Developing improved durum wheat germplasm by altering the cytoplasmic genomes

Ghavami F., Bassi F. M., Burciaga R., Soltani A., Noyszewski A., De Jimenez M.K.M., Gu Y.Q., Meinhardt S., Elias E.M., Kianian P.M.A., Mergoum M., Schivcharan S. M., Kianian S. F.

in

Porceddu E. (ed.), Damania A.B. (ed.), Qualset C.O. (ed.).
Proceedings of the International Symposium on Genetics and breeding of durum wheat

Bari : CIHEAM
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 110

2014
pages 177-187

Ghavami F., Bassi F. M., Burciaga R., Soltani A., Noyszewski A., De Jimenez M.K.M., Gu Y.Q., Meinhardt S., Elias E.M., Kianian P.M.A., Mergoum M., Schivcharan S. M., Kianian S. F. **Developing improved durum wheat germplasm by altering the cytoplasmic genomes .** In : Porceddu E. (ed.), Damania A.B. (ed.), Qualset C.O. (ed.). *Proceedings of the International Symposium on Genetics and breeding of durum wheat.* Bari : CIHEAM, 2014. p. 177-187 (Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 110)

Developing improved durum wheat germplasm by altering the cytoplasmic genomes

Farhad Ghavami[1], Filippo M. Bassi[1,2], Ryan Burciaga[1], Ali Soltani[1], Andrzej Noyszewski[1], Monika K. Michalak De Jimenez[1], Yong Q. Gu[3], Steven Meinhardt[1], Elias M. Elias[1], Penny M.A. Kianian[1], Mohamed Mergoum[1], Schivcharan S. Maan[1], Shahryar F. Kianian[3]

[1] North Dakota State University, USA
[2] ICARDA, Morocco
[3] USDA-ARS, USA

Abstract. In eukaryotic organisms, nuclear and cytoplasmic genomes interact to drive cellular functions. These genomes have co-evolved to form specific nuclear-cytoplasmic interactions that are essential to the origin, success, and evolution of diploid and polyploid species. Hundreds of genetic diseases in humans and phenotypic variations in plants are known to be the result of alterations affecting nuclear-mitochondrial (NM) communication. The genetic bottleneck in the nuclear genome of modern polyploid wheat species is mirrored by the homogeneity of cytoplasmic genomes in durum and bread wheat cultivars. This lack of variation is illustrated by our data indicating that the mitochondrial genome of durum wheat is almost identical to that of published bread wheat genome. The data by our group and others clearly illustrate that genes affecting NM interactions are directly or indirectly related to hybrid compatibility. Therefore, their manipulation and use would permit wider usage of alien germplasm and more efficient introgression. Thus, we have embarked on a series of studies to: 1) isolate, characterize and manipulate genes involved in NM interaction; 2) better understand the influence of cytoplasmic genome by analyzing the vast collection of wheat alloplasmic lines; and 3) determine the extent of mitochondrial genome variability in *Triticeae* and *Aegilops* species in order to generate more cytoplasmically variable, and agronomically adapted cultivars.
Utilizing traditional genetic mapping and radiation hybrid mapping, we located a gene in durum wheat (*T. turgidum* L. var. *durum*) involved in NM compatibility to a chromosome segment of a few hundred Kb in size. Isolation and characterization of this gene will provide us the ability to understand and manipulate regulatory mechanisms responsible for a number of developmental processes in durum wheat, including embryo/seed development and plant vigor. In parallel, we have demonstrated that variation in the cytoplasmic genome can influence plant-pathogen response such as the interaction with *Pyrenophora tritici-repentis* (tan spot) and *Puccinia triticina* (leaf rust). Sequencing the mitochondrial genome of an alloplasmic wheat line indicated a great amount of sequence and structural changes in the genome, and at a much higher frequency than is observed in evolutionarily distant species. Additionally, our data indicated paternal leakage, heteroplasmy and stoichiometric changes in the mitochondrial genomes. These results have important implications in terms of the potential to manipulate plant mitochondrial genomes and select for changes that are critical to plant development and adaptation**.**

Since plants cannot escape from adverse environmental conditions, adaptation is paramount to species survival. Cytoplasmic genomes play a critical role in adaptation, and possibly speciation. Therefore, manipulation of mitochondrial genomes and creation of new cytoplasmic variability may provide a further mechanism for stress tolerance.

Keywords. Cytoplasmic variability – Alloplasmic – Stress tolerance – Mitochondria – Breeding.

Amélioration génétique du blé dur à travers la modification des génomes cytoplasmiques

Résumé. *Dans les organismes eucaryotes, les génomes nucléaires et cytoplasmiques interagissent pour diriger les fonctions cellulaires. Ces génomes ont co-évolué pour produire des interactions nucléaires-cytoplasmiques spécifiques qui sont essentielles pour l'origine, le succès, et l'évolution des espèces diploïdes et polyploïdes. Des centaines de maladies génétiques chez l'homme et des variations phénotypiques chez les plantes sont connues pour être le résultat des altérations de la communication nucléaire-mitochondriale (NM). Le goulot d'étranglement génétique au niveau du génome nucléaire des espèces de blé polyploïdes modernes est reflété par l'homogénéité des génomes cytoplasmiques chez les cultivars de blé dur et de blé tendre. Cette absence de variabilité est illustrée par nos données indiquant que le génome mitochondrial du* *blé dur est presque identique à celui du blé tendre publié. Les données de notre groupe et d'autres groupes montrent clairement que les gènes intervenant dans les interactions NM sont directement ou indirectement liés à la compatibilité de l'hybride. Par conséquent, leur manipulation et leur utilisation permettrait d'exploiter davantage le matériel génétique étranger et de mieux réussir l'introgression. Ainsi, nous avons entrepris une série d'études pour : 1) isoler, caractériser et manipuler les gènes impliqués dans l'interaction NM ; 2) mieux comprendre l'influence du génome cytoplasmique à travers l'analyse de la vaste collection de lignées de blé alloplasmiques ; et 3) déterminer l'importance de la variabilité du génome mitochondrial des espèces Triticeae et Aegilops afin d'obtenir des cultivars plus variables du point de vue cytoplasmique et adaptés sur le plan agronomique.*

En utilisant la cartographie génétique traditionnelle et la cartographie des hybrides d'irradiation, nous avons localisé un gène dans le blé dur (T. turgidum L. var. durum) impliqué dans la compatibilité NM sur un segment chromosomique de quelques centaines de Kb. L'isolement et la caractérisation de ce gène nous permettra de comprendre et de manipuler des mécanismes de régulation responsables d'un certain nombre de processus de développement du le blé dur, y compris le développement embryon/semence et la vigueur de la plante. Parallèlement, nous avons démontré que la variation du génome cytoplasmique peut influencer la réponse plante-pathogène comme dans le cas de l'interaction avec Pyrenophora tritici-repentis ('helminthosporiose) et Puccinia triticina (rouille des feuilles). Le séquençage du génome mitochondrial d'une lignée de blé alloplasmique a mis en évidence de nombreux changements des séquences et des structures du génome, et ce, avec une fréquence beaucoup plus élevée par rapport aux espèces distantes du point de vue évolutif. En outre, nos données indiquent une perte de génome paternel, des changements de l'hétéroplasmie et des changements stœchiométriques dans les génomes mitochondriaux. Ces résultats sont importants dans la mesure où ils offrent un élan potentiel à la manipulation des génomes mitochondriaux des plantes et à la sélection des changements qui sont essentiels pour le développement et l'adaptation des plantes.

Comme les plantes ne peuvent pas échapper aux conditions de milieu défavorables, l'adaptation est primordiale pour la survie des espèces. Les génomes cytoplasmiques jouent un rôle fondamental dans l'adaptation et, probablement, la spéciation. Par conséquent, la manipulation des génomes mitochondriaux et la création d'une nouvelle variabilité cytoplasmique peuvent fournir un mécanisme supplémentaire pour la tolérance au stress.

Mots-clés. *Variabilité cytoplasmique – Alloplasmique – Tolérance au stress – Mitochondries – Sélection.*

I – Introduction

Wheat belongs to the Triticeae tribe of grasses, a group comprising some 300 species (Matsuoka, 2011). Wheat is widely adapted, grown on more land than any other agricultural plant, and - with rice and maize - vies yearly for the greatest tonnage of worldwide production (Shewry, 2009). Wheat also is one of the oldest crops, as established from abundant archaeological, religious (e.g., Biblical stories), and historical evidence indicating its importance to human civilization (Fuller, 2007). Wheat cultivation occurred around 10,000 years ago when human beings started to shift from hunting and gathering to self-production (Shewry, 2009). The early species cultivated by man were mostly diploid einkorn (AA) and tetraploid emmer (AABB) wheat (Dubcovsky and Dvorak, 2007). The *Triticum-Aegilops* species diverged from each other around 3 million years ago (Chalupska *et al*., 2008; Dvorak and Akhunov, 2005) (Fig. 1). This divergence followed changes in chromosome number as a result of two episodes of allopolyploidization leading to the formation of hexaploid cultivated bread wheat (Fig. 1). The first hybridization leading to the formation of cultivated tetraploid wheat (i.e., durum or pasta wheat) occurred ~0.5 million years ago and the second event leading to the formation of hexaploid wheat happened ~8,000 years ago (Fig. 1) (Chalupska *et al*., 2008; Dvorak and Akhunov, 2005). These events created genetic bottlenecks, which excluded potentially valuable alleles from the polyploid forms (Dubcovsky and Dvorak, 2007).

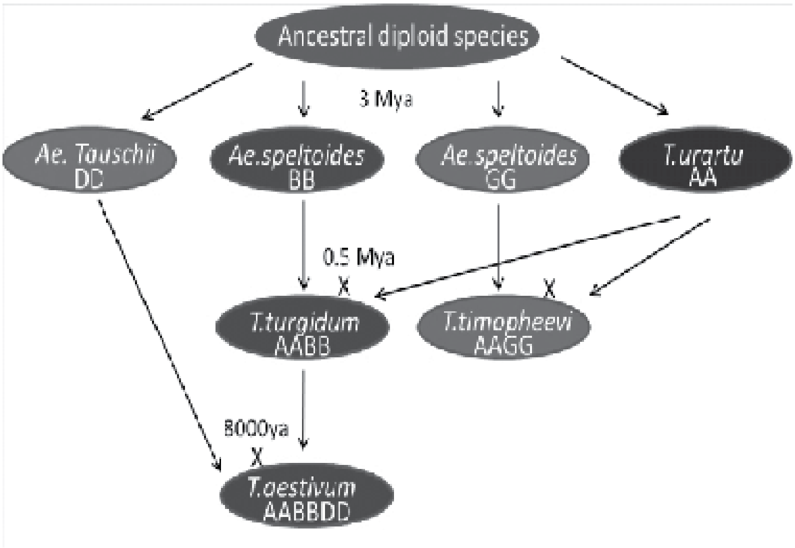

Figure 1. Evolutionary relationship of *Aegilops* and *Triticum* species leading to the formation of tetraploid pasta and hexaploid bread wheat. The wheat B genome donor is extinct, and is believed to be best represented by the S genome of *Ae. speltoides*. The cytoplasmic component of the genome is identified by ellipses filled with different shades of gray while the genomes are identified by their designation (e.g. DD for *T. tauschii*). MYA= million years ago.

It seems less than 15% of the *Ae. tauschii* and 30% of wild emmer wheat genetic variability is present in the D and A+B genomes of hexaploid wheat, respectively. However, the genetic diversity present in cultivated emmer wheat is 58% of the wild emmer wheat (Dubcovsky and Dvorak, 2007). The genetic bottleneck in the nuclear genome of modern polyploid wheat species is mirrored by the homogeneity of cytoplasmic genomes in durum and bread wheat cultivars. The hybridization event leading to the formation of tetraploid wheat was a rare event. The cytoplasmic genome, derived from an extinct species related to *Ae. speltoides*, coming from the female parent went through a similar reduction in variation (Fig. 1). Early in wheat research, Kihara (Kihara, 1954) showed that in crosses of polyploids with diploids, viable seed is more likely when the former is used as a female parent. Therefore, as the nuclear genome recovered some of its variation through introgressions from wild species, the cytoplasmic genome remained homogeneous due to uni-directional interspecific cross incompatibilities (Maan and Endo, 1991). This bottleneck was reiterated when the tetraploid wheat hybridized with Ae. tauschii forming the hexaploid wheat (Fig. 1). Lack of cyptoplamic variation is illustrated by a study that compared the mitochondrial genomes of 29 tetraploid and hexaploid wheat accessions with 21 microsatellite loci indicating that they are all the same (Ishii *et al*., 2006). Recently, we sequenced mitochondrial genomes of several Triticum species using 454 GS FLX technology. The *T. turgidum* mitochondrial genome is 451,925 bp in size and is almost identical in size to that of T. aestivum genome (452,528 bp) (Ogihara *et al*., 2005). The two genomes showed only 40 single nucleotide polymorphisms (SNPs) as compared with 605 SNPs between *T. aestivum* and *T. tauschii*.

In eukaryotic organisms, nuclear and cytoplasmic (mitochondria and plastids/chloroplast) genomes interact to drive cellular functions and biomass production. These genomes have co-evolved to form specific nuclear-cytoplasmic interactions that are essential to the origin, success, and evolution of diploid and polyploid species (Woodson and Chory, 2008). Plastids are known for their contribution to photosynthesis and storage of biomolecules such as carbophydrates, aminoacids and hormones. Therefore, the appropriate function of plastids in maintaining plant development and physiological process depends on the efficiency of the communication between nucleus and plastids in the cell (Jung and Chory, 2010). Mitochondria are also essential in the cell by providing the cellular energy through production of ATP needed for daily functions. Coordination of gene expression between the nuclear and mitochondrial genomes is critically important for all eukaryotic cells (Woodson and Chory, 2008). Plant mitochondria are not only vital for cell respiration, but are also involved in many important physiological functions such as oxidative stress (Mittler, 2002), alternative oxidase pathway (McDonald, 2008), programmed cell death (Vianello *et al.*, 2007) and cytoplasmic male sterility (Hanson, 1991). Therefore, the lack of variability of the cytoplasmic genome in polyploid wheat has significantly reduced our ability to develop valuable germplasm for crop improvement.

Although synthetic wheat production has been utilized to increase the spectrum of nuclear genome variability in wheat (Dubcovsky and Dvorak, 2007) wheat breeders have yet to utilize cytoplasmic variability. Alloplasmic lines (lines with alien cytoplasm) are created by replacing the nucleus of one species, through substitution backcrossing, with that of another species (Tsunewaki *et al.*, 1996). Thus, in an alloplasmic line, a new combination of nucleus and cytoplasm is created. A large collection of alloplasmic lines has been created in wheat (Tsunewaki *et al*., 1996; Tsunewaki *et al*., 2002). All alloplasmic lines are derived by using cytoplasmic donors as the female parent and a wheat or a bridging species as the nuclear donor parent followed in certain situations by embryo rescue of the resulting hybrid (Tsunewaki *et al*., 1996). In order to better understand the role of cytoplasmic genomes in wheat development and to increase their variability in modern cultivars, we have embarked on a series of studies to: 1) isolate, characterize and manipulate genes involved in Nuclear mitochondrial (NM) interaction; 2) better understand the influence of cytoplasmic genome by analyzing the vast collection of wheat alloplasmic lines; and 3) determine the extent of mitochondrial genome variability in *Triticeae* and *Aegilops* species in order to generate cytoplasmically more variable, and agronomically more adapted cultivars.

II – Genes involve in nuclear cytoplasmic (NC) compatibility

Over evolutionary time, many mitochondrial genes have been transferred to the nuclear genome, making proper NM interaction essential for cell function (Woodson and Chory, 2008). Changes in nuclear or mitochondrial genomes may interrupt intracellular communication, resulting in nuclear cytoplasmic (NC) incompatibility. The results of NC may include cytoplasmic male sterility (CMS), stunted growth, or seed abortion (Chase, 2007; MIchalak de Jimenez *et al.*, 2013. Key genes involved in NC compatibility are of critical importance for alloplasmic wheat production in the breeding programs (Maan, 1992a). According to the *scs* hypothesis, each diploid genome has at least one copy of the gene in the nucleus that facilitates NC compatibility (Maan, 1992b). These genes were named species-cytoplasm specific (*scs*) genes by Maan (Maan, 1975) or later as nuclear-cytoplasm compatibility (*Ncc*) genes (Asakura *et al*., 1997). *Ncc* and *scs* genes were found to be located on chromosome 1 group of *T. timopheevii* and *Ae. tauschii* which are also present in the D genome of wheat (Anderson and Maan, 1995; Asakura *et al*., 2000; Maan *et al*., 1999).

Maan (1992b) observed that tetraploid wheat is more sensitive to cytoplasm substitution than hexaploid wheat . The nucleus of hexaploid wheat (*T. aestivum*) was fully compatible with the cytoplasm of *Ae. longissima* (S1S1; 2n=2x=14) or *Ae. tauschii* but not the nucleus of tetraploid wheat (*T. turgidum*). However, male sterile alloplasmic lines of durum wheat in *Ae. longissima* or *Ae. tauschii* cytoplasm could be viable by transferring the whole or part of chromosome 1A from *T.timopheevi* or chromosome 1D from *T. aestivum* (Asakura *et al*., 1997; Asakura *et al*., 2000; Hossain *et al*., 2004b; Maan, 1992b). The *scs* genes originating from *T. timopheevii* Chromosome 1A and *T. aestivum* chromosome 1D designated as scs^{ti} and scs^{ae}, respectively in the durum wheat background. The positions of these genes were mapped in the alloplasmic lines of durum wheat having *Ae. longissima* cytoplasm or simply (lo) durum line using genetic mapping (Simons *et al.*, 2003) and radiation hybrid mapping (Hossain et al., 2004a). The two mapping strategies have been further implemented to identify potential candidate genes. Recently, by using the radiation hybrid mapping and designing gene based markers with the help of synteny between wheat, rice and *Brachypodium* the location of *scs* locus could be narrowed to a 1.1 Mb segment (MIchalak de Jimenez *et al*., 2013). The genetic mapping population also increased to 5,932 lines facilitated the fine mapping of the region on 1A for *scs*^{ti} (Ghavami *et al.*, 2010). Our results show that *scs*^{ti} and *scs*^{ae} are homoeoalleles (Fig. 2).

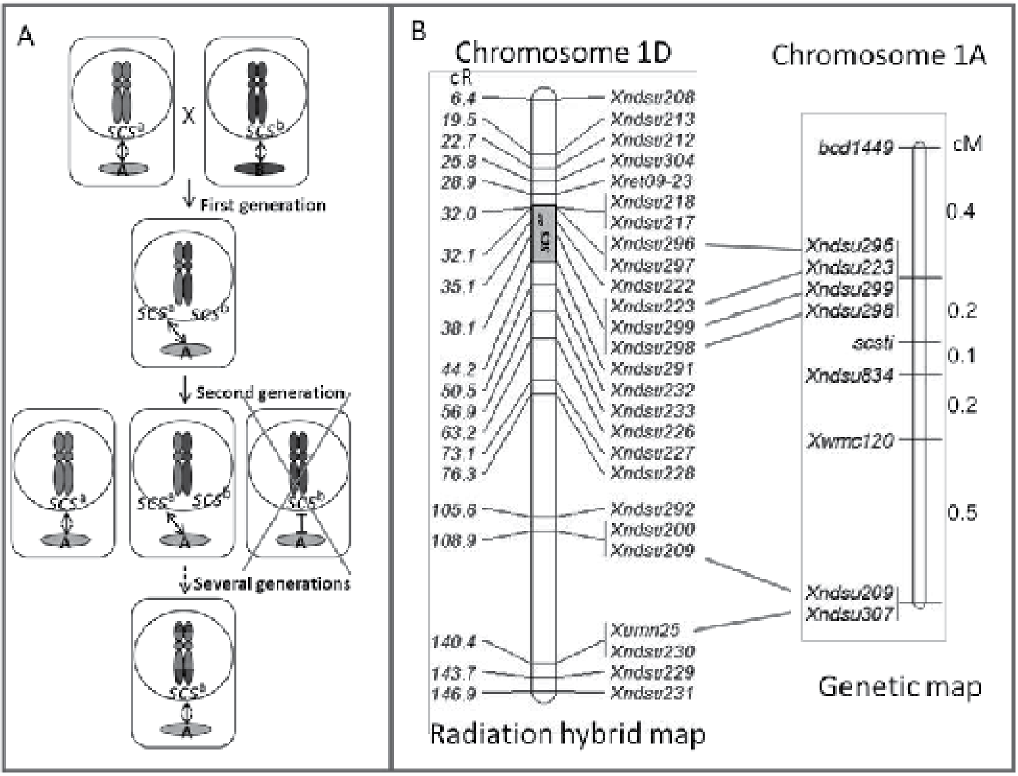

Figure 2. The hypothetical action of the *scs* gene during self- pollinated plant evolution (A) and the location of the *scs* homoeoalleles on chromome 1A and 1D mapped in an alloplasmic line of *T. turgidum* with *Ae. longissima* cytoplasm. The *scs* gene facilitates nuclear-cytoplasmic compatibility and during the evolution needs to be preserved. The *scs*^{ae} which can restore the compatibility between the *T. turgidum* nucleus and the *Ae. longissima* cytoplasm, was mapped to the long arm of chromosome 1D via radiation hybrid mapping. The genetic map also revealed the location of the *scs*^{ti} (derived from *T. timopheevii*) to almost the same location on chromosome 1A (B). For more information regarding the *scs* gene and marker sequences, see De Jimenez *et al*. (2013).

The homoeologous relationship between *Ncc-tmp1A* (from *T. timopheevii* 1A chromosome) and *Ncc-tmp1G* (from *T. timopheevii* 1G chromosome) has been confirmed before (Asakura *et al.*, 2000). It is very likely that common wheat carries three different *scs* gene homoeoalleles present in A, B and D genomes; however, only the *scs* on chromosome 1B is responsible for the NC compatibility, since both the cytoplasm and the B genome originated from *Ae. speltoides* (Fig. 1). The other *scs* gene homoeoalleles on chromosomes 1A and 1D of *T. aestivum* ensure NC compatibility when combining a nucleus of common wheat with different species carrying other plasmon types. Consequently, *T. aestivum* is compatible with *Ae. tauschii* as well as *T. urartu* (Tsunewaki, 1980). The presence of the three different *scs* gene homoeoalleles in wheat is explained by the fact that *T. aestivum* is more compatible in regards to cytoplasm substitution than durum wheat, which has only two different *scs* gene homoeoalleles. This hypothesis could also explain why it is not possible to produce alloplasmic durum lines with the *Ae. tauschii* cytoplasm, whereas this was not an issue with nuclear genome of common wheat. Cloning of the *scs* gene could facilitate development of further alloplasmic line with durum wheat nucleus with additional species in *Triticum-Aegilops* tribe that are thus far have failed.

III – Analyzing the mitochondrial genomes from the bread wheat ancestors

In most plant species including wheat, mitochondrial DNA (mtDNA) is transmitted to the progeny maternally. However, minor paternal leakage has been observed in some cases, especially in alloplasmic lines of wheat (Tsukamoto *et al*., 2000). Although size of the wheat mitochondrial genome (mt genome) is less than 0.5 Mb (Ogihara *et al.*, 2005), sequencing mitochondrial genome is difficult due to the presence of multiple copies of mt genome in the cell having different rearrangements due to recombination (Burger *et al*., 2003). Most of the previous works on mt genome variation among *Triticeae-Agilops* species were based on restriction fragment length polymorphism (RFLP) analysis (Skuza *et al*., 2007; Wang *et al*., 2000) or PCR based markers (Wang *et al*., 1997).

The full-length sequence of wheat (*T. aestivum* cv. Chinese Spring) mt genome became available in 2005 (Ogihara *et al*., 2005). Since then, additions to the mt genome are limited to a single other wheat cultivar (*T. aestivum* cv. Chinese Yumai;) (Cui *et al*., 2009) and an alloplasmic line of wheat with *Ae. kotschyi* (Liu *et al*., 2011) cytoplasm. The sequence of the Chinese Yumai cultivar was almost identical to the Chinese Spring with a few single nucleotide polymorphisms (SNPs) in non-coding regions (Cui *et al*., 2009). The size of the alloplasmic mtDNA originating from *Ae. kotschi* was larger than *T. aestivum* (647 kb compared with 452 kb) and there were differences in gene structure and significant changes in non-coding regions of the genome (Liu *et al.*, 2011). The mt genome sequence and structure of wheat ancestors have not been reported. Recently, we sequenced the mt genome of *T. turgidum* var. durum and *Ae. tauschii* using the 454 GS FLX sequencing technology to gain insight into the variation and evolutionary changes that have occured in *Triticum-Aegilops* species (unpublished data). All genes previously described in *T. aestivum* mt genome (Ogihara *et al*., 2005) were present in both species. However, major gene differences in atp6, nad6, nad9, rps19-p, cob were found between *Ae. tauschii* and the other two *Triticum* species. Only five SNPs were identified in the gene space, and 40 SNPs in total between the two *Triticum* species. When mt genome of *Ae. tauschii* was compared with *T. aestivum*, 27 SNPs were found in gene space and 679 SNPs in total. Comparison of gene order showed multiple rearrangements between diploid *Ae. tauschii* and tetraploid and hexaploid wheat (Fig. 3). An alloplasmic line of durum wheat carrying the cytoplasm of *Ae. longissima* [(lo) durum line] was also sequenced and compared to the sequence of its parents. The mt genome of the alloplasmic line was significantly different from its maternal parent *Ae. longissima* , indicating accelerated evolutionary changes as a possible result of nuclear genome substitution. Accelerated evolution in mt genome of alloplasmic lines from other species (Allen *et al.*, 2007; Bentolila and Stefanov, 2012) emphasizes the importance of alloplasmic lines for enhancing the variation of cytoplasmic genomes that exist in the nature. The amount of changes observed in mitochondrial gene structure of *Ae. tauschii* as compared with *T. turgidum* may explain why the production of this alloplasmic condition failed in durum wheat as it likely interrupts the NM interaction.

Figure 3. The arrangement of genes in the mitochondrial genome of *T. aestivum*, *T. turgidum* and *Ae. tauschii* indicating various rearrangements. The increased amount of rearrangements observed between *T. turgidum* and *Ae.tauschii* as compared with *T. aestivum* is not surprising considering the evolutionary distance.

IV The influence of cytoplasmic genome on wheat cultivar performance

Effect of cytoplasm on several morphological traits were studied in alloplasmic lines of wheat. (Tsunewaki *et al*., 2002) could classify the forty-six plasmons from *Triticum-Aegilops* species into 17 distinct groups based on their effects on 21 wheat characters. The classification based on phenotypic effects was in agreement with the plasmon genotyping, based on RFLP analysis (Wang *et al*., 2000). Therefore, it can be concluded that the diversity in cytoplasmic genomes is mirrored by diversity in the phenotype of the plants. Plasmon changes affected the number of selfed seed (male fertility), in contrast to female sterility, which was not affected by cytoplasm exchange. Wang's paper illustrates the significant effect on all studied characters, indicating the indispensable role that the mitochondria and chloroplast genomes have in plant development.

Alloplasmic condition in wheat has also proven valuable in improving plant responses to biotic and abiotic stresses (Hou *et al*., 2000; Klimov *et al*., 2005; Liu *et al.*, 2002). A number of these alloplasmic lines also show improved vigor and higher yield relative to parental controls (Tsunewaki *et al*., 2002). We analyzed differential responses of various alloplasmic lines to wheat foliar pathogens *Puccinia triticina* (*Pt*, leaf rust) and *Pyrenophora tritici-repentis* (*Ptr*, tan spot), both major disease problems worldwide. In this study some alloplasmic lines of tetraploid durum wheat (*T. turgidum*) 56-1, and hexaploid wheat (*T. aestivum*) cultivars 'Chris' and 'Selkirk' were tested for disease response to *Ptr* isolates BR15 and Pti2 (Table 1). The experiment was conducted with multiple replications under conditions that promote disease growth (unpublished data). The alloplasmic lines of the same *T. aestivum* cultivar also showed different responses to leaf rust. The *T. dicoccoides* cytoplasm confers resistance to tan spot, making it a candidate source for cytoplasmic substitution in both hexaploid and tetraploid wheat.

Table1. Responses of alloplasmic lines of *T. aestivum* cv. Selkirk, *T. aestivum* cv. Chris and *T. turgidum* var. durum line 56-1 to two different isolates of *Pyrenophora tritici-repentis* as compared with their euplasmic donors.

Cytoplasm	Nucleous	BR15 isolate	Pti2 isolate
Original (euplasmic)	Se kirk	Moderately susceptible	Resistant
Ae. cylindrica	Se kirk	Susceptible	NSD
Ae. mutica	Se kirk	Resistant	NSD
T. dicoccoides	Se kirk	Resistant	NSD
Ae. bicornis	Se kirk	Resistant	NSD
Original (euplasmic)	Chris	Moderately susceptible	Moderately susceptible
Ae. crassa	Chris	Susceptible	NSD
Ae. variabilis	Chris	Resistant	Resistant
Ae. heldreichii	Chris	Resistant	Resistant
Ae. squarrosa	Chris	Resistant	NSD
Original (euplasmic)	56-1	Susceptible	Moderately susceptible
Ae. longissima	56-1	NSD	Resistant
Ae. sharonensis	56-1	Resistant	NSD
Ae. variabilis	56-1	Resistant	Susceptible
T. dicoccoides	56-1	Resistant	Resistant

NSD=Not significantly different

The importance of cytoplasm effect on resistance to fungal diseases is not new, and is well established (Mullaney, 1981; Voluevich and Buloychik, 1992). Wu *et. al.* (1998b) found *Ae. ventricosa* cytoplasm substitution in wheat cultivars delivers strong and stable resistance to alloplasmic wheat cultivars against wheat scab. Durum wheat breeding programs lack good wheat scab resistance sources (Buerstmayr *et al*., 2009).

Cytoplasmic substitution can be an alternative approach for enhancing durum wheat germplasm in this regard.

Many alloplasmic lines exhibit prolonged plant life span and delayed flowering (Tsunewaki *et al.*, 2002). However, there are some NC combinations that combine earliness with large ears (Wu *et al*., 1998a). This raises the possibility of using alloplasmic lines to improve yield. We examined a number of alloplasmic lines of hexaploid wheat cultivars 'Selkirk' and 'Chris' with *Ae. mutica* and *Ae. cylindrica* cytoplasm along with their euplasmic lines and also their hybrid progeny (Selkirk×Chris and Chris×Selkirk) for dry matter weight (unpublished data). Maternal cytoplasm (MC), nuclear-maternal cytoplasm (N×MC) interaction and maternal cytoplasm-paternal cytoplasm (MC×PC) each show significant effect on dry matter weight. Therefore, not only cytoplasm itself is important but also the proper combination with the nucleus makes a significant difference in improving certain characters (Table 2).

Table 2. Analysis of variance for organelle effect on dry mater weight in alloplasmic lines of wheat that carry cytoplasms of *Ae. mutica* and *Ae. cylindrica.*

Effects	DF	Mean Square	F Value	Pr > F
Replication (Rep)	9	206.45274	0.65	0.5926
Nucleus N	2	878.80567	2.79	0.0883
N×Rep	18	315.40490	1.27	0.2549
Maternal cytoplasm (MC)	1	13058.89861	120.21	<.0001
N×MC	2	1188.82826	10.94	0.0003
N×MC×Rep	27	108.62990	0.44	0.9877
Paternal cytoplasm (PC)	1	83.56247	0.34	0.5652
N×PC	2	9.84394	0.04	0.9612
MC×PC	1	4680.47174	18.81	<.0001
N×MC×PC	2	5991.62809	24.08	<.0001

V – Conclusions

Different studies conducted during the last fifty years have shown that the mitochondrial genome in wheat can be changed by nuclear genome substitution, creating new variations that can be exploited for germplasm enhancement and crop improvement. The advantage of these lines as a source for biotic and abiotic stress tolerance is that their integration into a cultivar improvement program is relatively simple for it merely requires their use as female in a backcrossing scheme. This method eliminates the need for making large, bi/multi-parental populations and recurrent selection in the breeding program. The most difficult aspect of this strategy is to establish an array of alloplasmic lines in improved backgrounds of the modern durum and bread wheat cultivars. Once established, the alloplasmic line of interest can be used in a recurrent backcrossing scheme to develop additional cultivars (Wu *et al*., 1998). Xiaoshan2134 is the only alloplasmic wheat cultivar released in China, and had 20% increase in yield over the control checks in testing over 1991 to 1996 (Wu *et al*., 1998a). In conclusion, using alloplasmic wheat may be an efficient alternative approach in plant breeding that justifies more attention.

References

Allen J.O., Fauron C.M., Minx P., Roark L., Oddiraju S., Lin G.N., Meyer L., Sun H., Kim K., Wang C.Y., Du F.Y., Xu D., Gibson M., Cifrese J., Clifton S.W., Newton K.J., 2007. Comparisons among two fertile and three male-sterile mitochondrial genomes of maize. *Genetics,* 177, pp. 1173-1192.

Anderson J.A., Maan S.S., 1995. Interspecific nuclear-cytoplasmic compat bility controlled by genes group-1 chromosomes in durum-wheat. *Genome,* 38, pp. 803-808.

Asakura N., Nakamura C., Ohtsuka I., 1997. A nuclear compat bility gene, Ncc-tmp, of *Triticum timopheevi* for the cytoplasm of *Aegilops squarrosa*. *Genes Genet. Systems,* 72, pp. 71-78.

Asakura N., Nakamura C., Ohtsuka I., 2000. Homoeoallelic gene Ncc-tmp of *Triticum timopheevii* conferring compatibility with the cytoplasm of *Aegilops squarrosa* in the tetraploid wheat nuclear background. *Genome,* 43, pp. 503-511.

Bentolila S., Stefanov S., 2012. A re-evaluation of rice mitochondrial evolution based on the complete sequence of male-fertile and male-sterile mitochondrial genomes. *Plant Physiology,* 158, pp. 996-1017.

Buerstmayr H., Ban T., Anderson J.A., 2009. QTL mapping and marker-assisted selection for *Fusarium* head blight resistance in wheat: a review. *Plant Breeding,* 128, pp. 1-26.

Burger G., Gray M.W., Lang B.F., 2003. Mitochondrial genomes: anything goes. *Trends in Genetics,* 19, pp. 709-716.

Chalupska D., Lee H.Y., Faris J.D., Evrard A., Chalhoub B., Haselkorn R., Gornicki P., 2008. Acc homoeoloci and the evolution of wheat genomes. *Proc. Nat. Acad. Sci. USA,* 105, pp. 9691-9696.

Chase C.D., 2007. Cytoplasmic male sterility: a window to the world of plant mitochondrial-nuclear interactions. *Trends in Genetics,* 23, pp. 81-90.

Cui P., Liu H., Lin Q., Ding F., Zhuo G., Hu S., Liu D., Yang W., Zhan K., Zhang A., Yu J., 2009. A complete mitochondrial genome of wheat (*Triticum aestivum* cv. Chinese Yumai), and fast evolving mitochondrial genes in higher plants. *J. Genet.,* 88, pp. 299-307.

Dubcovsky J., Dvorak J., 2007. Genome plasticity a key factor in the success of polyploid wheat under domestication. *Science,* 316, pp.1862-1866.

Dvorak J., Akhunov E.D., 2005. Tempos of gene locus deletions and duplications and their relationship to recombination rate during diploid and polyploid evolution in the A*egilops-Triticum* alliance. *Genetics*, 171, pp. 323-332.

Fuller D.Q., 2007. Contrasting patterns in crop domestication and domestication rates: Recent archaeobotanical insights from the old world. *Ann. Bot*., 100, pp. 903-924.

Ghavami F., Michalak M.K., Riera-Lizarazu O., Gu Y.Q., Thilmony R., Maan S.S., Kianian S.F., 2010. Plant and Animal Genome XVIII, San Diego, California, USA.

Hanson M.R., 1991. Plant mitochondrial mutations and male-sterility. *Annual Review of Genetics,* 25, pp. 461-486.

Hossain K.G., Riera-Lizarazu O., Kalavacharla V., Vales M.I., Maan S.S., Kianian S.F., 2004a. Radiation hybrid mapping of the species cytoplasm-specific (scsae) gene in wheat. *Genetics,* 168, pp. 415-23.

Hossain K.G., Riera-Lizarazu O., Kalavacharla V., Vales M.I., Rust J.L., Maan S.S., Kianian S.F., 2004b. Molecular cytogenetic characterization of an alloplasmic durum wheat line with a portion of chromosome 1D of *Triticum aestivum* carrying the scs(ae) gene. *Genome,* 47, pp. 206-14.

Hou N., Wu Y.W., Liu C.G., Zhang C.L., Zhang Y., 2000. Studies of salt tolerance of alloplasmic wheat. In: Yi Chuan Xue Bao (Eds.) *Acta Genetica Sinica,* 27, pp. 325-30.

Ishii T., Takahashi C., Ikeda N., Kamijima O., Mori N., 2006. Mitochondrial microsatellite variability in common wheat and its ancestral species. *Genes Genet. Systems,* 81, pp. 211-214.

Jung H.S., Chory J., 2010. Signalling between chloroplasts and the nucleus: can a systems biology approach bring clarity to a complex and highly regulated pathway? *Plant Physiology,* 152, pp. 453-459.

Kihara H., 1954. Considerations on the evolution and distribution of *Aegilops* species based on the analyser-method. *Cytologia,* 19, pp. 336-357.

Klimov V.V., Astakhova N.V., Alieva G.P., Salnikov E.B., Trunova T.I., Morozova Z.A., Semenov O.G.. 2005. Effect of alien cytoplasm of goatgrass on biological and physiological properties of alloplasmic wheats. *Biology Bull*., 32, pp. 234-239.

Liu C.G., Wu Y.W., Hou H., Zhang C., Zhang Y.,. McIntosh R.A., 2002. Value and utilization of alloplasmic common wheats with *Aegilops crassa* cytoplasm. *Plant Breeding,* 121, pp. 407-410.

Liu H.T., Cui P., Zhan K.H., Lin Q., Zhuo G.Y., Guo X.L., Ding F., Yang W.L., Liu D.C., Hu S.N., Yu J., Zhang A.M., 2011. Comparative analysis of mitochondrial genomes between a wheat K-type cytoplasmic male sterility (CMS) line and its maintainer line. *Bmc Genomics*, pp. 12.

Maan S.S., 1975. Cytoplasmic variability and speciation in *Triticinea.* In: *A Multiple Review.* Wali M. K. (ed) Univ. North Dakota Press, Grand Forks, ND., pp. 225-281

Maan S.S., 1992a. A Gene for Embryo-Endosperm Compatibility and Seed Viability in Alloplasmic *Triticum turgidum*. *Genome,* 35, pp. 772-779.

Maan S.S., 1992b. Transfer of a species cytoplasm specific (scs) gene from *Triticum.timopheevi* to *Triticum. turgidum*. *Genome,* 35, pp. 238-243.

Maan S.S., Endo T.R., 1991. Nucleocytoplasmic interactions stabilize ploidy level in wheat interspecific hybrids. *Genome,* 34, pp. 983-987.

Maan S.S., Joppa L.R., Kianian S.F., 1999. Linkage between the centromere and a gene producing nucleocytoplasmic compatibility in durum wheat. *Crop Sci*., 39, pp. 1044-1048.

Matsuoka Y., 2011. Evolution of polyploid *Triticum* wheats under cultivation: The role of domestication, Natural Hybridization and allopolyploid speciation in their diversification. *Plant Cell Physiology,* 52, pp. 750-764.

McDonald A.E., 2008. Alternative oxidase: an inter-kingdom perspective on the function and regulation of this broadly distributed 'cyanide-resistant' terminal oxidase. *Functional Plant Biology,* 35, pp. 535-552.

MIchalak de Jimenez M.K., Bassi F.M., Ghavami F., Simons K., Dizon R., Seetan R.I., Alnemer L.M., Denton A.M., Dogramaci M., Simkova H., Dolezel J., Seth K., Luo M.C., Dvorak J., Gu Y.Q., Kianian S.F., 2013. A radiation hybrid map of chromosome 1D reveals synteny conservation at a wheat speciation locus. *Functional & Integrative Genomics,* 13, pp. 19-32.

Mittler R., 2002. Oxidative stress, antioxidants and stress tolerance. *Trends in plant science,* 7, pp. 405-410.

Mullaney E.J., 1981. Increased resistance to *Septoria nodorum* in alloplasmic lines of a wheat cultivar. *Phytopathology,* 71, pp. 244-244.

Ogihara Y., Yamazaki Y., Murai K., Kanno A., Terachi T., Shiina T., Miyashita N., Nasuda S., Nakamura C., Mori N., Takumi S., Murata M., Futo S., Tsunewaki K., 2005. Structural dynamics of cereal mitochondrial genomes as revealed by complete nucleotide sequencing of the wheat mitochondrial genome. *Nucleic Acids Res*., 33, pp. 6235-6250.

Shewry P.R., 2009. Wheat. *J. Exptl. Bot*., 60, pp. 1537-1553.

Simons K.J., Gehlhar S.B., Maan S.S., Kianian S.F., 2003. Detailed mapping of the species cytoplasm-specific (*scs*) gene in durum wheat. *Genetics,* 165, pp. 2129-2136.

Skuza L., Rogalska S.M., Bocianowski J., 2007. RFLP analysis of mitochondrial DNA in the genus *Secale*. *Acta Biologica Cracoviensia Series Botanica,* 49, pp. 77-87.

Tsukamoto N., Asakura N., Hattori N., Takumi S., Mori N., Nakamura C., 2000. Identification of paternal mitochondrial DNA sequences in the nucleus-cytoplasm hybrids of tetraploid and hexaploid wheat with D and D2 plasmons from *Aegilops* species*. Curr, Genet.,* 38, pp. 208-17.

Tsunewaki K., 1980. Genetic diversity of the cytoplasm in *Triticum* and A*egilops. Japan Soc. Promotion of Science*, Tokyo, Japan, pp. 290.

Tsunewaki K., Wang G.Z., Matsuoka Y., 1996. Plasmon analysis of *Triticum* (wheat) and *Aegilops* .1. Production of alloplasmic common wheats and their fertilities. *Genes & Genetic Systems,* 71, pp. 293-311.

Tsunewaki K., Wang G.Z., Matsuoka Y., 2002. Plasmon analysis of *Triticum* (wheat) and *Aegilops*. 2. Characterization and classification of 47 plasmons based on their effects on common wheat phenotype. *Genes & Genetic Systems,* 77, pp. 409-427.

Vianello A., Zancani M., Peresson C., Petrussa E., Casolo V., Krajnakova J., Patui S., Braidot E., Macri F., 2007. Plant mitochondrial pathway leading to programmed cell death. *Physiologia Plantarum,* 129, pp. 242-252.

Voluevich E.A., Buloychik A.A., 1992. Nuclear-cytoplasmic interactions in wheat resistance to fungi pathogens .6. nuclear genome influence of alloplasmic lines on quantitative resistance of seedlings to clones. *Genetika,* 28, pp. 68-74.

Wang G.Z., Miyashita N.T., Tsunewaki K.. 1997. Plasmon analyses of *Triticum* (wheat) and *Aegilops*: PCR single-strand conformational polymorphism (PCR-SSCP) analyses of organellar DNAs. *Proc. Natl. Acad. Sci., USA,* 94, pp. 14570-14577.

Wang G.Z., Matsuoka Y., Tsunewaki K.,. 2000. Evolutionary features of chondriome divergence in *Triticum* (wheat) and *Aegilops* shown by RFLP analysis of mitochondrial DNAs. *Theor. Appl. Genet*. 100, pp. 221-231.

Woodson J.D., Chory J., 2008. Coordination of gene expression between organellar and nuclear genomes. *Nature Reviews Genetics,* 9, pp. 383-395.

Wu Y., Zhang C., Liu C., Ren Sh., Zhang Y., 1998a. Breeding technology of alloplasmic wheat. *Science in China Series C: Life Sciences,* 41, pp. 449-458.

Wu Y.W., Ren S.X., Liu C.G., Hou N., Zhang C.L., Zhang Y., 1998b. Influence of alloplasm on resistance to wheat scab. *Chinese Science Bulletin,* 43, pp. 1038-1041.