The progeny from the [(T. turgidum X Dasypyrum villosum) amphiploid X Triticum aestivum] hybridization is an effective source of new durum wheat inbred lines

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The progeny from the [(*T. turgidum* X *Dasypyrum villosum*) amphiploid X *Triticum aestivum*] hybridization is an effective source of new durum wheat inbred lines

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Abstract. The potential of recombination and chromosome elimination in progenies from homoploid hybridization between an amphiploid $A^dA^dB^dB^dVV$ and *Triticum aestivum* $A^aA^aB^aB^aDD$ leading to novel durum wheat inbred lines, remains largely unknown. Here, we report the results of homoploid hybridization performed among three $A^dA^dB^dB^dVV$ amphiploids ('M × V', 'C × V', 'Cr × V_B' obtained, after chromosome doubling of the hybrids from crossing *Dasypyrum villosum* (*Dv)* to the *T. turgidum* ssp *durum* cvs 'Modoc', 'Capeiti'and 'Creso', respectively), and five $A^aA^aB^aB^aDD$ bread wheat varieties ('Agadir', 'Chinese Spring' ('CS'), 'Provinciale', 'Sagittario', and 'Salgemma') and one inbred line ('41-3').
The average floret fertility upon controlled hybridization was 0.55 (1 caryopsis with F_1 embryo every 2 pollinated florets). After selfing, the F_1 plants produced caryopses with F_2 embryo in 40% of the florets. The chromosome number of the F_2 seedlings ranged from 2n=28 to 2n=42. The expected proportion of viable F_2 seedlings with 2n=28 was 3.72×10^{-9} while the observed proportion (0.065) was about 7 orders of magnitude higher. The observed frequency of the F_2 seedlings with chromosome number 2n>42 was below 0.03, which was in line with the expectation that F_2 zygotes with 2n>42 had a very low viability. The expected proportion of such zygotes was 0.57 determining an expected F_1 floret fertility of 43%, amazingly close to the observed average floret fertility of the F_1 plants. Seven new durum wheat inbred lines have been derived from the 'M × V' × 'CS' hybridization, two of them ('5-04' and '13-04') showing satisfactory agronomic and grain quality traits.

Keywords. Intergeneric hybridization – Breeding methods – Grain yield – Grain quality – Amphiploids – *Dasypyrum villosum* –*Triticum turgidum* L. ssp *durum.*

La descendance de l'hybridation [(T. turgidum X Dasypyrum villosum) amphiploïde X Triticum aestivum] est une source efficace de nouvelles lignées consanguines de blé dur

Résumé. *Le potentiel de recombinaison et d'élimination des chromosomes chez les descendants d'une hybridation homoploïde entre un amphiploïde AdAdBdBdVV et Triticum aestivum AaAaBaBaDD, conduisant à de nouvelles lignées de blé dur consanguines, reste encore largement inconnu. Nous allons illustrer les résultats d'une hybridation homoploïde réalisée avec trois amphiploïdes AdAdBdBdVV ('M x V', 'C × V', 'Cr × VB' issus du doublement des chromosomes des hybrides du croisement Dasypyrum villosum (Dv) et T. turgidum ssp durum cvs Modoc, 'Capeiti' et Creso', respectivement), et cinq variétés de blé tendre AaAaBaBaDD ('Agadir', 'Chinese Spring' ('CS'), 'Provinciale', 'Sagittario', et 'Salgemma') et une lignée consanguine ('41-3').*

Le fertilité moyenne des épillets lors de l'hybridation contrôlée était de 0,55 (1 caryopse avec un embryon F1 toutes les 2 épillets pollinisés). Après autofécondation, les plantes F1 ont produit des caryopses avec des embryons F2 dans 40% des épillets. Le nombre de chromosomes des semis F2 variait de 2n = 28 à 2n = 42. La proportion attendue de semis F2 viables avec 2n = 28 était de 3,72 × 10-9 tandis que la proportion observée (0,065) était environ sept fois plus élevée. La fréquence observée des semis F2 avec un nombre de chromosomes 2n> 42 était inférieure à 0,03, confirmant l'hypothèse avancée selon laquelle les zygotes F2 à 2n> 42 avaient une très faible viabilité. La proportion attendue de ces zygotes est de 0,57 et elle détermine une fertilité attendue des épillets F1 de 43%, étonnamment proche de la fertilité moyenne des

épillets observée chez les plantes F1. Sept nouvelles lignées consanguines de blé dur ont été obtenues par l'hybridation 'M x V' × 'CS' et, deux d'entre elles («5-04» et '13 -04 ') ont montré des caractères agronomiques et de qualité du grain satisfaisants.

Mots-clés. *Hybridation intergénérique – Méthodes de sélection – Rendement en grain – Qualité du grain – Amphiploïdes – Dasypyrum villosum – Triticum turgidum L. ssp durum.*

I – Introduction

In most regions where agriculture began, primary crops such as wheat, were domesticated only once or very few times (Blumler 1998) starting from local wild gene-pool. In the Fertile Crescent area, the A genomes of the diploid species *T*. *urartu* (Dvorak *et al*. 1993) in combination with a species that belonged to the lineage of the current wild wheat species, *Aegilops speltoides* (SS genome), initiated the evolution of the tetraploid AABB and AAGG genome species less than 0.5 million years ago (Matsuoka, 2011). Harlan and Zohary (1966) suggested that a large-seeded race of wild emmer wheat (*T. dicoccoides*), from the vicinity of the Upper Jordan Valley, was the likely progenitor of cultivated emmer.

Population genetic studies based on molecular data, indicated that also the northern populations of the Fertile Crescent area had an important role in the domestication of emmer wheat, although evidence for the site of domestication remains inconclusive (Matsuoka, 2011). The non-brittle rachis mutant phenotype had a role in the domestication of the hulled emmer wheat as well as the genotypic change from *qqTgTg* to *QQtgtg* which was essential for the emergence of the free-threshing phenotype in tetraploid wheats. Free-threshing durums derived from domesticated hulled emmer wheats migrated northeastward in association with the spread of agriculture across and beyond the Fertile Crescent region. Kihara (1944), McFadden and Sears (1946), and Kihara and Lilienfeld (1949) evidenced as the spontaneous hybridization between individuals of the populations of the hulled tetraploid wheat with those of the sympatric populations of the wild diploid species *T. tauschii*, the donor of the D genome, gave rise to the hexaploid wheat *T. aestivum*. However, Dvorak *et al* (2012) proposed that the tetraploid parent of hexaploid wheat was not hulled emmer but the free-threshing form of tetraploid wheat. In Armenia and the south west coastal area of the Caspian Sea and a corridor between the two areas, the "strangulata" genepool of *T. tauschii* hybridized with the free-threshing tetraploid and fertile hexaploid amphiploids were produced by self-pollination of the triploid hybrids (Dvorak *et al* 1998a) due to high production of unreduced gametes (Kihara *et al.* 1950). Once a single free-threshing amphiploid was established, alleles contributed by subsequent intercrossing with hulled/spelt hexaploids from wild hulled tetraploids and *T. tauschii* hybridization, were particularly disadvantaged in the fields of free-threshing wheat and were lost because their adhering glumes would tend to eliminate them during threshing (Dvorak *et al.* 1998b).

This brief history of wheat domestication indicated that: (a) because the majority of accessions of ancestral hulled emmer wheat species were not involved in free-threshing speciation, many of their unique genes may not be present in the released *Td* varieties (Reif *et al.* 2005; Warburton *et al,* 2006); (b) free-threshing speciation caused a genetic bottleneck for adaptive traits that hinder resilience of the current wheat germplasm to pressures from global warming; (c) the differences in ploidy levels between *Td* and *Ta*, may have caused divergence in gene expression and gene evolution, especially for quantitative trait loci (QTL) in the AB genomes of tetraploids and hexaploid wheat species (Zhang *et al*, 2012); (d) under cultivation, the AB genomes of the restricted gene-pool of free-threshing tetraploid and hexaploid wheats evolved independently but followed a common domestication path: new alleles were generated by mutation and novel allele combinations formed through recombination which were selected by early farmers, resulting in landraces adapted to specific local climatic conditions.

Since the beginning of the domestication of the free-threshing durum and bread wheat in the eastern Mediterranean region (Feldman and Kislev 2007, Luo *et al*. 2007), the crop varieties were obtained from shuffling and selecting the genes inherited from the restricted number of free-threshing landraces that moved along the farmers while agriculture gradually diffused. During the last century the traditional landraces were continually replaced by modern wheat elite cultivars with a dual result of erosion of wheat genetic resources (van de Wouw *et al.* 2009) and a further reduction of genetic diversity in the cultivated gene pools. This exposed wheat farmers to the risk of yield reduction due to epidemics and vulnerability to environmental changes and the effect of global warming.

Different approaches are being pursued to introgress new genes in the cultivated durum wheat gene-pool to enlarge the genetic diversity necessary for further adaptations and yield increase.

One approach is the hybridization of the tetraploid durum wheat [*Triticum turgidum* L. spp*. durum* (Desf.) Husn. (=*Td*); chromosome constitution $A^dA^dB^dB^d$; 2n=4x=28] with the hexaploid bread wheats [*T. aestivum* L.) (=*Ta*); chromosome constitution $A^aA^aB^aB^aDD$; 2n=6x=42]. In this case, it is expected that (i) the genetic enhancement of *Td* occur by recombining the shared, but evolutionary divergent, $A^aA^dB^aB^d$ tetraploid chromosome complement, (ii) transfer the desirable D genome loci into durum (Boggini *et al.*, 2000), and (iii) the loss of the majority of the D chromatin. Kihara (1982) observed that the pentaploid F_1 plants from *Ta* × *Td*, contained 35 chromosomes consisting of 14 bivalents and seven univalents. In successive generations, plants divided into an 'increasing group', which included plants that returned to the hexaploid state and a 'declining group', which lost all D genome chromosomes, resulting in a tetraploid state. Recombination events after durum × bread wheat hybridization and their impacts on the selection and performance of new durums are well documented (Gilbert 2000, Wang *et al.* 2005, Lanning *et al.* 2008).

Another approach point to unlock the genetic variation concealed in the AB genome of bread wheat was coupled to recombination with the V genome of *Dasypyrum villosum* (*Dv*). This methodology may provide the necessary novel allele combinations for durum wheat trait enhancement and is based on the use of *T. turgidum* ssp *durum* × *Dasypyrum villosum (Dv)* amphiploid (genomes $A^dA^dB^dB^dVV$) instead of a *Td* parent, in the cross to *Ta* (De Pace *et al.* 2011a). The additional expectation from this method was the genetic enhancement of *Td* by the potential transfer of desirable V genome loci into *Td* genome complement. Genes from the V chromosomes have already been demonstrated to contribute to the improvement of grain yield and grain quality performance when introgressed in the wheat genomes (De Pace *et al.* 2011b).

The main objective of this study was the production of a set of progenies from the homoploid 'A^dB^dV-amphiploid' × '*Ta'* hybridization in order to assess (i) the average floret fertility of the parental amphiploid upon controlled hybridization with *Ta* pollen, (ii) the average fertility of the florets of the F_1 plants and the chromosome number of the F_2 seedlings, (iii) the expected and observed proportion of viable F_2 seedlings with 2n=28, (iv) the proportion of plants of the 'A^dB^dV-aphiploid' × '*Ta'* progeny that 'declined' to the durum chromosome number, and (v) the field performance of the derived new durums containing the $A^aA^dB^aB^d$ genomes and putative introgressed D or V genome loci.

II – Material and methods

1. Material

Three $A^dA^dB^dB^dVV$ amphiploids, 'M × V', 'C × V', and 'Cr × V_B' were obtained after crossing *Dv* to the *T. turgidum* ssp *durum* cvs 'Modoc', 'Capeiti' and 'Creso', respectively, followed by chromosome doubling after colchicine treatment of the seedlings from the F_1 embryos cultured *in vitro* ('M × V' amphiploid; Jan *et al.* 1986) or as a consequence of the union of unreduced gametes on the

untreated F_1 plants from normal and rare caryopses developed in the spike of the durum wheat female parent after the cross pollination with *Dv* pollen ('C × V' and 'Cr × V_B' amphiploids; De Pace et al. 2003). A multi-hybridization experiment was conducted in the last ten years among those amphiploids and *Triticum aestivum* $A^aA^aB^aB^aDD$ wheat varieties 'Agadir', 'Chinese Spring' ('CS'), 'Provinciale', 'Sagittario', and 'Salgemma', and the inbred line '41-3'. A total of 9 $A^aA^dB^aB^dDV$ F_1 progenies were produced (Table 1 A). Seven new durum wheat lines ('1-04', '5-04', '13-04', '1/07a', '1/07b', '2/07', and '3/07'), were selected and tested in the field.

Table 1. Cross-combinations among *T. aestivum* entries and three $A^dA^dB^dB^dVV$ amphiploids, and floret fertility expressed as proportion of the emasculated florets that produced caryopses with hybrid embryo.

Female parent		$A^aA^aB^aB^aVDD$ *T. aestivum* (Male parent)					
		'Agadir'	**'CS'**	**'Provinciale'**	**'Salgemma'**	**'Sagittario'**	**'41-3'**
(A) Cross-combination							
$A^dA^dB^dB^dVV$ amphiploid	'M × V'		X			X	
	'C × V'	X					x
	'Cr × V_B'		X	X	X	X	
(B) Proportion of the emasculated florets that produced caryopses with hybrid embryo							
$A^dA^dB^dB^dVV$ amphiploid	'M × V'		0.49			0.38	
	'C × V'	0.11					0.04
	'Cr × V_B'		0.55	0.24	0.45	0.03	

2. Methods

A. Root-tip preparation for chromosome counting

Seminal roots were treated with α-bromonaphtalene for 6 hours, fixed in ethanol-glacial acetic acid 3:1 (v / v) and stored at 4°C before enzyme treatment. The root-tips were washed with a citrate buffer (sodium citrate 6 mM and citric acid 4 mM, pH 4.6), for 20 minutes at room temperature under stirring. The root-tips were then treated with a solution of pectinase 6% and cellulase 10% in citrate buffer for 60 to 90 at 37°C, and squashed under a coverslip in a drop of 60% acetic acid. The coverslip was removed by the dry ice method and the preparations were dried overnight at 37°C. The chromosomes were fixed with paraformaldehyde 4%, washed with 2xSSC and 4xSSC/ Tween 20, and stained with a 2% solution of DAPI (4,6-diamidino-2-phenylindole) in McIlvaine buffer pH 7.0.

B. Estimate of the expected chromosome number of the F_2 embryos formed upon self-fertilization of the $A^aA^dB^aB^dDV$ F_1 plant

Considering that during meiosis occurring in florets of the $A^aA^dB^aB^dDV$ F_1 plant, the homologous chromosomes of the A and B genomes pair regularly during prophase I, only the 7 D and 7 V univalents are expected to migrate randomly at one or the other pole during anaphase I. The binomial expectation for the frequency of gamete types can be determined using the formula:

$$P_k = \frac{n!}{k!(n-k)!} p^k q^{(n-k)}$$

where k is the number of D and V univalents that migrate to the same pole at anaphase I, ranging from 0 to 14; n = 14 is the total number of univalents; p=1/2 is the probability that a given univalent is pulled to one pole and q=1/2 is the probability that the same univalent is pulled towards the other pole. The expected probability (P_z) to find each type of F_2 zygote resulting from the random union of one of the possible female gametes (set with probability $P_{k(f)}$) and one of the possible male gametes ($P_{k(m)}$), is $P_z = P_{k(f)} \times P_{k(m)}$ (see Table 2).

Table 2. The expected probability (P_z) of each type of F_2 zygotes resulting from the random union of one of the possible female gametes (P_k(f)) and one of the possible male gametes (P_k(m)).

k (number of D and V univalents)	Binomial coeff.	Chrom. Number in gametes	Male →	n − 14+0	n − 14+1	n − 14+2	n − 14+3	n − 14+4	n − 14+5	n − 14+6	n − 14+7	n − 14+8	n − 14+9	n − 14+10	n − 14+11	n − 14+12	n − 14+13	n − 14+14
		Female ↓	P_n	0.00000	0.00080	0.00555	0.02222	0.06110	0.12219	0.18329	0.20947	0.18329	0.12219	0.06110	0.02222	0.00555	0.00080	0.00000
0	1	n = 14+0	0.00000	0.0000000037	0.00000035	0.00000034	0.000001	0.000004	0.000007	0.000011	0.000013	0.000011	0.000007	0.000004	0.000001	0.000000	0.000000	0.000000
1	14	n − 14+1	0.00080	0.0000000[illegible]	0.00000[illegible]	0.00000[illegible]	0.000019	0.000052	0.000104	0.000157	0.000175	0.000157	0.000104	0.000052	0.000019	0.000005	0.000001	0.000000
2	91	n 14+2	0.00555	0.0000003900	0.00000475	0.00000305	0.000123	0.000339	0.000679	0.001010	0.001163	0.001010	0.000679	0.000339	0.000123	0.000031	0.000005	0.000000
3	364	n = 14+3	0.02222	0.0000135501	0.00001838	0.00012340	0.000494	0.001357	0.002715	0.004072	0.004654	0.004072	0.002715	0.001357	0.000494	0.000123	0.000019	0.000001
4	1001	n 14+4	0.06110	0.0000037262	0.00005271	0.00003634	0.001359	0.003733	0.007467	0.011199	0.012798	0.011199	0.007467	0.003733	0.001359	0.000339	0.000052	0.000004
5	2002	n = 14+5	0.12219	0.0000074603	0.00010441	0.00067858	0.002716	0.007466	0.014931	0.022396	0.025596	0.022396	0.014931	0.007466	0.002715	0.000679	0.000104	0.000007
6	3003	n = 14+6	0.18329	0.0000111904	0.00015662	0.00101837	0.004072	0.011198	0.022396	0.033594	0.038394	0.033594	0.022396	0.011198	0.004072	0.001018	0.000157	0.000011
7	3432	n 14+7	0.20947	0.0000127920	0.00017899	0.00116385	0.004654	0.012798	0.025596	0.038394	0.043879	0.038394	0.025596	0.012798	0.004654	0.001163	0.000179	0.000013
8	3003	n 14+8	0.18329	0.0000111904	0.00015662	0.00101837	0.004072	0.011198	0.022396	0.033594	0.038394	0.033594	0.022396	0.011198	0.004072	0.001018	0.000157	0.000011
9	2002	n − 14+9	0.12219	0.0000074603	0.00010441	0.00067858	0.002716	0.007466	0.014931	0.022396	0.025596	0.022396	0.014931	0.007466	0.002715	0.000679	0.000104	0.000007
10	1001	n 14+10	0.06110	0.0000037262	0.00005221	0.00003634	0.001357	0.003733	0.007467	0.011199	0.012798	0.011199	0.007467	0.003733	0.001357	0.000339	0.000052	0.000004
11	364	n = 14+11	0.02222	0.0000135501	0.00001838	0.00012340	0.000494	0.001357	0.002715	0.004072	0.004654	0.004072	0.002715	0.001357	0.000494	0.000123	0.000019	0.000001
12	91	n − 14+12	0.00555	0.0000033900	0.00000475	0.00003085	0.000123	0.000339	0.000679	0.001018	0.001163	0.001018	0.000679	0.000339	0.000123	0.000031	0.000005	0.000000
13	14	n = 14+13	0.00080	0.0000005215	0.00000073	0.00000475	0.000019	0.000052	0.000104	0.000157	0.000179	0.000157	0.000104	0.000052	0.000019	0.000005	0.000001	0.000000
14	1	n = 14+14	0.00000	0.0000000373	0.00000005	0.00000034	0.000001	0.000004	0.000007	0.000011	0.000013	0.000011	0.000007	0.000004	0.000001	0.000000	0.000000	0.000000
			1.0000															

Expected P_z for F_2 zygotes with 2n > 42	0.57	Expected P_z for F_2 zygotes with 2n = 28	3.7E-09
Expected P_z for F_2 zygotes with 2n =< 42	0.43	Observed P_z for F_2 zygotes with 2n = 28	0.065
		Odds in favour of 2n = 28 zygotes	1.7E+07

C. Field performance of the new durum wheat lines

The three new durum wheat lines '1-04', '5-04', and '13-04' were compared in 1 × 1 m plots arranged in a randomized block field design replicated twice at the experimental field of University of Tuscia (Viterbo) and CRA-SCV (S. Angelo Lodigiano, Lodi) in 2006 and 2007. The 'Modoc', 'M × V', and 'CS' parents and the durum wheat cultivar 'Creso' and line '4.5.1' were used as controls. Plants were evaluated for heading time (days from Jan. 1st), culm length (cm), response to air-born inoculum of *Blumeria graminis* f.sp. *tritici* (the causal agent of powdery mildew) and *Puccinia triticina* (leaf rust) (symptoms were scored as percentage of the leaf area covered by pustules). Grain quality traits (hardness, protein content, sodium-dodecyl-sulfate sedimentation volume, and specific sedimentation volume) were evaluated using the methodologies reported in Vaccino *et al.* (2010).

D. Technological quality analyses

The semolina required for the technological quality analyses of the seven new durum lines and controls grown at the Experimental farm of University of Tuscia, Viterbo, in 2011, was prepared using a Chopin CD2 laboratory mill and Chopin Semolina Purifier (Chopin Technologies, Villeneuve-la-Garenne, France). The yellow index (Minolta b*) of the durum wheat varieties and breeding lines was recorded using a Minolta CR-300 chroma meter (Minolta Camera Co. Ltd., Osaka, Japan).The wet gluten content and the gluten index of each durum wheat sample was determined on the basis of the ICC 158 standard method using a Perten Glutomatic 2200 instrument and a Perten 2015 Centrifuge (Perten Instruments AB, Hägersten, Sweden). Zeleny sedimentation volume was analysed by ICC 116/1 method. Crude protein content was determined by Kjeltec 1035 Analyzer (ICC105/2) from whole grain meal. Samples were analysed for total- (TOT-AX) and water-extractable- arabinoxylane (WE-AX) content with the pentosan method of Douglas (1981). The total amount of mixed-linkage β-glucan was determined using a Megazyme kit (ICC 168, AACC Method 32-23). Amylose and amylopectin content of starch were measured by the Megazyme method which is a modification of a Con A method developed by Yun and Matheson (1990).

E. Data analyses

Descriptive statistics, ANOVA, and Bonferroni's method for multiple comparison tests of the means, were performed using the GenStat 16 ed. (VSN International Ltd) software.

III – Results and discussion

The average floret fertility upon controlled hybridization was 0.55 and ranged from 0.03 ('Sagittario' × 'Cr × V_B') to 0.8 ('Chinese Spring' × 'M × V'). The average spikelet fertility of the F_1 plants was low for the 'Sagittario' × 'Cr x V_B' and '41-3' × 'C × V' hybrids, while it was the highest for the 'Salgemma' × 'Cr x V_B' and 'CS' × 'M × V' hybrids (Table 1B). The largest progenies (number of F_2 caryopses) were obtained from the F_1 'M × V' × 'CS' and 'C × V' × '41.3'.

Homologous pairing and recombination between the A and the B genome chromosomes of durum and bread wheat and the random assortment of the chromosomes of the D and V genomes occurred at first division of meiosis of the F_1 plants. This determined the formation of diads and gametes with constant AB chromosome number (7 $A^{a/d}$ plus 7 $B^{a/d}$) plus various inclusion (from 0 to 14) of the 14 D and V univalents, including the very rare configurations of the euploid parental genomes A^aB^a, A^dB^d, A^aB^d, A^dB^a, A^aB^aV, A^dB^dV, A^aB^dV, A^dB^aV, A^aB^aD, A^dB^dD, A^aB^dD, A^dB^aD, A^aB^aDV, A^dB^dDV, A^aB^dDV, and A^dB^aDV. The expected chromosome number (n) in the gametes ranged from 14 (7 A and 7 B chromosomes) to 28 (7A, 7B and 1 to 14 D and/or V-univalents (Table 2). Fifteen gamete types differing in chromosome number were expected, the variation being attributed to

the number of univalents (k) included in each of them. Their respective frequency was equal to their probability (P_k) of being set in the male or female germline. The expected absolute frequency of the F_2 zygotes formed by the random union of those gametes was estimated by the product (P_z) of the probability of the uniting gametes. The expected frequency of the F_2 embryos with 2n=28 was 3.7×10^{-9}. The chromosome number detected in the root-tip of a sample of 62 F_2 seedlings, ranged from 2n=28 to 2n=42 (Caceres *et al.* 2011). The proportion of the F_2 seedlings displaying 2n=28 ($A^{a/d}A^{a/d}B^{a/d}B^{a/d}$) was examined in the largest ('M × V' × 'CS') of the nine F_1 progenies, and 2n=28 was detected in 4 out of 62 F_2 seedlings, an absolute frequency (0.065) which is about 7 order of magnitude higher then the expected frequency (3.7×10^{-9}). Three additional F_2 seedlings with 2n=28 were found in a further sample of 41 F_2 seedlings from the same hybrid progeny.

The cumulative expected probability of F_2 zygotes with chromosome number 2n ≤ 42 was 0.43 and the cumulative expected probability of F_2 zygotes with 2n > 42 was 0.57 (Table 2). In the F_2 seedlings examined by Caceres *et al.* (2011), the proportion of the F_2 seedlings with chromosome number 2n > 42 was below 0.03, which fitted the expectation that the F_2 zygotes with 2n > 42 had a very low viability. Therefore when the probabilities in Table 2 are converted to frequencies, than 57% of the F_2 zygotes with 2n > 42 are expected to be unviable, causing an F_1 floret fertility of 43%. F_2 caryopses were formed in 609 of the 1522 florets examined in the spikes of the nine F_1 hybrids (Table 3) providing an observed F_1 floret fertility of 0.40, which meant that 60% of the F_1 florets did not set F_2 caryopses and 40% of the F_1 florets formed F_2 caryopses, a proportion of non-fertile vs fertile floret that was amazingly close to the expected proportion under the hypothesis that almost all the zygotes with 2n > 42 were unable to live or develop normally.

Table 3. Floret fertility in F_1 plants from some cross-combinations among *T. aestivum* entries and $A^dA^dB^dB^dVV$ amphiploids.

	'Cr × V_B'			'M × V'			'C × V'		
T. aestivum	**Florets No.**	**Caryo-pses No.**	**Floret fertility**	**Florets No.**	**Cary-opses No.**	**Floret fertility**	**Florets No.**	**Caryo-pses No.**	**Floret fertility**
'Provinciale'	68	16	0.24						
'Salgemma'	62	28	0.45						
'Sagittario'	62	2	0.03	129	39	0.30			
'CS'	54	11	0.20	513	226	0.44			
'Agadir'							72	41	0.57
'41-3'							562	246	0.44
Total	246	57	0.23	642	265	0.41	634	287	0.45

Overal floret fertility 0.40.

Table 4. Chromosome number assessed in F_3 seedling from F_2 plants obtained by crossing the amphiploid 'M x V' and *T. aestivum* cv 'CS' and displaying durum wheat spike and kernel morphology. The karyological events observed by Caceres et al. (2011) in the embryo from which the F_2 mother plant was rised, are also reported.

F_3 seedlings analyzed No.	Chromosome No.	Karyological event*
5	28	None
6	28	None
4	28	None
6	28	None
6	28	A-B Recombination
2	28	None
4	28	A-B Recombination
2	42	None

**Karyological event observed by GISH in the root-tips of the embryo from which the F_2 mother plant was risen.*

Table 5. Analysis of variance for six traits recorded on plants of 3 new durum wheat lines obtained from crossing the amphiploid 'M × V' and 'CS' ('1-04', '5-04', and '13-04') and 5 controls (the parental entries 'Modoc', 'CS', and 'MxV' amphiploid, and the durum wheats cv 'Creso' and inbred line '4.5.1'), grown for two years (2006 and 2007) according to a randomized block field design replicated twice at the Experimental farms of Univ. of Tuscia (Viterbo) and CRA-SCV (S. Angelo Lodigiano, Lodi).

Source of variation	d.f.	Heading time (days from 1st Jan)		Culm length (cm)		Protein content (% dry weight)		Grain Hardness		Sedimentation volume (mL)		Specific Sedim. volume (mL)	
		MS	F prob.	MS	F prob.	MS	F prob.	MS	F prob.	MS	F prob.	MS	F prob.
Line	7	101.4	<.001	2084.3	<.001	54.13	<.001	8598.1	<.001	228.7	<.001	2.2	0.009
Year	1	2334.4	<.001	501.6	0.003	153.28	<.001	682.9	<.001	280.6	<.001	8.4	0.001
Location	1	79.8	0.002	1686.5	<.001	0.01	0.944	425.7	<.001	33.0	0.093	4.6	0.013
Line x Year	7	16.0	0.062	39.6	0.583	1.06	0.755	18.0	0.511	21.3	0.10	0.6	0.558
Line x Loc	7	20.8	0.020	419.6	<.001	2.08	0.348	54.2	0.024	9.3	0.558	0.2	0.918
Year x Loc	1	51.6	0.012	78.0	0.215	6.79	0.059	23.1	0.288	17.1	0.222	2.9	0.046
Line x Year x Loc	7	7.0	0.483	50.8	0.421	2.49	0.238	53.1	0.026	16.1	0.216	0.4	0.80
Residual	32	7.3		48.7		1.78		19.8		11.0		0.7	
Total	63												

The chromosome counting in root tip from the F_3 embryos formed by self-fertilization of the F_2 plants with 2n=28 confirmed the 2n=28 chromosome number, except in one instance where the F_3 progeny was made by a mixture of 2n=28 and 2n=42 embryos (Table 4). In this case, an accidental kernel mixture during threshing of the F_2 spikes cannot be excluded.

The seven F_3 progenies with 2n=28 provided new durum wheat inbred lines that were tested in field trials to ascertain their agronomical and grain technological performance.

The new durum wheat lines '1-04', 5-04' and 13-04' were compared to the parental 'Modoc', 'M × V', and 'CS' entries and to the durums 'Creso' and '4.5.1' for two years and in two locations. It was ascertained that the interactions of the entries with the different yearly and location climatic conditions were not significant (Table 5). There were significant differences among the entries, but the new durum wheat lines were similar, or even better, than the 'Modoc' and 'Creso' durum wheat checks for several of the examined traits (Table 6). In each year and location the line '1-04' expressed high resistance to powdery mildew and leaf rust, while 'M × V' expressed immunity to powdery mildew (due to genes inherited from *Dv*), and 'CS' was resistant to leaf rust. The resistance to powdery mildew in '1-04' can be explained by the introgression of the *Dv* gene for resistance from 'M × V.

Table 6. Multiple comparison tests of the means, performed using Bonferroni's method, for six traits recorded on plants of 3 new durum lines ('1-04', '5-04', and '13-04') and 5 controls (the parental entries 'Modoc', 'Chinese Spring', and 'M × V' amphiploid, and the durum wheats cv 'Creso' and inbred line '4.5.1'). Means are overall years (2006 and 2007) and locations (Experimental farms of Univ. of Tuscia, Viterbo, and CRA-SCV S. Angelo Lodigiano, Lodi) of the trials.

Heading time (days from 1st Jan)		Culm length (cm)		Protein content (% dry weight)		Grain Hardness		Sedimentation Vol. (mL)		Specific Sedim. Vol. (mL)	
123	bc	105.7	b	16.3	b	105.3	b	37.5	cd	2.4	ab
116	a	77.0	a	14.0	a	106.7	b	33.5	bc	2.8	ab
122	bc	73.3	a	14.3	ab	104.4	b	39.0	cd	3.1	b
116	a	76.7	a	14.3	ab	103.9	b	28.8	ab	2.2	ab
118	ab	109.7	b	15.3	ab	32.2	a	42.3	d	3.1	ab
116	a	102.2	b	21.4	c	34.3	a	26.3	a	1.7	a
125	c	81.0	a	13.5	a	102.7	b	37.0	cd	3.2	b
120	ab	69.7	a	13.8	a	100.8	b	37.0	cd	2.9	ab

Significant differences were detected for seven traits related to grain quality recorded on plants of 7 new durum wheat lines, 2 parental checks, and four durum wheat checks (Table 7). The wet gluten content of the lines carrying alien genetic material ranged between 28.8% and 36.5% (Table 8). Two of the lines ('5-04' and '13-04') had significantly higher wet gluten content than 'Creso' and two further lines exceeded the value of 'Modoc'. The gluten structure – measured by gluten index – was excellent (>85) in most lines, one line had good and one further line ('1/07') had below average gluten strength. The yellow index values were very low in the whole experiment (14.0–18.9) in part due to the unfavorable climatic conditions during harvest, however, the yellow index of two lines was even higher than that of the 'Creso' variety. Falling number values were high, while the protein content and the Zeleny sedimentation volume of most of the lines were comparable with that of the durum wheat controls (Table 8).

Table 7. Analysis of variance for seven traits related to grain quality, recorded on plants of 7 new durum wheat lines, 2 parental controls, and four durum wheat controls.

Source of variation	d.f.	Wet gluten content (%)	Gluten index	Yellow Index	Amylose content	β-glucan	Arabinoxylan (Total)	Arabinoxylan (We)
Entry	12	34.4	718.4	22.85	5.5	1.4	116.4	7.0
Residual	13	0.2	5.5	0.09	1.3	0.1	3.6	0.2
Total	25							

Table 8.Multiple comparison tests of the means, performed using Bonferroni's method, for seven traits recorded on plants of 7 new durum wheat lines obtained from crossing the amphiploid 'M × V' x 'CS', the two parental *Triticum* cvs 'Modoc' and 'CS', and four additional *T. turgidum* ssp *durum* cultivars used as controls. The variables were: wet gluten content (WGC), gluten index (GI), yellow index(YI), amylose content(AC), β-glucan content (βGC), Arabinoxylan Tot. content (ATC) arabinoxylan We. content (AWe). Falling number (FN), Zeleny sedimentation test (ZST ml), and protein content (PC%) were evaluated in one sample only.

ENTRY	WGC (%)		Gi		YI		AC		β-GC		ATC		AWe		FN	ZST	PC
'M × V' × 'CS'	$\dot{X}^1$	MC*	$\dot{X}^1$	MC*	$\dot{X}^2$	MC*	$\dot{X}^3$	MC*	$\dot{X}^3$	MC*	$\dot{X}^4$	MC*	$\dot{X}^4$	MC*	$\dot{X}^5$	$\dot{X}^5$	$\dot{X}^5$
'1/07a'	**36.5**	f	42.0	A	**18.9**	f	25.7	ab	4.1	cd	21.2	bcd	**6.2**	de	510	16	**16.1**
'1/07b'	28.8	abc	85.4	C	14.8	bc	26.2	ab	3.2	ab	21.9	cde	**5.8**	Cd	377	17	15.1
'2/07'	30.9	cde	86.2	cd	15.8	de	26.8	b	3.2	A	18.4	B	**6.0**	Cd	371	28	14.7
'3/07'	29.4	bcd	94.2	cde	15.4	cd	27.0	B	3.7	abcd	20.1	bcd	**6.1**	De	390	20	14.0
'1-04'	35.4	f	74.9	B	**18.2**	f	26.5	Ab	4.2	Cd	20.3	bcd	4.6	Ab	676	28	15.8
'5-04'	**32.5**	e	**92.8**	cde	**16.0**	de	25.5	Ab	3.9	abcd	19.2	Bc	4.0	A	**433**	20	**16.1**
'13-04'	**31.9**	e	**86.5**	cd	**16.5**	e	23.8	A	3.6	abcd	21.6	bcd	**5.7**	Cd	**352**	25	**14.4**
Parental check																	
'Modoc'	28.4	ab	96.1	de	13.9	b	25.0	Ab	**4.1**	Cd	21.1	bcd	4.8	B	369	26	14.6
'CS'	42.3	g	45.0	A	7.4	a	23.6	A	**5.4**	E	15.0	A	4.9	B	503	21	16.0
Other durum wheat check																	
'Creso'	30.9	cde	100.0	E	16.2	de	26.9	B	**4.0**	bcd	28.9	G	6.8	Ef	538	23	15.6
'Duilio'	31.4	de	85.6	C	14.8	bc	25.6	Ab	**3.5**	abcd	22.7	De	5.3	Bc	376	27	14.9
'Simeto'	27.1	a	97.2	E	16.0	de	26.8	B	**3.5**	abc	25.1	Ef	7.4	F	572	27	14.6
'4.5.1'	28.3	ab	97.0	E	14.0	b	24.5	Ab	**4.3**	D	28.3	Fg	6.3	De	322	22	14.5
Grand mean	31.8		83.3		15.2		25.7		3.9		21.8		5.7		445	23	15.1

1 Mean of two replicates; 2: Mean of three replicates; 3: Mean of four replicates; 4: Mean of eight replicates; 5: No replicates
** Multiple comparisons test of significance of 91 pair of means using an experiment-wise error rate of 0.05 and a comparison-wise error rate of 0.0006*
Values which are significantly better or differently by chance from either 'Modoc' or the best durum check 'Creso', are highlighted in bold

In order to get some information about the health related properties of the studied genotypes, the amylose content of the starch, the β-glucan content of the seed and the quantity of the total- (TOT-AX) and water-extractable-arabinoxylan (WE-AX) were measured (Table 8). These components contribute to the total dietary fibre content of the wheat. As the results show, none of the lines had significantly higher amylose (23.8-27.0%), β-glucan (3.2-4.2 mg/g) or total-arabinoxylan content (18.4-21.9 mg/g) than the control 'Modoc' or 'Creso', but there was a significant difference in the water extractability of the arabinoxylan. Five of the seven 'M × V' × 'CS' lines had significantly higher WE-AX content (5.7-6.2 mg/g) than the Modoc (4.8 mg/g) control.

Compared to the 'CS' control, all the studied lines had significantly high TOT-AX content. Altogether we can say that health related properties of the new lines were improved through the increased level of the arabinoxylan.

IV – Conclusions

Our results showed large variability among hexaploid genotypes for their ability to produce viable progeny when crossed to 'A^dB^dV-amphiploid', and suggested that 'Chinese spring' wheat may be a good bridge variety for homoploid crosses.

Forty-three percent of the F_1 florets were fertile and 57% were sterile. This last percentage coincided with the proportion of the expected F_2 embryos with 2n > 42 suggesting an association between high chromosome number (>42) and reduced F_2 zygote viability.

The proportion of the F_2 seedlings displaying 2n=28 ($A^{a/d}A^{a/d}B^{a/d}B^{a/d}$) was about 7 order of magnitude higher than the expected frequency (3.7×10^{-9}), indicating that the progeny from the [(*T. turgidum* x *Dasypyrum villosum*) amphiploid x *Triticum aestivum*] hybridization is an effective source of new durum wheat inbred lines where each A and B chromosome is a chimera ($A^{a/d}$ and $B^{a/d}$) of the genes in the A^dB^d genomes of durum wheat and A^aB^a genomes of bread wheat as consequence of homologous pairing and recombination of the A and B chromosome in the 'A^dB^dV-amphiploid' x '*Ta*' F_1.

The agronomic performance of some of the new durums was similar or even better then either wheat parents and in one case ('1-04') there was evidence of the gene transfer for disease resistance from the V chromosome of the parental amphiploid.

The parameters measuring the value of the grain for technologically complex traits of two lines ('5-04' and '13-04') were superior to the parental durum 'Modoc' and very similar to that of 'Creso'.

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