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BIOLOGY

Genetic stability in synthetic wheat accessions: cytogenetic evaluation as a support in breeding programs

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ABSTRACT: *Synthetic wheat is developed by crossing tetraploid species (**Triticum turgidum**, AABB) with a diploid species (**Aegilops tauschii**, DD), followed by chromosome duplication through the use of colchicine to restore the resultant sterile hybrid to a fertile hexaploid plant. The main importance of producing synthetically improved wheat is to increase their genetic variability and to incorporate genes that code for resistance to biotic and abiotic stressors. This study aimed to evaluate the presence of micronuclei (MN) and the meiotic index (MI) in the tetrad phase in synthetic wheat accessions and cultivars (**Triticum aestivum**) stored at the Germplasm Bank of Embrapa Trigo (Brazil), in order to identify and select genetically stable accessions for plant improvement. Five plants were collected by genotype, prior to anthesis, and the tissues were fixed in Carnoy solution. Cytological slides were prepared by the smash method, and the cells were dyed with 1% acetocarmine and observed under an optical microscope. Presence of MN was observed in all genotypes analyzed, and variability of genetic stability was reported in the two years of analysis. In 2014, the highest MI of synthetic wheat accessions was 96.86% and the lowest was 46.32%. In 2015, the highest MI was 96.60% and the lowest was 47.96%. Based on the results, some genotypes were considered meiotically stable and suitable for use in wheat breeding programs.*
Key words: ***Aegilops tauschii**, meiotic index, micronuclei.*

Estabilidade genética em acessos de trigos sintéticos: avaliação citogenética como apoio em programas de melhoramento genético

RESUMO: *Trigos sintéticos são resultados do cruzamento entre uma espécie tetraploide (**Triticum turgidum**, AABB) e uma espécie diploide (**Aegilops tauschii**, DD) originando um híbrido estéril, seguido por duplicação cromossômica, por meio de colchicina, para restabelecer um trigo hexaploide fértil. A principal importância do uso de trigos sintéticos no melhoramento é que possibilitam aumentar a variabilidade genética, bem como introgredir genes de resistência a estresses bióticos e abióticos. O presente estudo teve como objetivo avaliar a presença de micronúcleos (MCN) e índice meiótico (IM) na fase de tétrades, em acessos de trigos sintéticos e cultivares testemunhas (**T. aestivum**), armazenadas no Banco de Germoplasma da Embrapa Trigo, a fim de selecionar os acessos estáveis geneticamente e disponibilizar ao melhoramento. Cinco plantas por genótipos, na fase anterior à antese, foram coletadas e fixadas em solução Carnoy. As lâminas citológicas foram preparadas pelo método de maceração e a coloração das células com carmim acético 1%. As observações foram em microscópio óptico. Observou-se a presença de micronúcleos em todos os genótipos analisados e foi encontrado variabilidade quanto à estabilidade genética nos dois anos de análises. Em 2014, o percentual máximo de IM dos acessos de trigos sintéticos foi de 96,86% e o mínimo de 46,32%. Em 2015, o percentual de IM máximo foi de 96,60% e o mínimo de 47,96%. Com base nos resultados, alguns genótipos foram considerados meioticamente estáveis e, poderão ser utilizados em programas de melhoramento genético de trigo.*
Palavras-chaves: ***Aegilops tauschii**, índice meiótico, micronúcleo.*

INTRODUCTION

Wheat (***Triticum aestivum*** L.) is a staple food grain that will play an increasingly important role as human populations continue to grow. Wheat plant originated millions of years ago from the natural hybridization between two diploid species, ***Triticum urartu*** Gandylian and ***Aegilops speltoides*** Á. Löve, creating the tetraploid wheat ***Triticum dicoccoides*** Schrank. About 10,000 years later, the domestication of this cereal had contributed to the evolution of ***Triticum durum*** Desf., which was hybridized with the wild species ***Aegilops tauschii*** Coss to produce the hexaploid wheat ***T. aestivum*** (AABBDD) (MUJEEB-KAZI et al., 1996).

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Synthetic hexaploid wheat plants are produced by artificial crossbreeding between tetraploid species, such as ***Triticum turgidum*** (genome AABB, 2n = 28), and the diploid wild relative ***Ae. tauschii*** (genome DD, 2n = 14), and successively undergoing chromosome doubling with colchicine to create a hexaploid genome (AABBDD) (TRETHOWAN & VAN GINGEL, 2009). The genetic diversity reported in the D genome of ***Ae. tauschii*** is much higher than that in the common wheat (REIF et al., 2005; NAGHAVI & MARDI, 2010).

Cytogenetic studies compare wild and cultivated populations on the basis of their chromosome characteristics during cell division. Regardless of an adequate supply of germplasm, breeding programs required knowledge of the reproductive mode, number of chromosomes, and meiotic behavior within and between compatible species in order to select strains for crossbreeding (MENDES-BONATO et al., 2006). Wild and crossbred plants have variable meiotic behavior among genotypes. As such, genotypes may be anomalous (e.g., produce micronuclei (MN)) in the meiotic and post-meiotic phases, resulting in limited fertility of the male gametes (DAMASCENO JUNIOR et al., 2010).

Calculation of the meiotic index (MI) is a simple procedure for evaluating the regularity of the meiotic process, and since it is a quick cytological technique, the degree of meiotic stability is readily reported (LOVE, 1951). In this context, this study evaluated the genetic stability of synthetic wheat accessions stored at the Active Germplasm Bank (BAG) of the Empresa Brasileira de Pesquisa Agropecuária (Embrapa Trigo), Passo Fundo, RS, Brazil, by analyzing the MN and MI, in order to identify superior accessions for use in crossbreeding programs aiming to increase genetic variability and diversity, as well as to introgress genes with resistance to biotic and abiotic stressors.

MATERIALS AND METHODS

Twenty synthetic wheat accessions from the International Maize and Wheat Improvement Center (CIMMYT, Mexico) were evaluated and stored at the BAG of Embrapa Trigo; namely, CIGM88.1351-OB, CIGM90.896, CIGM90.909, CIGM92.1629, CIGM92.1666, CIGM92.1680, CIGM92.1696, CIGM92.1698, CIGM92.1706, CIGM92.1713, CIGM92.1849, CIGM93.200, CIGM93.205, CIGM93.225, CIGM93.268, CIGM93.294, CIGM93.298, CIGM93.302, CIGM93.403, and CASW94Y00054S. Six traditional varieties of wheat also stored at the BAG were witness cultivars; namely, 'BR 18 Terena', 'BRS Guamirim', 'BRS 194', 'BRS 179', 'Frontana', and 'Sumai 3'. The test was carried out in an experimental field of Embrapa Trigo (latitude 28° 15′ 46″ S, longitude 52° 24′ 24″ W, and altitude 684m) in 2014 and 2015.

Cytogenetic analyses were performed in the Laboratory of Biotechnology, Cytogenetic Department at Embrapa Trigo. The randomized block design included 26 treatments and three replications. Each genotype was sown in a part composed of a 5-m line with 60 suitable seeds per linear meter. The test was carried out according to the wheat and triticale production manual (REUNIÃO, 2014).

Five ears of wheat per genotype (treatment) were randomly collected prior to anthesis. The ears were fixed in Carnoy fixative (absolute ethanol:glacial acetic acid, 3:1), for 24 hours at room temperature, and stored in 70% ethanol at -20°C. Slides were prepared for cytological evaluation using three anthers of a same flower and the medial portion of an ear. The anthers were crushed and stained with 1% acetocarmine. Variables analyzed were normal tetrads and presence of MN. Each treatment was carried out with five replicates; an ear and a slide represented each repetition. The first 200 entire tetrads were counted and analyzed for the presence or absence of MN. A Zeiss Axio Lab optical microscope (×400 magnification) and Pinnacle Studio Plus software were used for the analysis.

The MI was calculated according to LOVE (1949), where MI = (number of normal tetrads/ number of tetrads analyzed) × 100. Cultivars for which the MI was >90% were considered meiotically stable. In 2014, the accession CIGM92.1629 was not statistically analyzed for tetrads with MN and MI, because the tetrad phase was not reported. In 2015, however, this genotype was statistically analyzed. After angular transformation (arcsine square root of the proportion) and checking variance homogeneity with the Cochran test at 1%, statistical analysis was performed using the software Genes (CRUZ, 2013), first by analysis of variance and then with the Scott-Knott test at 5% probability.

RESULTS AND DISCUSSION

Analysis of variance of the 2014 and 2015 test results showed significant differences between treatments with respect to the presence of MN and the MI, according to the F-test at a significance level of 5% (Table 1 and 2). Pearson's product-moment correlation coefficient was also highly significant (r = 0.52). As such,

Table 1 - Analysis of variance and Pearson correlation coefficients for the presence of micronuclei in 2014 and 2015, Embrapa Trigo, Passo Fundo/RS.

----------ANOVA MN 2014----------					----------ANOVA MN 2015----------			
FV	GL	SQ	QM	F	GL	SQ	QM	F
Treatments	24	420,07017	17,5029	7.9268**	251	3,9155	0,156618	12,1147**
Residue	100	220,8083	2,20808		104	1,3445	0,012928	
Total	124	640,8785			129	5,2599		
Mean			2,92808				0,33304	
CV (%)			50,7486				34,1408	
r=0.52								

r = correlation coefficient of Pearson at 5% probability.

the magnitude of the correlation coefficient may indicate the importance of the genotype–environment interaction effect, taking into account differences in environmental conditions between years (interaction effect year *versus* genotype). It is noteworthy to point out the genotypes that outperformed in both years of evaluation.

In 2014, the variable grain with MN formed three statistically different groups (Table 3). Twenty-one wheat genotypes showed tetrads with <20% MN (group C). Two synthetic wheat accessions showed between 30% and 40% MN (group B), and another two accessions had between 50% and 55% (group A). The MI of 21 genotypes, including witness cultivars and synthetic wheat accessions, showed a percentage of >80% (group A). However, only two accessions had a MI of <70% in group B, and two accessions had a MI of <50% in group C.

In 2015, the variability in the MN and MI percentages of tetrads was higher. With regard to the MN percentages, four groups were distinct. The first group (group A) had genotypes with >40% MN; the second group (group B) had genotypes with between 25% and 31% MN; and the third group (group C) had genotypes with between 10% and 25% MN (Table 4). Genotypes with minor abnormalities below these values were grouped into group D. For the MI, 15 accessions had a value >90% (including six cultivars from group A), seven accessions had a value between 89% and 75% (Group B), two accessions had a value between 75% and 70% (Group C), and two accessions had values of <60%.

In 2014 and 2015, six witness cultivars were in the group with superior MI values. However, synthetic wheat accessions with higher MI had different MI values. In 2014, accession

Table 2 - Analysis of variance and Pearson correlation coefficients for meiotic index in 2014 and 2015, EmbrapaTrigo, Passo Fundo/RS.

----------ANOVA MI 2014----------					----------ANOVA MI 2015----------			
FV	GL	SQ	QM	F	GL	SQ	QM	F
Treatments	24	5,3647	0,223531	9,0918**	251	3,9154	0,156615	12,1163**
Residue	100	2,4586	0,024586		104	1,3443	0,012926	
Total	124	7,8233			129	5,2597		
Mean			1,2595				1,2378	
CV (%)			12,4496				9,1854	
r=0.52								

r = correlation coefficient of Pearson at 5% probability.

Table 3 - Cytogenetic evaluation in accessions of synthetic wheats and control cultivars: percentage of tetrad with micronucleus (MN) and meiotic index (IM) in 2014, Embrapa Trigo, Passo Fundo/RS.

Genotype	MN	Group	Genotype	IM	Group
CIGM92.1706	55,46	a	BRS 179	98,98	a
CIGM93.268	53,68	a	BRS Guamirim	97,96	a
CIGM93.205	38,10	b	Sumai 3	97,58	a
CIGM92.1680	30,20	b	CIGM93.403	96,86	a
CIGM90.896	19,20	c	CIGM92.1666	96,68	a
CIGM93.294	18,96	c	Trigo BR 18	96,56	a
CIGM93.302	13,44	c	CIGM93.298	96,34	a
CIGM90.909	12,96	c	CASW94Y00054S	96,12	a
CIGM93.200	11,56	c	BRS 194	94,70	a
CIGM93.225	10,50	c	CIGM92.1696	94,08	a
Frontana	10,28	c	CIGM88.1351-OB	92,82	a
CIGM92.1849	09,36	c	CIGM92.1698	91,80	a
CIGM92.1713	09,28	c	CIGM92.1713	90,72	a
CIGM92.1698	08,20	c	CIGM92.1849	90,64	a
CIGM88.1351-OB	07,18	c	Frontana	89,72	a
CIGM92.1696	05,92	c	CIGM93.225	89,50	a
BRS 194	05,30	c	CIGM93.200	88,44	a
CASW94Y00054S	03,88	c	CIGM90.909	87,04	a
CIGM93.298	03,66	c	CIGM93.302	86,56	a
Trigo BR 18	03,44	c	CIGM93.294	81,04	a
CIGM92.1666	03,32	c	CIGM90.896	80,80	a
CIGM93.403	03,14	c	CIGM92.1680	69,82	b
Sumai 3	02,42	c	CIGM93.205	61,90	b
BRS Guamirim	02,04	c	CIGM93.268	46,32	c
BRS 179	01,02	c	CIGM92.1706	44,54	c

Averages followed by the same letter do not differ statistically by the Scott and knott test at 5% probability.

CIGM93.403 had a MI of 96.86%, and in 2015 accession CIGM93.294 had a MI of 96.60%. This study found MN in all the genotypes. In a study with synthetic wheat accessions, MN were the major abnormality reported in dyad and tetrad genotypes (REZAEI et al., 2010).

MN may be defined as structures resulting from entire chromosomes or chromosome fragmentation that are lost in cell division, and; therefore, are not included in the nuclei of daughter cells, remaining in the cytoplasm of interphase cells (HEDDLE, 1973). During telophase, the nuclear envelope is formed around the entire chromosome or missing chromosome, which decondenses and gradually takes on the morphology of the interphase nucleus, except for size (since it is much smaller than its core, hence the name "micronucleus") (FENECH, 2000). These abnormalities in hexaploid synthetic wheat species, which undergone high genotype–environment interactions (REZAEI et al., 2010).

Cytomixis, which is the migration of genetic material, gene products, and organelles among meiocytes through cytoplasmic connections or cytomictic channels (FALISTOCCO et al., 1995), may cause MN formation, especially when associated with prophase (BOLDRINI, PAGLIARINI& VALLE, 2006).

A possible explanation for the high rates of MN in synthetic wheat is; however, because this germplasm results from artificial hybridization. In addition, the synthetic wheat genome is a combination of ***T. turgidum*** × ***Ae. tauschii*** (REZAEI et al., 2010), causing the meiotic cycle to vary in different genomes, which may cause meiotic instability (OETTLER, 2005).

In addition to these genetic reasons, abiotic factors may also cause the fragmentation of the genetic material, producing cells with MN (DIEGUES et al., 2015). Although cellular repair mechanisms are efficient, MN formation is due to chromosomal

Table 4 - Cytogenetic evaluation in accessions of synthetic wheats and control cultivars: percentage of tetrad with micronucleus (MCN) and meiotic index (IM) in the year 2015, Embrapa Trigo, Passo Fundo/RS.

Genotype	MN		Genotype	MI	
CIGM90.896	52,04	a	BRS 179	99,54	a
CIGM92.1629	43,62	a	Trigo BR 18	97,06	a
CIGM93.268	30,38	b	CIGM93.294	96,60	a
CIGM93.205	26,94	b	Frontana	96,20	a
CIGM92.1696	22,50	c	BRS Guamirim	96,08	a
CIGM92.1680	16,66	c	CIGM93.302	95,84	a
CIGM88.1351-OB	15,42	c	CIGM92.1713	95,30	a
CIGM92.1698	14,58	c	CASW94Y00054S	92,50	a
CIGM90.909	14,08	c	CIGM92.1849	92,50	a
CIGM92.1706	12,74	c	BRS 194	92,04	a
CIGM93.298	11,58	c	CIGM92.1666	91,56	a
Sumai 3	09,36	d	CIGM93.200	91,14	a
CIGM93.225	09,02	d	CIGM93.403	91,12	a
CIGM93.403	08,88	d	CIGM93.225	90,98	a
CIGM93.200	08,86	d	Sumai 3	90,64	a
CIGM92.1666	08,44	d	CIGM93.298	88,42	b
BRS 194	07,96	d	CIGM92.1706	87,26	b
CIGM92.1849	07,50	d	CIGM90.909	85,92	b
CASW94Y00054S	07,50	d	CIGM92.1698	85,42	b
CIGM92.1713	4,70	d	CIGM88.1351-OB	84,58	b
CIGM93.302	4,16	d	CIGM92.1680	83,34	b
BRS Guamirim	3,92	d	CIGM92.1696	77,50	b
Frontana	3,80	d	CIGM93.205	73,06	c
CIGM93.294	3,40	d	CIGM93.268	69,62	c
BR 18 Terena	2,94	d	CIGM92.1629	56,38	d
BRS 179	0,46	d	CIGM90.896	47,96	d

Averages followed by the same letter do not differ statistically by the Scott and knott test at 5% probability.

abnormalities, often related to environmental factors (MAJER et al., 2001).

In 2014, for example, a synthetic wheat accession with higher MN percentage was CIGM92.1706 (55.46%), which was collected for tetrad analysis in the second half of October, when the minimum and maximum temperatures and relative humidity were above normal. Likewise, in 2015, synthetic wheat accessions with a higher MN percentage (group A) were those undergoing the tetrad phase at the end of October, when the average minimum and maximum temperatures and relative humidity were again above normal. The relative humidity was 9.6% above normal for that month.

Relative humidity variations may affect MN formation in the tetrad phase (SPÓSITO et al., 2015). In a study with wheat, OMIDI et al. (2014) noted that varieties had the lowest amounts of abnormalities in normal environmental conditions. Several varieties had significant increases in meiotic changes as the temperature increased.

Accessions with a high incidence of MN are not recommended for use in crossbreeding, since the material contained in these MN may ultimately influence gene expression in nuclear ribosomal DNA, mitochondrial DNA, and chloroplast DNA. This fact may also cause very important evolutionary implications, alter the variability of produced meiospores, and have an effect on the viability and ploidy level of pollen grains (DIEGUES et al., 2015). In this study, the MN percentage in group C averaged 7.86%, the lowest in 2014. In 2015, group D had an average of 6.06% MN. Other MN patterns, lower than those reported in this study, have already been found in synthetic wheat accessions. This may be a further indication of environmental influences, since the characteristics of the evaluated sites differed greatly.

With regard to the MI, 15 out of the 19 synthetic wheat accessions were in group A, which was the group with superior MI percentages in 2014 (average 90.63%). Four genotypes were in the second and third groups (B and C), with an average MI of <70%. In 2015, nine synthetic wheat accessions in group A had an average MI of 93.13%. Seven accessions in group B had an average MI of 84.63%. Four synthetic accessions of the remaining two groups had an average MI of <72.5%. Genotypes with a MI of <90% may result in reproductive issues when involved in crossbreeding, because they are considered cytogenetically unstable, and this may ultimately make chromosome pairing difficult (LOVE, 1951).

Both in 2014 and 2015, the wheat cultivar 'BRS 179' had maximum MI and minimum MN percentages. The average MI in cultivars was 95.91% and 95.26% in 2014 and 2015, respectively, both in group A. Comparatively, studies with species of the ***Caricaceae*** family had higher MI percentages in cultivated than in wild species (DAMASCENO JUNIOR et al., 2010). The MI of 19 genotypes of synthetic wheat accessions ranged from 44.54% to 96.86% in 2014. In 2015, the maximum and minimum MI of synthetic wheat was 96.60% and 47.96%, respectively. In both years, variability of the genetic stability of the wheat products was noted. In an Iranian study with synthetic wheat accessions, the lowest MI was 99% (ARABBEIGI et al., 2010).

When interspecific hybrids are the object of study, such as synthetic wheat, it is essential to know the meiotic behavior of the species before using them in crossbreeding. The meiotic behavior in plants is related to their degree of fertility, and formation of functional gametes is controlled by genes that guarantee a normal meiotic process (PAGLIARINI, 2000). The identification of more stable genotypes using cytological analysis; therefore, permits both the planning of seed production of cultivars to be launched, and support their potential use to produce new wheat populations (POZZOBON et al., 2011).

CONCLUSION

This study found variability (due to genetic and environmental factors) in the genetic stability of synthetic wheat accessions in both years that the experiment was carried out. Thus, only meiotically stable genotypes should be incorporated in crossbreeding studies involving hybridization, with the aim to increase the genetic variability of wheat plants and introgression of genes with resistance to biotic and abiotic stressors.

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REFERENCES

ARABBEIGI, M. et al. Meiotic behavior of wild, synthetic and cultivated wheats. **Cytologia**, Tokyo, v. 75, p. 169-175, 2010. Available from: <https://www.jstage.jst.go.jp/article/cytologia/75/2/75_2_169/_pdf>. Accessed: Jan. 12, 2016. doi: 10.1508/cytologia.75.169.

BOLDRINI, K. R. et al. Cell fusion and cytomixis during microsporogenesis in ***Brachiaria humidicola*** (***Poaceae***). **South African Journal of Botany**, Scottsville, v. 72, p. 478-481, 2006. Available from: <http://ac.els-cdn.com/S025462990600038X/1-s2.0-S025462990600038X-main.pdf?_tid=f26caf5e-eb79-11e5b45400000aab0f01&acdnat=1458134649_129739d109a99edb603d1fca478c2497>. Accessed: Nov. 15, 2015. doi: 10.1016/j.sajb.2005.11.004.

CRUZ, C. D. GENES - a software package for analysis in experimental statistics and quantitative genetics. **Acta Scientiarum**, Maringá, v. 35, p. 271-276, 2013. Available from: <http://periodicos.uem.br/ojs/index.php/ActaSciAgron/article/view/21251/pdf_1>. Accessed: Oct. 12, 2015. doi: 10.4025/actasciagron.v35i3.21251.

DAMASCENO JUNIOR, P. C et al. Meiotic behavior of ***Carica papaya*** and ***Vasconcelle amonoica***. **Caryologia**, Firenze, v. 63, p. 229-236, 2010. Available from: <http://www.tandfonline.com/doi/s/10.1080/00087114.2010.10589732#aHR0cDovL3d3dy50YW5kZm9ubGluZS5jb20vZG9pL3BkZi8xMC4xMDgwLzAwMDg3MTE0LjIwMTAuMTA1ODk3MzJAQEAw>. Accessed: Sept. 14, 2015. doi: 10.1080/00087114.2010.10589732.

DIEGUES, P. I. et al. Meiotic behavior and pollen viability in the species ***Jatropha curcas*** L. **Ciências Agrárias**, Londrina, v. 36, p. 141-150, 2015. Available from: <http://www.uel.br/revistas/uel/index.php/semagrarias/article/view/14926/16036>. Accessed: Oct. 17, 2015. doi: 10.5433/1679-0359.2015v36n1p141.

FALISTOCCO, E.et al. Cytomixis in pollen mother cells of diploid Dactylis, one of origins of 2n gametes. **Journal of Heredity**, Washington, v. 86, n. 6, p. 448-453, 1995.

FENECH, M. The *in vitro* micronucleus technique. **Mutation Research**, Amsterdan, v. 455, p. 81-95, 2000. Available from: <http://ac.els-cdn.com/S0027510700000658/1-s2.0-S0027510700000658-main.pdf?_tid=3acd9d06-eb7c-11e5-896500000aacb35e&acdnat=1458135630_cb3a7cbb2adfcae4030dc024f7af08f0>. Accessed: Nov. 18, 2015. doi: 10.1016/S0027-5107(00)00065-8.

HEDDLE, J. A. A rapid *in vitro* test for chromosomal damage. **Mutation Research**, Amsterdam, v. 18, n. 2, p. 187-190, 1973. Available from: <http://ac.els-cdn.com/0027510773900353/1-s2.0-0027510773900353-main.pdf?_tid=a6b678f8-eb7c-11e5-a01c-00000aacb35f&acdnat=1458135811_0cdaf8406ffac2239fa62b20d7972bdc>. Accessed: Nov. 22,2015. doi: 10.1016/0027-5107(73)90035-3.

LOVE, R. M. Varietal differences in meiotic chromosomes behavior of Brazilian wheats. **Agronomy Journal**, Madison, v. 43, n. 2, p. 72-76, 1951.

MAJER, B. J. et al. Use of the micronucleus assay with exfoliated epithelial cells as a biomarker for monitoring individuals at elevated risk of genetic damage and in chemoprevention Trials. **Mutation Research**, Amsterdam, v. 489, p. 147-172, 2001. Available from: <http://ac.els-cdn.com/S1383574201000680/1-s2.0-S1383574201000680-main.pdf?_tid=38f4fe0a-eb7e-11e5-bd3800000aacb35d&acdnat=1458136485_4963b0e96d72fd1d5cca4f037ce017dc>. Accessed: Nov. 21, 2015. doi: 10.1016/S1383-5742(01)00068-0.

MENDES-BONATO, A. B. et al. Chromosome number and meiotic behavior in ***Brachiaria jubata*** (***Gramineae***). **Journal of Genetics**, Bengaluru, v. 85, p. 83-87, 2006. Available from: <file:///C:/Users/citogenetica/Downloads/83%20(1).pdf>. Accessed: Nov. 18, 2015. doi: 10.1007/BF02728976.

MUJEEB-KAZI, A. et al. Conservation of the genetic variation of ***Triticum tauschii*** in synthetic hexaploid wheats and its potential utilization for wheat improvement. **Genetic Resources and Crop Evolution**, Dordrecht, v. 43, p. 129-134, 1996. Available from: <https://link.springer.com/article/10.1007%2FBF00126756#page-1>. Accessed: Nov. 25, 2015. doi: 10.1007/BF00126756.

NAGHAVI, M. R.; MARDI, M. Characterization of genetic variation among accessions of ***Aegilops tauschii***. **Journal of Molecular Microbiology and Biotechnology**, Switzerland, v. 18, p. 93-96, 2010. Available from: <http://www.msmbb.org.my/apjmbb/html181/181u.pdf>. Accessed: Nov. 29, 2015. doi: 10.1270/jsbbs.61.347.

OETTLER, G. The fortune of a botanical curiosity triticale: past present and future. **Journal of Agricultural Science**, Toronto, v. 143, p. 329-346, 2005. Available from: <https://www.researchgate.net/publication/231847496_The_fortune_of_a_botanical_curiosity_Triticale_Past_present_and_future>. Accessed: Jan. 12 2016. doi: 0.1017/S0021859605005290.

OMIDI, M. et al. The influence of terminal heat stress on meiosis abnormalities in pollen mother cells of wheat. **Cytologia**, Tokyo, v. 79, p. 49-58, 2014. Available from: <https://www.researchgate.net/publication/289537748_The_Influence_of_Terminal_Heat_Stress_on_Meiosis_Abnormalities_in_Pollen_Mother_Cells_of_Wheat>. Accessed: Dec. 18, 2015. doi: 10.13140/RG.2.1.2721.2887.

PAGLIARINI, M.Q.S. Meiotic behavior of economically important plant species: the relationship between fertility and male sterility. **Genetics and Molecular Biology**, Ribeirão Preto, v. 23, n. 4, p. 997-1002, 2000. Available from: <http://www.scielo.br/pdf/gmb/v23n4/6262.pdf>. Accessed: Nov. 03, 2015. doi: 10.1590/S1415-47572000000400045.

POZZOBON, M. T. et al. Meiosis and pollen viability in advanced pepper strains. **Horticultura Brasileira**, Brasília, v. 29, p. 212-216, 2011. Available from: <http://www.scielo.br/pdf/hb/v29n2/a13v29n2.pdf>. Accessed: Oct. 23, 2015.

REIF, J. C. et al. Wheat genetic diversity trends during domestication and breeding. **Journal Theoretical and Applied Genetics**, Warsaw, v. 110, p. 859-864, 2005. Available from: <https://link.springer.com/article/10.1007%2Fs00122-004-1881-8#/page-1>. Accessed: Oct. 25, 2015. doi: 10.1007/s00122-004-1881-8.

REZAEI, M. et al. Meiotic behaviour of tetraploid wheats (***Triticum turgidum*** L.) and their synthetic hexaploid wheat derivates influenced by meiotic restitution and heat stress. **Journal of Genetics**, Bengaluru, v. 89, p. 401-407, 2010. Available from: <https://link.springer.com/article/10.1007%2Fs12041-010-0058-2#/page-1>. Accessed: Nov. 12, 2015. doi: 10.1007/s12041-010-0058-2.

SPÓSITO, J. C. V. et al. Genetic instability in plants associated with vehicular traffic and climatic variables. **Ecotoxicology and Environmental Safety**, v. 120, p. 445-448, 2015. Available from: <http://ac.els-cdn.com/S0147651315300014/1-s2.0-S0147651315300014-main.pdf?_tid=dc73ba44-eb83-11e5-95b400000aacb361&acdnat=1458138907_b4d62e407cce0b363494e85bbab52ed0>. Accessed: Nov. 17, 2015. doi: 10.1016/j.ecoenv.2015.06.031.

TRETHOWAN, R.M.; VAN GINKEL, M. Synthetic wheat – an emerging genetic resource. In: CARVER, B. **Wheat - Science and trade**. Ames: John Wiley & Sons. 2009. p.369-386.