pollen grain viability have been described in triticale (Shkutina and Khvostova, 1971; Falcão et al., 1981; Jung et al., 1985; Hohmann, 1993). There are several hypotheses regarding the cause of meiotic irregularities in this crop, including structural dissimilarity between wheat and rye chromosomes (Varghese and Lelley, 1983; Jung et al., 1985; Lelley, 1992), insufficient time for chiasmata formation in rye chromosomes during synapse (Oettler, 2005), premature disjunction of bivalents (Lelley, 1974), difficulties in cytoplasmic interactions or in generating univalent chromosomes (Sisodia and McGinnis, 1970) and problems during chromosome paring (Lelley, 1992).

Many irregularities were observed in the meiotic process in the present study, as was a high incidence of micronuclei during the tetrad stage, which compromised the meiotic index. The presence of micronuclei in *Vicia faba* L. is typically caused by irregularities during the second meiotic division, notably the irregular separation of univalents, which results in a reduction in the meiotic index (Sjödin, 1970). Although meiotic abnormalities are always present in triticale cultures, the high rate of abnormalities observed in the present study is likely directly related to and caused by male sterility.

The pollen viability values observed in several groups of plants were higher than their corresponding meiotic index values (Table 3). This result could potentially be related to a degeneration of irregular tetrads prior to the flowering stage with mature pollen grains, which would favor the analysis and increase the viability percentage. This apparent degeneration process was observed by Baptista-Giacomelli et al. (2000) in *Avena sativa* L.

During the summer and fall of 2007, phenotypic analyses of the plants established in greenhouses allowed for the characterization of fertility phenotypes. Although most plants showed a male fertility phenotype, but sterility phenotypes were observed in most genotypes after the occurrence of intense frosts at the location. The phenotypic changes observed in the present study are similar to those proposed by Bodanese-Zanettini et al. (1983), who suggested that climatic changes, especially in temperature, influence the degree of irregularities in wheat culture, with a preponderant role in the emergence of lagging chromosomes, and consequently, in crop sterility. The results from the phenotypic evaluations suggest that low temperatures and the incidence of frosts may have caused sterility and the expression of this trait in plants. Even though pollen viability has not been evaluated under controlled temperature conditions, the hypothesis that low temperature has contributed for higher pollen sterility was based upon the observations made in triticale (Sisodia and Mcginnis, 1970), chickpea (Clarke and Siddique, 2004) and rice (Oliver et al., 2005).

Similar phenotypic changes were noted in the field in 2007, where some plants exhibited the male sterility phenotype in one spike and the male fertility phenotype in the other (i.e., tiller(s) with the male-fertile phenotype and tiller(s) with the male-sterile phenotype in the same plant). A possible explanation was that the environment was sufficiently different during the development of these tillers to produce this variation. Adverse environmental factors causing male sterility have been described by Matsui et al. (1999 and 2000) in *Oryza sativa* L. and *Hordeum vulgare* L. and by Goetz et al. (2001) in *Nicotiana tabacum*.

The phenotypic evaluation conducted in the field in 2007 determined that 6 % of the plants from the F_2 population and 3 % of the plants derived from backcrosses exhibited the male sterility phenotype. These results show a high degree of restoration of the male fertility phenotype, and the control cultivar genome probably contributed to this restoration because crosses and backcrosses were performed between plants from the male-sterile population and male-fertile controls. Whether this restoration of fertility was due to one or several genes present in the control cultivars, or whether it was due to an overall contribution of their genomes, cannot be determined.

Cytological analyses were performed on flowers obtained from the F_2 population, backcrosses and control cultivars that were used as parents in the crosses (Table 4). The analysis of the F_2 population determined that the highest percentage of normal cells was 44 %, while the highest meiotic index was 41 % and the highest pollen grain viability was 82 %, all of which were observed in the F_2 population that had the BR 4 cultivar as the parent. The BR 4 cultivar displayed reduced meiotic stability and pollen fertility when compared to the Fundacep 48 cultivar, which was the parent to the other F_2 population under assessment. Cytological analyses of the backcrosses revealed 45 %, 61 % and 93 % as the maximum values for normal cells, meiotic index, and pollen grain viability, respectively. The highest values observed for the control cultivars under assessment were 54 % normal cells in meiosis (Fundacep 48), 67 % meiotic index (Fundacep 48), and 93 % pollen grain viability (Embrapa 53).

Table 4 – Meiotic analysis, meiotic index, and pollen viability in triticale F_2 plants, backcrosses and controls.

Generation	Phen. 2005	Phen. 2007	Parent Backcr	Diakinesis/ Metaphase I[a]	Anaphase/ Telophase I[b]	Metaphase II[b]	Anaphase/ Telophase II[b]	No. Eval.	Normal Cells	No. Eval.	M.I.	No. Eval.	Pollen Fert.
									%		%		%
F2	NP Me	F	BR 4	41(40)	183(251)3*	42(82)	214(260)	5	44.16	5	40.95	2	82.27
F2	NP Und	F	Fundacep 48	29(23)	187(252)2*	30(36)	173(287)	6	41.15	6	39.02	9	76.63
Backcr	NP Me	F	Fundacep 48	44(53)	306(378)	36(76)	400(453)	8	45.30	6	59.18	9	91.28
Backcr	NP Me	F	Embrapa 53	1(0)	19(29)	5(9)	73(104)	2	37.17	2	60.91	1	92.89
Backcr	NP Me	F	Iapar 54	13(16)	93(75)	9(5)	29(41)	3	39.07	4	56.82	4	84.17
Backcr	NP Und	F	Fundacep 48	19(26)	218(432)2*	31(68)	99(223)	5	33.67	7	49.67	-	-
						Control							
BR 4	-	-	-	3(5)	56(66)1*	3(2)	12(20)	1	44.05	1	54.04	1	83.81
Fund 48	-	-	-	4(9)	25(18)	20(16)	76(63)	1	54.11	1	67.28	1	96.03
Emb 53	-	-	-	0(0)	18(20)	14(14)	118(117)	1	49.83	1	55.72	1	96.36
Iapar 54	-	-	-	2(5)	61(53)	17(12)	29(26)	1	53.17	1	50.68	1	90.69

P: Protected; NP Me: Unprotected with male-sterile phenotype; NP Und: Unprotected with undefined phenotype; F: Fertile; S: Sterile; No. Eval.: Number of evaluated plants; Backcr: Backcrosses; M.I: Meiotic index; [a]Number of normal cells (outside the parentheses); number of cells with chromosomes I, III, IV or multiple unidentified associations (between the parentheses); [b]Number of normal cells (outside the parentheses); number of cells containing irregularities (between the parentheses); *Number of recorded bridges.

Backcrosses displayed higher meiotic indexes and pollen fertilities than plants from the F_2 populations (Table 4). Moreover, no improvements in the percentage of normal meiotic cells were detected in these backcrosses when compared to the F_2 populations. Crossing plants with the male-sterile phenotype to male-fertile controls allowed for a rapid and effective reduction in the expression of the male-sterile phenotype.

Comparisons between the meiotic analyses of F_2 individuals and backcrosses with control cultivars determined that the best indexes were obtained from control cultivars, followed by the results from the backcrosses (Table 4). These effects are likely associated with the fact that the F_2 and backcross populations still present some degree of male sterility, suggesting that this characteristic is heritable, despite having a cytogenetic cause. Meiosis is under genetic control (Phadnis et al., 2011) and any mutation, even minor ones, in the genetic pathways controlling it may affect the meiotic behavior. From our data it was not possible to determine the heritability of male sterility or identify any major genetic alteration causing it, since it was originated from a male-fertile triticale cultivar. Anyway, the male sterility in the population was inheritable, given that it passed on from one generation to another. Sisodia and Mcginnis (1970) suggested the existence of an inheritable variation for meiotic instability in triticale, where the progeny, with rare exceptions, show abnormality frequencies that are very similar to those exhibited by the mother plant, which could be the case in the studied population.

Cytogenetic analysis of the backcrosses revealed relatively low proportions of normal cells in meiosis and intermediate meiotic index values when compared to the high proportions of pollen viability. These results suggest that the final stages of gametogenesis might be more easily restored than the initial stages (i.e., despite the presence of irregularities in the meiotic process, the microspores that are able to form will give rise to viable pollen grains). Among other factors, the restoration of the ability to form viable pollen most likely depends on the formation of tetrads with a smaller proportion of irregularities (i.e., normal tetrads that are not degenerated).

The phenomenon of male sterility in plants has been explored in great detail, especially in breeding programs that aim to produce hybrid seeds, and it is a key feature because it prevents the process of self-fertilization, thereby forcing cross-pollination (Chase, 2006). However, in this study, sterility within the population appears to act as a negative factor, given that plants with the male-sterile phenotype displayed lower spike fertility (Table 2), a higher proportion of meiotic irregularities, and reduced pollen fertility (Table 3) when compared to male-fertile plants. The cytogenetic analyses allow us to infer that the reduced fertility of male-sterile plants is associated with a cytogenetic component, which is probably under genetic control. However, reduced fertility is also subject to strong environmental effects, as indicated by the observation of both male-fertile and male-sterile tillers in the same plant in 2007.

Due to the high meiotic instability and reduced pollen fertility associated with the reduced spike fertility in the male-sterile population and considering that these characteristics are inheritable and highly influenced by the environment, this new source of male sterility should be avoided in breeding programs because it may contribute to increased meiotic instability within derived populations, which would likely compromise grain yield.

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