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Prospect for the use of *Hordeum chilense* in durum wheat breeding

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SUMMARY – *Hordeum chilense* is a wild species, native to Chile and small area in Argentina, which has been successfully crossed with diploid, tetraploid and hexaploid wheats and the intergeneric amphiploids obtained from them have been named tritordeums. Hexaploid tritordeum can be used as bridge species to introgress genetic material from this wild barley into durum wheat. Addition lines of *H. chilense* on tetraploid wheat have been also obtained with this purpose. Resistance to pests and diseases have been tested on *H. chilense* and its amphiploids. *Hordeum chilense* is resistant to rusts and powdery mildew, to *Septoria tritici*, to common and karnal bunt, to the smuts *Ustilago nuda* and *U. tritici*, to *Pyrenophora teres* and *P. tritici-repentis* and to *Rhynchosporium secalis*. *H. chilense* is also known to possess resistance to the aphids *Diuraphis noxia*, *Rhopalosiphum padi* and *Schizaphis graminum* and to nematodes *Meloidogyne* spp. *H. chilense* confers to tritordeum certain degree of tolerance to drought and salt. *H. chilense* could be also a source of quality traits for wheat breeding. Extreme variabilty have been found for endosperm storage proteins. *H. chilense* confers to tritordeum high carotenoid pigment content. STSs have being developed to be used in marked assisted selection. *In situ* hybridization with pAs1 repetitive sequence have been found to be valuable for *H. chilense* chromosome identification in durum wheat genetic background. Chromosome variants plus molecular markers will be useful tools for the introgression of *H. chilense* chromatin on durum wheat.

Key words: *Triticum*, *Hordeum chilense*, tritordeum, interspecific hybridization.

RESUME – *"Perspectives d'utilisation de* Hordeum chilense *en amélioration du blé dur".* Hordeum chilense *est une espèce sauvage, autochtone du Chili et d'une petite région en Argentine, qui a eu un grand succès en croisement avec des blés diploïdes, tétraploïdes et hexaploïdes. L'amphiploïde inter-générique obtenu de ce croisement a été nommé Tritordeum. L'hexaploïde tritordeum peut être utilisé comme une espèce pont pour l'introduction de matériel génétique, en provenance de cette orge sauvage, dans le blé dur. D'autres lignées chromosomiques d'addition de l'* H. chilense *dans du blé dur ont été obtenues dans le même but. La résistance de l'*H. chilense *et de ses amphiploïdes aux ravageurs et aux maladies a été testée. L'*H. chilense *est résistant à la rouille et à l'oïdium, à* Septoria tritici, *aux caries* Ustilago nuda *et* U. tritici, *à* Pyrenophora teres *et* P. tritici-repentis *et à* Rhynchosporium secalis. *Il est connu aussi que* H. chilense *est résistant aux aphidiens :* Diuraphis noxia, Rhopalosiphum padi *et* Schizapis graminum *ainsi qu'aux nématodes* Meloidogyne *spp. L'*H. chilense *transmet au tritordeum un certain degré de tolérance à la sécheresse et au sel. Il pourrait aussi être une source de caractères de qualité pour l'amélioration du blé, car il y a été trouvé une grande variabilité dans les protéines emmagasinées dans l'endosperme. L'*H. chilense *transmet au tritordeum un haut contenu de pigment carotène. Des STSs ont été développés pour être utilisés dans la sélection assistée par marqueurs. Il a été trouvé que l'hybridation in situ avec la séquence répétitive pAs1, est valable pour l'identification des chromosomes de l'*H. chilense *dans le background génétique du blé dur. Les variants chromosomiques plus les marqueurs moléculaires peuvent être des instruments valables pour l'introgression de la chromatine de* H. chilense *dans le blé dur.*

Mots-clés : Triticum, Hordeum chilense, *tritordeum, hybridation inter-spécifique.*

Crossability and variability of *Hordeum chilense*

Hordeum chilense Roem. et Schult. is a native South American diploid wild barley included in the section *Anisolepis* (Bothmer *et al.,* 1980). *H. chilense* occurs exclusively in Chile and Argentina and is extremely polymorphic both at the morphological and biochemical levels. After *H. vulgare* and *H. bulbosum*, we believe *H. chilense* is the species of the genus *Hordeum* with the highest potential for cereal breeding purposes, given its high crossability with other members of the Triticeae tribe and its agronomically interesting characteristics.

H. chilense has been successfully crossed with Triticeae species of the genera *Aegilops*, *Agropyron*, *Dasypyrum*, *Hordeum*, *Secale*, *Triticum* and *Triticosecale*. (see Martín *et al.,* 1998 for a review).

Tritordeums are amphiploid obtained after chromosome doubling of hybrids between *Triticum* and *Hordeum* spp. Hexaploid tritordeum (2n=6x=42, AABBH^{ch}H^{ch}), the amphiploid obtained after chromosome doubling of the hybrid *T. turgidum* conv. *durum* and *H. chilense* is the subject for a breeding program with the goal of creating a new crop (Martín, 1988), given the high chromosome stability and its good field performance. In addition, hexaploid tritordeum can be used as bridge species to introgress genetic material from this wild barley into durum wheat. Addition lines of *H. chilense* on tetraploid wheat have been obtained with this purpose.

In the breeding program of tritordeum the first step was to build up a wide genetic base of the amphiploid. We set up a 200 populations *H. chilense* collection wich was assessed for several morphological and physiological traits. Some grouping on basis of habitats of origin (wet vs. dry), spike shape (flat vs. cylindrical), secondary flower fertility, avoidance to leaf rust fungi and cytoplasm. The two main groups were related to H1 and H7, the two first *H. chilense* accessions used in wheat crosses. Fertile amphiploids have been obtained with 103 *H. chilense* accessions after colchicine treatment of the hybrids. Up to now 251 primary amphiploids have been obtained. Two of them are tetraploids, 172 are hexaploids and 77 are octoploids. Therefore, throughout these amphiploids a huge genetic variability from *H. chilense* is available for wheat breeding. Up to now resistance to diseases and quality traits (see Martín and Álvarez in this volume) are the best candidates for such introgression.

Reaction to biotic and abiotic stresses

H. chilense is resistant to the barley and wheat brown rusts (*Puccinia hordei* and *P. recondita* f.sp. *tritici*, respectively). *H. chilense* lines may be susceptible to *P. recondita* f.sp. *agropyrina*, to wheat and barley yellow rusts (*P. striiformis* ff.ssp. *tritici* and *hordei*, respectively) (Rubiales and Niks, 1992) and to the wheat stem rust (*P. graminis*). Tritordeum behaves in all instances as the wheat parental (Rubiales *et al.,* 1991, 1993b) therefore making useless the transfer of the *H. chilense* gene/s responsible for increasing wheat resistance because most probably they will be inactivated (suppressed) on wheat background.

H. chilense is resistant to the wheat powdery mildew (*Erysiphe graminis* f.sp. tritici). There is a quantitative contribution to the resistance of tritordeum (Rubiales *et al.,* 1993a). *H. chilense* and tritordeum were resistant to the barley, oat and rye powdery mildew (*E. graminis* ff.ssp. *hordei*, *avenae* and *secalis,* respectively) isolates studied (Rubiales *et al.,* 1993a). Several resistance mechanism acting at different phases of the infection proccess might be operative as some haustoria formation and colony development is allowed in some lines but not in others. These complex control of resistance make unreliable to transfer the resistance to wheat maintaining the agronomical performance of wheat unchanged.

Both *H. chilense* and tritordeum are resistant to *Septoria tritici*. Hexaploid tritordeums were immune whatever their stature and lateness. Studies with *H. chilense* chromosomal addition and substitution lines on bread wheat indicated that resistance is mainly controlled by factors on crhomosomes 4 H^{ch} and in a minor extend by chromosome 5 H^{ch}. *H. chilense* and tritordeum may be susceptible to *S. nodorum*. Some tritordeums were more resistant than their wheat parental lines, although others were not (Rubiales *et al.,* 1996a). There was a slight dilution of the resistance at higher ploidy level, but not as clear as for *S. tritici*. Therefore resistance to *Septoria* is a trait amenable to chromosome manipulation.

Tritordeum as a crop can be regarded as being susceptible to *Fusarium culmorum*. The level of resistance to colonization by Fusarium is on average higher in tritordeum than in wheat. Some *H. chilense* genotypes (H7, H17, H56, H61) enhanced the wheat resistance to *F. culmorum* in its tritordeum offspring, others (H1, H11, H12, H13) did not (Rubiales *et al.,* 1996a).

H. chilense is resistant to common bunt (*Tilletia caries*). Resistance is conferred by chromosome 7, and in a minor extend by chromosome 6 (Rubiales and Martín, 1999). Resistance is expressed in tritordeum. No infected spikes of *H. chilense* or hexaploid tritordeum were found in bunt field tests at Córdoba. Octoploid tritordeum lines did, however, display some bunted spikes but much less than their respective bread wheat parental line (Rubiales *et al.,* 1996b).

H. chilense posses resistance also to the smuts *Ustilago nuda* and *U. tritici* (Nielsen, 1987), to karnal bunt (*Tilletia indica*) (Chauhan and Singh, 1997), to take-all (*Gaeumannomyces graminis*) (Jorgensen and Jensen, 1976), to *Pyrenophora tritici-repentis* and *P. teres*, and to *Rhynchosporium secalis*. Susceptibility in *H. chilense* has been reported to *Typhula ishikariensis* and to *Pseudocercosporella herpotrichoides* (unpublished results).

H. chilense is resistant to the root-knot nematodes *Meloidogyne naasi* (Person-Dedryver *et al.,* 1990), and to the Columbia root-knot nematode (*Meloidogyne chitwoodi*) (Jensen and Griffin, 1994). The resistance to the root-knot nematode was is located on the short arm of the chromosome $1H^{ch}$.

Varying levels of resistance to aphids such as Schizaphis *graminum* (Castro *et al.,* 1994), *Diuraphis noxia* (Clement and Lester, 1990) and *Rhopalosiphum padi* (Weibull, 1987) have been described in *H. chilense*, but not simple and therefore transferable genetic control have been found.

Addition lines of *H. chilense* in bread wheat were used to locate genes for tolerance to salt on chromosomes $1H^{ch}$, $4H^{ch}$ and $5H^{ch}$ (Foster *et al.,* 1990). The complexity of the genetic control of this trait makes difficult to think on *H. chilense* as a source of salt tolerance on wheat breeding.

Introgression of hordeum chilense into durum wheat

The availability of addition lines in *T. aestivum* allowed us to develop specific markers for H*. chilense* chromosomes (Hernández *et al.,* 1996, 1999a,b). Those markers that were conserved across distant accessions were used to assist the development of a new set of addition lines in bread and durum wheat (non-published results). In the same way these markers are valuable tools to track the chromatin of *H. chilense* in wheat backgroung.

In addition to marker assisted selection, *in situ* hybridization is a tool which we have used successfully for identifying *H. chilense* chromosomes (or chromosome translocation) in wheat background. Cabrera *et al.* (1995) found that the pAs1 repetitive DNA probe isolated from *T. tauschii* by Rayburn and Gill (1986) hybridize to specific sites along the chromosomes of *H. chilense*, allowing the identification of all seven pairs.

The hexaploid tritordeum collection synthesized with 103 different *H. chilense* accessions is the basic material for the introgression of characters of interest into wheat. With this purpose we crossed *Triticum monococcum* (2n=2x=14, A^mA^m) and hexaploid tritordeum (2n=6x=42, AABB H^{ch} H^{ch}). The hybrid A^mAB H^{ch} (chromosome number 28) is male sterile but after backcrossing to durum wheat (2n=4x= 28, AABB) set some seeds. The chromosome number of the backcrossing progeny is always close to 35 and occasionally close to 42. Most probably these backcrosses have the genome constitution $AABBH^{ch}$ (2n=35) and A^mAABBH^{ch} (2n=42) respectively. Clearly functional females gametes produced for the hybrid A^mABH^{ch} are unreduced gametes, ABH^{ch} (in which A is a mixture of A^m and A) or A^mABH^{ch}. Therefore, the chance of obtaining introgression of *H. chilense* into durum wheat is quite low, given the need of backcrossing to restore fertility.

After this failure, alternatively crosses have been made between chromosome addition lines $4H^{ch}$ and $7H^{ch}$, which are the lines of interest for introgressing septoria resistance and carotenoids content, with *T. urartu* (2n=2x=14, AA). The hybrid AAB plus one H^{ch} chromosome is easily obtained and backcrosses, again, indicated the production of unreduced gametes.

A new approach we are working on is using as male parental in crosses of addition lines of *H. chilense* to durum wheat of the D-genome substitution of durum wheat (Joppa and Williams, 1977), in this way we expect to avoid the problem of unreduced gametes in the previous crosses. The objective of this cross is to have for group 4 and 7 a hybrid with only one chromosome of the three genomes B, D and H^{ch} and therefore there is chance for the recovery of translocation involving the H^{ch} chromosome.

We have synthesized amphiploids between *H. chilense* and durum and bread wheat carrying *Ph1* (pairing homoeologous) gene mutants. Until now they have not been used for promoting recombination between barley and wheat chromosomes, given that the pairing between *H. chilense* and wheat genomes is nil even in the absence of 5B chromosome in hybrids of *H. chilense* with N5B T5D bread wheat (Martín and Sánchez-Monge, 1980). Nevertheless, spontaneous translocations, terminal, interstitial or centromeric, are frequently observed on the progeny of the hybrid bread wheat x hexaploid tritordeum, $AABBDH^{ch}$. This material could be an alternative source for *H. chilense* introgressions in wheat.

Conclusions

Hordeum chilense could be a useful source of genes of resistance to biotic and abiotic stresses and quality for wheat breeding. Tritordeum, the amphiploid barley-wheat, is the basic genetic material for using the genetic variability of *H. chilense* in durum wheat breeding. Over 100 *H. chilense* accessions have been used to synthesize 251 tritordeums. The genetic diversity of H. *chilense* on resistance to diseases of cultivated Triticeae deserves more attention to this species. Resistance to septoria, located on chromosome 4$\mathbf{H}^{ch}$ and high carotenoid pigment content and resistance to common bunt, both located on chromosome 7$\mathbf{H}^{ch}$ are among the traits of interest to be transferred to durum wheat.

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References

Bothmer, R. von, Giles, B.R. and Nicora, E. (1980). Revision of *Hordeum* sect. *Anisolepis* Nevski. *Bot. Notiser,* 133: 539-554.
Cabrera, A., Friebe, B., Jiang, J. and Gill, B.S. (1995). Characterization of *Hordeum chilense* chromosomes by C-banding and in situ hybridization using highly repeated DNA probes. *Genome,* 38: 435-442.
Castro, A.M., Martín, L.M., Martín, A., Arriaga, H.O., Tobes, N. and Almaraz, L.B. (1994). Screening for Greenburg Resistance in Hordeum chilense Roem et Schult. *Plant Breeding,* 112: 151-159.
Chauhan, R.S. and Singh, B.M. (1997). Resistance to Karnal bunt in *Hordeum chilense* and its amphiploids with *Triticum* species. *Euphytica,* 96: 327-330.
Clement, S.L. and Lester, D.G. (1990). Screening wild *Hordeum* species for resistance to russian wheat aphid. *Cereal Res. Comm.,* 18: 173-177.
Foster, B.P., Philips, M.S., Miller, T.E., Baird, E. and Powell, W. (1990). Chromosome location of genes controlling tolerance to salt (NaCl) and vigour in *Hordeum vulgare* and *H. chilense*. *Heredity,* 65: 99-107.
Hernández, P., Hemmat, M., Weeden, N.F., Dorado, G. and Martín, A. (1999b). Development and characterization of *Hordeum chilense* chromosome-specific STS markers suitable for wheat introgression and marker-assisted selection. *Theoretical and Applied Genetics,* 98: 721-727.
Hernández, P., Martín, A. and Dorado, G. (1999a). Development of SCARs by direct sequencing of RAPD products: A practical tool for the introgression and marker-assisted selection of wheat. *Molecular Breeding,* 5: 245-253.
Hernández, P., Rubio, M.J. and Martín, A. (1996). RAPDs as molecular markers for the detection of Hordeum chilense chromosomes in wheat addition lines and in tritordeum. *Plant Breeding,* 115: 52-56.
Jensen, K.B and Griffin, G.D. (1994). Resistance of diploid Triticeae species and accessions to the columbia root-knot nematode, *Meloidogyne chitwoodi. J. Nematology,* 26: 635-639.
Joppa, L.R., Williams, N.D. (1977). D-Genome Substitution-Monosomics of Durum Wheat. *Crop Science,* 17: 772-776.
Jorgensen, J.H. and Jensen, H.P. (1976). Screening of *Hordeum* species for resistance to the take-all fungus, *Gaeumannomyces graminis.* Z. *Pflanzenzüchtg.,* 76: 200-203.
Martín, A. (1988). Tritordeum: The first ten years. *Rachis,* 7: 12-15.
Martin, A., Martin, L.M., Cabrera, A., Ramirez, M.C., Gimenez, M.J., Rubiales, D., Hernandez, P. and Ballestros, J. (1998). The potential of *Hordeum chilense* in breeding Triticeae species. In: *Triticeae III,* Jaradat, A.A. (ed.). Science Publishers, Inc., Enfield, NH, USA, pp. 377-386.
Martín, A. and Sánchez-Monge Laguna, E. (1980). Effects of the 5B system on control of pairing in *Hordeum chilense* x *Triticum aestivum* hybrids. Z. *Pflanzenzüchtg,* 85: 122-127.
Nielsen, J. (1987). Reaction of *Hordeum* species to the smut fungi *Ustilago nuda* and *U. tritici. Can. J. Bot.* 65: 2024- 2027.
Person-Dedryver, F., Jahier, J. and Miller, T.E. (1990). Assessing the resistance to cereal root-knot nematode, *Meloidogyne naasi* in a wheat line with the added chromosome arm 1HchS of *Hordeum chilense. J. Genet. Breed.* 44: 291-296.
Rayburn, A.L. and Gill, B.S. (1986). Isolation of a D-genome specific repeated DNA sequence from *Aegilops squarrosa. Plant. Mol. Biol. Rep.* 4: 102-109.
Rubiales, D. and Martín, A. (1999). *Hordeum chilense* chromosome location and expression of common bunt resistance in wheat addition lines. *Euphytica* (in press).

Rubiales, D. and Niks, R.E. (1992). Histological responses in *Hordeum chilense* to brown and yellow rust fungi. *Plant Pathol.,* 41: 611-617.
Rubiales, D., Ballesteros, J. and Martín, A. (1991). The reaction of X Tritordeum and its *Triticum* spp. and *Hordeum chilense* parents to rust diseases. *Euphytica,* 54: 75-81.
Rubiales, D., Brown, J.K.M. and Martín, A. (1993a). *Hordeum chilense* resistance to powdery mildew and its potential use in cereal breeding. *Euphytica,* 67: 215-220.
Rubiales, D., Niks, R.E. and Martín, A. (1993b). Genomic interactions in the resistance to mildew and rust fungi in hybrids and amphiploids involving the genera *Triticum, Hordeum* and *Secale. Cereal Res. Comm.,* 21: 187-194.
Rubiales, D., Nicholson, P., Snijders, C.H.A. and Martín, A. (1996a). Reaction of tritordeum to *Septoria nodorum* and *Fusarium culmorum. Euphytica,* 88: 165-174.
Rubiales, D., Ramírez, M.C. and Martín, A. (1996b). Resistance to common bunt in *Hordeum chilense* x *Triticum* spp. amphiploids. *Plant Breeding,* 115: 416-418.
Weibull, J. (1987). Screening for resistance against *Rhopalosiphum padi* (L.). 2. *Hordeum* species and interspecific hybrids. *Euphitica,* 36: 571-576.