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Review

Status of remote hybrids in the *Poaceae*: problems and prospects

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Abstract. *Wide hybridization as a tool of classical breeding is a method which is successfully applied in the development of breeding programs in cultural plants. Its application is a particularly effective means to overcome the effects of biotic and abiotic stress in cultural species in Poaceae. Through wide hybridization genes for resistance to phytopathogens, insect pests, tolerance to the toxicity of aluminum, copper, cadmium are successfully transferred. The method has also high efficiency in breeding with regard to cold resistance, drought tolerance, lodging, plant height. This was achieved thanks to the phylogenetic proximity of bread wheat (Triticum aestivum) with wild species as representatives of Aegilops, Haynaldia, Agropyron, Elytrigia, Elymus, Leymus. Wide hybridization is useful in order to obtain haploid plants resulting in valuable homozygous lines. The utilization of crosses as Triticum x Zea, Triticum x Imperata, Triticum x Pennisetum allows rapid elimination of the paternal chromosomes in the meiotic division. The method of wide hybridization was successfully used to combine the genomes of valuable cultivars. Thus amphidiploid cultural species as Triticale (Triticum x Secale) and Tritordeum (Triticum x Hordeum) are successfully obtained. However, wide hybridization is related to the problems of heredity. The low percentage of crossability due to incompatibility of the stigmas of maternal plants and pollen of the paternal plants set the large number of crosses that should be handled. In many crosses there are different types of genomic incompatibility – preembryonic (receiving reduced seedset), embryonic (low percentage of germination of the seeds) postembryonic (high degree of sterility of the obtained hybrids). Low viability and the hybrid necrosis is also an essential disadvantage of wide hybrids. Using techniques such as colchicine treatment, anther culture and embryo culture are means to overcome difficulties in wide hybridization and make it a promising method of breeding in the Poaceae.*

Keywords: *Poaceae*, resistance, tolerance, wide hybrids, winter wheat

Introduction

The family of the cereals *Poaceae* covers many cultural plant species. Numerous representatives of the genera *Triticum*, *Hordeum*, *Secale*, *Zea*, *Panicum*, *Sorghum*, *Oryza*, allow the obtaining of various products to meet the greatly varied food needs of man. It is known that the production of cereals has a share of over 60% of the world crop, which is indicative to the importance of this group. This is due to peculiarities of the physiology and morphology of grain cereals, and the relatively simple farming practices thereof. The feature of cultural cereals is that they are determined by a high content of vital components of food (Terziev, 2006), and high ecological plasticity, which allows they to be widely cultivated (Popov, 1964). The features that determine the prevalence and effective use of these crops by humans should be associated with the evolution of the family *Poaceae*. The typical phylogenetic development of the group and the associated specific ontogenetic development of the grain cereals is the cause of similarities in terms of agrotechnology, direction of use, breeding programs as well.

However, with the grain cereals, in the process of their phylogenetic specialization, phytopathogens, insects and weeds inevitably have accompanying agroecosystems that have also evolved. This arises the necessity to seek solutions to overcome the influences of biotic factors. Economically important diseases of cereals, which could significantly reduce yields and decrease the quality characteristics are powdery mildew (*Erysiphe graminis*), species of rust (*Puccinia* sp.), smuts and bunts species (*Tiletia* sp., *Ustilago* sp., *Urocystis* sp.), ergot (*Claviceps* sp.), *Fusarium* head blight (*Fusarium* sp.), *Septoria* leaf blotch (*Septoria tritici*, *Stagonospora nodorum*). The group of insect pests is significantly differentiated in relation to species of the group of cereals, and there are significant differences of enemies on *Pooidae* (*Triticum*, *Hordeum*, *Secale*, *Avena*), *Panicoidae* (*Panicum*, *Zea*, *Sorghum*) and *Ehrhartoidae* (*Oryza*). Except biotic factors it is essential to seek effective tools for combating the adverse abiotic environmental factors which, by their dynamic values, significantly affect the productive potential of the crops. Especially harmful effects are due to damage from high and low temperatures, logging, and heavy metal toxicity. All these factors imply a considerable amount of breeding work, which is necessary for the development of resistant and tolerant forms, ensuring production which is capable to meet the increasing and growing worldwide demand.

An effective method for overcoming the influence of biotic and abiotic stress factors is wide hybridization. As an instrument of classical selection, the typical feature of this method is the potential transfer of valuable genes encoding different types of resistance and tolerance to environmental factors (Stoyanov et al., 2011), and also to combining heritable material by creating amfidiploid cultivars. Wide hybridization uses as starting breeding material different plant species belonging to the same genus (interspecific hybridization, such as *Triticum turanicum* x *Triticum timopheevi*) and between plant species belonging to different genera (intergeneric hybridization, such as *Triticum aestivum* x *Aegilops cylindrica*). As a factor determining species formation, heredity is a barrier to the development and obtaining of wide hybrids and hybrid forms. This is due to interspecific incompatibility related to the unique physiology and morphology of new plant organism. Difficulties in hybridization could occur; on the crossing level: caused by the incompatibility of pollen and stigmas of the two plant species, at fertilization and embryo development level (preembryonic incompatibility) caused

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by the incompatibility of gametes and their inability to create a functional zygote; at germination level (embryonic incompatibility) caused by the inability of the embryo to develop as a result of reduced physiological incompatibility between it and the endosperm; at germinated plant level (postembryonic incompatibility) due to the difficulties in physiology (hybrid necrosis) and meiotic disturbances (high degree of sterility). The occurrence of incompatibility is strictly dependent on combinations of maternal and paternal components in the cross. As species are phylogenetically more distant, the crossability between them is lower and hybridization is more laborious.

Wide hybridization is an effective method and it is also connected with solving other problems of classical breeding. Via crossing the embryonic incompatible species such as *Triticum aestivum* x *Zea mays*, *Triticum aestivum* x *Imperata cylindrica*, haploid plants are obtained because of the elimination of the chromosomes from the pollinator after a few cell divisions of the original zygote. The resulting haploid could be effectively used for producing genetically aligned lines without holding an extended genotype selection.

Overcoming the difficulties associated with wide hybridization is an issue the solution of which allows the diversification of gene transfer in cultural plants. Somatic hybridization is an efficient process to overcome the problems associated with low rates of crossability and preembryonic incompatibility. Thus, even the somatic hybrids *Triticum* x *Pisum* and *Oryza* x *Pisum* (Bajaj, 1983) have been created at the cellular level. In relation to embryonic incompatibility numerous techniques have been developed using tissue cultures. By setting isolated embryos or seeds in a suitable growing medium a fairly good percentage of germination is obtained (Kolev, 1978). Postembryonic incompatibility associated with high levels of sterility of the resulting hybrid plants due to disturbances in the processes of meiosis are effectively overcome by employing doubling of the chromosome number via different chemical compounds. Effective in this case are colchicine, orizalyne, trifluraline (Ayala, 1987). These methods outline the wide hybridization and wide hybrids as a viable source for improving agricultural production by increasing the quantitative and improving the qualitative indicators of cultivated plants.

Creation of wide hybrids aiming transferring biotic and abiotic stress resistance and tolerance genes

In general

Wide hybridization and products of its implementation - intergeneric and interspecific hybrids are a convenient method for transferring valuable genes for resistance and tolerance in grain cereal species. There are different methods to create and apply the properties of wide hybrids in practical breeding. Using multiple backcrosses of F1-plants with pollen from cultivars is related to the advantage that doubling of the chromosome set of the hybrid is not necessary. The fact that sterility in hybrid generation could occur in two forms male and female, a direct backcross of hybrids with non-doubled chromosome number, may be affected largely by the high percentage of female sterility in pollinated plants. This requires the use of a large number of crosses and is a laborious and slow process. The discovery of colchicine and other chemical agents which double the chromosome set, allows fertility of the offspring to be significantly increased as a product of wide hybridization. Increasing the fertility in this case varies widely and it is directly dependent on both parental components involved in the crosses and the concentration and type of working chemical solution. Spetsov et al. (2009) reported that obtaining a seedset in crossing with various wheat forms with *Aegilops tauschii*, is directly related to maternal chromosome number of components. Stoyanov et al. (2011) reported a high degree of sterility of hybrids between *Triticum aestivum*, *Triticum dicoccon* and *Aegilops cylindrica* treated with colchicine solution. In backcross of such plants with the pollen of bread wheat female sterility within 95.0-99.5% was recorded. In non-colchicined plants of the same hybrid, the female sterility was 100%. This is probably due to the strong gametocyde role of *Aegilops cylindrica* (Hohmann et al., 1995). Similar results were obtained by Shoenenberger et al. (2006) and Rehman et al. (2006) and female sterility was 99.40-100%. With very low female fertility are the hybrids *Zea mays* x *Tripsacum dactyloides* (Pesqueira et al., 2006). Leblanc et al. (1995), Sokolov et al. (2000), Molina et al. (2005) reported receiving a number of viable seeds in backcrosses of such hybrids.

The creation of genetically stable amphidiploids is another method that could be used to transfer valuable genes into cultivars. This method is based on the creation of synthetic wheat forms (2n = 6x = 42, AABBDD) between tetraploid wheat species with AABB (2n = 4x = 28) genome and wild *Aegilops tauschii* (2n = 2x = 14, DD). The genome of synthetic forms is largely similar to that of common bread wheat *Triticum aestivum* (2n = 6x = 42, AABBDD). Backcross of synthetic forms with varieties of bread wheat is facilitated in comparison with backcross of other genomic-differentiated hybrid plants. This is due to assimilation of the evolutionary process of natural polyploidisation of bread wheat (Stoyanov et al. 2010). However, the creation of synthetic forms is not a uniform process because of the wide variation in the genotype of wild species and many varieties of tetraploid wheat, which can be maternal component.

Sources of transferable genes for disease resistance in crops in wide hybridization could be both cultural forms and their wild representatives. In the grain cereals group the most widely studied are hybrid plants of different wheat species (*Triticum* sp), and less wide hybrids of barley (*Hordeum vulgare*), oats (*Avena sativa*), maize (*Zea mays*), pearl millet (*Pennisetum americanum*) and rice (*Oryza sativa*). Excluding the crosses of wheat species, in other genera the wide hybridization is limited to obtaining interspecific hybrids only. Table 1 presents data for wide crosses in which representatives of different genera and species are involved. The table shows that the most widely represented are crosses and research into the genetics of bread wheat. This is due to the fact that the probable phylogenetically close families of other cultural representatives are not as well studied as those of the genus *Triticum*.

Transferring phytopathogens resistance

Resistance to the negative impact of phytopathogens is one of the major problems that are solved by wide hybridization. The transfer of genes encoding resistance and tolerance is the basis of receiving lines and varieties of crop plants, in order to retain their productive potential in relation to biotic stress. For cultures of *Poacea*, major economically important diseases are presented in Table 2. From the table, it is clear that major diseases are economically important for bread wheat. This requires more and deeper research on the sources of resistance genes in wild species and cultural species. At the present stage of development of classical breeding, genes for disease resistance to most of the economically important diseases have been successfully transferred into bread wheat. Especially hard is the work on the

Table 1. Comparison of species taking part in wide hybridization

Maternal parent	Paternal parent	
	Interspecific hybridization	Intergeneric hybridization
Triticum sp.	*Triticum urartu, Triticum beaoticum, Triticum monococcum, Triticum dicoccoides, Triticum diccocon, Triticum timopheevi, Triticum turanicum, Triticum durum, Triticum polonicum, Triticum aestivum, Triticum macha, Triticum vavilovii, Triticum sinskajae, Triticum militinae, Triticum soveticum, Triticum karamischevii, Triticum fungicidum, Triticum borisovii, Triticum petropavlovsyi*	*Aegilops tauschii, Aegilops cylindrica, Aegilops speltoides, Aegilops ovata, Aegilops neglecta, Aegilops vaentricosa, Aegilops crassa, Aegilops kotschyi, Agropyron elongatum, Agropyron cristatum, Elytrigia repens, Elymus arenarius, Leymus mollis, Imperata cylindrica, Pennisetum americanum, Secale cereale, Hordeum chilense, Haynaldia villosa, Zea mays,*
Hordeum vulgare	*Hordeum bulbosum, Hordeum murinum, Hordeum chilense*	
Avena sativa	*Avena strigosa, Avena byzantina, Avena hirtula, Avena abyssinica*	*Zea mays*
Pennisetum americanum	*Pennisetum glaucum, Pennisetum orientale*	
Zea mays	*Zea mexicana*	*Tripsacum dactyloides*
Oryza sativa	*Oryza glaberrima, Oryza nivara, Oryza australiensis, Oryza minuta, Oryza logistiminata, Oryza brachiantha, Oryza officinalis*	

resistance to powdery mildew, brown rust and fusarium head blight. In recent years, due to increased risk of occurrence and mass multiplication of septoria leaf blight, research on the cause of the disease has begun. Resistant species to the cause of rye ergot *Claviceps purpurea* are still not found (Mirdita et al., 2008).

Table 2. Economically important diseases on species of Poaceae (Stancheva, 2002)

Cultural species	Economically important diseases	
	Name	Caused by
Triticum aestivum	Powdery mildew	*Erysiphe graminis*
	Leaf rust	*Puccinia recondite*
	Stem rust	*Puccinia graminis*
	Stripe rust	*Puccinia striformis*
	Fusarium head blight	*Fusarium graminearum*
	Septoria leaf blight	*Septoria tritici*
	Stagonospora blight	*Stagonospora nodorum*
	Loose smut	*Ustilago tritici*
	Common bunt	*Tiletia sp.*
Hordeum vulgare	Barley stripe	*Dreschlera gramiea*
	Powdery mildew	*Erysiphe graminis*
Avena sativa	Crown rust	*Puccinia coronata*
	Powdery mildew	*Erysiphe graminis*
Secale cereale	Ergot	*Claviceps purpurea*
	Snow mould	*Fusarium culmorum*
Zea mays	Corn smut	*Ustilago zeae*
	Head smut	*Sorosporium reilianum*
	Maize leaf fleck	*Maize leaf fleck virus*
Oryza sativa	Downy mildew	*Sclerospora macrospora*
	Rice blast	*Piricularia orizae*
	Fusarium head blight	*Fusarium oxysporum*

Powdery mildew *Erysiphe graminis* is a pathogen that is the most widely studied in the world due to the large area of distribution and the serious losses that result in some years, especially in susceptible varieties. The transfer of resistance genes in bread wheat, barley and oats is well-advanced and at this stage varieties of these crops with high resistance to most races of the pathogen and pathotypes are derived. Successfully are transferred genes for resistance to powdery mildew in bread wheat from *Triticum aestivum*, *Triticum timopheevi* (Maxwell et al., 2009), *Triticum dicoccoides* (Ji et al., 2007; Ji et al., 2008), *Triticum urartu* (Qiu et al., 2005), *Aegilops speltoides* (Jia et al., 1995), *Triticum militinae* (Jacobson et al., 2006), *Aegilops tauschii* (Miranda et al., 2006; Miranda et al., 2007), *Agropyron* (*Thinopyrum*, *Elytrigia*) *intermedium* (He et al., 2009 ; Luo et al., 2009), *Aegilops umbellulata* (Zhu et al., 2006), *Haynaldia villosa* (Xie et al., 2011), *Aegilops variabilis* (Stoilova and Spetsov, 2005), *Aegilops ovata* (Spetsov, 2004), *Aegilops kotschyi* (Spetsov, 2004). Resistance of barley Hordeum vulgare to powdery mildew has been successfully transferred from *Hordeum bulbosum* (Pickering et al., 1995). Genes for resistance to powdery mildew have been transferred into oats *Avena sativa* by crossing it with *Avena macrostachya* (Yu, 2005). Resistance to powdery mildew exhibit the hybrids between *Tr. aestivum* and *Aegilos cylindrica* (Stoyanov et al., 2011), *Aegilops tauschi* (Spetsov et al., 2008), *Agropyron glaucum* (Tsitsin, 1978). Resistance to the pathogen show amphidiploids bred in DZI-General Toshevo in the period 1950-1990, namely *Ae. tauschii* x *Tr. boeoticum*, *Ae. tauschii* x *H. villosa*, *Triticosecale* x *S. cereale*, *Tr. boeoticum* x *Tr. dicoccon*, *Tr. dicoccon* x *Ae. tauschii*, *Tr. timopheevi* x *Ae. tauschii*, *Tr. polonicum* x *Tr. boeoticum*, *Tr. durum* x *S. cereale*, *Tr. durum* x *Ae. speltoides*, *Tr. aestivum* x *S. cereale*, *Tr. sphaerococcum* x *S. cereale*, *Tr. aestivum* x *Ae. speltoides*, *Ae. tauschii* x *Tr. paleocolchicum* x *Elymus giganteus* x *Tr. dicoccon*, *Tr. aestivum* x *Ae. variabilis*, *Tr. aestivum* x *Ae. kotschyi*, *Tr. aestivum* x

Ae. ovata, *Tr. aestivum* x *Ae. columnaris* (Spetsov and Savov, 1992)

Brown (leaf) rust in wheat is the most important pathogen in economic terms, because under certain conditions it can cause severe damage to the crop yield (Stancheva, 2002). Pathogen of the disease is characterized by a large number of races and pathotypes that define a wide range of genes which have to be studied in relation to resistance. Sources of resistance to brown rust are most species of the genera *Aegilops*, *Agropyron* and *Haynaldia villosa*. Genes for resistance to this pathogen have been successfully transferred to bread wheat from species such as *Aegilops triuncialis* (Kuraparthy et al., 2011), *Triticum dicoccoides* (Marais et al., 2005), *Triticum timopheevi* (Lalkova et al., 2004), *Aegilops tauschii* (Lalkova et al., 2004), *Secale cereale* (Singh et al., 2012), *Agropyron elongatum* (Schachermayr et al., 1995), *Aegilops umbelullata* (Chhunea et al., 2007), *Aegilops speltoides* (Cherukuri et al., 2005). Hybrid plants of *Triticum aestivum* x *Aegilops cylindrica* cross exhibit good results in relation to brown rust resistance (Stoyanov, 2009). Good resistance to leaf rust pathogen possess amphidiploids created at DZI-General Toshevo during the period 1950-1990 (Spetsov and Savov, 1992). Suitable species for transferring genes for resistance via the method of wide hybridization exhibit the species *Aegilops triuncialis*, *Aegilops ovata*, *Aegilops biuncialis*, and some samples of the species *Triticum diccocon* (Ivanova, 2012). It decreased the occurrence of the black (stem) rust in recent decades due to the cultivation of resistant varieties (Stancheva, 2002). However, its harmful potential remains. Therefore, it is hard work to create wide hybrids that possess genes of resistance to the pathogen. Resistance to the pathogen is transferred to bread wheat from species *Aegilops searsii* (Liu et al., 2011), *Haynaldia villosa* (Qi et al,. 2011), *Aegilops speltoides* (Mago et al., 2009), *Aegilops cylindrica*, *Triticum erebuni*, *Triticum dicoccoides* x *Aegilops tauschii* (Babaiants et al., 2011). Yellow rust on wheat has limited spreading under specific environmental conditions. Its harmfulness is due to its high level of aggressiveness. Resistance to the yellow rust pathogen possess amphidiploids *Agropyron elongatum* x *Triticum aestivum* (Ma et al., 2000), wide hybrids of *Triticum aestivum* with *Aegilops sharonensis* (Marais et al., 2009) and *Triticim dicoccoides* (Gerechter-Amitai and Stubbs, 1970). Crown rust on oats is a disease that could greatly reduce yields. Resistance to disease demonstrate wide hybrids of *Avena sativa* with *Avena strigosa* (Rines et al., 2007) and *Avena fatua* (Sebesta and Kuhn, 1990).

Fusarium head blight caused by *Fusarium graminearum* is the subject of intense study because of its harmful character, occurring with a strong decrease in yields and also with the presence of toxic substances that impair the quality of grain. At present stage studies on *Fusarium* resistance are conducted with amphidiploids as *Triticum aestivum* x *Agropyron elongatum* (Oliver et al., 2006), and hybrids involving *Aegilops sharonensis* (Olivera et al., 2007).

Septoria leaf blight caused by *Septoria tritici* and stagonospora blight caused by *Stagonospora nodorum* are diseases affecting mainly bread wheat and possess a dangerous potential harmful effect, especially in years with wet spring and in susceptible varieties. Wide hybridization as a method is poorly studied with regard to the transfer of resistance to both pathogens. Studies were carried out with amphidiploids such as *Triticum aestivum* x *Agropyron elongatum* (Oliver et al., 2006) and hybrids involving *Aegilops tauschi* (Murphy et al., 2000). From hybrids of *Hordeum chilense* a gene for resistance to *Stagonospora nodorum* is successfully transferred (Rubiales et al., 2000). Potential sources of resistance to septoria leaf blight are *Triticum diccocon*, *Aegilops speltoides*, *Aegilops tauschii* (Van Ginkel and Rajaram, 1999), *Aegilops umbelullata* (Maximov et al., 2006).

The smuts and bunts on cereals are more serious diseases that are caused by many pathogens (*Tilletia caries*, *Tilletia contraversa*, *Tilletia laevis*, *Ustilago tritici*, *Ustilago nuda*, *Ustilago hordei*, *Ustilago nigra*). Most of the pathogens are stored in the seed or germ of the wheat, so the resistance is to be manipulated more difficult than other pathogens. It was found that these pathogens also efficiently infect wild species of the genera *Aegilops*, *Agropyron*, *Haynaldia*, *Secale*, *Triticum*, *Hordeum*. The choice of source starting breeding material for wide hybridization is very limited. Galaev et al. (2006) reported the successful transfer of genes for resistance to common bunt *Tilletia caries* in the genome of bread wheat from *Aegilops cylindrica*. Potential source of resistance to bread wheat and barley is a wild species *Hordeum chilense*, because of its resistance to all of these pathogens, as well as in barley stripe (Martín et al., 2000). Diseases on rice caused by Rice grassy stunt virus and *Xanthomonas oryzae* are successfully overcome through the development of wide interspecific hybrids of *Oryza sativa* with *Oryza nivara*, *Oryza minuta*, *Oryza brachyantha* (Abbasi et al., 2010).

Transferring insect pests resistance

Insect pests as part of the biotic effects of the environment could significantly affect the productive potential of cereals. Breeding aimed resistance to pests associated with wide hybridization is on considerably lower stage than breeding to the resistance of phytopathogens. This is due to the specifics of the plants enemies and the inability of plants to respond in a similar way like the response to phytopathogens. Factors that determine the resistance of the plant organism to insect attack are determined by indirect effects on the insect body.

Some wild species similar to bread wheat respond differently to insect pests. For example, genes for resistance to Hessian fly (*Mayetiola destructor*) are successfully transferred into bread wheat by species such as *Aegilops triuncialis* (Martin-Sanchez et al. 2003), *Aegilops tauschii* (Wang et al., 2006), *Secale cereale* (Friebe et al., 1990), *Triticum dicocoon* (Liu et al. 2004), and in most cases good resistance is caused by the action of the 'gene for gene' theory (Bouhssini et al., 2008). In relation to aphids (e.g. *Schyzaphis graminum*) resistance genes were transferred into bread wheat from species such as *Aegilops tauschi* (Azhaguvel et al. 2012). In rice similar effects are observed in distant hybrids with some wild species. Hybrids of *Oryza sativa* with *Oryza longistaminata* exhibit strong resistance to attack by various pests on rice (*Nilaparvata lugens*, *Sogatella furcifera*) (Abbasi et al., 2010).

Transferring abiotic factors tolerance

Abiotic environmental factors are described by a strong difficult predictability and dynamism in their values. While some impacts on the environment have a wider range of occurrence and distribution (effect of climatic factors), others (ion, metal toxicity) have too local character. For this reason, wide hybridization as a specific process is intended to overcome specific environmental factors. Often cultural plant species and their varieties, resulting in strong genetic uniformity, are not able to respond to the specific abiotic factor. This is especially demonstrated when a plant organism was introduced directly into the environment with such factors. This creates the necessity to seek opportunities in wide hybridization for transferring genes for tolerance to the effects of low temperatures, low humidity, metal toxicity, salinity (Mujeeb-Kazi et al., 2009). It was found that wild members of the group *Triticum-Aegilops-Haynaldia*, possess very good adaptability to the above factors (Plamenov, 2003). This makes them a suitable source of starting breeding material for wide hybridization in bread wheat. In barley medium of such genes is *Hordeum chilense* (Martín et al., 2000), in oats – *Avena strigosa*, in maize – *Zea mexicana* and *Tripsacum dactyloides*, in rice – *Oryza australiensis* and *Oryza officinalis*. It was found that the resulting hybrids involving these species possess high levels of tolerance to the above factors. Landjeva et al. (2003) reported high levels of resistance to copper toxicity in species such as *Aegilops triuncialis* followed by *Aegilops ovata*, *Aegilops cylindrica*, *Haynaldia villosa*. Successful hybrids were obtained with transferred drough tolerance into bread wheat from *Aegilops tauschii* (Gupta et al., 2010). Genes for tolerance to low temperatures are localized in the bread wheat hybrids with *Aegilops ovata* (Klimov et al., 2006). With great potential hybrids of bread wheat with *Agropyron elongatum* (Fredkin et al., 2012) and hybrids involving *Elymus arenarius* and *Leymus mollis* are emerged (Anamthawat-Jonsson, 1995). Problematic in some areas of cultivation is also the salinity of the soil caused by high levels of NaCl. High levels of salinity tolerance show wide hybrids of maize *Zea mays* with *Tripsacum dactyloides* (Pesqueira et al., 2008). Similar results were observed in crosses of maize with teosinte (*Zea mexicana*). Salinity tolerance occurs in hybrids of bread wheat with *Agropyron elongatum* (Fredkin et al., 2012) and hybrids involving *Elymus arenarius* and *Leymus mollis* (Anamthawat-Jonsson, 1995). Good levels of salinity tolerance show plant accessions of the species *Triticum macha* and *Triticum timopheevi*, which seem to be a good source for inclusion in breeding programs of bread wheat (Badridze et al., 2009). Good source of genes for salinity tolerance and water stress appeared in some synthetic forms of wheat (Mujeeb-Kazi et al., 2009).

Wide hybrids and dihaploidy

Dihaploid selection is an effective method of obtaining homozygous plants. Dihaploid plants can successfully reproduce through self-pollination and can provide valuable varieties or lines possessing high levels of resistance to biotic and abiotic factors. Obtaining dihaploid plants is associated with obtaining haploids via various methods. In practice, two methods are widely used – by anther culture or by wide hybridization using very phylogenetically distant species. In the history crosses as *Oryza sativa* x *Pennisetum* sp., *Saccharum* x *Sorghum, Oryza sativa* x *Sorghum bicolour*; *Triticum aestivum* x *Zea mays, Pennisetum americanum* x *Zea mays, Triticum aestivum* x *Sorghum bicolour*; *Hordeum vulgare* x *Zea mays* is known (Ahmad and Comeau, 1990). In bread wheat anther culture is not a good method because the resulting plant regenerants are often with very low viability and show a lack of chlorophyll (albinism). On the other hand, wide hybrids, in the process of multiple division after pollination and fertilization, pollinator's chromosomes are completely eliminated from the embryo and these hybrids appear to be a very promising source to obtain haploid regenerants. Researches on the haploids derived by wide hybridization with bread wheat have been made with species such as *Zea mays*, *Tripsacum dactyloides*, *Hordeum bulbosum*, *Imperata cylindrica*, *Pennisetum glaucum*. There is a differentiation in relation to the crossability of the diferent types of wheat with plants mentioned above. For example, crossing of common wheat *Triticum aestivum* and durum wheat *Triticum durum* with bulbous barley *Hordeum bulbosum* results in a lower rate of crossability compared to other types of crosses in tetraploid wheat. This is due to the presence of genes in bread and durum wheat which strongly suppress crossability with other species (O `donoghue and Bennet, 1993). In crosses of bread wheat with *Tripsacum dactyloides*, there are significant differences in the values of crossability and the formation of a viable embryo. Crossability values in this case are between 27 and 59%, and the formation of a viable embryo is between 0.5 and 59% (Li et al., 1996). Significant difference in the crossability of wheat and corn were observed. Laurie and Bennet (1988) have reported crossability below 1%, and the formation of a viable embryo was within 27%. The same authors reported that after crossing wheat and corn, 20% of the pollinated flowers pollen does not reach the egg, in 2% pollen tubes highly damaged the embryo, in 33% the sperm nucleus does not reach the egg, in 16% sperm nucleus reaches the egg but died, and the remaining 29% the sperm nucleus successfully reaches wheat egg. In general, pollination varies from 14 to 30% (Laurie and Bennet, 1987). With the treatment of maternal spikes after successful pollination with 2,4 D dichlordipheniloxyacetic acid, values of viable embryo formation vary from 18 to 32% and the percentage of regenerated plants is up to 84% (Suenaga and Nakajima, 1989). Ahmad and Comeau (1990) reported a higher rate of pollination between bread wheat and pearl millet *Pennisetum glaucum* 80%, compared to maize - 56%. Good results at this stage show the crosses between bread wheat and silver grass *Imperata cylindrica* (Chaudhary, 2008).

Cultural amphidiploids

The combination of valuable qualities of two or more cultures was achieved in relation to polyploidy. Wide hybridization is an effective method for artificial creation of polyploids in the group of cereals. This is due to the fact that crops such as bread wheat and oats are the result of natural polyploidisation. Creating cultures that combine valuable features of crops begin with hybridization between wheat and rye in the country (Kolev, 1978) and the first reports of successful cross are made by Wilson and Barnard in the period 1875-1886. The most important cultural amphidiploids obtained as a result of wide hybridization prove to be triticale, tritordeum and perrenial wheat.

Triticale is an annual cultural amphidiploid obtained by hybridization of wheat with rye. The hybrid is hexaploid or octoploid depending on the type of wheat used in hybridization. At the present stage of breeding various forms of triticale are created. It is used as food and fodder crops (Terziev, 2006).

Tritordeum is also an amphidiploid annual crop resulting from hybridization between durum wheat and *Hordeum chilense*. The hybrid was created by Antonio Martin in 1977. The plant is hexaploid and possesses resistance to most pathogens of bread wheat. It is still in introduction into agriculture practice as a food crop (Knuepffer, 2009).

Perrenial wheat is a perennial cultural amphidiploid resulting from wide hybridization between bread wheat and *Agropyron intermedium*. The species was created by Nikolai Tsitsin, in 1948, and it is octoploid and cross-pollinated. It has considerable resistance to fungal pathogens. It is not used in practice because of the rapid thinning of crops due to low temperatures and strong affinity of crossability with bread wheat (Tsitsin, 1978).

Problems of wide hybridization and methods to overcome

The problems of wide hybridization are covered by the theory of species formation and the practical impossibility of different plant types to cross each other (Ayala, 1987). However, there are natural hybrids among cereals. Stoyanov (2009) reported about hybrids of *Triticum aestivum* x *Aegilops cylindric*, discovered in natural ways and the wild species in culture agrocenosis to be established. The studies of the discovered hybrid plants show a high degree of sterility and among 50 tested spikes there were found only 10 kernels, and thereafter one of them germinates. The next generation features even higher sterility reaching 100%, which is apparent even at flowering - plant flowers in "open type". Often the incompatibility in the crossing of several plant species is determined both by purely morphological and physiological factors (length, shape and structure of stigma and pistil, a period of ear formation (head formation) and flowering, shape and openness of flowers, etc.) and by the specific chromosome number and plant genome as a whole (chromosome number, presence of crossability genes, presence of homology, etc.) (Genchev, 1969).

The low percentage of crossability is one of the factors which define the difficulties of wide hybridization. The presence of crossability genes in bread and durum wheat (*Kr1*, *Kr2*, etc.) are associated with the inability of foreign pollen to make an effective pollination of wheat stigmas, and these genes were first identified in crosses between wheat species and rye (O`donoghue and Bennet, 1993). Stoyanov et al. (2011), Shoenenberger et al. (2005) and Rehman et al. (2006), Cifuentes and Benavente (2009) reported high levels of crossability of bread wheat with *Aegilops cylindrica* and lower levels when the maternal component is tetraploid wheat. However, Spetsov et al. (2008) reported higher crossability in hybrids involving *Ae.tauschii* and bread wheat than crossing with tetraploid wheat species. This is probably due to the presence of D-genome in wild species, which facilitates the formation of a number of homologous pairs of chromosomes. Tsitsin (1978) describes varying rates of crossability when crossing bread wheat with different types of wheatgrass (*Agropyron*), the highest crossability is reported in crosses with *Agropyron intermedium,* lower with *Agropyron glaucum* and the lowest type with *Elytrigia repens*. The same author reported increased crossability of wheat with hybrids *Agropyron intermedium* x *Agropyron glaucum* and *Agropyron glaucum* x *Agropyron intermedium*. Zero crossability occurs between bread wheat and crested wheatgrass *Agropyron cristatum* (Spetsov, personal communication). Bozhanova et al. (2009) reported high variation in crossability rates between durum wheat and other species in the group *Triticum-Aegilops*. With the lowest crossability are determined crosses *Triticum durum* x *Aegilops juvenalis* (3.2%), *Triticum durum* x *Aegilops kotschyi* (3.6%), medium crossability of *Triticum durum* x *Triticale* (19%) and high crossability of *Triticum durum* x *Triticum dicoccoides* (50.9%). Naskidashvili (1984) in interspecific hybridization of bread wheat with different types of diploid and tetraploid wheat found that crossability depends on the ploidy of wheat, and the existence and/or absence of a concrete genomic constitution. Usually, overcoming the problems associated with low crossability could be solved by the method of somatic hybridization, by performing a large number of crosses or treatment of pollinated plants with a solution of 2,4 dichlordipheniloxiacetic acid.

Low germination of wide hybrids and their inability to germinate prove to be the main problem in development of grain cereals breeding programs. Interspecific and intergeneric incompatibility determine the difficulties in the germ formation at embryonic stage of hybrids development. Difficult germination or lack thereof is often provoked by physiological inability of the embryo to feed by the endosperm due to differences in its chemical compounds. For example, difficult germination or lack of germination of seeds is to be found in crosses *Triticum durum* x *Elymus arenarius*, *Triticum durum* x *Elymus giganteus* (Kolev, 1978), *Hordeum jubatum* x *Secale cereale* (Kolev, 1978), *Triticum durum* x *Agropyron intermedium* (Stoyanov, 2009), *Triticum aestivum* x *Hordeum vulgare* (Sethi et al., 1986). Overcoming the effects of immature germ or its low viability is achieved by using embryo rescue techniques. By applying the embryo rescue method in crosses of durum wheat with *Aegilops cylindrica*, *Agropyron cristatum*, *Triticosecale*, *Triticum macha*, *Triticum spelta*, Bozhanova et al. (2006), different degrees of recovery of hybrid embryos and varying degrees of adapted plants are established, which depends on the type of pollinator component and the accession used. Thus, using four accessions of *Aegilops cylindirca*, ranges the percentage of the regenerated plants from 30 to 75%. By using other types of paternal component, the same authors described regeneration between 33 and 50%. The same study determines the influence of composition of the medium on the regeneration of hybrid embryos into plants.

Postembryonic incompatibility is a major problem in modern breeding because the hybrid plants are lost. This type of incompatibility displays very high sterility of hybrid offspring, occurrence of hybrid necrosis or unviability of the hybrid plants resulting from disturbances in their development or death. Often, the last two incompatibilities are caused by lack of homologous chromosomes, which is evidenced by the specific development of haploid plant regenerants. High sterility exhibit all plant hybrids in *Poaceae* (95%) where there is no presence of homologous chromosomes, but also because of cytoplasmic male sterility (Gotsov, 1980). The sterility problem of the hybrids is solved by the method of chromosome number doubling. According to Kostoff (1938) the most effective method for chromosome number doubling is treatment of plants in tillering phase with weak aqueous solutions of colchicine. Tsitsin (1978) reported prevailing fertility in backcross with pollen of bread wheat in wheat-wheatgrass hybrids by homologous pairing of chromosomes of similar subgenomes originating from *Agropyron elongatum*. Spetsov et al. (2008), Stoyanov et al. (2010, 2011) Plamenov et al. (2011) reported the recovery of normal meiotic processes in the cross *Triticum dicoccon* with *Aegilops tauschii* or *Aegilops cylindrica*. In hybrids involving *Aegilops cylindrica* more frequent disturbances in meiosis occurred. Hybrid necrosis is also a problem in some plants which is solved by proper selection of starting breeding material.

Perspectives in front of the wide hybrids in Poaceae

The prospects for the wide hybrids at this moment are promising due to the discovery of more and new tools of molecular and tissue biology aiming at the elimination of the problems associated with species incompatibility. Discovery of new sources of resistance and tolerance to biotic and abiotic stress is an opportunity to develop new hybrids and amphidiploids with valuable qualities for breeding programs in cereals. Ever-increasing consumption requires creation of high-quality wheat cultivars for satisfaction of market demand but at the same time they must be with low cost and to be developed relatively easily.

The use of wide hybrids in breeding programs of cereals is widely used both at home and abroad. The creation of new varieties of cereals which are resistant to pathogens, insect pests and tolerance to abiotic stress are the main priorities of the breeding organizations and institutes. Worldwide, many varieties of winter wheat were created by wide hybridization and adapted successfully to environmental conditions. In this regard, first place take the wheat varieties created by using synthetic hexaploid lines (Spetsov et al., 2008). In countries like Mexico and Spain (Spetsov et al. 2009) and Australia (Limin et al., 2006) the methods of wide hybridization are widely used. Of growing proportion are hybrids involving *Aegilops tauschii*, considering the transfer of resistance to the phytopathogens in common winter wheat (Plamenov and Spetsov, 2011). There are new varieties and lines that have resistance to powdery mildew, which are obtained from wild species of the group of *Triticum-Aegilops* (Plamenov, 2003). Wide hybridization has particularly important role in creating new varieties of triticale, and at this moment, new lines and varieties of tritordeum. (Knuepffer, 2009). There is hard work to create alternative crops, using the method of amphidiploidisation. At this stage of development of plant breeding, species such as senaldiya project DDRR (combining the genomes of *Aegilops tauschii* and rye, for obtaining a plant biomass with high production of lignocellulosic alcohol), project TRITOL (creating triticale varieties for bioethanol) will be used as crops in agriculture.

Remote hybridization as a method of classical breeding should have both scientific and practical character. This requires the creation and more research on hybrids that have the prospect to production. At the same time new and improved existing methods should be developed to overcome the problems resulting from interspecific and intergeneric incompatibility. To create hybrids effectively, at first, the appropriate starting breeding material must be selected consistent with the purpose of breeding and characteristics of plant species. Innovations in breeding work should be introduced and used and the hybrids thus developed to meet the planned breeding purposes. At the present stage of development, techniques such as tissue culture and embryo rescue shall be widespread in order to successfully avoid the difficulties arising from highly incompatible crosses.

Upgrading wide hybridization as a breeding method requires its integration (co-operation) with other branches of biological sciences. Research in cellular and genetic engineering and transgenic crops are increasingly implemented to improve the wide hybrids (Vasil, 1999). Studies with transgenic lines are made with wheat (Vasil and Vasil, 1999), barley (Lemaux et al., 1999), rice (Datta, 1999), maize (Gordon-Kamm et al., 1999), sorghum (Castillo and Casas, 1999), rye (Castillo and Casas, 1999), oats (Somers, 1999) and especially important for wide hybridization – in triticale and tritordeum (Barcelo et al. 1999). The resulting transgenic lines, and lines resulting from the genetic transformations (Klein and Jones, 1999; Komari and Kubo, 1999; Båga et al., 1999), could be effectively used as starting breeding material for wide hybridization.

Conclusion

Remote hybrids allow transfer of genes from wild species, encoding resistance to biotic and abiotic stress in the genome of cultural plants.

Problems that arise by the wide hybridization could successfully be eliminated by applying the methods of molecular and tissue biology.

It is expected that wide hybrids will be a promising good plant material to be applied in the breeding programs of cultivated plants, but for its successful use it is necessary to organize modern molecular laboratories in breeding institutes in the country.

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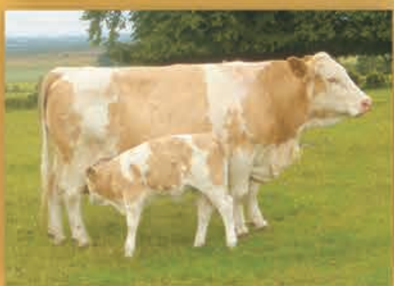

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