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MORPHO-AGRONOMIC, CYTOGENETIC AND MOLECULAR CHARACTERIZATION OF SYNTHETIC WHEAT ACCESSIONS AS A POTENTIAL GERMPLASM FOR PLANT BREEDING

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Abstract: Synthetic hexaploid wheat (AABBDD) is produced by using the tetraploid *Triticum turgidum* var. *durum* (AABB) and the diploid *Aegilops tauschii* (DD) as parents. This cross has allowed genetic variability to be transferred from parental to other wheat hexaploid accessions, lines or cultivars. This transference may occur not only of variable disease resistance genes but also traits involving quality and tolerance to abiotic stresses. We evaluated and characterized the genetic diversity and stability of synthetic wheat accessions that can be used in breeding programs due to the presence of desirable characteristics. Fifty synthetic accessions were investigated regarding their morphological and agronomic characteristics, presence of micronuclei in tetrads and genetic similarity based on molecular markers. Four commercial cultivars were used as controls. Depending on their proposed use, some genotypes were characterised as suitable for breeding programs based on their morphological traits, genetic stability or genetic diversity, or because of a combination of these factors.
Keywords: Meiotic index, microsatellite, genetic diversity, morphology, genetic stability.

INTRODUCTION

Hexaploid wheat was produced by the crossing of *Triticum urartu* (the A-genome donor) and an unknown grass thought to be related to *Aegilops speltoides* (the B-genome donor), producing a tetraploid emmer wheat (AABB, *T. dicoccoides*) which hybridized again with *Ae. tauschii* (the D-genome donor) produced the modern bread wheat (*T. aestivum*). It is believed that only a few accessions of the donor species were involved in the evolution of common wheat. Consequently, wheat has both a diverse background, originating from the intercrossing of three distinct grasses, and a narrow base, as just a few individuals within each grass species contributed their genes to wheat (Van Ginkel; Ogbonnaya, 2007; Yang et al., 2009).

The presence of diverse, and often adverse, environmental conditions, such as diseases and abiotic stresses, means that genetic variability is essential for wheat production. When the same wheat cultivar is used for many years, or the same parental cultivars are employed for breeding, genetic erosion can occur due to bottlenecks in diversity levels at the crop, variety and allele levels of crop integration (Van de Wouw et al. 2010). Genetic erosion narrows the genetic potential of wheat and negatively affects wheat improvement programs. The limited genetic diversity of crops also complicates wheat production, which has been hindered by climate change and the high degree of wheat pathogen specialization (Li et al., 2018; Masood et al., 2016).

The genetic diversity can be explored in the wild relatives of wheat and this genetic diversity can be introduce the in commom wheat by the use of synthetic hexaploid wheat (SHW) (Li et al., 2018; Yang et al., 2009). SHW is developed in two main steps: first is wide hybridization of *T. turgidum* with *Ae. tauschii*, resulting in triploid hybrid with genome ABD. The second step is amphidiploidization, generating a fertile hybrid with genome AABBDD (Rosyara et al., 2019; Yang et al., 2009). SHW lines have no significant reproduction barrier therefore are useful in introducing agronomically needed traits into common wheat from wild genetic resources (Li et al., 2018).

The International Maize and Wheat Improvement Center (Centro Internacional de Mejoramiento de Maíz y Trigo, CIMMYT) since the late 1980s, has developed more than 1000 SHW lines with valuable diversity for traits related to agronomic features, abiotic

stress tolerance and biotic stress resistance (Das et al., 2016; Rosyara et al., 2019). The current challenge is to make the best use of this new diversity in developing varieties for farmers and consumers. (Van Ginkel; Ogbonnaya, 2007).

The variability between plants can be assessed using morphological characteristics, such as height or the growth cycle pattern, or agronomic parameters like grain traits (colour, diameter, hardness and weight) (Rasheed et al., 2014). Molecular markers can also be used to assess variability, with probably the most widely applied methodology being microsatellites or Simple Sequence Repeats (SSR) analysis, due to its rapidity, simplicity, rich polymorphism profile and stability (Qi-Lun et al., 2008; Ramu et al., 2013; Sajib et al., 2012).

In addition to variability, genetic stability is also needed for a plant to be a suitable breeding parental, because an unstable genotype may be infertile and chromosomal instability can lead cells to rapidly mutate and evolve (Fenech et al., 2011). Cytogenetic stability is often assessed by the frequency of micronuclei in tetrads. During the metaphase and anaphase phases of the cell cycle a micronucleus (MN) can form from either a whole chromosome which is lagging or an acentric chromosome fragment detaching from a chromosome after breakage. Estimates of genetic diversity and genetic stability are therefore essential for successful breeding programs.

In our study, we evaluated the genetic diversity of 50 synthetic wheat accessions by characterizing and evaluating their agronomic traits and cytogenetic approaches to estimate the genetic stability. We also used molecular markers to analyse the genetic similarity.

MATERIAL AND METHODS

Fifty synthetic hexaploid wheat (2n=6X=42, AABBDD) accessions from the Wheat Active Germplasm Bank at the Brazilian Agricultural Research Corporation – Embrapa were analysed. The accessions were sent by CIMMYT and originally produced by using the tetraploid *T. turgidum* var. *durum* (2n=4X, AABB) as the maternal parent and the diploid *Ae. tauschii* (2n=2X=DD) as the paternal parent. Controls were four *T. aestivum* bread Brazilian wheat stable cultivars: BR 23, IAS 54, Jacuí and BR 18 (Terena).

Plants were grown between June and October in the experimental area at Embrapa Trigo in Passo Fundo/Rio Grande do Sul, the southernmost state in Brazil. Plants were grown in complete randomised blocks. For agronomic characterization, we planted 60 seeds of each accession in individual 1m ($0{,}3m^2$) plots of a clayey-textured humic Haplustox soil. For every 20 plots of synthetic wheat we planted one plot of the 4 controls, plus one plot at the beginning and one at the end of the experimental area, giving a total of 5 replicate control plots. The plants were visually evaluated for the following vegetative traits: habit (1 = prostrate or creeping, 2 = semi-creeping, 5 = intermediate, 4 = semi-erect leaves, 5 = erect leaves); level of ear, stalk or sheath waxiness (1 = very high, 2 = high, 3 = medium, 4 = low or 5 = absent); emergence cycle (silking and emergence to flowering); plant height; and grain weight. All measurements were compared to the control cultivars.

The genetic diversity between genotypes was estimated from the Euclidean distance and the accessions grouped by the unweighted pair-group method with averages (UPGMA) (Sokal; Michener, 1958). Dendrograms were constructed based on the data. The NTSYS-pc program (Rohlf, 1998) was used for the analysis.

Cytogenetic evaluations for meiotic index (MI) were made by collecting five immature spikes per accession at the 'boot' stage, Feeks

growth stage 10 (Large, 1954). The spikes were fixed in Carnoy's solution at room temperature (20 ºC) for 24 hours, transferred to 70% ethanol and kept at -20 ºC until use. For each spike we recorded the percentage of normal tetrads in between 200 and 527 tetrads per accession and the MI values calculated as MI = (number of normal tetrads/total number of tetrads) x 100 (Love, 1951). The presence of micronuclei in each accession was assessed by squashing six anthers in 8µL of acetic carmine (1% w/v) and viewing the preparations in an Olympus model BX50 optical microscopic at 400X magnification.

For molecular marker analysis, the DNA from each accession was extracted from seedling leaf tissues according to the CTAB protocol (Doyle; Doyle, 1990) and 54 wheat primers, covering wheat genomes A, B and D, were selected from the literature, of these primers, 20 were used in the analysis (Table 1). Polymerase chain reactions (PCR) were prepared using 0.2 mM of each forward and reverse primer, 0.2 mM of each dNTP, 2.5 mM of $MgCl_2$, 0.75 U of Taq-DNA polymerase, 1X Taq buffer and 100 ng of accession DNA. The amplification protocol consisted of one denaturation cycle at 94 °C for 3 minutes followed by 45 cycles of 94 °C for 1 minute, 60 °C for 1 minute and 72 °C for 1 minute, with a final extension at 72 °C for 10 minutes. The PCR amplified fragments were separated in 2 % (w/v) ultrapure agarose gel (Invitrogen) for 90 minutes at 120 volts, stained with ethidium bromide and visualized under ultraviolet light using GelDoc XR+ equipment (Bio-Rad).

The genetic similarity of the accessions was estimated from the Jaccard coefficient and the accessions grouped by the UPGMA method (Sokal; Michener, 1958). Dendrograms were constructed based on the data. The NTSYS-pc program (Rohlf, 1998) was used for the analysis.

RESULTS AND DISCUSSION

It was possible to verify genetic diversity in the tested genotypes based on the evaluated morphological characters (Table 1). The grouping of data by the UPGMA method generated two large groups. Group 1 with 46 SHW accessions showing high genetic diversity and with the accessions forming a large number of smaller groups with distinct characteristics, the diversity varying from ~18% to 75%. Group 2 contained four accessions (dendrogram code numbers (DCN) 29, 39, 40 and 49) plus the four controls and a range of percentage diversity varying from ~25% to 78% (Figure 1).

Agronomic characterization varied for all the features evaluated. In general, the control cultivars used in our study possess erect or semi-erect leaves and short-cycled cultivars with early or mid-maturity that are the most suitable for crop system for southern Brazil. Waxy plant surfaces protect against the entrance of pathogens (Souza; Marcos-Filho, 2001), so the waxy stalk, sheath or ear traits are desirable along with semi-erect or erect leaves and a short or mid cycle of less than 140 days emergence to flowering (Table 2).

In synthetic wheat, variability in traits like height and yield are not normally of agronomic importance, however variation in these traits can be useful for transferring target genes to bread wheat (Jafarzadeh et al., 2016). SHW itself cannot be used as a cultivar because it shows a number of undesirable traits. Therefore, it is necessary develop synthetic derivative lines to remove these characters and select for agronomically desirable traits (Li et al., 2018; Rosyara et al., 2019).

Genetic similarity estimated by SSR analysis also placed the accessions into two major groups, with group 1 being formed by 16 accessions and group 2 containing 34 accessions and all four controls. The genetic similarity between all accessions was less than

SSR	Forward	Reverse	Chromosome
WMC 25	TCTGGCCAGGATCAATATTACT	TAAGATACATAGATCCAACACC	2BS. 2DS
WMS95	GATCAAACACACACCCCTCC	AATGCAAAGTGAAAAACCCG	2AS
WMS112	CTAAACACGACAGCGGTGG	GATATGTGAGCAGCGGTCAG	3B.4B. 7B
WMS114F	ACAAACAGAAAATCAAAACCCG	ATCCATCGCCATTGGAGTG	3D
WMS118	GATGTTGCCACTTGAGCATG	GATTAGTCAAATGGAACACCCC	4AL.5BL
WMC167	AGTGGTAATGAGGTGAAAGAAG	TCGGTCGTATATGCATGTAAAG	2DL
WMS 193	CTTTGTGCACCTCTCTCTCC	AATTGTGTTGATGATTTGGGG	6BS
WMC215	CATGCATGGTTGCAAGCAAAAG	CATCCCGGTGCAACATCTGAAA	5D. 5AL. 3A
WMS219	GATGAGCGACACCTAGCCTC	GGGGTCCGAGTCCACAAC	6B
WMS261	CTCCCTGTACGCCTAAGGC	CTCGCGCTACTAGCCATTG	2D
WMS272	TGCTCTTTGGCGAATATATGG	GTTCAAAACAAATTAAAAGGCCC	5DL
WMC331	CCTGTTGCATACTTGACCTTTTT	GGAGTTCAATCTTTCATCACCAT	4DL
WMS408	TCGATTTATTTGGGCCACTG	GTATAATTCGTTCACAGCACGC	5BL
WMS471	CGGCCCTATCATGGCTG	GCTTGCAAGTTCCATTTTGC	7A.7B
WMS499	ACTTGTATGCTCCATTGATTGG	GGGGAGTGGAAACTGCATAA	5BL
WMS508	GTTATAGTAGCATATAATGGCC	GTGCTGCCATGATATTT	6BS
WMS539	CTGCTCTAAGATTCATGCAACC	GAGGCTTGTGCCCTCTGTAG	2D
WMS604	TATATAGTTCAATATGACCCG	ATCTTTTGAACCAAATGTG	1BS.5BL
WMS617	GATCTTGGCGCTGAGAGAGA	CTCCGATGGATTACTCGCAC	5A.6A
WMS664	CAGTCAGTGCCGTTTAGCAA	AGCTTTGCTCTATTGGCGAG	3DL.4B

Table 1. Simple sequence repeat (SSR) molecular markers selected for the genetic similarity assessment.

		Morphological and agronomic characteristics									Cytogenetic data		
Dendrogram Code Number (DCN)	Accession number	Habit*	†Waxy sheath	†Waxy Stalk	†Waxy ear	Emergence time to silking (days)	Emergence time to flowering (days)	Height (cm)	Grain weight (g)	Meiotic index (%)	Micronuclei (%)	Normal cells (%)	Number of cells assessed
1	CASS02B00002S	2	2	1	1	60	62	69	16.2	27.3	72.8	27.3	400
2	CASS02B00004S	3	3	2	2	70	74	91	31.7	50.6	49.4	50.6	500
3	CASS02B00010S	3	1	3	1	82	85	69	29.3	72.0	28.0	72.0	500
4	CASS02B00011S	3	1	1	1	73	77	83	43.9	43.0	57.0	43.0	400
5	CASS02B00012S	4	3	1	1	81	85	86	25.2	42.6	57.4	42.6	500
6	CASS03GH00001S	3	3	2	1	82	85	72	29.8	74.4	25.6	74.4	500
7	CASS03GH00003S	3	3	1	1	84	88	49	13.3	74.6	25.4	74.6	500
8	CASS03GH00012S	3	2	1	1	82	88	63	13.3	47.4	52.6	47.4	500
9	CASS03GH00019S	4	3	3	1	64	67	69	19.2	77.0	23.0	77.0	500
10	CASS03GH00029S	2	2	3	1	75	78	76	18.2	52.2	47.8	52.2	500
11	CASS03GH00056S	3	2	3	3	76	79	66	20.8	55.2	44.8	55.2	500
12	CASS03GH00058S	2	4	2	1	82	86	64	14.1	53.8	46.2	53.8	500
13	CASS03GH00059S	2	4	2	1	75	77	58	16.9	27.6	72.4	27.6	500
14	CASS03GH00060S	2	3	4	2	75	78	73	21.6	44.0	56.0	44.0	500
15	CASS03GH00061S	3	5	4	3	71	73	72	17.3	50.0	50.0	50.0	300
16	CASS03GH00063S	3	4	3	1	79	82	70	10.0	70.2	29.8	70.2	500
17	CASS03GH00064S	1	5	2	1	90	92	75	13.7	65.2	34.8	65.2	500
18	CASS03GH00067S	3	4	2	1	73	76	73	15.9	70.2	29.8	70.2	500

19	CASS03GH00068S	2	4	3	3	81	83	67	11.8	33.4	66.6	33.4	500
20	CASS03GH00114S	3	4	2	1	89	92	85	9.6	32.5	67.5	32.5	400
21	CASW00GH00014S	5	1	1	1	80	83	57	24.6	39.2	60.8	39.2	500
22	CASW00GH00026S	3	3	1	2	83	89	69	7.6	46.6	53.4	46.6	399
23	CASW00GH00044S	1	4	3	3	82	84	62	8.4	68.8	31.2	68.8	500
24	CASW00GH00053S	2	2	1	1	92	95	49	13.0	50.4	49.6	50.4	500
25	CASW00GH00058S	3	4	3	3	79	84	73	12.7	33.2	66.8	33.2	500
26	CASW00GH00060S	2	4	2	1	80	84	74	14.5	43.0	57.0	43.0	400
27	CASW00GH00062S	4	3	2	2	72	74	75	17.9	49.6	50.4	49.6	500
28	CASW00GH00067S	3	4	4	2	64	68	76	49.4	68.2	31.8	68.2	500
29	CASW01GH00004S	5	5	5	5	62	69	80	29.3	75.5	24.5	75.5	200
30	CASW02GH00008S	3	2	2	3	88	91	70	3.6	35.2	64.8	35.2	500
31	CASW02GH00010S	2	3	3	1	79	82	60	15.8	75.5	24.5	75.5	200
32	CASW02GH00012S	2	3	2	1	72	75	77	24.9	88.5	11.5	88.5	480
33	CASW02GH00016S	3	2	3	1	84	89	73	18.0	84.0	16.0	84.0	300
34	CASW94Y00014S	2	3	2	1	78	81	67	7.0	58.9	41.1	58.9	492
35	CIGM.90909	3	2	3	2	66	69	74	34.0	83.0	17.0	83.0	500
36	CIGM.93.403	5	1	2	2	64	70	98	37.7	51.0	49.0	51.0	500
37	CIGM88.1239-3B	5	2	1	1	67	70	80	38.7	82.8	17.2	82.8	500
38	CIGM88.1239-2B	5	3	2	1	66	68	95	14.7	56.8	43.2	56.8	500
39	CIGM92.1666	4	5	5	5	63	69	70	48.8	26.0	74.0	26.0	500
40	CIGM92.1696	2	5	5	5	66	70	83	69.1	41.2	58.8	41.2	500
41	CIGM92.1706	3	4	1	2	78	80	76	27.4	54.2	45.8	54.2	500
42	CIGM92.1713	3	2	2	1	78	80	78	35.9	59.0	41.0	59.0	500
43	CIGM93.177	4	3	2	2	69	72	65	12.1	29.0	71.0	29.0	300
44	CIGM93.200	4	3	3	1	73	75	57	5.6	56.7	43.3	56.7	300
45	CIGM93.225	5	5	5	1	64	70	71	56.0	90.0	10.0	90.0	500
46	CIGM93.242	2	4	2	1	74	77	70	23.0	78.7	21.3	78.7	300
47	CIGM93.267	2	2	2	1	76	79	82	26.1	66.6	33.4	66.6	500
48	CIGM93.268	3	2	2	2	74	77	80	18.5	29.4	70.6	29.4	500
49	CIGM93.294	3	3	1	1	79	81	76	9.0	41.6	58.4	41.6	500
50	CIGM93.406	3	2	1	2	81	85	70	6.5	71.5	28.5	71.5	527
51	BR 23	4	2	5	5	67	69.2	85.4	204.5	92.4	7.6	92.4	500
52	IAS 54	5	4	3	3	69	71.2	96	203.0	97.4	2.6	97.4	500
53	Jacuí	4	5	5	5	62	68	100	209.0	95.6	4.4	95.6	500
54	BR 18 (Terena)	5	5	5	4	70	73	86.2	205.1	95.4	4.6	95.4	500

*1 = prostrate or creeping, 2 = semi-creeping, 3 = intermediate, 4 = semi-erect leaves, 5 = erect leaves.

†Waxy sheath, stalk, ear: 1 = very high wax, 2 = high wax, 3 = medium wax, 4 = low wax, 5 = no wax.

Table 2. Morphological and agronomic evaluations of 50 synthetic wheat accessions (numbers 1 to 50) plus four control *Triticum aestivum* bread wheat stable cultivars (numbers 51 to 54) normally grown in the Brazilian state of Rio Grande do Sul.

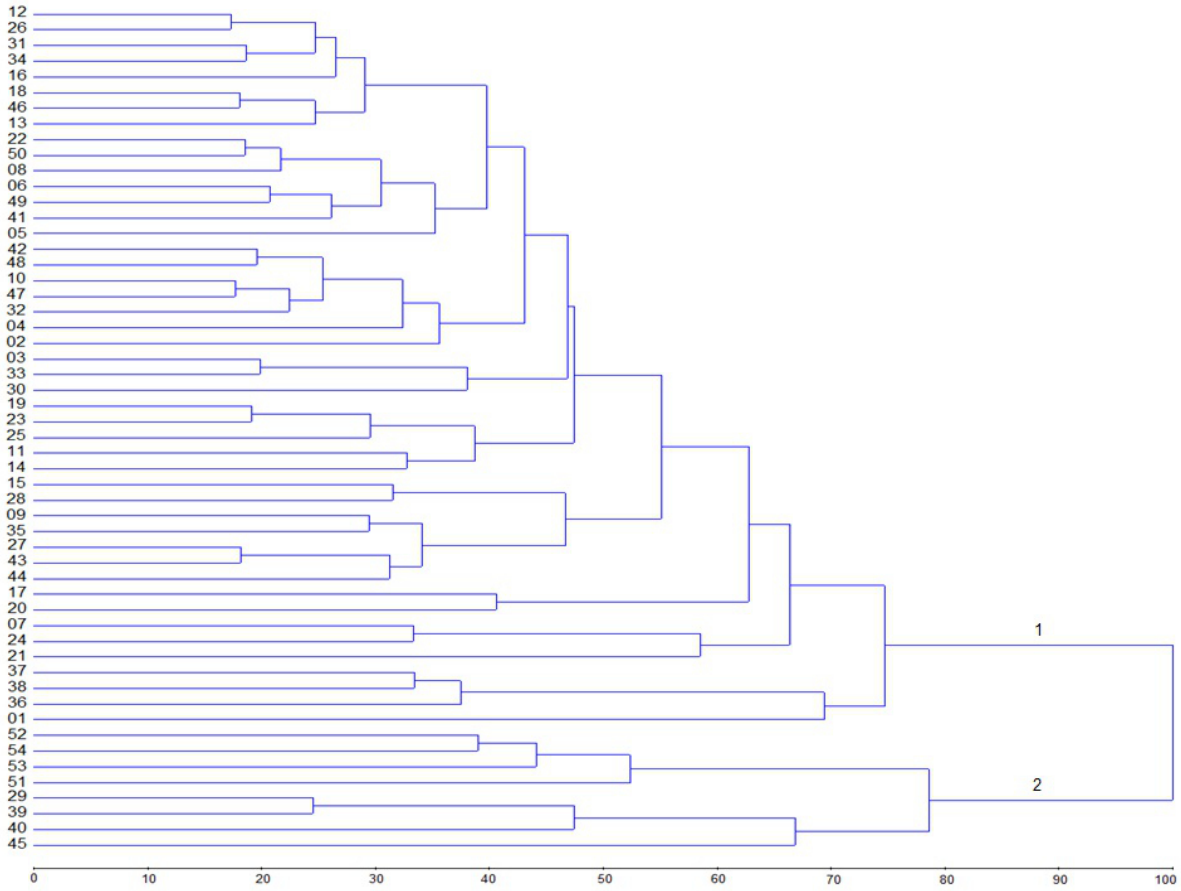

Figure 1. Euclidian distance between accessions based on morphological and agronomic evaluations.

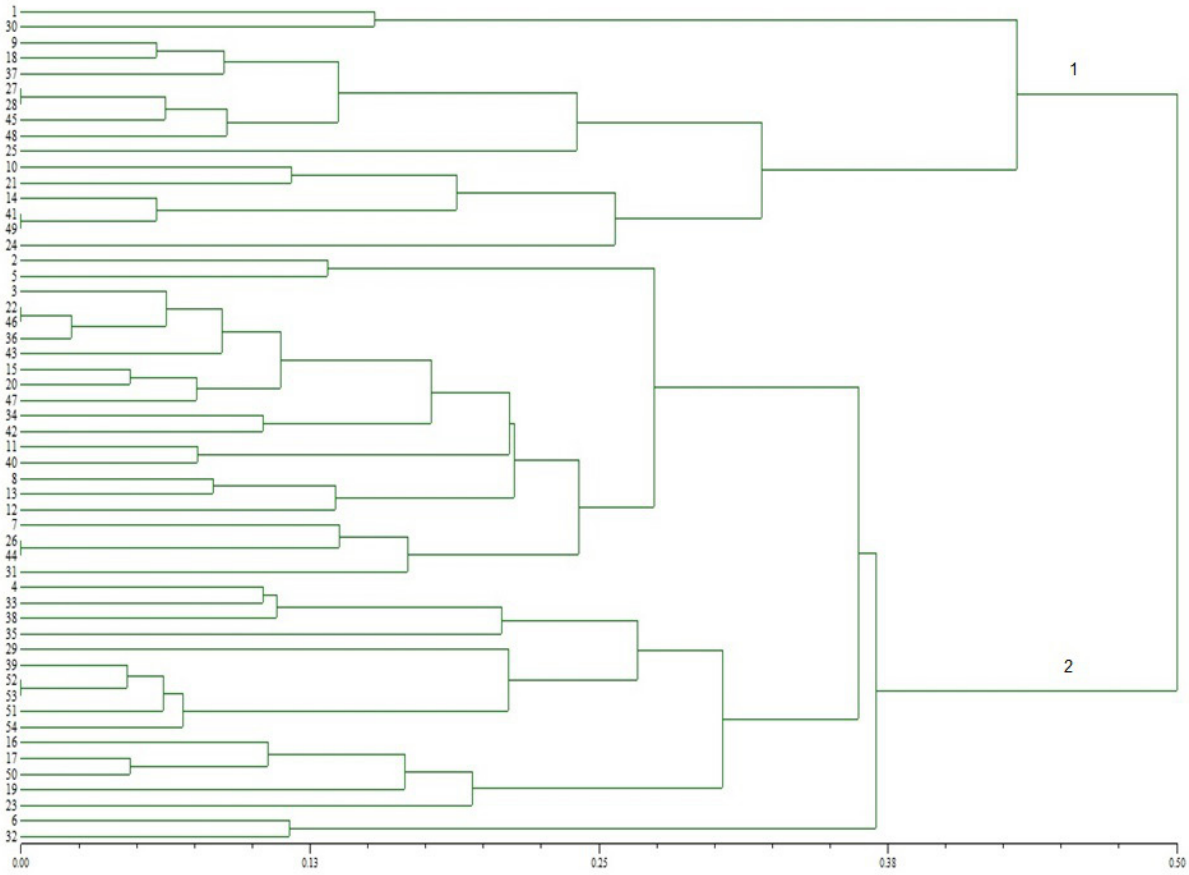

Figure 2. Genetic similarity between accessions based on single sequence repeat analysis using the Jaccard coefficient.

50% (Figure 2). Polymorphism was shown by 20 of the 54 primers used (Table 2). The data of SSR analysis and agronomic characterization confirm the high genetic diversity between accessions.

In our study, the control cultivars BR 23, IAS 54, Jacuí and BR 18 (Terena) (DCN 51, 52, 53 and 54, respectively) are widely grown in Brazil because they possess suitable agronomic features (Table 1). We found that accessions CASW01GH00004S (DCN 29, group 2 in Fig. 1 and 2) and CIGM92.1666 (DCN 39, group 2 in Fig. 1 and 2) showed most similarity to the controls as assessed by our agronomic and SSR evaluations. However, since these two accessions are genetically similar to the controls they are not adequate sources of genetic diversity, but genotypes from group 1 in Fig. 1 and 2 are suitable sources for genetic diversity as they are genetically diverse from the control cultivars.

Using morphologic descriptors to assess diversity is a useful approach, but its use as a single approach is debatable. Gerrano et al. (2014) used different approaches to access the genetic diversity, confirming that the morphological characterization and evaluation of accessions only produces reliable results when it involves multiple strategies of analysis. Casassola et al. (2013) also indicated that using morphological descriptors as well as molecular markers gave a better understanding of genetic diversity because molecular markers estimated the genetic diversity with higher accuracy.

Using SSR markers to assess genetic diversity is very useful, because these markers show co-dominant expression, *multiallelism*, high polymorphism information content, are frequently and randomly distributed (Casassola et al., 2013). The presence or absence of an allele allows the generation of matrices and estimation of genetic similarity or diversity (Lamara et al., 2013). The SSR markers were effective for the assessment of genetic variation in SHW accessions evaluated by Das et al. (2016), the cluster analyses showed distinct groups indicating presence of substantial genetic diversity among the SHW accessions studied.

Bottlenecks are known to occur in the genetic diversity of wheat, and this causes concern among breeders due to the fact that genetic resources are finite and genetic uniformity increase the vulnerability of crops to diseases (Girma, 2017). The wild relatives of wheat have potential to improve disease resistance in wheat (Rosyara et al., 2019), including resistance genes for leaf, stem, and stripe rusts (Casey et al., 2016; Periyannan et al., 2013; Singh; Mujeeb-Kazi; Huerta-Espino, 1998). Also, new resistance/tolerance genes were found for other biotic stress as insect pest (Azhaguvel et al., 2012; Weng et al., 2005) and abiotic stress as adaptation to drought conditions (Trethowan; Mujeeb-Kazi, 2008), heat tolerance (Van Ginkel; Ogbonnaya, 2007), salt tolerance (Jamil et al., 2016) and tolerance to pre-harvest sprouting (Imtiaz et al., 2008). SHW also been reported to contribute to yield (Cooper et al., 2012) and as an important source for developing biofortified wheat (Xu et al., 2011).

Interspecific hybrids, such as SHW and wild wheat species are useful genetic resources that can be used to transfer needed agronomically important genes to increase the diversity and performance of wheat crops (Li et al., 2018). To now more than 60 synthetic derivative lines have been registered as cultivars around the world and have been released for breeding programs (Li et al., 2018). China is one of the major countries that takes full advantage of SHW as a genetic resource, four cultivars derived for SHW, Chuanmai 38, Chuanmai 42, Chuanmai 43 and Chuanmai 47 have been generated and and released to farmers for large-scale growth (Li et al., 2018; Yang et al., 2009).

But, for its use is important the evaluation of genetic stability of interspecific hybrids to avoid reproductive problems. One way to evaluate the genetic stability is the presence of micronuclei, SHW without micronuclei represents excellent germplasm for use in the pre-breeding program of *T. aestivum*. Micronuclei are formed by the enclosure of lagging chromosome fragments during the reformation of nuclear membranes at the end of mitosis (Vásquez-Diez et al., 2016), and can occur when a hybrid embryo is formed by the elimination of a uniparental chromosome (Gernand et al., 2005; Rezaei; Arzani; Sayed-Tabatabaei, 2010). The lower the number of micronuclei the more genetically stable is the genotype.

Our cytogenetic analysis showed that the percentage of micronuclei (MC%) of SHW ranged from 10% to 74% and for controls ranged from 2.6% to 7.6% (Table 1). Accessions CASW02GH00012S (DCN 32), CASW02GH00016S (DCN 33), CIGM.90909 (DCN 35), CIGM88.1239-3B (DCN 37) and CIGM93.225 (DCN 45) were the most genetically stable genotypes, they exhibited less than 20% micronuclei and are thus possible stable sources for use in wheat breeding studies.

Meiotic instabilities, including the loss of genetic material as indicated by the presence of micronuclei, can lead to infertility or reproductive problems such as morphological alterations which may include unbalanced pollens. Such alterations have also been reported in synthetic wheat in comparison to parental plants (Arabbeige; Arzani; Saeidi, 2010). Plants showing a mitotic index of less than 90% are problematic in wheat breeding programs because if they are used as parental accessions their descendants are generally genetically unstable (Love, 1951), which demonstrates the importance of this type of analysis for breeding programs. In our study, the meiotic index ranged from 26% to 90%, with most values being less than 90%. Other than the control cultivars, only accession CIGM93.225 (DCN 45) showed a meiotic index of 90% (Table 1), indicating that it was the most suitable accession for use in breeding trials in terms of reproduction.

High levels of micronuclei in SHW may be the result of artificial hybridization between *T. turgidum x Ae. tauschii*, the interspecific crossing can cause variation in the duration of the meiotic cycle in different genomes inducing meiotic instability (Oettler, 2005; Rezaei; Arzani; Sayed-Tabatabaei, 2010). In addition to genetic causes, environmental factors can lead to fragmentation of genetic material and a high micronucleus index (Diegues et al., 2015). Variation in humidity and increase in temperature can lead to a greater number of meiotic alterations changes (Omidi et al., 2014; Spósito et al., 2015).

CONCLUSIONS

The use of morphological and agronomic traits combined with cytogenetic and molecular marker analysis showed that different genotypes may be useful dependent on the purpose of a specific breeding program. Diversity is essential to produce new gene combinations which may result in desirable agronomic traits and new disease resistance profiles. If one of the aims of a breeding is to allow the receiving genotype to retain morphologically suitable traits they can be modified or restored by backcrosses. Genomic stability is also important, with infertile genotypes obviously being useless for plant improvement programs. For SHW accessions with genetic breeding desirable characteristics but with meiotic index below 90%, it is recommended to perform backcrosses with commercial cultivars of *T. aestivum* aiming to increase genetic stability besides the to incorporate interest genes. Therefore, the use

of combined analytical techniques allowed efficient characterization of the accessions and permits a better and more efficient wheat selection process.

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