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CHARACTERISTICS OF SECOND GENERATION HYBRIDS BETWEEN WHEAT SPECIES (*TRITICUM* SP.) AND *AEGILOPS CYLINDRICA* HOST.

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Abstract

Hybrids resulting from the wide hybridization in Poaceae, posses valuable qualities such as resistance to biotic and abiotic stress factors and are important for breeding programs of wheat culture. Six different second generation hybrid accessions resulting from wide hybridization between different types of wheat (Triticum aestivum, Triticum dicoccon) and Aegilops cylindrica were examined. Three of the accessions are obtained after self-pollination of the colchicined initial hybrids (a, e, f), and the other three (b, c, d)-through backcross of colchicined hybrids with bread wheat (Triticum aestivum) pollen. Only six of all hybrid seeds germinated and five survived to adult plants. Each accession exhibits a heterogeneous morphology and/or physiology, depending on the participating parental components. While in the first hybrid generation, a partial fertility in colchicined plants and complete sterility in non-colchicined plants was observed, in the examined second generation, all tested plants are fully sterile. The complete sterility was proven by the determined heterogeneous karyotype in an observation of metaphase chromosomes during the mitotic cell division. The number of chromosomes was often uneven and varies depending on the type of hybrids (selfed, backcross) and maternal component (Triticum aestivum, Triticum dicoccon). All plant hybrid accessions of the second generation that reached maturity were completely resistant to the pathogens of powdery mildew (Erysiphe graminis) and brown rust (Puccinia recondita). This makes the hybrids involving Aegilops cylindrica valuable plant resources of resistance which could be introduced into the genome of bread wheat.

Key words: *Aegilops cylindrica, Triticum sp., wide hybrids.*

INTRODUCTION

Wide intergeneric hybridization is a classical breeding method, which is used to improve bread wheat using wild species which are phylogenetically close to the genus *Triticum*. Such species are representatives of the genus *Aegilops*, *Haynaldia*, *Agropyron*, *Elytrigia*, *Elymus*, *Hordeum*, *Secale* and others. This allows valuable genes encoding resistance and tolerance of biotic and abiotic stress to be transferred into the wheat genome (Spetsov, 1998). Furthermore, through this method cultural amphidiploids as *Triticale* (Kolev, 1978) and *Tritordeum* (Knuepfer, 2009) and also synthetic hexaploid wheats (Spetsov et al., 2008; Spetsov et al., 2009) are developed. Despite major work opportunities, wide hybridization meets a number of obstacles such as low or zero crossability, germ incompatibility, hybrid necrosis in plants derived from hybrid seeds, high sterility of hybrid plants (Stoyanov, 2012). The use of colchicine to double the chromosome number and combination of biotechnological methods solves some of the problems of wide hybridization and makes it an effective method of creating varietal diversity (Ayala and Kiger, 1987).

Aegilops cylindrica ($2n = 4x = 28$, CCDD) is one of the closest to bread wheat species, which is coextensive with it in the process of the evolutionary shaping the *Poaceae* family (Matsuoka, 2011). Both are donors of the D genome, which with regard to their phylogenetic development, is derived from *Aegilops tauschii* ($2n = 2x = 14$, DD) (Nakai, 1981). This generates conditions for higher crossability and stability of hybrid plants of hexaploid species *Triticum aestivum* ($2n = 6x = 42$, AABBDD) with *Aegilops cylindrica*, than the tetraploid wheat species *Triticum durum* and *Triticum dicoccon* ($2n = 4x = 28$, AABB) with *Aegilops cylindrica* (Stoyanov et al., 2011). However, hybrids involving *Aegilops cylindrica* are characterized by extremely high or 100% sterility (Stoyanov et al., 2011). For such sterility of obtained hybrids reports Cifuentes and Benavente (2009), Shoenenberger et al. (2006), Rehman et al.

(2006), regardless of species and parental origin of the involved component of *Triticum*.

Aegilops cylindrica is a donor of high resistance to some races of the pathogen of powdery mildew (*Erysiphe graminis*) and brown rust (*Puccinia recondita*), and complete resistance to pathogen of septoria leaf blight (*Mycosphaerella graminicola* / *Septoria tritici*) (Spetsov et al., 2006). Such resistance also exhibit hybrids where the wild species is involved. These characteristics as well as the ability to transfer resistance to a number of other stress factors such as metal toxicity (Landzheva et al., 2003), drought tolerance (Plamenov, 2003), make them a valuable resource for gene transfer in bread wheat. At the same time the strong gametocydial effect of *Aegilops cylindrica* occurring in the first generation of hybrid plants (Hohman et al., 1995) hampers breeding work. This requires careful studying the morphological, physiological and cytological characteristics of the different generations of the hybrids, in order to be properly used as a starting breeding material. The similarities and differences should also be set out in relation to the impact of different accessions involved in the crosses.

The aim of this report is to be described some morphological (coleoptile color, type of the habitus, spikes fragileness, seedset), physiological (flowering type, fertility, resistance to powdery mildew, brown rust and septoria leaf blight) and cytological (chromosome number) characteristics of the second generation hybrid plants between different types of wheat and *Aegilops cylindrica* and to assess their suitability as a starting material to be included in the breeding programs of the bread wheat.

MATERIALS AND METHODS

Seeds obtained from six second generation hybrid of wheat species with *Aegilops cylindrica* were used. Hybrids and seeds number are presented in Table 1. Scheme of obtaining hybrids is presented on Figure 1. Seeds of each hybrid were germinated in Petri dishes in an incubator at a temperature of 20ºC within 5 days. Young plants were acclimatized for 3 days at a room temperature for better development of the germ roots. Plants are planted in ceramic pots per hybrid types and are grown under unregulated greenhouse conditions. Second generation hybrid plants are not treated with colchicine solution.

Table 1. Hybrids and seeds between Triticum sp. and Aegilops cylindrica

No	Code	Hybrid	Type	Seeds
1	a	C1 (91683 x ACC46)	sp	1
2	b	F1 (F1k (91683 x ACC46) x Yanitsa)	bc	4
3	c	F1 (F1k (Yanitsa x ACC46) x KO/4-2)	bc	2
4	d	F1 (F1k (Goritsa x ACC46) x Enola))	bc	1
5	e	C1 (Goritsa x ACC46)	sp	1
6	f	C1 (Goritsa x ACC46)	sp	1

sp – selfed, bc – backcrossed.

Seeds of each hybrid were germinated in Petri dishes in an incubator at a temperature of 20ºC within 5 days. Young plants were acclimatized for 3 days at a room temperature for better development of the germ roots. Plants are planted in ceramic pots per hybrid types and are grown under unregulated greenhouse conditions. Second generation hybrid plants are not treated with colchicine solution.

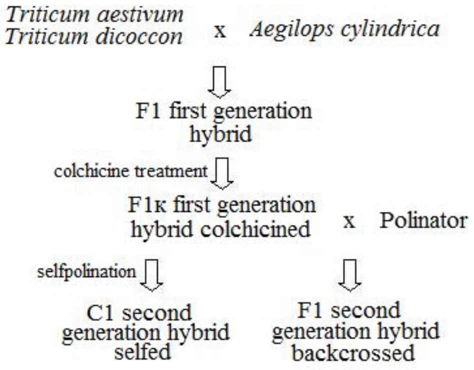

Figure 1. Scheme of hybrids' obtaining

Before planting from any offspring have been taken two of the three germ roots 1-1.5 cm long for cytological studies. Roots are dried on filter paper and fixed in 96% ethanol and glacial acetic acid (3:1) overnight (12 hours) at 4-5ºC. The fixed material was stained with 0.5% acetocarmine following a standard methodology (Georgiev et al., 2008). To establish the chromosome number, microscopic slides are prepared using standard methodology developed in Dobrudzha Agricultural Institute-General Toshevo. For each hybrid microscopic slides were prepared from which chromosomes of 10 well structured and maintained metaphase groups were counted using 400-fold zooming in. The number of chromosomes was averaged per hybrids.

Determination of phytopathogens attack was carried out under conditions of unregulated greenhouse and natural occurrence of pathogens: for powdery mildew (EG) using the methods of Stoilova and Spetsov (2006) for brown rust (PR) methodology of Ivanova (2012); for septoria leaf blight (ST) using the methods of Eyal et al. (1987). Reporting is done by established resistant (R), medium resistance (M) and sensitive (S) accessions. Evaluation is carried out in accordance with standard varieties for susceptibility to powdery mildew (*Erysiphe graminis*)-Sadowska ranozreyka, brown rust (*Puccinia recondita*)-Michigan Amber, septoria leaf blight (*Septoria tritici*)-Enola.

For each hybrid plant coleoptile color of young plantlets and the type of habitus-retracted, open, intermediate were defined, it is also made a comparative characteristics between different types of hybrids. The spike fragileness was estimated in comparison with the spikes of *Aegilops cylindrica*. An assessment of the flowering of the second generation hybrid plants-open or closed was also made.

From each hybrid plants two spikes were selected, which are isolated with paper isolators for selfing and determining male fertility/sterility. Two other spikes were castrated and baccrossed with pollen of wheat variety Enola for determining the female sterility/fertility. The other spikes are left without isolators to examine the natural possibility of receiving grains of the second generation hybrids. For each hybrid an analysis of male, female and natural sterility/fertility is made by counting the seedset.

An analysis of the normal course of meiosis in the previous generation is made and the chromosome coefficient for each hybrid is calculated as a ratio of the real average chromosome number (RChN) to the expected number of chromosomes (EChN) in the second generation hybrids. The expected chromosome number is calculated based on parental forms involved in the crosses.

RESULTS AND DISCUSSIONS

The results of the morphological characteristics of second generation hybrids involving *Aegilops cylindrica*, are presented in Table 2 and Table 3. From the tables it can be established that according to the studied properties coleoptile colour (CC), seedset (SS), habitus type (HB), spike fragileness (SF), flowering type (FT) and (stamens mode) SM, the plants grown do not differ significantly. This is probably due to the identity of the cross (Gorica x ACC46). Although one of the studied accession is selfed and the rest are baccrossed with bread wheat pollen, the morphology of pollinator apparently remains discreet. In the first generation hybrids the impact of maternal component on the morphological characteristics of plants is clearly highlighted (Stoyanov et al., 2011).

Table 2. Germination characteristics of hybrid plants

No	Hybrid	SSN	GSN	SvPN	CC
1	a	1	1	1	green
2	b	4	4	4	green
3	c	2	0	-	-
4	d	1	0	-	-
5	e	1	0	-	-
6	f	1	1	0	green

SSN – sown seed number, GSN – germinated seed number, SvPN – survived plants number, CC – coleoptile colour.

A characteristic feature of all grown second generation hybrids plants is that their flowering is an open type but stamens apparently do not burst. They are small, distorted, when grinding they do not release normally developed pollen, which indicates the presence of variations in the generative processes, and in particular variations in meiosis. Lack of seedset in all collected spikes of hybrid plants also involves abnormal generative processes.

Open habitus type and fragile spikes are typical of the first generation hybrid plants (Stoyanov et al., 2011; Stoyanov, 2010). The similarities between the two generations and the dominance of the morphological features of the paternal component in the crosses (ACC46) even into the backcrossed accessions, is indicative of the possibility of amphiploidisation, but due to its strongly gametocydial action of the imported genetic material from *Aegilops cylindrica*, the subsequent reproducing is prevented.

Table 3. Morphologic characteristic of hybrid plants

No	Hybrid	HB	SF	SS	FT	SM
1	1b	open	fragile	0	open	unburst
2	2b	open	fragile	0	open	unburst
3	3b	open	fragile	0	open	unburst
4	4b	open	fragile	0	open	unburst
5	f	open	fragile	0	open	unburst
6	ACC46	open	very fragile	0	closed	burst

HB – habitus type, SF – spike fragileness, SS – seedset, FT – flowering type, SM – stamens mode.

Rehman et al. (2006) reported zero seedset in hybrids *Triticum aestivum* x *Aegilops cylindrica* first generation and the inability to obtain second generation hybrids. Shoenenberger et al. (2005, 2006) succeeded in producing seeds in F1 and obtaining the second and third generation hybrids, but using the reverse cross (*Aegilops cylindrica* x *Triticum aestivum*) and backcrossing the hybrid plants with pollen of *Aegilops cylindrica*. The same authors state that the third generation self-pollinated hybrids are possible to obtain. Such results highlight the depressant effect on wheat chromosomes of the genes from *Aegilops cylindrica*. The stabilization of the genome of the wild species by repeatedly baccrossing of the quoted authors allows more consistent generation of hybrids to be achieved.

With regard to the sterility of second generation hybrids, all plants are 100% male and female sterile. Seedset is not observed either in isolated spikes or in baccrossed with pollen from Enola. Natural sterility, which is seen as the complex influence of all factors on the growing conditions is also 100%. This indicates that the studied plants are completely unable to reproduce. The data are presented in Table 4. From the data, and from morphological characteristics can be concluded that in the studied hybrids deviations in the mechanism of generative processes are observed.

Shoenenberger et al. (2006), in a second inbred generation hybrids succeed to achieve fertility in two samples-0.16% and 5.21% respectively. The resulting fertility rates are again in relation to baccrossed hybrids with *Aegilops cylindrica* from the reverse cross (*Aegilops cylindrica* x *Triticum aestivum*). The authors point to the very high levels of fertility (up to 87.7%) in the third generation hybrids after self-pollination. Wang et al. (2005) also reported the presence of fertility in baccrossed second generation hybrids and selfed-ones in the third generation.

Table 4. Physiological characteristics of hybrid plants

No	Hybrid	MS,%	FS,%	NS,%	TS,%	EG	PR	ST
1	1b	100	100	100	100	R	R	R
2	2b	100	100	100	100	R	R	R
3	3b	100	100	100	100	R	R	R
4	4b	100	100	100	100	R	R	R
5	f	100	100	100	100	R	R	R
6	ACC46	-	-	-	-	M	M	R
7	SR	-	-	-	-	S	M	M
8	MA	-	-	-	-	M	S	M
9	EN	-	-	-	-	M	M	S

MS – male sterility, FS – female sterility, NS – natural sterility, TS – total sterility, EG – powdery mildew, PR – brown rust, ST – septoria leaf blotch, SR – Sasovska Ranozreika, MA – Michigan Amber, EN – Enola.

The high degree of sterility of the observed second generation hybrids is directly dependent on the reduced chromosome number and the presence of variation in chromosome number reported in microscopic observations. Table 5 presents data on the cytology features of the three germinated hybrid accessions. The table shows that in all investigated samples is observed as an even and odd number of chromosomes, which is indicative of unequal cell division, and thus the physiological difficulties most clearly expressed in the accession ‘a’, which died in phase ‘first leaf’. Although all three samples hybrids were obtained in different ways and with different maternal parental forms, heterogeneous set kariotypes were formed under the influence of the same accession of *Aegilops cylindrica*. The difference between the expected chromosome number and reported real chromosome number defined the wrong course of meiosis in the first generation hybrid plants. This is emphasized by the chromosomal coefficient that clearly differs from 1.00 i.e. normal course of cell division (ChC ≠ 1.00).

Shoenenberger et al. (2006) also described a strong variation in chromosome number in the second generation hybrids. While the first generation hybrid plants all had 35 chromosomes, in the second their number is between 30 and 84.

Table 5. Cytological characteristics of hybrid plants

No	a	b	f
1	56	49	68
2	55	47	67
3	55	49	69
4	56	47	69
5	54	48	70
6	53	48	70
7	56	49	68
8	56	48	69
9	55	49	68
10	55	47	69
AChN	55	48	69
EChN	56	49	70
ChC	0.9821	0.9796	0.9857
SD	0.9944	0.8756	0.9487

AChN – average chromosomes number, EChN – expected chromosomes numer, ChC – chromosome coefficient, SD – standard deviation.

The presence of the same chromosome number is not indicative of the presence of sterility, as these authors reported partial fertile and fully sterile plants, each possessing 41 chromosomes. The same study reported no difference in the chromosome number of the same hybrid and the same microscope slide. In the hybrids studied by us, different numbers of chromosomes were observed even at the cellular level. These results identify significant deviations in generative processes of hybrids a, b and f. Crémieux (2000) reported 28 to 49 chromosomes in second generation backcrossed hybrids. For similar results reported Cifuentes and Benavente (2009) in hybrids involving *Triticum durum*. Sears (1943) states that in *Triticum* hybrids with *Aegilops cylindrica*, deviations resulting in a reduction of chromosome number in the second generation backcrossed hybrids are mainly due to improper interaction between wheat genome and that of *Aegilops cylindrica*, and misallocation of bivalents and univalents in meiotic division in the first generation hybrids.

Despite the negative impact of the genome of the wild species, the studied second generation hybrids showed very good pathogen resistance. At all stages of their development to their maturity all grown accessins were completely resistant to the pathogen of powdery mildew, brown rust and septoria leaf blight in the native represented races and forms into the growing conditions. These results are proved by the presence of typical symptoms of the three diseases on standard varieties for susceptibility to the pathogens.

CONCLUSIONS

From these results the following conclusions could be drawn:

- Second generation hybrids between wheat species and *Aegilops cylindrica*, are characterized by similar morphology resembling that of wild species.
- Flowering of the all hybrids plants is an open type, and stamens do not burst, which defines zero seedset.
- Studied hybrids are 100% sterile, as evidenced by the non-homogeneous chromosome number.
- Although differences in their obtaining and involved mother component, the chromosomal aberrations are caused by improper interaction between wheat genome and that of the wild species.
- Despite their negative qualities, all studied accessions are characterized by complete resistance to the pathogen of powdery mildew, brown rust and septoria leaf blight, which makes them a good starting material for inclusion into the bread wheat breeding programs.

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