

ITEMS FROM SPAIN

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CONSEJERÍA DE INFRAESTRUCTURAS Y DESARROLLO TECNOLÓGICO

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Effects of introgressed 4N^v Aegilops ventricosa chromosome on yield and yield components in bread wheat.

The wild grass *Ae ventricosa* is an allotetraploid (2n=28; genomes D'D'N^vN^v) and has attracted considerable attention as a source of genes for resistance (RG) to pathogens such as insect and fungi. Genetic material from *Ae. ventricosa* has been transferred to hexaploid wheat through an intermediate self-sterile hybrid between *T. turgidum* and *Ae. ventricosa*, which was backcrossed using pollen from hexaploid wheat. The progeny were repeatedly selfed to obtain 42-chromosome, stable lines. One line, H-93-33 (4D/4N^v substitution), carried the genes *Pm* and *H27*, which confer resistance to powdery mildew and Hessian fly, respectively (Mena et al. 1988; Delibes et al. 1997). Introduction of these RGs from line H-93-33 into the commercial wheat cultivars Adalid and Astral was by backcrossing. Marker-assisted selection used the isozyme *Acph-N^v1*, which is linked to genes *H27* and *Pm* on 4N^v chromosome (Delibes et al. 1987, 1997). BC₄₋₆F₄-BC₄₋₆F₉ lines were evaluated against Hessian fly in Azuaga (38°14'N, 5°40'W) from 2000 to 2006; and BC₄₋₆F₆ lines were evaluated against powdery mildew in 2002 in Giménells (41°39'N, 0°25'E). Lines with the *Acph-N^v1* marker were resistant to both Hessian fly and powdery mildew.

These lines, with and without *Acph-N^v1* marker, also were evaluated from 2000 to 2007 for grain yield in several Spanish localities under irrigated and unirrigated conditions. Averaged across the different genetic backgrounds and 18 different environments, the 4N^v introgression decreased grain yield by 17%. The effect of 4N^v introgression on grain yield, yield components, (evaluated as described by Bell and Fisher 1994), and quality was studied over five growing seasons (2000–05) in Giménells under irrigated conditions. Averaged across the different genetic backgrounds and years, the 4N^v introgression decreased the fertile spike number/m² by 12.8 %, and kernels/spike by 7.8 % but increased kernel weight by 9.3 % and protein content by 12.4%. Bread making (determined by alveograph parameters, W, P, L, and P/L) was not affected significantly by the introgression. The isolines also differed in heading date. Lines without the introgression were 1 to 2 days earlier than those without.

The effects of *H27* and insecticide treatment for the control of Hessian fly were compared. Three pairs of NILs differing at the *H27* gene were evaluated with and without insecticide Diazinon. The field trial was conducted in the 2005–06 growing season in Azuaga. Hessian fly damage was estimated visually by incidence of broken tillers on the second spring generation. The effect of insecticide on lines with *H27* gene was not significant. Moreover, lines carry-

ing *H27* gene had a lower incidence of broken tillers ($P < 0.01$) than respective isolines without RG, thus *H27* was more effective on the control of flies than insecticide treatment.

The effects of *Pm* and fungicide treatment to control powdery mildew on yield, yield components, and quality also were compared. The same three pairs of NILs used above, which differ at the *Pm* gene, were evaluated with and without fungicide (Cyproconazole plus Tiophanate-methyl). Field trials were conducted in the 2005–06 and 2006–07 growing seasons in Giménez. Grain yield was 35.8% greater in 2007 than in 2006. In both years, treated plots yielded more than untreated plots. The decrease in yield in plots untreated (in relation with treated) was lower (4.7%) in lines with the 4N^v introgression than in lines without introgression (8.7%). As expected, the *Pm* gene had some effect in controlling disease. Protein content was not affected by fungicide treatment, but it was affected positively by the introgression. Bread making was not affected by the introgression or fungicide treatment.

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CONSEJO SUPERIOR DE INVESTIGACIONES CIENTIFICAS

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Peroxidase expression in a cereal cyst nematode (Heterodera avenae) resistant hexaploid wheat line.

The incompatible interaction between plant and pathogen is often determined by the hypersensitive reaction (HR). This response is associated with accumulation of reactive oxygen species (ROS), which results in adverse growth condi-

tions for pathogens. Two major mechanisms involving either NADPH oxidases or peroxidases have been proposed for generation of ROS. Peroxidases (PER, EC 1.11.1.7), present in all land plants, are members of a large multigenic family with high number of isoforms involved in a broad range of physiological processes.

PER genes, which are expressed in nematode feeding sites, have been identified in several plant species (Zacheo et al. 1997). A strong correlation between HR and PER activities at four and seven days post nematode infection, was detected in roots of wheat lines carrying *Cre2*, *Cre5* (from *Ae. ventricosa*) or *Cre7* (from *Ae. triuncialis*) *Heterodera avenae* resistance genes (Andrés et al. 2001; Montes et al. 2003, 2004).

We have studied changes in root of peroxidase mRNAs levels after infection by *H. avenae* of a wheat/*Ae. ventricosa* introgression line (H-93-8) carrying *Cre2* (Delibes et al. 1993). We also report and classify the predicted protein sequences derived from complete peroxidase transcripts.

Materials and Methods. Seedlings from the resistant line (H-93-8), obtained from the cross [(*T. turgidum* cv. Rubroatum, H-1-1/*Ae. ventricosa*, AP-1)//*T. aestivum* cv. Almatense, H-10-15] (Delibes et al. 1993) were inoculated with pathotype Ha71 of *H. avenae*. Root sections and leaves were harvested 4 and 7 days-after-infection; uninoculated tissues served as controls. Total RNA was extracted using the method of Båga et al. (1995). PER cDNAs were synthesized using 3'RACE a Superscript™ one-step RT-PCR kit (Invitrogen Life Technologies, San Diego, CA) and a 5'RACE SMART™ RACE cDNA Amplification Kit (Clontech Laboratories, Inc, Mountain View, CA) kit according to the manufacturer's recommendations. Primers from conserved regions of plant peroxidase genes were used for second cDNA synthesis and PCR. Preferential amplification of different PER sequences was obtained with primers designed from low-sequence-homology areas. Amino acid sequences were derived from the coding regions and aligned using MultAlign program (Corpet 1988). A distance-based tree was constructed by NEIGHBOR Joining with MEGA version 3.1 (Kumar et al. 2004).

The expression levels of each PER group in inoculated roots and uninoculated controls were determined by qRT-PCR. Primers for each peroxidase cluster were designed using Primer Express 2.0 software (PE Applied Biosystems, Foster City, CA). PCRs were performed using Power SYBR® Green PCR Master Mix (PE Applied Biosystems, Foster City, CA) according to the manufacturer's instructions in a ABI PRISM 7300 Detection System and software (PE Applied Biosystems, Foster City, CA).

Results and Conclusions. Comparative analysis of the amino acid sequences predicted from cDNAs revealed that they contain conserved structural features and activity sites of typical class III peroxidases. The distance tree of wheat line H-93-8 peroxidases was organized in five major clusters of homologous genes (*Pox1*, *Pox2*, *Pox3*, *Pox4*, and *Pec1*; Fig. 1), strongly supported by Bootstrap values. Interestingly, two members from rice peroxidase group IV (BAC79531.1, BAC83103.1, Passardi et al. 2004), which resulted equivalent to pathogen inducible proteins (Chittoor et al. 1997), were closely related to *Pox1*, *Pox2*, and *Pox3*.

Both with and without attack, all PER groups showed weak expression profiles in leaves. PER classified as *Pox1*, *Pox2*, and *Pox3* exhibited enhanced expression in infected roots when compared to noninoculated controls. Nematode infection apparently did not alter the expression pattern of *Pox4*, *Pec1*, and *Putper* in roots. The *Pox3* cluster showed the highest levels of transcription, independently of attack.

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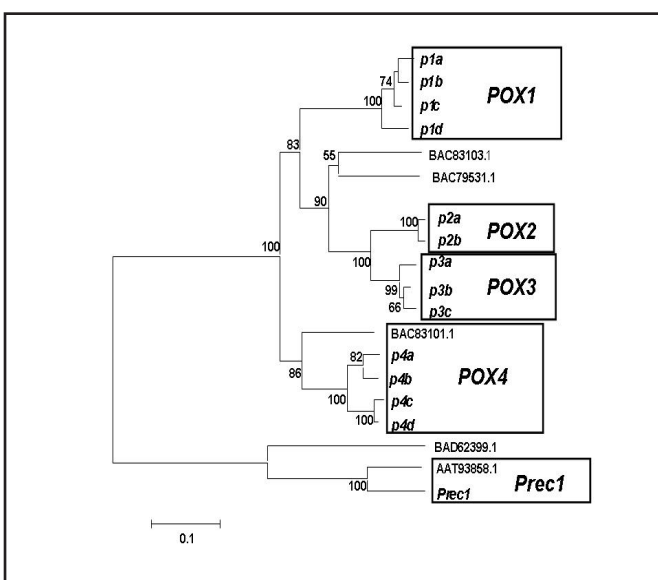


Fig. 1. Unrooted phylogenetic tree of wheat line H-93-8 PER based on predicted protein sequences and its relationships with representative rice peroxidases (GenBank accession numbers: AAT93858.1, BAD62399.1, BAC83101.1, BAC79531.1, BAC83103.1). The tree was constructed by the NEIGHBOR joining method. Values at nodes indicate Bootstrap supports greater than 50 %.

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Release of Mapeña spring bread wheat.

Mapeña is a spring bread cultivar released in 2007 carrying the *Cre7* resistance gene to *H. avenae* transferred from *Ae. triuncialis* (Romero et al. 1998). The cultivar was developed from the cross 'TR-353/Betres//Alcotán/3/Rinconada/4/3*Betres' under the designation ID-2181. Mapeña is a high-yielding, medium maturing, semidwarf cultivar with moderate resistance to leaf rust, yellow rust, powdery mildew, and Septoria. This cultivar is better adapted to the southern and northeastern wheat growing regions of Spain. Mapeña has good quality properties for baking industry and is registered in the Spanish Catalogue of Commercial Plant Varieties (BOE, 2008).

Coöperation with other institutions.

We are coöperating with Agrosa Semillas Selectas SA.

Personnel.

Dr. Guillermo Briceño-Felix left the bread wheat program in the UdL-IRTA Center. Dra. María Dolores Romero has just retired from Consejo Superior de Investigaciones Científicas.

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Karyotype characterization of wheat breeding lines carrying resistance genes from *Aegilops ventricosa*.

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We have used in situ hybridization combining genomic and repeated DNA fluorescent probes to determine the karyotype composition of two bread wheat introgression lines: H-93-33, which carries the gene *H27* for resistance to the Hessian fly *M. destructor* (Delibes et al. 1997); and H-93-8, carrying the gene *Cre2* which confers resistance to the cereal cyst nematode *H. avenae* (Delibes et al. 1993). Both introgression lines had been derived from an earlier cross between *T. aestivum* subsp. *aestivum* (2n=42; genome composition AABBDD) and a semi-fertile hybrid between *T. turgidum* subsp. *turgidum* (2n=28; genome composition AABB) and the wild grass *Ae. ventricosa* (2n=28; genome constitution D^vD^vN^vN^v). We also have examined several resistant advanced lines that were obtained from H-93-33 (lines ID-2151, ID-2193, Ma-1612-a and Ma-1612-b) or H-93-8 (line ID-2150) after 3 to 5 backcrosses with commercial wheats.

The ISH protocol was essentially as described in Sánchez-Morán et al. (2001). Three different DNA probe combinations were separately hybridized on mitotic slides from each of those breeding lines. The first mix contained differentially labelled A- and S-genome DNA probes, and D-genome DNA blocking. A second mix contained differentially labelled A- and D-genome DNA probes, and S-genome DNA blocking. These two probe combinations revealed the number of chromosomes belonging to the A and B genomes of wheat and to the D genome from either wheat or *Ae. ventricosa*. The third mix was primarily designed to reveal the suspected presence of N^v-genome chromosomes in those lines, which contained chromosome pairs that had been blocked by any of the two former probe combinations. This mix contained differentially labelled D- and N-genome DNA probes with durum wheat (AB) DNA was added as blocking. This mix also included the ribosomal DNA probe pTa71 and the repeated DNA probe pAs1 (Rayburn and Gill 1987). The latter probe provides a distinctive ISH pattern for individual D-genome chromosomes in wheat (Pedersen and Langridge 1997) and *Ae. ventricosa* (Badaeva et al. 2002). A summary of the karyotype findings in the lines examined is described here (Tables 1 and 2).

H-93-33 and derived lines. The ISH analysis confirmed the existence of a 4N^v(4D) substitution in H-93-33, which had been proposed from earlier biochemical and cytological analy-

Table 1. Chromosome constitution of the bread wheat breeding lines. The D genomes of wheat and *Ae. ventricosa* are pooled in column D. An * indicates the genome includes a D-N translocation).

Line	Genome			
	A	B	D	N
H-93-33	14	14	12	2
ID-2151	14	14	14	0
ID-2193	14	14	14*	2*
Ma-1612-a	14	14	12	2
Ma-1612-b	14	14	14	0
H-93-8	12	14	12	4
ID-2150	14	14	14	0

Table 2. Identification of individual chromosomes in the breeding lines. A + indicates presence and a – indicates absence; T¹ is the translocation 4DS-4NS.4NL and T² is the translocation 5DS.5DL-5D^vL.

Line	Wheat							<i>Ae. ventricosa</i>	
	1D	2D	3D	4D	5D	6D	7D	D ^v	N ^v
H-93-33	+	+	+	-	-	+	-	3D ^v , 5D ^v	4N ^v
ID-2151	+	+	+	+	+	+	+	0	0
ID-2193	+	+	+	T ¹	+	+	+	0	T ¹
Ma-1612-a	+	+	+	-	+	+	+	0	4N ^v
Ma-1612-b	+	+	+	+	+	+	+	0	0
H-93-8	+	+	-	-	T ²	+	-	3D ^v , 4D ^v , T ²	5N ^v , 7N ^v
ID-2150	+	+	+	+	+	+	+	0	0

ses (Mena et al. 1989). Comparison between the ISH patterns of pAs1 found in this line and those reported by Pedersen and Langridge (1997) and Badaeva et al. (2002) undoubtedly demonstrated the presence of additional *Ae. ventricosa* introgressed chromosomes, i. e., a 5D^v(5D) substitution and the replacement of wheat 7D by its nonhomoeologous 3D^v. None of these D^v genome introgressions is maintained in any of the Hessian fly resistant lines derived from H-93-33 that were checked. However, the 4N^v(4D) substitution has been transmitted to line Ma-1612-a, and a large part of the long arm of this alien chromosome is still present in a 4D-4N^v translocation detected in line ID-2193. These findings confirm former data indicating that gene *H27* is linked to Acph-N^v1, a molecular marker located on 4N^v (Delibes et al. 1997).

H-93-8 and derived lines. Previous results had proposed a double substitution in line H-93-8: 5N^v(5A) and 7N^v(7D) (Mena et al. 1993). The ISH analysis has demonstrate the presence of 5N^v and 7N^v and the absence of 7D in this introgression line, although it could not be confirmed that 5A is the A-genome pair absent in this line. Two additional substitutions (3D^v(3D) and 4D^v(4D)) and a 5D-5D^v translocation that were not previously detected by molecular marker approaches have been also cytologically evidenced (Fig. 1B). None of these alien chromosomes or translocations appears in the advanced line ID-2150, whose ISH karyotype is indistinguishable from that of bread wheat.

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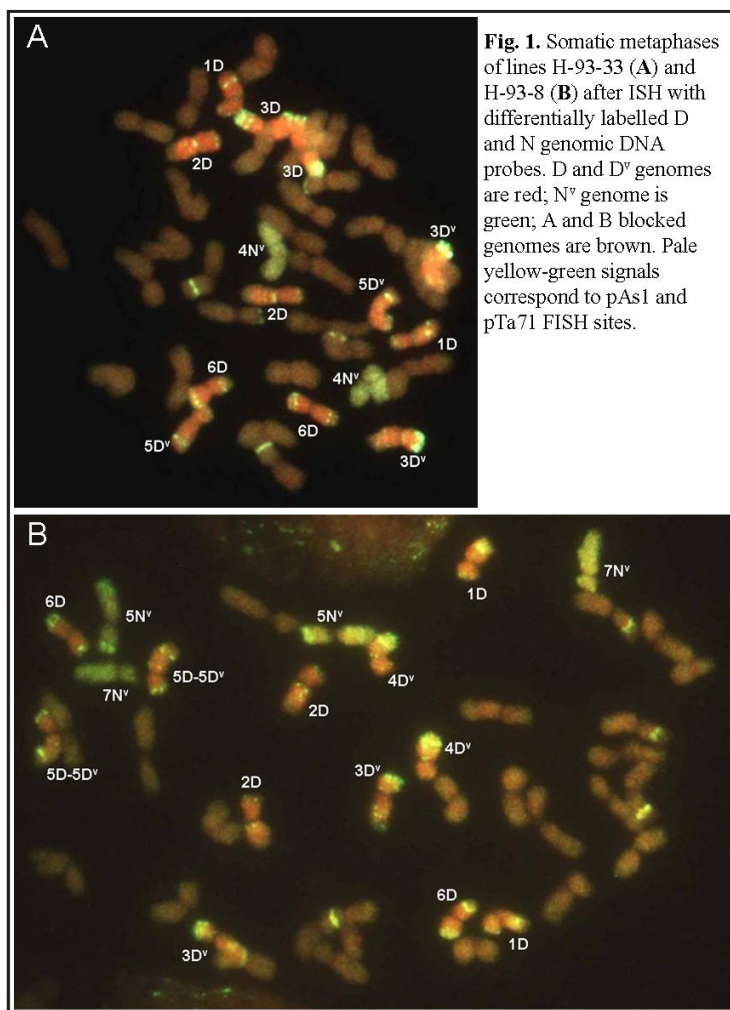


Fig. 1. Somatic metaphases of lines H-93-33 (A) and H-93-8 (B) after ISH with differentially labeled D and N genomic DNA probes. D and D^v genomes are red; N^v genome is green; A and B blocked genomes are brown. Pale yellow-green signals correspond to pAs1 and pTa71 FISH sites.

Characterization of endosperm proteins and bread-making quality in wheat breeding lines carrying resistance genes for *Mayetiola destructor* and/or *Heterodera avenae*.

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The experimental material included thirteen bread wheat-breeding lines that carry genes for resistance to *M. destructor* and/or *H. avenae*. The sources of these resistances are the wild species *Ae. triuncialis* and *Ae. ventricosa* (lines TR and H-93, respectively) (Delibes et al. 1993, 1997; Romero et al. 1998). We have determined the composition in HMW-glutenin subunits (related with bread-making quality), puroindoline proteins (related with hardness of grain), and waxy proteins (related with starch viscosity). In addition to, we analysed the bread-making quality and some agronomic parameters of the lines. A previous analysis of prolamins by electrophoresis SDS-PAGE indicated the homogeneity of the lines.

Glutenins were extracted from crushed endosperm (Singh et al. 1991) and the extracts fractionated by SDS-PAGE electrophoresis (Payne et al. 1980). Waxy proteins were extracted from the flour, and electrophoresis was performed as described by Rodríguez-Quijano et al. (1998). Puroindoline allelic composition was obtained by DNA isolation (Dellaporta et al. 1983) and PCR amplification of pinA and pinB coding regions with specific primers (Giroux and Morris 1997).

Gluten strength was estimated by the SDS-sedimentation (SDSS) test (Mansur et al. 1990). Protein was measured with a NIR spectroscope (Infra-lyzer 300). Mixing time (MT), and resistance to breakdown (BDR) were determined using 10 g of flour and a National Manufacturing Co. Mixograph apparatus (Lincoln, NE), as described by Finney and Shogren (1972). Starch viscosity was analysed by a Rapid Visco Analyser (RVA-3D, Newport Scientific, Pty. Ltd.) and the viscosity peak (VP) parameter was derived from the RVA curve. All parameters were measured twice. Line Ma-99-75-5 (H93) was not tested because the amount of material was insufficient.

The results indicate variability for proteins in the breeding lines (Table 3). Regarding to bread-making quality, four lines stand out for their high dough strength: ID-2193, ID-2151, ID-2004 and Ma-99-93-1 (Table 3). Lines ID-2193 and Ma-99-93-1 are resistant for *M. destructor*, line T-2004 carries resistance genes for both *H. avenae* and *M. destructor* while line ID-2151 lacks resistance genes.

According to their composition in puroindoline proteins, the lines were identified as having a 'soft' or 'hard' endosperm (Table 3). This is an important classification to determine their final use since the hard wheat varieties are the most valuable in bread-making industry. Among the lines with good bread-making quality, three are hard and one is soft (Table 3).

The waxy protein analysis has revealed that two lines possess the null allele (*b*) for the *Wx-B1* locus. Yamamori et al. (1992) related the presence of null alleles with less amylose content on bread wheat starch. The

ratio amylose/amylopectin is very important in relation to the end use of any variety. Oda et al. (1980) determined that the noodles made of flour that are low in amylose were the favourites of Japanese consumers. High viscosity peak from

Table 3. Genetic composition of the breeding lines.

Breeding line	Locus and HMW-glutenin subunits				Locus and alleles of waxy proteins		
	<i>Glu-A1</i>	<i>Glu-B1</i>	<i>Glu-D1</i>	Hardness	<i>Wx-A1</i>	<i>Wx-D1</i>	<i>Wx-B1</i>
ID-2181 (TR)	2*	17+18	5+10	Hard	<i>a</i>	<i>a</i>	<i>a</i>
T-2003 (TR)	1	7*+9	5+10	Hard	<i>a</i>	<i>a</i>	<i>a</i>
T-2004 (TR)	2*	6+8	5+10	Hard	<i>a</i>	<i>a</i>	<i>b</i>
T-2105 (TR)	2*	17+18	5+10	Hard	<i>a</i>	<i>a</i>	<i>b</i>
ID-2193 (H93)	1	7*+8	2+12	Hard	<i>a</i>	<i>a</i>	<i>a</i>
ID-2150 (H93)	1	7*+9	5+10	Hard	<i>a</i>	<i>a</i>	<i>a</i>
ID-2151 (H93)	1	7*+8	2+12	Soft	<i>a</i>	<i>a</i>	<i>a</i>
Ma-1612a (H93)	1	7*+8	2+12	Hard	<i>a</i>	<i>a</i>	<i>a</i>
Ma-1612b (H93)	1	7*+8	2+12	Hard	<i>a</i>	<i>a</i>	<i>a</i>
Ma-99-75-5 (H93)	1	7*+8	2+12	Hard	<i>a</i>	<i>a</i>	<i>a</i>
Ma-99-93-1 (H93)	1	7*+8	2+12	Hard	<i>a</i>	<i>a</i>	<i>a</i>
Ma-99-41-6 (H93)	Null	7*+8	2+12	Soft	<i>a</i>	<i>a</i>	<i>a</i>
Ma-99-104 (H93)	1	7*+8	2+12	Soft	<i>a</i>	<i>a</i>	<i>a</i>

RVA correlates with a lower content in amylose. The highest values for the VP parameter are found in the lines ID-2150, Ma-99-93-1, ID-2181, T-2004 and T-2105 (Table 4). These two latter lines are those having the null allele at the *Wx-B1* locus (Table 3, p. 140).

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Table 4. Quality parameters of the breeding lines.

Breeding lines	% Protein (14% Hum)	SDSS (mm)	Mixograph analysis		Starch viscosity VP (RVU)
			MT (s)	BDR (%)	
ID-2181 (TR)	9.9	71.0	156.0	15.4	306.6
T-2003 (TR)	10.5	67.0	120.0	27.7	275.6
T-2004 (TR)	11.4	86.5	165	13.3	301.9
T-2105 (TR)	9.9	79.0	90.0	17.2	313.9
ID-2193 (H93)	9.5	94.0	110.0	18.3	280.7
ID-2150 (H93)	8.9	65.5	150.0	15.4	313.9
ID-2151 (H93)	10.6	79.0	140.0	13.43	266.5
Ma-1612a (H93)	10.2	57.0	130.0	18.2	254.7
Ma-1612b (H93)	8.9	67.5	210.0	13.4	187.9