

CYTOGENETIC AND AGRO-MORPHOLOGICAL STUDY OF SUBSTITION LINES BETWEEN BREAD WHEAT AND *AEGILOPS UMBELLULATA*

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Abstract. Introgression of genetic material from wheat wild relatives into the common wheat genome remains important. This is a natural and inexhaustible source of enrichment of the wheat gene pool with genes that improve wheat's adaptive potential. In bread wheat (*T. aestivum* L.) breeding, introgression of useful genes via intergeneric hybridization is a powerful strategy for improving the crop productivity. *Ae. umbellulata* shows great potential in terms of useful traits; however, little is known about the cytogenetic and agronomic characteristics of intergeneric hybrids between these two species. Here, we examine the cytogenetic and agronomic characteristics and relationships between the two in intergeneric F₁ hybrids between bread wheat line - 171ACS and *Ae. umbellulata* originated and collected from Azerbaijan and their morphological characteristics. Meiotic analysis showed in F₁ plants have 28 chromosomes, as expected. The fertility of the hybrids was low. FISH identification of the chromosomes of the F₃ hybrid plant belonging to the combination 171ACS × *Ae. umbellulata* and observed 1U and 5U chromosomes of *Ae. umbellulata*. This information on intergeneric F₁ hybrids between bread wheat line and *Ae. umbellulata* will contribute to effective utilization of *Ae. umbellulata* in bread wheat breeding.

Keywords: Bread wheat, *Ae. umbellulata*, meiosis, fertility, GISH, FISH.

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1. Introduction

Wheat (*Triticum aestivum* L.) is one of the most important cereal grains and has been a stable food source for humans for ~ 10,000 years since agriculture begun in Neolithic times (Matsuoka, 2011). It is widely grown in the world due to its broad adaptation to varied environmental conditions. Continued improvement in agronomic management and plant breeding has led to a steady increase in grain yield over the years. The average global wheat production during 2010-2014 amounted to ~ 655-700 million metric tons (MMT) per year (<http://www.statista.com/topics/1668/wheat>). Wheat is an important crop worldwide, occupying 17% of global cultivated lands and providing 30% of global calorie consumption (Shewry, 2009). However, abiotic stresses, such as drought and salinity, have a significant impact on its yield, particularly under changing climate conditions.

Ae. umbellulata included in section *Aegilops* (2n=14, genome formula UU), it has been found to be an excellent source of resistance to various diseases in previous studies (Valkoun *et al.*, 1985; Dhaliwal *et al.*, 1991; Singh & Dhaliwal, 2000). Chhuneja (2008)

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reports the introgression of leaf rust and stripe rust resistance into hexaploid wheat from *Ae. umbellulata* acc 3732. It is resistant to leaf rust, stripe rust (Sears, 1956; Bansal *et al.*, 2017), stem rust (Edae *et al.*, 2016) and powdery mildew (Zhu *et al.*, 2006), salt and drought stresses (Cakmak *et al.*, 1999) have many desirable traits for hexaploid wheat improvement such as tolerance. Also, have high zinc and iron content (Wang *et al.*, 2011) and grain quality (Wang *et al.*, 2017). Wheat wild relatives (WWRs) serve as an important source of stress resiliency traits for wheat improvement. *Aegilops umbellulata* Zhuk. is one such diploid ($2n=2\times=14$) WWR with a large and repetitive U-genome similar to other *Triticeae* genomes 1:2. *Ae. umbellulata*, first reported by Zhukovsky (1928), is a self-pollinated annual grass species that grows primarily in the subtropical ecosphere. It is distributed more prominently in Turkey but has also spread to other West Asian countries along the Fertile Crescent, including Iraq, Lebanon, Iran (West), Syria (North) and Caucasus region - Azerbaijan and Armenia (Eldarov *et al.*, 2015).

Interspecific hybridization, plays a major role in improving yield, disease resistance and adaptability to abiotic stresses in bread wheat cultivars. Despite the importance of the *Ae. umbellulata* germplasm in wheat breeding, the large genetic distance between bread wheat and *Ae. umbellulata* results in cross-incompatibility, a major constraint in generating intergeneric hybrids. Further constraints arise from the difficulty of distinguishing between genuine intergeneric hybrids and self-progeny. However, crosses between bread wheat lines and *Ae. Umbellulata* have been less successful (Riley, 1966; Yu & Jahier, 1992). Successful intergeneric hybridization between bread wheat lines and *Ae. umbellulata* could therefore help further these advances in wheat production. Intergeneric hybridization is a challenging but powerful tool for broadening the genetic base in polyploid crop breeding, having resulted in new crops such as Triticale (Wilson, 1876) and Tritordeum (Martin & Chapman, 1977). Understanding chromosome behavior in intergeneric hybrids, such as chromosome addition, elimination and unreduced gametes resulting from different coexisting chromosomes, is important for practical application in introgression programs.

In addition to cytogenetic characteristics, agronomic traits of intergeneric hybrids are also important in identifying useful and undesirable genes. In wheat \times rye intergeneric hybridization, disease resistance genes from rye were introduced into wheat; however, the resulting breadmaking quality of the hybrids was inferior to that of wheat (Lowry, 1984; Macri, 1986). However, information on the potentially valuable agronomic characteristics of intergeneric F₁ populations between wheat and *Ae. umbellulata* are limited, possibly due to the very low success rate of this hybridization. Moreover, the relationship between these cytogenetic and agronomic characteristics also remains unknown, further hindering the effective utilization of intergeneric hybrids in wheat breeding.

In this study, we report, the cytogenetic and agronomic characteristics and relationships between the two in intergeneric F₁ hybrids between modern bread wheat lines and *Ae. umbellulata*. The detailed findings will contribute to effective application of *Ae. umbellulata* as a potential gene source in wheat breeding.

2. Material and methods

The plant materials used in this study were common wheat line 171ACS and *Ae. umbellulata* from a collection of Molecular cytogenetics department of Azerbaijan Genetic Resources Institute. Field works were carried out at the Absheron Research

Station of GRI. No embryo rescue or hormone treatment was applied for the production of F₀ seeds. In order to obtain intergeneric hybrids between wheat and *Aegilops* standard methods were used. Recipient plants were emasculated according to commonly accepted rules and pollinated by donor plants' pollen (Gorin *et al.*, 1968). The number of seeds set on bread wheat spikes was counted 16-20 days after pollination. Hybrid seeds were cultivated in Petri dishes to check germination ability in the autumn. After the germination ability of the seeds was identified, seedlings were transplanted into the experimental field and phenological observations were made on hybrid plants during the vegetation period. For cytological investigation of meiosis spikes from hybrid plants with anthers containing pollen mother cells were fixed (Tikhomirova, 1990). PMC was prepared by means of the standard Carnoy fixative and acetocarmine squash method.

The study of meiosis in pollen mother cells was carried out as follows: anthers were taken from the spikelet flower and placed in special containers which contained acetocarmine solution for coloring. This material was stored in the fridge and was heated several times during the day. Then, temporary slides were made from colored anthers and were studied on a light microscope Motic (China). The results obtained during the study of the meiosis process have been mathematically and statistically developed (Dospekhov, 1979; Lakin, 1990).

Mitotic chromosome preparations were made from the root tips of F₃ plants according to Molnár *et al.* (2011). Genomic DNA was isolated with the use of Quick Gene-Mini80 (FujiFilm, Tokyo, Japan) according to the manufacturer's instructions. Sequential FISH on the lines was performed as described by Molnár *et al.* (2011). The pSc119.2 and Afa-family sequences were amplified and labelled with biotin-16-dUTP (Roche) and digoxigenin-11-dUTP (Roche), respectively, using PCR (Nagaki *et al.*, 1995; Contento *et al.* 2005). The pTa71 clone was labelled with 50% biotin-16-dUTP and 50% digoxigenin-11-dUTP. Digoxigenin and biotin were detected using anti-digoxigenin-rhodamine Fab fragments (Roche) and streptavidin-FITC (Roche), respectively. The slides were counterstained with DAPI (4',6-diamidino-2-phenylindole, Amersham). A Zeiss Axioskop-2 microscope was equipped with filter sets appropriate for DAPI (Zeiss filter set 02), FITC and Rhodamin (Zeiss filter set 24) and a Spot CCD camera (Diagnostic Instruments, Sterling Heights, Michigan, USA) was used to document the hybridization signals.

3. Results

Pollen mother cells (PMCs) for studies of meiotic chromosome behavior were prepared by means of the standard Carnoy fixative and acetocarmine squash method and observed 28 chromosomes during the metaphase I in F₁ plants, as expected. It can be seen from Figure 1 and Table 1 that the amount of ring and rod bivalents, in intergeneric hybrid were very low. Thus the amount of ring and rod bivalents in F₁ plants were between 0.31 and 0.62, respectively, for each PMC. This indicates that the *chromosome pairing* is not normal and thus causes *low fertility* in the *hybrids*. Multivalent associations such as trivalents have been found (0.33) in this combination. F₁ hybrid plants of 171ACS × *Ae. umbellulata* grew vigorously and the morphological traits of F₁ hybrids were intermediate between the parental species. Although the hybrids were in an intermediate position on the height of the plant, the length of the spike and the amount of spikes in the spike, they prefer parent forms according to the number of tillers. The shape of plants resembled the wild parent - *Ae. umbellulata*. The F₁ hybrid spikelets had a morphology similar to

wheat. The leaf length and peduncle length were intermediate between two parents. The fertility of F₂ and F₃ plant was 0.66 and 11.97, respectively. *Aegilops* contribute to the evolution of cultivated wheat and are important sources of genes for wheat improvement. There exists wide genetic variation among *Aegilops* accessions.

Table 1. Mean chromosome pairing of meiotic configurations at metaphase I in meiosis of wheat × *aegilops* hybrid

Cross combination	No. cells analysed	Chromosome number in PMCs(2n)	Univalents	Bivalents		Trivalents	No. of chiasmata per cell
				Ring	Rod		
171ACS × <i>Ae. umbellulata</i>	135	28	21,15±0,55	0,31±0,13	0,62±0,31	0,33±0,12	3,90±0,30

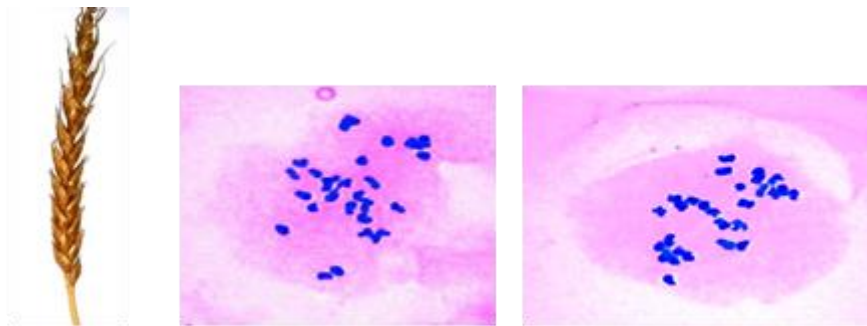


Figure 1. F₁ hybrid and its meiosis from combination 171ACS × *Ae. umbellulata*

Ae. umbellulata has been shown to carry useful genetic diversity that can be used for wheat breeding, in contrast to most diploid and allopolyploid Triticeae, that have preserved chromosome macrostructure, rye (*Secale cereale*), *Ae. umbellulata* and *Ae. caudata* genomes are highly rearranged (Danilova *et al.*, 2017). As a consequence of evolution, chromosome rearrangements in the genomes of wild relative species can disrupt the collinearity between the wild relative and the homoeologous wheat chromosomes (Zhang *et al.*, 1998). This can result in reduction or absence of meiotic chromosome pairing. Genomic rearrangements can be caused in Triticeae interspecific crosses due to gametocidal (*Gc*) chromosomes, also known as cuckoo chromosomes, which cause chromosome breakage, in the gametes that lack them

Traits related to plant and spike architecture, as well as yield, were investigated under field conditions (Table 2) for each genotypes. The spikes of the 1U(1D) substitution line have a compact structure but are significantly shorter than the spikes of the wheat parent (Figure 3, Table 2). These spikes have fewer spikelets than 171ACS, resulting in significantly lower grain numbers per spike and per plant. The plant height of the 1U(1D) substitution line is significantly shorter than that of the wheat parent and Its spikes are shorter, The number of seeds per main spikes and per plant, however, is comparable to the wheat parent, indicating good fertility.

Table 2. Morphological traits of 171ACS, wheat-*Ae. umbellulata* substitution 1U(1D), 5U(5A) and 5U(5D) lines grown in the field

Genotype	Plant height	Spike length	Spikelets per spike	Density of spike	Seeds	Spike weight	Seeds weight
171ACS	115,80±5,31	12,60±1,85	17,60±1,85	1,41±0,12	54,80±13,15	3,06±0,96	1,94±0,74
485-1U(1D)	92,8±2,99	10,40±2,08	17,40±3,61	1,70±0,35	27,00±10,88	1,02±0,07	0,78±0,19
489-1U(1D) 5U(5A)	96,0±3,40	13,0±0,45	15,80±0,75	1,21±0,05	23,00±8,00	1,48±0,37	0,70±0,26
491-1U(1A) 5U(5D)	146,8±3,97	18,60±1,43	24,20±2,23	1,30±0,09	73,00±7,45	4,14±0,53	2,70±0,24

**Figure 2.** Spike and seed morphology of 171ACS, 171ACS-*Ae. umbellulata* 1U(1D); 1U(1D), 5U(5A) and 1U(1A), 5U(5D) substitution lines

These differences in morphological traits were eliminated under high-input field conditions, with the exceptions of plant height. Although have high plant height disom substitution line (1U(1A) 5U(5D)) have more spikelets per spike thus Seeds weight from main spike was higher than wheat parent.

Plant height also has an impact on wheat yield and exploiting the role of dwarfing or reduced height (*Rht*) loci resulted in one of the most significant breakthroughs in modern plant breeding during the Green Revolution (Hedden, 2003) of the twentieth century. The *Rht1* and *Rht2*, are two of the most commonly used dwarfing genes in wheat, have been located on the 4BS and 4DS chromosome arms, respectively (Achard, 2006). In the present study, replacement of the chromosome 1D with 1U caused a 23 cm reduction in plant height in the substitution line, which may be attributed to the effect of a putative *Aegilops Rht* gene. Various plants belonging to this hybrid population were involved in molecular cytogenetic studies and the results of their GISH and FISH analyzes were comparatively analyzed. All the wheat chromosomes could be identified from the hybridization pattern of the pSc119.2 (green), Afa family (red) and pTa71 (yellow) probes.

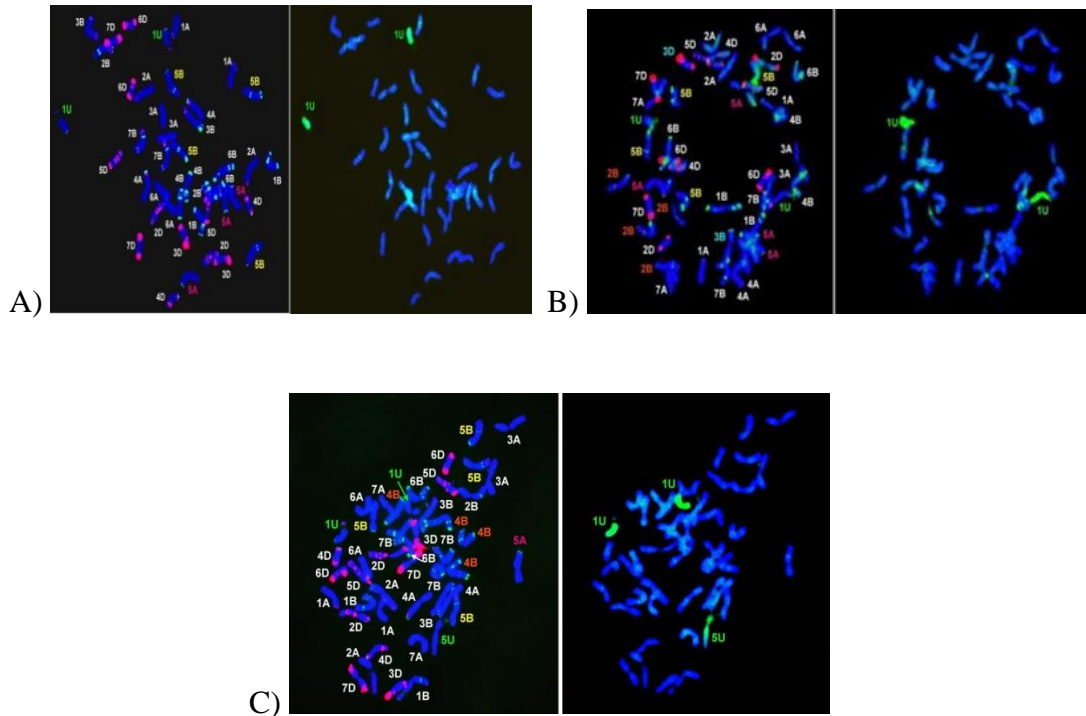


Figure 3. FISH image of a root-tip metaphase spread of chromosomes from line 171ACS \times *umbellulata* combination with probes for pTa71 (yellow) and pSc119.2 repeat (green), Afa family (red). A- $2n=43$ 1U(1D); B- $2n=46$ 1U(1D) 5U(5A); C- $2n=46$ 1U(1A) 5U(5D). Chromosomes were counterstained by DAPI (grey). Bar = 10 μ m

Based on the results of GISH and FISH analysis, hybrid plants No. 485 belonging to the combination 171ACS \times *Ae. umbellulata* ($2n=43$) were nullisomic for 7A and 1D chromosomes, trisomic for 5A, tetrasomic for 5B and also had 2 U chromosomes. Since one pair of 1U chromosomes replaces one pair of 1D chromosomes in them, those hybrid samples were considered as 1U(1D) with disomy substitution for 1U.

Another hybrid number 489 from this combination ($2n=46$) was found to have 2 U chromosomes, but it was nullisomic for 1D, monosomic for 3B and 3D and tetrasomic for 5A, 2B and 5B. Since a pair of 1U chromosomes replaced a pair of 1D chromosomes in the hybrid. It was considered 1U(1D) and 5U(5A) with a disomic substitution.

Hybrid No. 491 was ($2n=46$) were found to have 2 1U and 3 5U chromosomes, as well as being nullisome for 1D, 4D and 5D and heptasome for 5A. Since a pair of 1U chromosomes replaces a pair of 1D chromosomes in these plant, they can be considered 1U(1D) with disomy substitution for 1U. Moreover, they can be considered both disomic substitution 5U(5D) and monosomic addition according to 5U.

Lines with disomic replacement between chromosomes 1U(1B) and 1U(1D) were also obtained by Shepherd and a pair of chromosomes 1B and 1D belonging to the Chinese Spring variety of bread wheat, respectively, 1U chromosome of *Ae. umbellulata* (Shepherd, 1973). It was found that there is homeologous conjugation between chromosome 1U of *Ae. umbellulata* and chromosomes 1A, 1B and 1D of common wheat and chromosome 1U, which controls 7 subunits of gliadin, is closer to 1B and 1D than to 1A (Dvorak *et al.*, 1989). Chhuneja *et al.* (2008) found 21 bivalents, 19 ring and 2 rod, in meiosis analysis of F1 plants from a cross between the introgressed line 393-4 and the wheat variety PBW343, suggesting alien introgression. GISH analysis of line 315-5

revealed four *Ae. umbellulata* chromosomes, one submetacentric and three subterminal centromeres. GISH analysis of another introgressed line 333-4 revealed a chromosome with subterminal centromeres belonging to *Ae. umbellulata*. Du et al. (2019) obtained a wheat-*Ae. umbellulata* disom-addition line GN05 using the bread wheat variety Chinese Spring and the Y361 sample of *Ae. umbellulata*.

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