ISSN 1479-2621
Plant Genetic Resources: Characterization and Utilization (2011) **9**(2); 352–356
doi:10.1017/S1479262111000141

Deployment of either a whole or dissected wild nuclear genome into the wheat gene pool meets the breeding challenges posed by the sustainable farming systems

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Abstract

Deploying whole and dissected nuclear genome of wild *Triticeae* species in the homoeologous wheat genetic background through inter-specific hybridization and introgression is a lower cost and effective option to prepare wheat germplasm with unexploited genes for disease resistance and enhanced grain yield and quality traits. The whole nuclear genomes of *Dasypyrum villosum (Dv)* and *T. turgidum* var *durum* have been combined, and an homoploid derivative of the original amphiploid displayed typical 'farro' spike morphology, tough rachis and the adaptive traits of *Dv* such as high resistance to diseases (caused by *Tilletia tritici*, *Blumeria graminis* f. sp. *tritici*, *Puccinia triticina* and *P. graminis* f. sp. *tritici*), heading earliness and fortified caryopses (high protein and micronutrient contents). The dissection of the *Dv* genome by either '*Triticum aestivum* cv Chinese Spring (CS) × hexaploid amphiploid' or '(CS × *Dv*) × CS' hybridization and backcrossing provided wheat introgression breeding lines (IBLs) expressing one or more of the *Dv* adaptive traits. Molecular analyses revealed that either cryptic or Genomic In-situ Hybridization (GISH) detectable *Dv* chromatin introgression occurred in those IBLs. The IBLs, after 2 years of low-input field tests and genetic analyses in Italy and Hungary, showed simple inheritance, dominance and stability of the adaptive and disease resistance traits.

Keywords: *Dasypyrum* gene pool; gene transfer; introgression; wheat germplasm; wild plant species

Introduction

In order to meet the predicted global cereal grain demand for the next decades, efficient conventional and molecular cereal breeding programmes and proper wheat genetic resources are necessary for selecting the suitable breeding lines (Baenziger *et al.*, 2008).

Many dominant genes for adaptation and trait enhancement have been lost during cereal crop domestication, but they have been retained in the genome of the wild components of the *Triticeae* gene pools (Dwivedi *et al.*, 2008). In natural habitat, wild *Triticeae* species

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such as *Dasypyrum villosum (Dv),* whose genome was exposed to million of years of climatic and environmental changes, are now expressing increased heading earliness, density stands and plant biomass. Deploying whole and dissected *Dv* nuclear genome in the homoeologous wheat genetic background through interspecific hybridization and introgression could be a lower cost and effective option to help wheat breeders to merge and select the proper adapted gene pools to sustain the needed yearly grain yield increase. In this study, we show that combining the *Dv* genome with the *T. turgidum* var *durum* genomic background and deploying dissected *Dv* genome in *Triticum aestivum* provided wheat genetic resources with new trait enhancements.

Materials and methods

Combining the whole nuclear genomes of Dv and T. turgidum *var*. durum

A hulled and brittle rachis *Dv* ecotype ($2n = 2x = 14$; VV) collected near Bari (Puglia, Italy) was used as pollen parent in hybridization with the free-threshing and tough rachis *T. turgidum* var. *durum* cv 'Modoc' ($2n = 4x = 28$; AABB) (Jan *et al*., 1986). The resulting hexaploid amphiploid ($2n = 42$; AABBVV), labelled M × V-b_1, was fertile and showed brittle rachis (Supplementary Fig. S1, available online only at http://journals.cambridge.org). A non-brittle rachis mutated amphiploid plant (M × V-nb_1; AABBVV; $2n = 6x = 42$) was discovered in the M × V-b_1 plot grown in 1987 (De Pace *et al*., 2003). In the following years, other genetically stable variants ('Mut 7-04', 'Mut 12-04' and 'Mut 16-04') were found among the M × V-nb_1 plants.

*Dissection of the Dv genome by [*T. aestivum *cv 'Chinese Spring' (CS) × Dv] × CS hybridization and backcrossing*

We selected six *Dv*-introgressed wheat breeding lines (IBLs), sharing a common CS ($2n = 6x = 42$; A′A′B′B′ D′D′) genetic background, from a population of 150 aneuploid lines developed at the University of Tuscia, Viterbo, Italy, from the backcross (CS × *Dv*) × CS (Supplementary Fig. S2, available online only at http://journals.cambridge.org). The IBLs CS × V63 and CS × V32 contained a disomic addition of 6V and a disomic substitution 6V(6B), respectively, and CS_1B-1V line contained a pair of 1BL-1VS chromosomes from a spontaneous exchange between chromosome 1B of wheat and 1V of *Dv*. The IBLs CS × V58, CS × V59 and CS × V60 were morphologically different from CS, although they did not contain apparent GISH detectable V chromatin (Minelli *et al*., 2005; Caceres *et al*., 2008). The IBLs were evaluated for resistance to *Blumeria graminis* f. sp. *tritici* (*Bgt*), *Puccinia triticina* (*Pt*) and *P. graminis* f. sp. *tritici* (*Pgt*) isolates in Italy and Hungary, and for end-use grain quality traits.

Dissection of the Dv genome by hybridization of T. aestivum *cv CS and M × V-*nb_1 *hexaploid amphiploid*

An F_2-like breeding population with broad genetic diversity was obtained from selfing the F_1 plants obtained after crossing the hexaploid amphiploid M × V-nb_1 to CS (Supplementary Fig. S3, available online only at http://journals.cambridge.org). After two generations of selfing, three F_4 lines, named '8-1', '41-3' and 'Mut 3-04', were tested for two consecutive growing seasons

Table 1. Mean values for heading time, plant height, number of spikelets/spike, 1000 kernel weight and protein content in M × V-nb_1 hexaploid amphiploid and derived variants by spontaneous mutation in comparison with 'farro' species used as controls[a]

Entry	Heading time (days from 1st April)	Plant height (cm)	Number of spikelets/spike	1000 Kernel weight (g)	Protein content (% dry weight)
Hexaploid amphiploid					
M × V-nb_1	114	101	14.7	27.1	21.1
Derived variants from spontaneous mutation in the hexaploid amphiploid					
Mut 7-04	116	105	14.6	23.5	21.5
Mut 12-04	124	104	16.3	42.6	16.5
Mut 16-04	124	106	15.9	42.6	16.9
Control 'farro' species					
T. monococcum	144	109	17.7	20.0	20.0
T. dicoccoides	132	125	15.5	35.5	22.0
T. spelta	135	124	17.0	39.8	18.8

[a] Data were taken from plots at S. Angelo Lodigiano (Lodi, Italy) in 2007 and 2008.

https://doi.org/10.1017/S1479262111000141 Published online by Cambridge University Press

Table 2. Average values for test weight (TW), protein content (PC), SDS sedimentation volume (SSV), specific SDS sedimentation volume (SSSV = SSV/PC), gluten index, rheological flour dough properties (Brabender farinograph and Chopin alveograph) and bread quality, determined for three inbred breeding lines obtained from hybridization of *T. aestivum* cv CS and M × V-nb$_1$ hexaploid amphiploid[a]

							Brabender farinograph			Chopin alveograph			Bread test		
Line	Year	TW (kg/hl)	PC (%dm)	SSV (ml)	SSSV	Gluten index	Stab. (min)	Degree of softness (BU)	Water abs (%)	P (mm)	L (mm)	P/L	W ($\times 10^{-4}$J)	Vol (cm^3)	Height (mm)
8-1	2008	74.9	12.6	91.0	7.2	99	18.2	23	57.8	104	128	0.81	400	710	100
	2009	79.6	13.8	88.5	6.4	84	17.4	25	63.0	80	130	0.62	286	735	103
41-03	2008	76.4	12.3	89.0	7.2	98	14.6	25	57.7	92	119	0.77	334	685	101
	2009	79.8	14.5	87.5	6.1	80	17.8	21	62.6	72	119	0.61	234	770	105
Mut 3-04	2008	77.0	12.6	91.0	7.2	99	13.1	33	58.1	93	136	0.68	375	720	105
	2009	80.0	13.8	88.5	6.4	76	17.8	12	62.9	81	119	0.68	266	790	110
Control															
CS	2008	64.7	12.2	48.0	3.9	32	3.9	93	56.4	54	61	0.89	90	560	84
Bologna	2009	80.4	13.9	86.5	6.2	97	18.5	22	58.5	78	140	0.57	396	720	105
PR22R58	2009	76.4	12.1	87.5	7.2	97	19.0	21	54.5	56	115	0.49	219	695	98
Best check	2009													785	109

Stab., stability; BU, Brabender Units; abs, absorption; P, pression, measures the tenacity of the dough; L, length, measures the extensibility of the dough; W, work, measures the strength of the dough.

[a] The values are mean of plots raised at two locations (S. Angelo Lodigiano and Viterbo) in Italy for 2 years (2008 and 2009).

(2007/2008 and 2008/2009), in the field at two sites: S. Angelo Lodigiano, SAL, near Lodi in northern Italy, and Tolentino, TOL, near Macerata in central Italy. The plots were managed using low-input criteria. The hexaploid wheat cultivars 'Bologna' and 'PR22R58' were used as checks. Heading time (days from 1st April), yield components and rheological properties of flour dough were evaluated at both sites.

Results and discussion

Combining the whole nuclear genomes of Dv and T. turgidum *var* durum

The hexaploid amphiploid M × V-nb$_1$ and the derived homoploid variants displayed 'farro' traits (tenacious glumes but tough rachis) and the typical adaptive traits of *Dv,* such as high resistance to diseases (caused by *Tilletia tritici*, *Bgt*, *Pt* and *Pgt*), fortified caryopses (+17 to +21% protein contents and >+25% of Fe and Zn content, compared with CS) and heading earliness. When compared with the conventional 'farro' (*T. monococcum*, *T. dicoccoides*, and *T. spelta*), new 'farro' types expressed earlier heading and shorter culms (Table 1), and also caryopses (Mut 12-04 and Mut 16-04) with larger size.

Dissection and deployment of the Dv genome by '(CS × Dv) × CS' hybridization and backcrossing

This type of hybridization favoured the haploidization of the V genome in the heterohaploid A′B′D′V F$_1$, the random assortment (dissection) of V chromosomes in 7A′ + 7B′ + 7D′ + 1V gametes and the formation of wheat IBLs with the disomic addition of one of the V chromosome or V chromosome arm after backcrossing to CS. The IBLs CS × V32 and CS × V63 contained the chromosome 6V#4 (De Pace *et al*., 2011), conferring multiple disease resistance to virulent strains of *Bgt, Pt* and *Pgt.* The IBL CS_1BL-1VS expressed superior Sodium Dodecyl Sulfate (SDS) sedimentation and rheological properties of flour dough than CS. Other IBLs (CS × V58, CS × V59 and CS × V60) had cryptic *Dv* chromatin introgression exhibiting a −7 to −9 d earliness in heading time compared with CS in the field. Using specific primers for several DNA targets in the genome of these lines revealed *Dv* alleles, but not CS alleles, at two loci (*Vrn-A1* and *Vrn-B3*) involved in the vernalization response pathway (Caceres *et al*., 2008).

F$_3$ progenies derived from the hybridization of two disomic addition lines for chromosome 6V, CS × V63 (+6V#4) and CS + 6V#1 (produced by Sears, 1982, susceptible to *Bgt*, *Pt* and *Pgt*; Supplementary Fig. S4, available online only at http://journals.cambridge.org) expressed simple inheritance, due to *Dv*-derived Mendelian dominant genes governing resistance response to each of the *Bgt*, *Pt* and *Pgt* pathogen. The high SDS sedimentation value of CS_1BL-1VS was traced to the effect of one high-molecular weight glutenin subunit (1v in Supplementary Fig. S5, available online only at http://journals.cambridge.org) encoded at the *Glu-V1* locus in 1VS of CS_1BL-1VS (Vaccino *et al*., 2010).

Dissection and deployment of the Dv genome by hybridization of T. aestivum *cv CS and M × V-nb$_1$ hexaploid amphiploid*

'New' chromosome assortments were achieved using the AABBVV hexaploid amphiploid as a bridge to combine the A and B genomes from durum wheat; the A′, B′ and D′ genomes of bread wheat, and the V genome of *Dv*. Tetraploid AA′BB′ lines, hexaploid AA′BB′D′D′ lines, and aneuploid AA′BB′ lines with the addition of one or more V and D′ chromosomes were obtained (Supplementary Figs. S3 and S6, available online only at http://journals.cambridge.org). Promising outcomes of this breeding scheme were the high frequency of euploid segregants (Supplementary Fig. S3, available online only at http://journals.cambridge.org), the phenotypic uniformity observed in the progenies after only two generations of selfing that followed the CS × (M × V-nb$_1$) triparental hybridization and the good agronomic values of those lines. The three inbred breeding lines, '8-1', '41-3' and 'Mut 3-04', had chromosome counting of $2n = 42$ and were as good as the best check for yield components, grain quality and rheological flour dough properties (Table 2 and Supplementary Table S1, available online only at http://journals.cambridge.org).

In conclusion, it is suggested that a sustainable breeding response to mitigate the severe threat to the world's wheat supply is necessary (Hovmøller *et al*., 2010). We evidenced that deploying whole and dissected *Dv* nuclear genome in the homoeologous wheat genetic background, it is possible to prepare wheat germplasm with unexploited genes for rust disease resistance and enhanced grain yield and quality traits.

Acknowledgements

This research was funded by the 'Ministero delle Politiche Agricole Alimentari e Forestali', Italy, in the framework of the research project FRUMIGEN (D.M. 292/7303/05 issued oct. 12th, 2005).

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