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Effects of the 4N^v Chromosome from *Aegilops ventricosa* on Agronomic and Quality Traits in Bread Wheat

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Abstract: Advanced wheat lines carrying the Hessian fly resistance gene *H27* were obtained by backcrossing the wheat/*Aegilops ventricosa* introgression line, H-93-33, to commercial wheat cultivars as recurrent parents. The *Acph-N^{v}1* marker linked to the gene *H27* on the 4N^v chromosome of this line was used for marker assisted selection. Advanced lines were evaluated for Hessian fly resistance in field and growth chamber tests, and for other agronomic traits during several crop seasons at different localities of Spain. The hessian fly resistance levels of lines carrying the 4N^v chromosome introgression (4D/4N^v substitution and recombination lines that previously were classified by *in situ* hybridisation) were high, but always lower than that of their *Ae. ventricosa* progenitor. Introgression lines had higher grain yields in infested field trials than those without the 4N^v chromosome and their susceptible parents, but lower grain yields under high yield potential conditions. The 4N^v introgression was also associated with later heading, and lower tiller and grain numbers/m^2. In addition, it was associated with longer and more lax spikes, and higher values of grain weight and grain protein content. However, the glutenin and gliadin expression, as well as the bread-making performance, were similar to those of their recurrent parents.

Keywords: Hessian fly; introgression; plant breeding; powdery mildew; resistance gene

The wild grass *Aegilops ventricosa* is an allotetraploid species ($2n = 4x = 28$, genomes D^{v}D^{v}N^{v}N^v) and has been a particularly valuable source of diseases and pests resistance genes for wheat. Hessian fly (Hf, *Mayetiola destructor*, Say) is a destructive pest of wheat in many parts of the world. In SW Spain, losses caused by this insect have been estimated to be above 40% (Martín-Sánchez *et al.* 2003). The line H-93-33 ($2n = 42$ chromosomes), derived by repeated selfing from the cross [(*Triticum turgidum* H-1-1/*Aegilops ventricosa* AP-1)/*T. aestivum* H-10-15], was characterised by RFLP/isozyme markers and by Giemsa C-banding as a 4N^v(4D) substitution line (Mena *et al.* 1989, 1993). This introgression line carries the Hf-resistance gene *H27* that provides resistance to the biotype prevailing in SW Spain (Delibes *et al.* 1997), and eyespot (*Pch-1*) and powdery mildew (*Pm*) resistance genes (Mena *et al.* 1989, 1992). The *Pm* and *H27* genes were located on the 4N^v chromosome in the H-93-33 line, and both were linked to the isozyme marker *Acph-N^{v}1* (Delibes *et al.* 1987, 1997).

The aim of this paper is to analyse the effect of the 4N^v chromosome introgression on agronomic and quality traits in advanced wheat lines derived from H-93-33.

MATERIAL AND METHODS

Advanced wheat lines carrying Hf-resistance gene *H27* were obtained by backcrossing the H-93-33 line to the susceptible *T. aestivum* cvs Astral, Adalid and Cargifaro as recurrent parents, followed by 8–10 cycles of self-pollination. A total of 170 BCnF$_1$ plants (n = 2 to 6), derived from forty-three different initial crosses, were selfed, and their progenies were individually sown under irrigated conditions at Gimenells (Lleida, Spain). Lines were developed through use of the *Acph-N*^{v}*1* marker for marker assisted selection (MAS), and evaluated for Hf resistance in field and growth chamber tests, as described in Delibes *et al.* (1997).

Twenty-three agronomic trials were performed between 2000 and 2008 at different locations in Spain (Gimenells (Gi) and Foradada (Fo) in the Northeast; and Azuaga (Az), Maguilla (Ma) and Carmona (Ca) in the Southwest). With the exception of Gimenells, trials were carried out under rainfed conditions. In all trials, the experimental plots were 3 m^2, with an alpha-lattice design of three replicates and 25–30 entries. Agronomic and quality traits were determined from Gimenells trials, without Hf infestation. Biomass, harvest index and yield components were estimated on a random grab sample as well as on ten spikes from each plot as described by Bell and Fischer (1994).

Statistical analyses were done using SAS version 9.1 (SAS Institute, Cary, USA). An analysis of variance (GLM procedure) was first performed on each of the measured traits in each agronomic trial, and means were compared by Fischer's LSD test (P = 0.05). In order to determine the 4N^v-introgression effect on each agronomic and quality trait, the adjusted means from the analyses of variance of each genotype evaluated in each trial, were analysed according to a mixed model (MIXED procedure). Only the homozygous genotypes for the presence (NN) or absence (DD) of the *Acph-N*^{v} marker were included. The mixed model included: trial, marker, and trial × marker as fixed effects, and genotype, as random effect.

RESULTS AND DISCUSSION

Karyotypes of advanced lines (2n = 42) were determined by *in situ* hybridisation. All these lines carried the 4N^v introgression, almost equal amounts with a 4D(4N^v) substitution as those carrying a 4DS-4N^{v}S.4N^{v}L translocation, and all showed resistance to Hessian fly and powdery mildew. But in all cases the Hf-resistance level was lower than that of their *Ae. ventricosa* progenitor. The *Acph-N*^{v}*1* marker was effective for MAS in the process of obtaining Hf-resistant lines. However, this marker was lost progressively throughout the backcrossing procedure, as previously described by Joppa (1987) in gametes with a foreign chromosome.

Lines with the introgression (NN) yielded more than those without it (DD) and their susceptible parents in field trials with high Hf-infestation levels (more than 1.2 pupae per tiller). But, under slight (Azuaga and Maguilla), or no Hf-infestation conditions (Foradada and Gimenells), the 4N^v introgression usually had a negative effect on grain yield (Figure 1).

Results of analyses of effects of the introgression on sixteen agronomic and quality traits are summarised in Table 1. The 4N^v-introgression had a clear effect on date to heading: the NN lines had a slower development and headed 1-2 days later than DD lines. Snape *et al.* (2001) described the presence of genes controlling vernalization response on chromosome 4D (absent in our lines NN) and reported that grain yield as a trait strongly related to flowering time and life cycle duration in wheat.

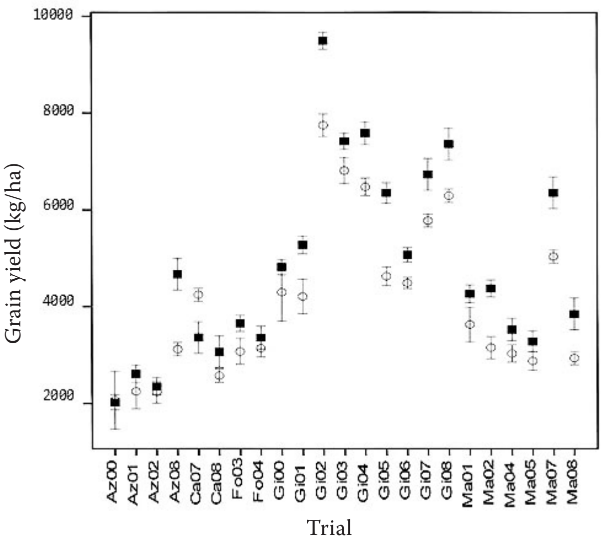

Figure 1. Means and standard errors of grain yield (kg ha) of advanced lines selected with (white circles) and without (black squares) the *Acph-N*^{v}*1*marker, across several Spanish locations between 2000 and 2008; letters indicate location: Az – Azuaga; Ca – Carmona; Fo – Foradada; Gi – Gimenells; Ma – Maguilla; and numbers indicate the two last digits of harvest year

Table 1. Effects of the $4N^v$ chromosome on sixteen agronomic and quality traits in advanced lines evaluated during seven growing seasons at Gimenells, under irrigated conditions and no Hessian Fly-attack

Traits	NN lines[a]		DD lines[b]		$Pr > F$[c]		
	mean	SE	mean	SE	trial	marker	trial × marker
Heading (days)	157.3	0.3	155.4	0.5	< 0.001	< 0.001	0.117
Height (cm)	87.7	3.2	92.0	3.3	< 0.001	0.022	0.084
Grain yield (kg/ha)	5 668	76	6 656	125	< 0.001	< 0.001	< 0.003
Biomass (kg/ha)	14 599	435	15 498	241	< 0.001	0.0816	0.009
Harvest index (%)	27.9	0.65	31.2	0.50	< 0.001	< 0.001	0.001
No. spikes/m^2	347	9.8	389	9.3	< 0.001	0.003	0.032
No. grains/m^2	13 219	304	16 229	281	< 0.001	< 0.001	0.544
No. grains/spike	48.0	0.95	51.5	0.90	< 0.001	0.009	0.002
Spike length (cm)	11.0	0.2	10.3	0.2	< 0.001	0.034	0.781
No. spikelets/spike	19.9	0.2	20.1	0.2	< 0.001	0.351	0.320
Spike density (%)	18.2	0.4	19.8	0.3	< 0.001	0.007	0.724
No. grains/spikelet	2.93	0.03	2.99	0.03	< 0.001	0.137	0.006
Grain weight (mg)	40.15	0.56	37.60	0.56	< 0.001	0.002	0.019
Test weight (g/l)	766.6	43.4	768.3	45.0	< 0.001	0.7984	0.102
Protein (% DM)	13.32	0.14	12.14	0.13	< 0.001	< 0.001	0.128
SDSS (ml)[d]	10.08	0.21	9.79	0.18	< 0.001	0.301	0.103

[a]NN lines – advanced lines carrying the $4N^v$ introgression ($4N^v$/4D substitution or $4N^vL.4N^vS$-4DS recombination); [b]DD lines – advanced lines carrying wheat chromosomes ($2n = 42$); [c]Probability > F from the analyses of mixed model; [d]SDSS – Sodium Dodecyl Sulphate Sedimentation volume

Among yield components, the number of spikes per unit area probably had the major effect on final grain yield. Calderini *et al.* (1995) considered this trait as the most relevant yield component in the Mediterranean area. So, the lower grain yield of those lines with the introgression should result in lower numbers of spikes/m^2 than in DD lines, and consequently, fewer grains per unit area (13 140 versus 16 277 grains/m^2). However, NN lines compensated with a lower number of grains per unit area with a greater grain weight. Furthermore, lower tillering (number of spikes/m^2) is normally related to bigger spikes and to higher grain yield per spike, which is also considered as a favourable trait under terminal drought stress conditions (Duggan *et al.* 2005). Thus, NN lines had longer spikes than DD lines, but the number of spikelets per spike and grains per spikelet was slightly lower than in lines DD. Consequently, NN lines showed a non-significant ($P > 0.05$) lower number of grains per spike than DD lines (48.0 v 51.5).

A strong association between the $4N^v$-chromosome introgression and high grain protein content (Table 1) was also found. This trait is controlled in wheat by multiple genes located on the three wheat genomes (Stein *et al.* 1992), and our data suggest that genes coding for grain protein content could be located on this introgressed chromosome as well. Recently, Mann *et al.* (2009) identified several QTLs associated with different traits related to bread making quality in different regions of the 4D chromosome. However, the substitution of this chromosome by the $4N^v$ in our breeding lines did not show any significant influence on test weight, sodium dodecyl sulphate sedimentation test (Table 1) and alveograph parameters (data not shown).

Most of the selected Hf-resistant lines, with a good agronomic performance, had cv. Adalid as a recurrent parent (4–6 cycles of backcrosses and 8–10 of self-pollination). These lines presented similar morphology and the same gliadin and glutenin patterns as their recurrent parent (data not shown), which is an acceptable composition for bread making performance.

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References

Bell M.A., Fischer R.A. (1994): Guide to plant and crop sampling: measurements and observations for agronomic and physiological research in small grain cereals. In: CIMMYT Wheat Special Report No. 32. CIMMYT, Mexico.

Calderini D.F., Dreccer M.F., Slafer G.A. (1995): Genetic improvement in yield wheat and associated traits. A re-examination of previous results and the latest trends. Plant Breeding, **114**: 108–112.

Delibes A., López-Braña I., Mena M., García-Olmedo F. (1987): Genetic transfer of resistance to powdery mildew and of an associated molecular marker from *Aegilops ventricosa* to hexaploid wheat. Theoretical and Applied Genetics, **73**: 605–608.

Delibes A., Del Moral J., Martín-Sánchez J.A., Mejías A., Gallego M., Casado D., Sin E., López-Braña I. (1997): Hessian fly-resistance gene transferred from chromosome $4M^v$ of *Aegilops ventricosa* to *Triticum aestivum*. Theoretical and Applied Genetics, **94**: 858–864.

Duggan D.L., Richards R.A., Van Herwaarden A.F., Fettell N.A. (2005): Agronomic evaluation of a tiller inhibition gene (tin) in wheat. Effect on yield, yield components, and grain protein. Australian Journal of Agriculture Research, **56**: 169–178.

Joppa L.R. (1987): Aneuploid analysis in tetraploid wheat. In: Heyne E.G. (ed.): Wheat and Wheat Improvement. ASA-CSSA-SSSA, Madison, 255–267.

Mann G., Diffey S., Cullis B., Azanza F., Martin D., Kelly A., Mcintyre L., Schmidt A., Ma W., Nath Z., Kutty I., Leyne P.E., Rampling L., Quail K.J., Morell M.K. (2009): Genetic control of wheat quality: interactions between chromosomal regions determining protein content and composition, dough rheology, and sponge and dough baking properties. Theoretical and Applied Genetics, **118**: 1519–1537.

Martín-Sánchez J.A., Gómez-Colmenarejo M., Del Moral J., Sin E., Montes M.J., González-Belinchón C., López-Braña I., Delibes A. (2003): A new Hessian fly resistance gene (*H*30) transferred from the wild grass *Aegilops triuncialis* to hexaploid wheat. Theoretical and Applied Genetics, **106**: 1248–1255.

Mena M., Orellana J., López-Braña I., García-Olmedo F., Delibes A. (1989): Biochemical and cytological characterization of wheat/*Aegilops ventricosa* addition and transfer lines carrying chromosome $4M^v$. Theoretical and Applied Genetics, **77**: 184–188.

Mena M., Doussinault G., Lopez Braña I., Aguaded S., García-Olmedo F., Delibes A. (1992): Eyespot resistance gene *Pch-1* in H-93 wheat lines. Evidence of linkage to markers of chromosome group 7 and resolution from the endopeptidase locus *Ep-D1b*. Theoretical and Applied Genetics, **83**: 1044–1047.

Mena M., Orellana J., Lopez-Braña I., García-Olmedo F., Delibes A. (1993): Characterization of wheat/*Aegilops ventricosa* introgression and addition lines with respect to the M^v genome. Theoretical and Applied Genetics, **86**: 197–204.

Snape J.W., Butterworth K., Whitechurch E., Worland A.J. (2001): Waiting for fine times: genetics of flowering time in wheat. Euphytica, **119**:185–190.

Stein I.S., Sears R.G., Hoseney R.C., Cox T.S., Gill B.S. (1992): Chromosomal location of genes influencing grain protein concentration and mixogram properties in ‘Plainsman V’ winter wheat. Crop Science, **32**: 573–580.