Evaluation and utilization of *Aegilops* Germplasm for biofortification of wheat for high grain iron and zinc content

Tiwari VK[1], Rawat N[1], Singh N[1], Randhawa GS[1], Singh Kuldeep[2], Chhuneja Parveen[2], Tripathi SK[1], Dhaliwal HS[1]

[1]Indian Institute of Technology, Roorkee - 247667 Uttarakhand, India

[2]Punjab Agricultural University, Ludhiana- 141 004, Punjab, India

ABSTRACT

Grains of 80 accessions of wild *Triticum* and seven *Aegilops* species with non-progenitor S, U and M genomes along with 15 semi-dwarf bread and durum wheat cultivars, grown over two years at IIT Roorkee, were analyzed for iron and zinc content. The wheat and durum cultivars had very low content and limited variability for iron and zinc content. The *Aegilops* species showed up to 2-3 fold higher grain iron and zinc content than the cultivars. There was a significant high positive correlation between flag leaf iron and grain iron (r=0.82) and flag leaf zinc and grain zinc (r=0.92) among selected donors. Fourteen synthetic amphiploids involving *Triticum aestivum* cultivars and different *Aegilops* accessions had nearly as high micronutrient content as that of their *Aegilops* parents. Putative amphiploids generated through meiotic restitution in F_1 hybrids between *Triticum durum* and *Ae. longissima accessions* had also two to three fold high grain iron and zinc content in comparison to the parental durum cultivars. The amphiploids are being used to transfer useful variability and development of alien addition and substitution lines in wheat background.

The sterile F1 hybrids between wheat and *Aegilops* species were extensively backcrossed with recurrent wheat cultivars to recover fertile introgression lines (ILs). Some fertile advance backcross ILs were found to have high grain iron and zinc content, confirming the transfer of superior genetic system(s) of the *Aegilops* donors for high micronutrient content. This was also confirmed on the basis of higher grain ash content and higher grain ash iron and zinc in the wild donors, amphiploids and ILs. The ILs with high grain iron and zinc content are being characterized by using GISH, FISH and SSR markers.

INTRODUCTION

Out of 22 minerals required by human beings for normal growth, dietary deficiency of iron and zinc is the most common and wide spread. About 60-80 % of the world population suffers from iron and more than 30 % from zinc deficiency[1]. Mineral density in staple food crops such as rice and wheat in Asia, maize in Sub-Saharan Africa and Latin America is very low. There is little existing variability for micronutrients in the cultivated germplasm of cereals for their improvement[2,3]. Losses during processing[4] and their reduced bioavailability due to the presence of various antinutritional factors such as phytic acid, tannins, lignins and food fibers further aggravate the problem [5,6]. Among various strategies such as biofortification, dietary diversification, fortification and supplementation; biofortification through genetic engineering and molecular breeding of plants for high mineral content in the grains is considered the most sustainable and cost effective approach [7,8]. To biofortify micronutrient-poor wheat cultivars through molecular breeding, the germplasm of related wild species has been evaluated for useful variability for higher grain and zinc.[9,10.]

This article deals with the evaluation of several non progenitor *Aegilops* species for higher grain iron and zinc content and their utilization for wheat biofortification through interspecific crosses and synthetic amphiploids.

MATERIALS AND METHODS

The experimental material comprising eighty accessions wild *Triticum* and non progenitor *Aegilops* and species of wheat from different geographical origins as well as 15 bread and durum wheat cultivars was obtained from the Wheat Germplasm Collection maintained at the Punjab Agricultural University, Ludhiana, India. The related wild species and bread and durum wheat cultivars were grown at the experimental fields of the Indian Institute of Technology, Roorkee for two consecutive seasons of 2004-05 and 2005-06. Grain samples of wild accessions, cultivars and amphiploids were digested as per the standard procedure described previously[11]. Micronutrient analysis was done using Atomic Absorption Spectrophotometer and the concentrations were expressed in parts per million. Interspecific crosses were made using Chinese Spring (Ph^I) as the female parent with selected *Aegilops* donors for inducing homoeologous chromosome pairing[12,13]. For meiotic analysis spikes of interspecific F_1 plants were fixed in Cornoy's solution for 24 hours and then transferred to 70% ethanol. Anthers of different hybrids, derivatives and amphiploids at various stages of meiotic division-I were squashed in 2% acetocarmine and the pollen mother cells (PMCs) were scored for chromosomal count and pairing. Grain ash and grain ash micronutrient content of some parents and backcross derivatives were also analyzed. Amphiploids were synthesized by immersing coleoptiles of germinating seeds of F_1 hybrids with 0.25% of colchicine (in 5 % DMSO solution) for 5 hours. The spikes with doubled

chromosomes and dehiscing viable pollen grains during anthesis were identified and tagged. Synthetic amphiploids of *T. durum – Ae. longissima* were obtained through unreduced gamete formation in the fertile F_1 hybrids without colchicine treatment.

RESULTS AND DISCUSSIONS

All the 15 bread and durum wheat cultivars recommended for commercial cultivation in northern India, possess low levels of grain iron (23-27 ppm) and zinc (18-22 ppm) content (Table 1), thus emphasizing the necessity for identification and utilization of wild germplasm for wheat biofortification. There are several reports of lower iron and zinc content among bread and durum wheat cultivars as compared to the wild and primitive *Triticum* species[3, 14]. Diverse wild diploid wheat (ssp *urartu*, ssp *boeoticum*,ssp *mococcum*), tetraploid wheat (ssp *dicoccoides*), *Ae.tauschii* and synthetic wheat (*T.dicoccoides*× *Ae.tauschii*) have been screened by various scientists[2,3,9,10,15] for grain iron and zinc content. Different accessions of *Ae. kotschyi, Ae. peregrina, Ae. longissima, Ae. geniculata, Ae. ventricosa* were found to have 2 to 2.5 times higher grain iron as well as zinc content. Secondary and tertiary gene pools of wheat are known to harbour many gene(s) for abiotic[16], biotic stress[17,18,19] and for other agronomically useful traits which have been utilized for wheat germplasm enhancement. The screened wild relatives in general and selected donors in particular, with high grain iron content were also found to have high grain zinc content, which strongly suggests similar mechanism(s) of uptake, translocation and deposition of the two micronutrients. High positive correlation in grain iron and zinc content were also reported by other scientists[20,21]. Most of the amphiploids having bolder grains than their durum and bread wheat parents had nearly as high grain iron and zinc content as that of the *Ae. kotschyi* or *Ae.longissima* parent indicating that the Aegilops species possess superior mechanism(s) for micronutrient biofortification. Cytological studies revealed that the wheat-*Ae.kotschyi* amphiploids having variable chromosome number were slightly less stable than *T. turgidum* ssp. *durum-Ae. longissima* amphiploid (Fig. 1). The useful variability from the U/S genome can be transferred to wheat through induced homoeologous

Table1. Range and mean of grain iron and zinc content of bread and durum wheat cultivars, wild *Triticum* and *Aegilops* species and their backcross derivatives and amphiploids.

S.N.	Species/derivative	No. of accessions	Genome	Iron (mg/kg)		Zinc (mg/kg)	
				Range	Mean ± S.D	Range	Mean ± S.D
1	*Triticum aestivum*	13	ABD	21.2-30.5	27.9 ± 1.2	14.8-19.3	22.1 ± 1.0
2	*T. durum*	2	AB	21.9-25.6	23.5 ± 0.9	13.6-19.6	18.7 ± 1.7
3	*T. monococcum*	4	A^m	20.6-22.4	21.3 ± 0.8	19.5-22.8	20.5 ±1.5
4	*T. boeoticum*	19	A^m	23.8-40.5	30.9 ± 0.6	22.1-39.0	29.2 ±2.0
5	*T. dicoccoides*	17	AB	27.6-42.6	32.9 ± 1.3	22.5-66.5	35.3 ± 0.6
6	*T. araraticum*	6	AG	23.1-59.0	29.8 ± 1.9	19.2-30.5	23.5 ± 0.7
7	*Ae. longissima*	5	S^l	59.1-81.5	73.2 ± 2.5	24.9-50.5	41.6 ± 2.8
8	*Ae. kotschyi*	14	US	22.9-90.9	67.4 ± 1.4	22.2-58.6	49.2 ± 2.1
9	*Ae. peregrina*	10	US	34.3-82.3	64.6 ± 0.8	33.3-49.4	59.5 ± 0.8
10	*Ae. cylindrica*	3	CD	52.2-93.2	66.7 ± 0.7	32.3-52.1	38.5 ± 1.1
11	*Ae. ventricosa*	3	DN	55.4-93.5	65.7 ± 1.2	24.0-39.0	33.8 ± 0.5
12	*Ae. geniculata*	3	UM	52.2-81.9	69.9 ± 1.8	31.9-40.8	37.7 ± 1.9
13	Amph. CS(Ph^I)-*Ae. kotschyi*	4	ABDUS	59.8-70.8	62.9 ± 1.3	29.5-42.7	34.2 ± 0.6
14	Amph. WL711-*Ae. kotschyi*	3	ABDUS	61.8-65.3	63.5 ± 1.5	30.6-33.3	32.0 ± 0.8
15	Amph. CS(Ph^I)-*Ae. peregrina*	2	ABDUS	65.4-67.8	66.3 ± 0.7	32.4-40.8	42.5 ± 1.0
16	Amph. *T. durum-Ae. longissima*	4	ABS	45.5-53.9	50.4 ± 0.4	28.6-53.5	50.3 ± 2.0
17	Backcross derivatives 0f *Ae. kotschyi*	8	ABDU/S	33.7-61.9	46.5 ± 2.0	29.0-4.2	36.9 ± 0.2
18	Backcross derivatives of *Ae. peregrina*	6	ABDU/S	31.5-61.2	43.1 ± 1.1	41.9- 64.2	49.4 ± 1.5

chromosome pairing using CS (Ph^I). The sterile F_1 hybrids of wheat/*Ae. kotschyi*, and wheat/*Ae. peregrina* with variable homoeologous chromosome pairing (Fig. 1) were backcrossed with recurrent parent. A number of BC_1 and $BC_2F_{2,3}$ progenies with nearly normal chromosome number and pairing, spike morphology and harvest index and high grain iron and zinc content were recovered. This may be attributed to *Aegilops* chromosome(s) transfer, substitutions and additions in wheat background. This unequivocally demonstrates the proof of the concept of the superior genetic systems of *Aegilops* donors for uptake, translocation which could be used for wheat biofortification. Morphological and SSR markers showed the association of homoeologous group 2 of *Ae. kotschyi* in $BC_2F_{2/3}$ progenies carrying with high grain iron and zinc content. Work to identify *Aegilops* alien addition, substitution and transfer lines using molecular cytogenetics is in progress.

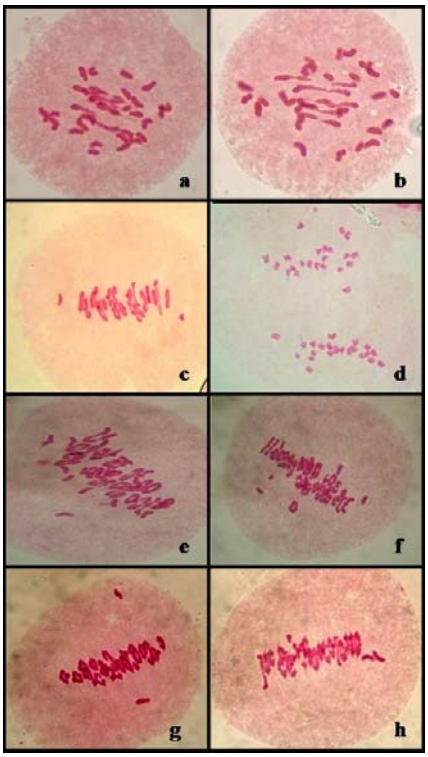

Fig.1. Chromosome pairing in wheat-*Aegilops* F_1 hybrids, introgressive derivatives, synthetic amhiploids and durum wheat-*Ae. longissima* amphiploids a. F_1 WL 711/*Ae.kotschyi* 391 (1 III +3 II+ 26 I) b. F_1 CS(Ph^I)/*Ae. kotschyi* 396 (6 II + 23 I) c. BC_2F_2 CS(Ph^I)/*Ae. kotschyi* 396 derivative (19 II+ 2 II) d. BC_1F_3 CS(Ph^I)/*Ae.kotschyi* 3790 derivative (21 II + 1 I) e. Amph. CS(Ph^I)-*Ae. kotschyi* 3774 (32 II + 8 I) f. Amph. WL711-*Ae. kotschyi* 3790 (32 II+3 I) g. Amph. PDW 233-*Ae. longissima* 28 (20 II + 2 I) h. Amph. PDW 274-*Ae. longissima* 3770 (20 II + 2I).

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