In vivo Conservation of Agro-Biodiversity: A Selective But Practical Approach for its Sustainable Utilization

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Abstract: PMB group of NIAB has created new agro-bio-diversity through conservation into commercial cultivars, genomes of 5 different accessions of *Aegilops geneculata* (syn. *ovata*). The objectives were to produce new agricultural bio-diversity for stressed lands and enhancing the existing one through the use of wild relatives of wheat. The accessions were collected from abroad and within the country and are no more available. However, the genomes of these accessions conserved in commercial cultivars are available for improvement of salinity and water deficiency tolerance in wheat. Compared to *in situ* and *ex situ*, *in vivo* conservation is selective yet two steps ahead of the former two. Firstly, genome conserved *in vivo* is known for its specific characters. Secondly, its cross-ability with wheat is also known. For *in-situ* and *ex-situ* conservation, first the germplasm is to be collected followed by screening for specific characters. The selected species/accession would then be crossed to transfer the required characters to the commercial cultivars if the two are crossable. *In-vivo* conservation is therefore, less cumbersome, economical, and more practical.

Keywords: *Aegilops geneculata*; amphiploid; bio-diversity; allopolyploid; genome

In vivo conservation of agro-biodiversity is a new concept in which we conserve whole genome of a particular species/accession in commercial cultivars that are crossable with this very accession. It is like of natural amphiploidy, where chromosomes in the F_1 inter-generic hybrid doubled automatically under the influence of maternal parent. The first ever natural amphiploid was reported about 9000 BP (Stebbins 1946) and was that of emmer wheat crossed naturally with *Aegilops. tauschii* giving birth to first hexaploid wheat that is *Triticum spelta*. The duplication of genomes (polyploidy) whether auto polyploidy (same genome), or allopolyploidy (different genome) is a major force of evolution that affect genome size and gene copy number (Shaked *et al*. 2001). In allopolyploidy, fixation of heterozygote (Liu *et al*. 2002) has the potential to offer substantial advantages (Allard *et al*. 1993). The biggest advantage is conservation of whole genome of a species into cultivar where (*i*) it can maintain its original characters, (*ii*) can change the character of the recipient parent (Wendel 2000), or (*iii*) it can behaves as a new species (Soltis & Soltis 2000). However, for successful establishment as new species, newly formed alloplyploids must overcome reduced fertility (Ozkan *et al*. 2001) resulting from improper chromosomes pairing and segregation (Shaked *et al*. 2001) that usually occur during first generation of amphiploids. PMB group of NIAB has created such a new diversity by conserving into commercial cultivars, whole genome of 5 different accession of *Ae. geneculata* (Synon. *Ae. ovata*): $2n = 4x = 28$ $C^uC^uM^oM^o$. These accessions were collecated from within the country but are no more available. However, the genome of these species conserved in commercial cultivars is available for improvement of salinity and water deficiency tolerance in wheat. In the present study, we are reporting for the first time, fully fertile allopolyploid: amphloid produced naturally during our effort of conserving genome of *Ae. geneculata* (*ovata*: $2n = 4x = 28$ $C^uC^uM^oM^o$) into *Triticum aestivum* ($2n = 6x = 42$ AABBDD) and *T. turgidum* ($2n = 4x = 28$: AABB) cultivars. The objectives were to

Table 1. Description of the material used in the study

No.	Material with genome	Accession/Cultivar	Origin
1	*Ae. genetulata* $C^uC^uM^oM^o$	F	Pakistan
2	*Ae. genetulata* $C^uC^uM^oM^o$	65	Italy
3	*Ae. genetulata* $C^uC^uM^oM^o$	330487	USDA, ARS
4	*Ae. genetulata* $C^uC^uM^oM^o$	266978	USDA, ARS
5	*Ae. genetulata* $C^uC^uM^oM^o$	369578	USDA, ARS
6	*Ae. variabilis* $C^uC^uS^vS^v$	A	CIMMYT
7	*Ae. variabilis* $C^uC^uS^vS^v$	B	CIMMYT
8	*Ae. variabilis* $C^uC^uS^vS^v$	E	CIMMYT
9	*Triticum aestivum* AABBDD	Pak-81	CIMMYT, Mexico
10	*Triticum aestivum* AABBDD	LU-26	UAF, Faisalabad, Pakistan
11	*Triticum turgidum* AABB	Durum	Selection from AARI

UAF: University of Agriculture; AARI: Ayub Agriculture Research Institute, Faisalabad

produce new agricultural bio-diversity for stressed lands and enhancing the existing one through the use of wild relatives of wheat.

MATERIAL AND METHODS

Material used in this study comprised 5 different accessions of *Ae. geneculata* obtained from within the country, Italy, and USDA, ARS, USA, 3 different accessions of *Ae. variabilis*, *Triticum turgidum* cv. Durum, and *T. aestivum* cv. Pak-81 and LU-26. Detail description of the material is given in Table 1.

Normal wide crosse breeding and manual emasculation and pollination procedure was adopted using wheat cultivars as female parents. Seed set

Table 2. Comparative frequency of natural and induced amphiploidy in *Ae. geneculata* and *Ae. variabilis*

No.	Combination	Seed set (%)
1	*T. turgidum* × *Ae. geneculata* accession F	108 (24) c*
2	*T. turgidum* × *Ae. geneculata* accession 65	15 (14.70) b*
3	*T. turgidum* × *Ae. geneculata* accession 330487	59 (16.72) b*
4	*T. turgidum* × *Ae. geneculata* accession 276978	40 (4.60) a*
5	*T. turgidum* × *Ae. geneculata* accession 369578	63 (25) c*
6	*T. turgidum* × *Ae. variabilis* accession A	0.00
7	*T. turgidum* × *Ae. variabilis* accession B	0.00
8	*T. turgidum* × *Ae. variabilis* accession E	0.00
9	*T. aestivum* cv. Pak-81 × *Ae. variabilis* accession A**	3 (9) 7***
10	*T. aestivum* cv. Pak-81 × *Ae. variabilis* accession B**	3 (50) 1***
11	*T. aestivum* cv. Pak-81 × *Ae. variabilis* accession E**	0.00
12	*T. aestivum cv. LU-26* × *Ae. variabilis* accession A**	12 (21) 2***
13	*T. aestivum* cv. LU-26 × *Ae. variabilis* accession B**	15 (14) 3***
14	*T. aestivum* cv. LU-26 × *Ae. variabilis* accession E**	4 (7) 10***
15	*T. aestivum* cv. Pak-81 × *Ae. geneculata accession* F**	79 (33) (7)***
16	*T. aestivum* cv. Pak-81 × *Ae. geneculata accession* 65**	50 (0.001) (1)***
17	*T. aestivum* cv. LU-26 × *Ae. geneculata accession* F**	180 (60) (2)***

*Natural amphiploid seeds
**Chromosomes doubling induced via chochicine
***Amphiploid seeds indeced via colcicine
Figures followed by same letters are not significantly different from each other at 5% level significance according to DMRT.

Table 3. Variation in morphological characteristics of a particular natural amphiploid

No.	Combination	Plant height (cm)	No. of spikes	Plant weight (g)	Grain weight/spike (g)
1	Durum/*Ae. geneculata* 65	99	21	62.8	0.20
2	Durum/*Ae. geneculata* 65	87	26	58.9	0.20
3	Durum/*Ae. geneculata* 65	96	16	20.3	0.30
Average		94	21	47.3	0.23

obtained for crosses between durum wheat and *Ae. geneculata* were allowed to mature on the plants. For all other combinations, F_1 plants were obtained through embryo culture followed by chromosome doubling via colchicine. For this purpose, F_0 seeds of various hybrids were treated with 0.1% aerated solution of colchicines for 2–3 h followed by washing the roots thoroughly with running tap water. After removing all the tillers, all the treated plants were transferred in potted soil to raise F_1 plants. F_0 seeds of crosses between *T. turgidum* × *Ae. genetulata* were germinated to raise F_1 plants. Both natural and induced amphiploidy was established through mitotic chromosome counts. Fertility of the amphiploids was established through chromosomes studies at meiosis. Second generation amphiploids were field tested to see the performance of *Ae. geneculata* genome conserved in *T. aestivum* and *T. turgidum*

RESULTS

Significant variations were observed for both natural and induced amphiploidy obtained from crosses between *T. aestivum* and *T. trugidum* with various accessions of *Ae. geneculata* and *Ae. variabilis* (Table 2). Natural amphiploidy was observed only in crosses between *T. turgidum* and *Ae. geneculata*. The frequency of natural amphiploid seeds varies between 25% (*T. turgidum* × *Ae. geneculata* accession 369578) to 4.60% (*T. turgidum* × *Ae. geneculata* accession 276978). No seed could be obtained for crosses between *T. turgidum* and any of the *Ae. variabilis* accessions. Natural amphiploid was also not observed in any of the crosses between *T. aestivum* and *Ae. geneculata* accessions F and/or 65. The induced amphiploid seeds however varies between 1and 7 (Table 2).

Significant variations were also observed with respect to plant height, plant weight or biomass, No. of spikes, and single grain weight (Table 3). For one particular combination of a natural amphiploid (*T. turgidum/Ae. geneculata:* accession 65), plant height varies between 87–99 cm, No. of spikes between 16–26, plant weight between 20.3 and 62.8 g and grain weight varies between 0.2–0.3 g (Table 3).

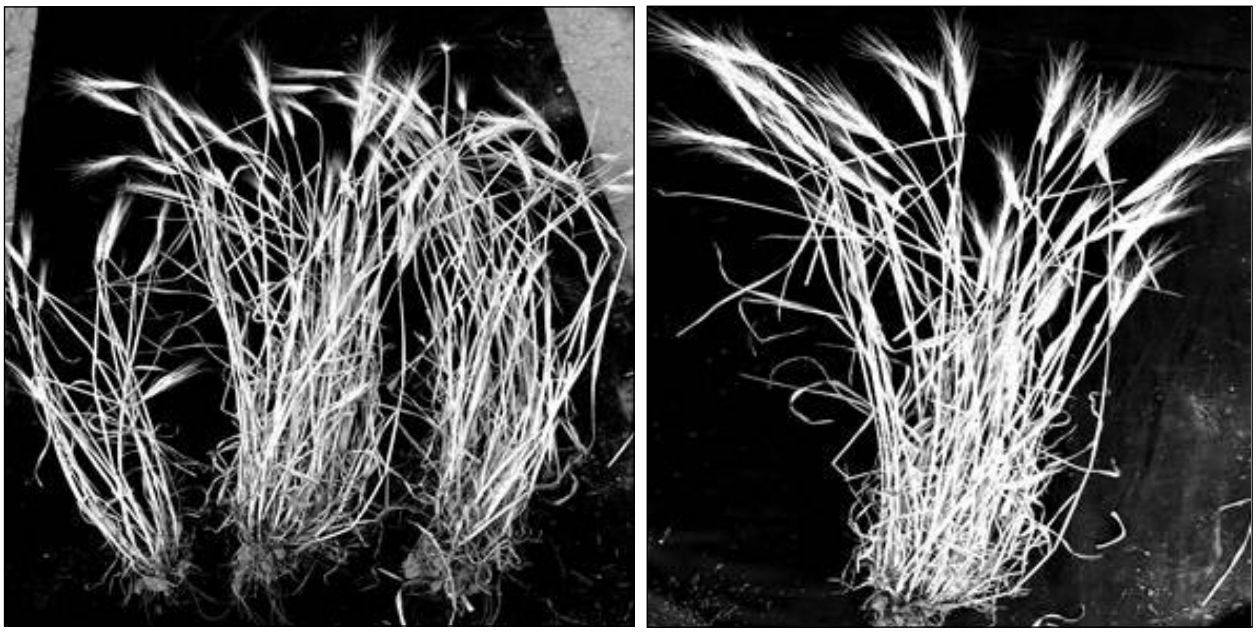

Figure 1. Differences in plant height, biomass, No. of spikes and No. of tillers/plant

Figure 2. Variation in no. of grains, grain weight/spike and no. of spikes/plant in natural amphiploids of *Triticum turgidum* × *Ae. geneculata*

For all other combinations, plant height ranges between 23 to 99 cm; no. of spikes between 4–35 (Figure 1); No. of grain/spike varies from 4–26, grain weight/spike from 0.2 to 6.7 g, and single grain weight ranges between 0.03 and 0.06 g (Figure 2). Single plant weight or plant biomass however, ranges between 7 and 70 gram (Figure 3) depending upon the accession used in crosses.

DISCUSSION

As mentioned earlier, polyploidy, is a prominent mode of speciation in plants (Masterson 1994) as many of the important crops including oat, coffee, potato, canola, soybean, sugarcane, tobacco, and cotton are examples of typical polyploids (Liu & Wendel 2000). Tribe *Triticeae* it self is formed of species with three polyploidy levels (diploid, tetraploid, and hexaploid) that are readily crossable and help transferring gene(s) among different species or can retain full genome of related wild species of different ploidy levels. It is thorough such events that *T. spelta* (first hexaploid wheat: Stebbins 1946), *T. aestivum* and *T. turgidum* came into existence (Feldman *et al*. 1995; Fieldman 2001). Not only that species of *Triticum* but also species of certain other genera can also be crossed with (or their full genome can be conserved in) *T. aestivum* or *T. turgidum*. The results of such crossing/conservation have either yielded viable hybrids/amphiploids such as *Triticale* (Wilson 1876); *Tritipyrum* (King *et al*. 1997); *Agroticum* (Martin *et al*. 1998); *Tritordium* (Martin *et al*. 1999; Soliman *et al*. 2001) or have been exploited for crop improvement (Multani *et al*. 1988; Schachtman *et al*. 1992; Pestsova *et al*. 2001).

Figure 3. Variation in plant weight/biomass, and spike morphology of natural amphiploids

In all these manipulations, however, hybrids have been regenerated through embryo culture and amphiploids have been produced through artificial doubling of chromosomes via chochicine. We are however, reporting here for the first time, 5 different amphiploids produced naturally as a result of crossing a local tetraploid wheat (*T. turgidum*) variety Durum with 5 different accessions of *Ae. geneculata* (*ovata*).

Benavente *et al*. (2002) has reported amphiploids between *Triticum turgidum* and *Aegilops ovata* (*Ae. geneculata*). But, these amphiploids were synthesized using a French tetraploid wheat (*T. turgidum*) cultivar Primadur that was homozygous for *Ph1*/*Ph1* locus, and a mutant line (Creso), that was homozogous for *ph1c*/*ph1c*. Hence the amphiploids were the results of either spontaneous doubling of chromosomes due to homoeologous paring promotion (amphiploid with Creso) or induced doubling of the chromosome by colchcine (Amphiploid with Primadur). None of these amphiploids was a natural amphiploid with full intact genome of *Ae. geneculata* like we are reporting in the present study. Also, the amphiploids reported by Benavente *et al*. (2002) were probably synthesized to facilitate genetic transfers from *Ae. ovata* to wheat using homoeologous chromosomes pairing promoter lines. This was probably one of the reasons that except for meiotic exchanges observed between chromosomes of *T. turgidum* and *Ae. ovata,* no other information regarding morphological data, fertility, grain yield and field performance of the amphiploids have been reported. We have not studied our amphiploids to see the possibility of genetic exchanges between chromosomes of *T. turgidum* and *Ae. genecualta* however, the fully fertile amphiploid ($2n = 8x = 56$: $AABBC^uC^uM^oM^o$) were twice field tested and found stable, which indicated that genome of *Ae. geneculata* has not gone through much of genetic exchanges and is likely to be intact. Hence these amphiploids can easily be used as a new crop in the areas beset with water deficiency or as a new species to be used for improvement of wheat for salinity and water deficiency tolerance.

The advantages of such genome conservation is that compared to other conservation such as *in situ*, *ex situ*, and *in vitro*, it is selective yet two step ahead of the former three. For *in situ*, *ex situ* and *in vitro* conservation of biodiversity, first we have to get the germplasm from the site, which is usually followed by screening for specific characters. The selected species would then be crossed with the cultivars to be improved provided: the two are crossable. Contrary to this, *Ae. geneculata* is already known for its specific characters and secondly, its cross-ability with wheat is also known (Farooq

et al. 1990). *In vivo* conservation is therefore, less cumbersome, economical, and more practical. The fully fertile F_1 hybrid (amphiploid) would stay for years and could be used for crop improvement for characters that are specific for species and/or accessions: the genome of which is conserved in the commercial cultivar. This way, not only that the precious genome would be conserved, but after interacting with different cultivars, it will also create new agro-biodiversity: a vital component of the sustainable agriculture system.

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