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CHARACTERISTICS OF NATURAL HYBRIDS BETWEEN *Aegilops cylindrica* HOST. AND COMMON WINTER WHEAT (*Triticum aestivum* L.)

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Abstract

Natural hybrids between wild species in Triticeae and common winter wheat (Triticum aestivum) are important part of breeding due to the mechanism of their creation, and because of the opportunity various qualities from the wild species to be more easily transferred into the genome of cultural plant. At the same time their detection in natural environment is difficult due to limited opportunities for interspecific and intergeneric pollination, fertilization and seed formation. One of the most common wide natural hybrids in wheat agrocenoses resulting from crosses between Aegilops cylindrica as a maternal component and bread wheat as a paternal component. In the survey conducted during 2008 the natural hybrid EXO F1 (Aegilops cylindrica x Triticum aestivum) was found, and its spikes were collected and analyzed for the presence of fertility. From the 10 found seeds only one seed germinated in 2009, and developed into second generation hybrid plant (EXO F2) which reached maturity. The flowering is an open type and continues for a long period of time. Obtained 11 spikes were observed and all of them were determined as completely sterile. Throughout the period of its growth EXO F2 does not give indications of attack by pathogens of powdery mildew and brown rust. There have not been monitored any symptoms and lesions of the same pathogens when collecting EXO F1. Natural hybrids were distinguished by resistance to herbicides transferred from cultural plant because EXO F1 was found in agrocenosis, where treatment against all weed groups was applied. Despite their negative specifications, natural hybrids are a valuable source of genes encoding resistance to biotic and abiotic stress and they could become a good source of breeding material.

Key words: *Aegilops cylindrica, natural hybrids, Triticum aestivum L.*

INTRODUCTION

Bread wheat (*Triticum aestivum* L.) is one of the most important food crops worldwide. The main task facing any economy is the sustainable production of this crop, coupled with continuous improvement in yield and quality of the production. This should be achieved through the development of new and improvement of existing technologies for growing, but also by using the classical breeding methods - creation of new varieties. In front of the modern breeders prime task is to develop lines and varieties that combine high yield and quality of bread wheat, but also the creation and introduction of genetic resistance to economically important pests. In this regard, searching, selection and use of suitable starting breeding material is the most serious problem for the wheat breeding. As promising in this regard are natural hybrids of bread wheat.

The occurrence of natural hybrids between wheat cultivars and their phylogeneticaly related species allows much more easily qualities of the wild type as resistance to pathogens and pests (Spetsov, 1998; Plamenov, 2003), cold resistance, metal toxicity (Landzheva et al., 2003) and others to be transferred into other cultures. Natural wide hybridization is a process in which the species incompatibility was eliminated which leads to biodiversity and a new combination of the genetic material. This process could be regarded as completely random, but on the other hand it could also be regarded as a mechanism of plant evolution (Ayala and Kiger, 1987). The result of such a process is the creation of bread wheat. By combining *Triticum urartu* (2n = 2x = 14, AA) and *Aegilops speltoides* (2n = 2x = 14, BB (SS)) is obtained tetraploid species *Triticum turgidum* ssp. *dicoccoides* (2n = 4x = 28, AABB), which is subsequently crossed with *Aegilops tauschii* (2n = 2x = 14, DD) which gives the beginning of the hexaploid wheat (Stoyanov et al., 2010). This multi-level process does not go through

intercourse alone and unilaterally, but over a long period of time. Along with the parallel polyploidisation similar species are separated (Matsuoka, 2011). This is the reason why the same subgenomes can be found in polyploid representatives of *Triticeae* (Nakai, 1981). The result of the natural process of wide hybridization is a wide variety of polyploid species in the same family. However, natural hybridization as a speciation mechanism should not be taken as strictly logical as the creation of new species is the result of a combination of other natural factors (natural mutation and natural disturbances in mitosis and meiosis, plant-plant interactions, plant- pests interactions, etc.).

In contrast, species formation, which occurs over a long period of time, natural hybridization occurs in agroecosystems more often, but relatively discreet. This is due to the fact that often the resulting natural hybrids are completely sterile, and if not, then the seed is unviable (Ayala and Kiger, 1987). Often hybrid in wheat agroecosystem is *Aegilops cylindrica* x *Triticum aestivum*, due to the prevalence of the wild species, especially in meadows, shelterbelts, ditches and around roads located near the wheat crop (Stoyanov, 2009; Stoyanov, 2012). The ability to transfer valuable genes from *Aegilops cylindrica* into bread wheat, but also the possibility of negative transfer of genes in wild species (Gandhii, 2006) (herbicide resistance) that might make it too invasive, is a prerequisite natural hybrids to be well researched, not only from breeding but also agronomic point of view.

The purpose of this report is to describe the morphological and physiological characteristics of first and second generation of natural hybrids between *Aegilops cylindrica* and *Triticum aestivum* and to assess their suitability as a starting breeding material and to determine the transferability of negative characteristics of bread wheat into the wild species.

MATERIALS AND METHODS

In the process of investigation for establishing the representation of wild grasses in the Bulgarian Black Sea coast in the period 2000-2008, during the expedition in 2008 natural hybrids in wheat agroecosystems are found. All plants were collected during June-July, since it is the period of maturity of the representatives of natural hybrids with bread wheat. When collecting samples as the primary method of distribution, visiting roadside habitats of wild-type and meadows is used where no explicit human activity and agricultural processes are found, but they are close to wheat agroecosystems. The discovery of natural hybrids is conducted by searching near or in fields of wheat, or at the border between lawn and meadow and steppe ecosystems and cultural plantations.

Plants from natural hybrids are evaluated for detection of pathogenic infection, for powdery mildew (EG) using the methods of Stoilova and Spetsov (2006) for brown rust (PR) using the methods of Roelfs et al. (1992) for early leaf blight (ST) using the methods of Eyal et al. (1987).

It is reported the presence/absence of wild type near the hybrid plants and assessment of the herbicide resistance of hybrids is made.

Collected spikes of natural hybrids are sorted and analyzed for the presence of pathogens. A structural analysis of the spikes is made and the number of spikelets in the spikes, number of flowers in spikes, number of grains in the spikes are reported. It is also reported the fertility of each spike. The data is averaged for the natural hybrid assemblies. The standard deviation and coefficient of variational are reported. The hybrid morphology of collected samples is described.

The collected seeds are germinated in Petri dishes. Young seedlings are planted in ceramic pots and grown under unregulated greenhouse conditions. During the vegetation the attack of powdery mildew, brown rust and septoria leaf blight are estimated following the methods stated above. A parallel with standard varieties of susceptibility is made - to powdery mildew (*Erysiphe graminis*) - Sadowsky ranozreyka, brown rust (*Puccinia recondita*) - Michigan Amber, septoria leaf blight (*Septoria tritici*) - Enola.

The spikes of second generation hybrid are collected in full maturity and analyzed for the same parameters as well as spikes found from the first generation hybrid plants.

RESULTS AND DISCUSSIONS

In the expeditions to establish the distribution of wild cereals, a natural hybrid of *Triticum aestivum* (wheat) and *Aegilops cylindrica*, is found labeled as EXO F1. Figure 1 shows the natural EXO F1 hybrid involving *Aegilops cylindrica* at wheat agroecosystems. Clearly are seen the differences between the hybrid plant and bread wheat. It was of a larger height, with a pronounced luxuriance. There is relatively good tillering.

Figure 1. Hybrid EXO F1 in wheat agroecosystem

Figure 2 presents the collected natural hybrid spikes from EXO F1, and in Figure 3 is a comparative characteristic between the spikes of the hybrid and both parental forms. In the spikes collected from the hybrid the length is apparently almost twice of that of bread wheat and *Aegilops cylindrica*. Moreover, the spikes are straight and cylindrical, like the wild species, but are also wider. Awns are concentrated at the top and they are short and straight. There are differences between single spikelets. In *Aegilops cylindrica* spikelets are slender cylindrical, with a cut top, while those of hybrids are more compact similar to these of synthetic hexaploid wheats (Spetsov et al., 2008; Spetsov et al., 2009; Stoyanov et al., 2010). Figure 4 presents comparative characteristics of the seeds from hybrid and both parental forms. The seeds of hybrid plants differ significantly from those of common wheat, but there are also differences from the seeds of *Aegilops cylindrica*. They are highly shriveled as a result of differentiation of the hybrid endosperm. The size of the seeds compared to those of bread wheat is considerably smaller. The grain of the hybrid plant is separated from the glumes more difficultly compared to the parental forms (especially wheat). Grain scutellum is round, like this of bread wheat, and opposed to oblong-elliptical shape of the grain scutellum of *Aegilops cylinrdica*. The central crease of the grains is more expressed compared with the seeds of wild species. Epidermal hairs of the hybrid grains are similar to these of bread wheat.

In 2008 there are found a large number of spikes of natural hybrid EXO F1 - 50, from which 10 seeds were obtained. Data from structural analysis of spikes are presented in Table 1. High degree sterility is established in relation to the studied hybrid - 99.22%. Similar data were reported by Shoenenberger et al. (2006), in deliberate crosses between *Aegilops cylindrica* with *Triticum aestivum* - 99.70-99.97%. With regard to morphological characteristics of the spikes, there is little variation within 13%, which demonstrates normal physiological processes occurring in the first hybrid generation. For high degree of sterility in similar hybrids reported Wang et al. (2000a), Cifuentes and Benavente (2009), Shoenenberger et al. (2005), Sears (1943).

On the collected plants any symptoms of pathogens attack of powdery mildew, brown rust and septoria leaf blight are not found that determines EXO F1, as completely resistant to these diseases.

Figure 2. Spikes of EXO F1

Table 1. Spike characteristics of EXO F1

No	SN	FN	OSN	Ft
1	14	28	0	0.00
2	12	24	0	0.00
3	13	26	0	0.00
4	12	24	0	0.00
5	12	24	0	0.00
6	11	22	0	0.00
7	16	32	1	3.13
8	14	28	0	0.00
9	14	28	0	0.00
10	15	30	1	3.33
11	13	26	0	0.00
12	13	26	0	0.00
13	16	32	2	6.25
14	14	28	0	0.00
15	12	24	0	0.00
16	12	24	0	0.00
17	16	32	1	3.13
18	13	26	0	0.00
19	16	32	0	0.00
20	15	30	1	3.33
21	11	22	0	0.00
22	14	28	1	3.57
23	12	24	0	0.00
24	14	28	0	0.00
25	14	28	0	0.00
26	12	24	0	0.00
27	12	24	0	0.00
28	14	28	0	0.00
29	15	30	1	3.33
30	14	28	0	0.00
31	14	28	0	0.00
32	11	22	0	0.00
33	14	28	0	0.00
34	13	26	0	0.00
35	12	24	0	0.00
36	11	22	0	0.00
37	13	26	0	0.00
38	14	28	1	3.57
39	9	18	0	0.00
40	10	20	0	0.00
41	12	24	0	0.00
42	11	22	0	0.00
43	13	26	0	0.00
44	11	22	0	0.00
45	11	22	0	0.00
46	11	22	0	0.00
47	12	24	0	0.00
48	14	28	1	3.57
49	11	22	0	0.00
50	12	24	0	0.00
Total	644	1288	10	0.78
AV	12.88	25.76	0.20	0.66
SD	1.65	3.30	0.45	1.49
VC	12.80%	12.80%	-	-

SN – spikelets number, FN – florets number, OSN – obtained seeds number, Ft – fertility; AV – average value; SD – standart deviation; VC – variation coefficient.

From the hybrid EXO F1 seeds set to germination, only one germinated producing EXO F2, and the remaining seeds have non-viable embryo. Similarly to EXO F1, EXO F2 during its vegetation exhibits no symptoms of attack by powdery mildew, brown rust and septoria leaf blight. In both hybrids, the resistance is due to the imported genome from the wild species.

Singh et al. (2004) state that *Aegilops cylindrica*, possesses the genes for resistance to brown rust, which could be successfully introduced into the genome of bread wheat. Simultaneously Stoyanov et al. (2011) reported resistance to powdery mildew in all phases of the development in hybrids of bread wheat with *Aegilops cylindrica*. In our observations during the period 2009-2012 *Aegilops cylindrica* was not attacked by septoria leaf blight (personal communication), which is indicative of the absence of infection by the pathogen on the hybrid plants.

Table 2. Spike characteristics of EXO F2

No	SN	FN	OSN	Ft
1	18	36	0	0.00
2	18	36	0	0.00
3	13	26	0	0.00
4	17	34	0	0.00
5	18	36	0	0.00
6	14	28	0	0.00
7	14	28	0	0.00
8	16	32	0	0.00
9	9	18	0	0.00
10	14	28	0	0.00
11	14	28	0	0.00
Total	165	330	0	0.00
AV	15	30	0	0.00
SD	2.76	5.51	0.00	0.00
VC	18.38%	18.38%	-	-

SN – spikelets number, FN – florets number, OSN – obtained seeds number, Ft – fertility; AV – average value; SD – standart deviation; VC – variation coefficient.

In relation to the morphology of the EXO F2 the spikes almost resemble that of EXO F1 (Figure 5). During the flowering, all florets of EXO F2 are opened, which assume an inability of the plant to self itself. In this case, the stamens are rudimentary and do not burst.

Without further pollination EXO F2 hybrid exhibits 100% male sterility. This is evidenced by the lack of seedset into the spikes. The data are presented in Table 2. There is low variation

in the morphology of the spikes as close to that of EXO F1.

Figure 3. Spikes of: *Aegilops cylindrica* (left); hybrid EXO F1 (center); *Triticum aestivum* (right).

Figure 4. Seeds of: Hybrid EXO F1 (up, left); *Aegilops cylindrica* (up, right); *Triticum aestivum* (down)

Figure 5. Spikes of EXO F2

An interesting feature of EXO F1 is that it is found in wheat agrocenosis but the wild type was observed quite far from the hybrid. This is indicative of treatment with herbicides to the crop, which excludes the presence of samples of *Aegilops cylindrica* close to hybrid plants. The hybrid is not affected which revealed imported resistance to herbicides from bread wheat. Perez-Jones et al. (2006) reported the ability to transfer a gene for resistance to herbicides based on imidazolin, because these genes are located in the D-genome of bread wheat. *Aegilops cylindrica* also possesses D-genome, allowing genes easily to be exchanged into the hybrid plants. Shoenenberger et al. (2006) reported high fertility of baccrossed hybrids with pollen of the wild species after subsequent self-pollination. Gandhi et al. (2006) indicate that the sterility of hybrid plants decreases proportionally to the generation of natural hybrids in the presence of a natural process of backcross with the wild species. This exhibits a high degree of risk of increased invasiveness of *Aegilops cylindrica* as a weed in wheat crops due to increased gene flow from bread wheat to the wild species (Morrison et al., 2001; Wang et al., 2000b) and genetic material interchange (Avsenin et al., 2003).

CONCLUSIONS

Considering those results, the following conclusions could be drawn:

Studied natural hybrids involving *Aegilops cylindrica* are characterized by a high degree of male sterility, which, depending on the hybrid generation, ranged from 99.22 to 100%.

Collected natural hybrids have alleged resistance to herbicides which is not proven, but it is potentially dangerous due to existing gene flow from bread wheat to *Aegilops cylindrica*.

Natural hybrids involving *Aegilops cylindrica* are characterized by complete resistance to the pathogen of powdery mildew brown rust and septoria leaf blight, making them a potentially good source material for inclusion into the bread wheat breeding programs.

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