© NIAB 2011
ISSN 1479-2621

Plant Genetic Resources: Characterization and Utilization (2011) 9(2); 313–316
doi:10.1017/S1479262111000402

Exploitation of nuclear and cytoplasm variability in *Hordeum chilense* for wheat breeding

Cristina Rodríguez-Suárez[1], María J. Giménez[1], María C. Ramírez[1],
Azahara C. Martín[1,2], Natalia Gutierrez[2], Carmen M. Ávila[2],
Antonio Martín[1] and Sergio G. Atienza[1]*

[1] *Plant Breeding Department, IAS-CSIC, Apdo. 4084, E-14080, Córdoba, Spain and*
[2] *Área Mejora y Biotecnología, IFAPA-Centro Alameda del Obispo, Córdoba, Spain*

Abstract

Hordeum chilense Roem. et Schultz. is a diploid wild barley native to Chile and Argentina. The high crossability of this species with other members of the Triticeae tribe promoted the development of the new species × *Tritordeum* Ascherson et Graebner. Hexaploid tritordeum was developed from the hybrid derived from the cross between *H. chilense* (used as female parent) and durum wheat. The interest of *H. chilense* is based on the presence of traits potentially useful for wheat breeding, including high endosperm carotenoid content, septoria tritici blotch resistance and abiotic stress tolerance. Besides, the variability at cytoplasm level is also important in this species. The development of common wheat–*H. chilense* alloplasmic lines (nucleus from wheat and cytoplasm from *H. chilense*) results in fertile or male sterile genotypes, depending on the accession donating the cytoplasm. Furthermore, these alloplasmic lines constitute an ideal system for deepening our knowledge on nuclear–cytoplasm interactions. In conclusion, *H. chilense* is an interesting source of variability for wheat breeding.

Keywords: cytoplasm; *Hordeum chilense*; plant breeding; plasmon; wild relatives

Wild relatives for crop breeding: potential of nuclear variability in *Hordeum chilense* Roem. et Schultz

The progressive narrowing of the genetic base in crops (Tanksley and McCouch, 1997; Warburton *et al.*, 2006) has promoted a renewed interest in wild relatives such as *Hordeum vulgare* ssp. *spontaneum* (Matus *et al.*, 2003; Inostroza *et al.*, 2009) or other distant relatives such as *Aegilops tauschii*, donor of the D genome of common wheat (van Ginkel and Ogbonnaya, 2007); or *H. chilense* Roem. et Schultz. (Atienza *et al.*, 2000; Atienza *et al.*, 2005b; Martin *et al.*, 2008b). The wild barley *H. chilense* shows a wide range of variation (at both morphological and molecular levels) distributed into two main groups plus an intermediate group, as revealed by molecular markers (Vaz Patto *et al.*, 2001; Castillo *et al.*, 2010). The high compatibility of *H. chilense* with the genomes of *Triticum* species gives rise to fertile and stable amphiploids and allows the transfer of traits to wheat (Martin *et al.*, 1998), such as resistance to septoria tritici, abiotic stress tolerance or endosperm storage proteins (Martin *et al.*, 1999; Atienza *et al.*, 2002), but probably the main interest of this species is its potential for increasing carotenoid content (Alvarez *et al.*, 1999; Atienza *et al.*, 2004; Atienza *et al.*, 2005a; Atienza *et al.*, 2007b). The *phytoene synthase 1* from *H. chilense* is a good candidate gene for the improvement of carotenoid content (Atienza *et al.*, 2007a), and, therefore, the cloning and characterization of this gene offer new possibilities for wheat breeding (Rodriguez-Suarez *et al.*, 2010). Similarly, the development of *H. chilense* durum

---

* Corresponding author. E-mail: sgatienza@ias.csic.es

wheat chromosome substitution lines will be useful for evaluating the substitution effect of durum wheat by *H. chilense* genes for carotenoid content. The use of barley expressed sequence tag (EST) markers (Hagras *et al.*, 2005a; Hagras *et al.*, 2005b; Nasuda *et al.*, 2005) has proven very useful for physical mapping in *H. chilense* (Atienza *et al.*, 2007c; Said and Cabrera, 2009; Cherif-Mouaki *et al.*, 2011). Besides, the development of the genetic linkage map using ESTs, conserved orthologous set (Bolot *et al.*, 2009) and *H. chilense*-specific diversity arrays technology markers will allow the establishment of precise relationships between *H. chilense* and related species genomes, thus providing more efficient tools for the use of this wild barley in wheat breeding.

Cytoplasm × nuclear variability in *H. chilense*–wheat interactions

The nuclear genome has a predominating role for the inheritance of most plant traits Nevertheless, cytoplasmic factors and cytoplasm × nucleus interactions are also important and still largely unexplored. Genetic information of eukaryotic organisms is divided into a nuclear genome in the nucleus and organelle genomes (sometimes referred to as plasmon) in the cytoplasm. Since the cytoplasm is maternally inherited in Triticeae species (Kihara, 1951), the best way to investigate nuclear–cytoplasm interactions is by developing alloplasmic lines, i.e. lines with the same nucleus but cytoplasms from different species.

H. chilense–wheat alloplasmic lines have been developed by repeated substitution backcross as described by Kihara (1951). First, amphiploids *H. chilense* × wheat are developed as described by Martin and Chapman (1977). This step is essential since the hybrids between *H. chilense* and wheat are sterile while the amphiploids are fertile. Backcrossing to the nucleus donor is repeated until *H. chilense* chromosomes are fully eliminated. After somatic chromosome counting, the cytoplasm origin has to be checked, since paternal inheritance of cytoplasm has also been reported (Soliman *et al.*, 1987; Laser *et al.*, 1997; Aksyonova *et al.*, 2005; Badaeva *et al.*, 2006). Indeed, we have observed this phenomenon with both *H. chilense* (Atienza *et al.*, 2007d) and *H. vulgare* cytoplasms (Martin *et al.*, 2008a) using the chloroplastic marker ccSSR4 (Chung and Staub, 2003).

Alloplasmic lines are very useful for elucidating plant phylogeny and determining the genetic effect of different plasmons. Furthermore, since the discovery of cytoplasmic male sterility (CMS) in wheat (Kihara, 1951), breeders have been very interested in CMS systems, looking for a viable procedure for hybrid wheat production (for a review, see Martin, 2009).

The development of *H. chilense*–common wheat alloplasmic lines gives rise to two types of lines: male-sterile when the line H1 is used as cytoplasm donor, or fully fertile when other *H. chilense* lines are used. Accordingly, a research line is being developed to investigate the potential of this new CMS source, designated msH1, to produce hybrid wheat. The male sterile line does not show any floral or developmental abnormalities, but reduced height and delayed heading (Martin *et al.*, 2008b). Fertility restoration is obtained when chromosome $6H^{ch}S$ from *H. chilense* line H1 is added (Martin *et al.*, 2008b). Further research allowed the obtaining of a fertile euplasmic line carrying the translocation T6HchS·6DL (Martin *et al.*, 2009). However, a single dose of this translocation is insufficient for fertility restoration, which suggests the presence of one or more inhibitors of fertility genes in chromosome 6DL (Martin *et al.*, 2009). More recently, a highly fertile line with 42 chromosomes plus an extra acrocentric chromosome has been obtained (Martin *et al.*, 2010), whose long arm is the $1H^{ch}S$ chromosome, as demonstrated by molecular markers and fluorescent *in situ* hybridization. It seems that this chromosome originated from a deletion of the distal part of chromosome $1H^{ch}L$ and that the restorer gene is located on the retained segment from the $1H^{ch}L$ (Martin *et al.*, 2010). The disomic addition of this acrocentric chromosome is fully fertile and thus constitutes an additional source of restoration for wheat hybrid production based on msH1 system.

On the other hand, fully fertile alloplasmic lines were also obtained (Atienza *et al.*, 2007d). Preliminary evidence suggested that phenotypic and metabolic variations in wheat are associated with different nuclear–cytoplasmic combinations (Atienza *et al.*, 2007c; Atienza *et al.*, 2008), including phenotypic traits such as height or quality traits like endosperm carotenoid content. In other cases, the use of either wheat or *H. chilense* cytoplasm did not result in any phenotypic variation in Tritordeum (Atienza *et al.*, 2007e). The genetic effects of the plasmon have been studied in several species affecting different traits including yield (Loessl *et al.*, 2000), disease or pest resistance (Voluevich and Buloichik, 1992; Matsui *et al.*, 2002) and tolerance to abiotic stresses (Uprety and Tomar, 1993; Shonnard and Gepts, 1994; Zhang *et al.*, 2003). Nevertheless, the most detailed studies have been performed in the *Triticum–Aegilops* complex (Tsunewaki *et al.*, 1996, 2002; Tsunewaki, 2009) and in teosinte–maize combinations (Allen, 2005).

Recently, parallel transcriptomic and metabolomic analyses have been carried out on three alloplasmic lines to investigate the effect of *H. chilense*, *Ae. uniaristata* and *Ae. squarrosa* cytoplasms on nuclear–cytoplasm interaction with common wheat (Crosatti *et al.*, 2010). The gas chromatography-mass spectrometer metabolic profiling of leaves revealed significant differences between the alloplasmic lines and their euplasmic control. Transcriptomic analyses using the Affimetrix 61k wheat gene chip showed that more than 500 genes modified their behaviour in the *H. chilense* alloplasmic line compared with the euplasmic control (Crosatti *et al.*, 2010). Most of them encoded for chloroplast/mitochondrion localized proteins. The simultaneous consideration of transcriptomic and metabolomic data underlined that the amino-acid biosynthetic pathways are highly dependent on the nuclear–cytoplasm interaction.

In conclusion, *H. chilense* is an interesting source of variability for wheat breeding and the study of the alloplasmic lines allows us to increase our understanding of how nuclear and cytoplasmic genomes interact. Thus, this may open up new opportunities for plant improvement through cytoplasm modification.

Acknowledgements

Our work in this area is supported by grants (to S. G. A.) AGL2008-03720, and P09-AGR-4817 from Spanish Ministry of Science and Innovation, Junta de Andalucía and FEDER. C. R.-S. acknowledges financial support from CSIC (JAE-Doc program).

References

Aksyonova E, Sinyavskaya M, Danilenko N, Pershina L, Nakamura C and Davydenko O (2005) Heteroplasmy and paternally oriented shift of the organellar DNA composition in barley–wheat hybrids during backcrosses with wheat parents. *Genome* 48: 761–769.

Allen JO (2005) Effect of teosinte cytoplasmic genomes on maize phenotype. *Genetics* 169: 863–880.

Alvarez JB, Martin LM and Martin A (1999) Genetic variation for carotenoid pigment content in the amphiploid *Hordeum chilense* × *Triticum turgidum* conv. *durum*. *Plant Breeding* 118: 187–189.

Atienza SG, Gimenez MJ, Martin A and Martin LM (2000) Variability in monomeric prolamins in *Hordeum chilense*. *Theoretical and Applied Genetics* 101: 970–976.

Atienza SG, Alvarez JB, Villegas AM, Gimenez MJ, Ramirez MC, Martin A and Martin LM (2002) Variation for the low-molecular-weight glutenin subunits in a collection of *Hordeum chilense*. *Euphytica* 128: 269–277.

Atienza SG, Ramirez CM, Hernandez P and Martin A (2004) Chromosomal location of genes for carotenoid pigments in *Hordeum chilense*. *Plant Breeding* 123: 303–304.

Atienza SG, Avila CM, Ramirez MC and Martin A (2005a) Application of near infrared reflectance spectroscopy to the determination of carotenoid content in tritordeum for breeding purposes. *Australian Journal of Agricultural Research* 56: 85–89.

Atienza SG, Satovic Z, Martin A and Martin LM (2005b) Genetic diversity in *Hordeum chilense* Roem. et Schult. germplasm collection as determined by endosperm storage proteins. *Genetic Resources and Crop Evolution* 52: 127–135.

Atienza SG, Avila CM and Martin A (2007a) The development of a PCR-based marker for PSY1 from *Hordeum chilense*, a candidate gene for carotenoid content accumulation in tritordeum seeds. *Australian Journal of Agricultural Research* 58: 767–773.

Atienza SG, Ballesteros J, Martin A and Hornero-Mendez D (2007b) Genetic variability of carotenoid concentration and degree of esterification among tritordeum (× *Tritordeum* Ascherson et Graebner) and durum wheat accessions. *Journal of Agricultural and Food Chemistry* 55: 4244–4251.

Atienza SG, Martin AC and Martin A (2007c) Introgression of wheat chromosome 2D or 5D into tritordeum leads to free-threshing habit. *Genome* 50: 994–1000.

Atienza SG, Martin AC, Ramirez MC, Martin A and Ballesteros J (2007d) Effects of *Hordeum chilense* cytoplasm on agronomic traits in common wheat. *Plant Breeding* 126: 5–8.

Atienza SG, Ramirez MC, Martin A and Ballesteros J (2007e) Effects of reciprocal crosses on agronomic performance of tritordeum. *Russian Journal of Genetics* 43: 865–868.

Atienza SG, Martín A, Pecchioni N, Platani C and Cattivelli L (2008) The nuclear–cytoplasmic interaction controls carotenoid content in wheat. *Euphytica* 159: 325–331.

Badaeva ED, Pershina LA and Bildanova LL (2006) Cytogenetic analysis of alloplasmic recombinant lines (*H. vulgare*)–*T. aestivum* unstable in fertility and viability. *Russian Journal of Genetics* 42: 140–149.

Bolot S, Abrouk M, Masood-Quraishi U, Stein N, Messing J, Feuillet C and Salse J (2009) The inner circle of the cereal genomes. *Current Opinion in Plant Biology* 12: 119–125.

Castillo A, Budak H, Martin AC, Dorado G, Borner A, Roder M and Hernandez P (2010) Interspecies and intergenus transferability of barley and wheat D-genome microsatellite markers. *Annals of Applied Biology* 156: 347–356.

Cherif-Mouaki S, Said M, Alvarez JB and Cabrera A (2011) Sub-arm location of prolamin and EST-SSR loci on chromosome 1H^{ch} from *Hordeum chilense*. *Euphytica*. doi 10.1007/s10681-010-0268-y.

Chung S-M and Staub JE (2003) The development and evaluation of consensus chloroplast primer pairs that possess highly variable sequence regions in a diverse array of plant taxa. *Theoretical and Applied Genetics* 107: 757–767.

Crosatti C, Quansah L, Atienza SG, Mare C, Fait A and Cattivelli L (2010) Exploitation of diversity in nuclear–cytoplasm interaction using alloplasmic wheat lines. 2nd International Symposium on Genomics of Plant Genetic Resources, Bologna, Italy, p. 86.

Hagras AAA, Kishii M, Sato K, Tanaka H and Tsujimoto H (2005a) Extended application of barley EST markers for the analysis of alien chromosomes added to wheat genetic background. *Breeding Science* 55: 335–341.

Hagras AAA, Kishii M, Tanaka H, Sato K and Tsujimoto H (2005b) Genomic differentiation of *Hordeum chilense* from *H. vulgare* as revealed by repetitive and EST sequences. *Genes and Genetic Systems* 80: 147–159.

Inostroza L, del Pozo A, Matus I, Castillo D, Hayes P, Machado S and Corey A (2009) Association mapping of plant height, yield, and yield stability in recombinant chromosome substitution lines (RCSLs) using *Hordeum vulgare* subsp. *spontaneum* as a source of donor alleles in a *Hordeum* *vulgare* subsp. *vulgare* background. *Molecular Breeding* 23: 365–376.

Kihara H (1951) Substitution of nucleus and its effects on genome manifestations. *Cytologia* 16: 177–193.

Laser B, Mohr S, Odenbach W, Oettler G and Kück U (1997) Parental and novel copies of the mitochondrial orf25 gene in the hybrid crop-plant triticale: predominant transcriptional expression of the maternal gene copy. *Current Genetics* 32: 337–347.

Loessl A, Goetz M, Braun A and Wenzel G (2000) Molecular markers for cytoplasm in potato: male sterility and contribution of different plastid–mitochondrial configurations to starch production. *Euphytica* 116: 221–230.

Martin AC (2009) Desarrollo de un nuevo sistema de androesterilidad en trigo (*Triticum aestivum* L.) utilizando la especie *Hordeum chilense* Roem. et Schultz. PhD Thesis, University of Córdoba, Spain.

Martin A and Chapman V (1977) A hybrid between *Hordeum chilense* and *Triticum aestivum*. *Cereal Research Communications* 5: 365–368.

Martin A, Martín LM, Cabrera A, Ramírez MC, Giménez MJ, Rubiales P, Hernández P and Ballesteros J (1998) The potential of *Hordeum chilense* in breeding Triticeae species. In: Jaradat AA (ed.) *Triticeae III*. Enfield, CT: Science Publications, pp. 377–386.

Martin A, Alvarez JB, Martin LM, Barro F and Ballesteros J (1999) The development of tritordeum: a novel cereal for food processing. *Journal of Cereal Science* 30: 85–95.

Martin AC, Atienza SG and Barro F (2008a) Use of ccSSR markers for the determination of the purity of alloplasmic wheat in different *Hordeum* cytoplasms. *Plant Breeding* 127: 470–475.

Martin AC, Atienza SG, Ramirez MC, Barro F and Martin A (2008b) Male fertility restoration of wheat in *Hordeum chilense* cytoplasm is associated with 6H(ch)S chromosome addition. *Australian Journal of Agricultural Research* 59: 206–213.

Martin AC, Atienza SG, Ramirez MC, Barro F and Martin A (2009) Chromosome engineering in wheat to restore male fertility in the msH1 CMS system. *Molecular Breeding* 24: 397–408.

Martin AC, Atienza SG, Ramírez M, Barro F and Martin A (2010) Molecular and cytological characterization of an extra acrocentric chromosome that restores male fertility of wheat in the msH1 CMS system. *Theoretical and Applied Genetics* 121: 237–240.

Matsui K, Yoshida M, Ban T, Komatsuda T and Kawada N (2002) Role of male-sterile cytoplasm in resistance to barley yellow mosaic virus and Fusarium head blight in barley. *Plant Breeding* 121: 237–240.

Matus I, Corey A, Filichkin T, Hayes PM, Vales MI, Kling J, Riera-Lizarazu O, Sato K, Powell W and Waugh R (2003) Development and characterization of recombinant chromosome substitution lines (RCSLs) using *Hordeum vulgare* subsp. *spontaneum* as a source of donor alleles in a *Hordeum vulgare* subsp. *vulgare* background. *Genome* 46: 1010–1023.

Nasuda S, Kikkawa Y, Ashida T, Rafiqul Islam AKM, Sato K and Endo TR (2005) Chromosomal assignment and deletion mapping of barley EST markers. *Genes and Genetic Systems* 80: 357–366.

Rodriguez-Suarez C, Gimenez MJ and Atienza SG (2010) Progress and perspectives for carotenoid accumulation in selected Triticeae species. *Crop Pasture Science* 61: 743–751.

Said M and Cabrera A (2009) A physical map of chromosome 4H^{ch} from *H. chilense* containing SSR, STS and EST-SSR molecular markers. *Euphytica* 167: 253–259.

Shonnard GC and Gepts P (1994) Genetics of heat tolerance during reproductive development of common bean. *Crop Science* 34: 1168–1175.

Soliman K, Fedak G and Allard RW (1987) Inheritance of organelle DNA in barley and *Hordeum* × *Secale* intergeneric hybrids. *Genome* 29: 867–872.

Tanksley SD and McCouch SR (1997) Seed banks and molecular maps: unlocking genetic potential from the wild. *Science* 277: 1063–1066.

Tsunewaki K (2009) Plasmon analysis in the *Triticum–Aegilops* complex. *Breeding Science* 59: 455–470.

Tsunewaki K, Wang G-Z and Matsuoka Y (1996) Plasmon analysis of *Triticum* (wheat) and *Aegilops*. 1. Production of alloplasmic common whets and their fertilities. *Genes and Genetic Systems* 71: 293–311.

Tsunewaki K, Wang G-Z and Matsuoka Y (2002) Plasmon analysis of *Triticum* (wheat) and *Aegilops*. 2. Characterization and classification of 47 plasmons based on their effects on common wheat phenotype. *Genes and Genetic Systems* 77: 409–427.

Uprety DC and Tomar VK (1993) Photosynthesis and drought resistance of *Brassica carinata* and its parent species. *Photosynthetica* 29: 321–327.

van Ginkel M and Ogbonnaya F (2007) Novel genetic diversity from synthetic wheats in breeding cultivars for changing production conditions. *Field Crops Research* 104: 86–94.

Vaz Patto MC, Aardse A, Buntjer J, Rubiales D, Martin A and Niks RE (2001) Morphology and AFLP markers suggest three *Hordeum chilense* ecotypes that differ in avoidance to rust fungi. *Canadian Journal of Botany* 79: 204–213.

Voluevich EA and Buloichik AA (1992) Nuclear-cytoplasmic interactions in wheat resistance to fungi pathogens: V. Quantitative resistance of alloplasmic line seedlings of Penjamo 672 variety to powdery mildew. *Genetika* 28: 82–88.

Warburton M, Crossa J, Franco J, Kazi M, Trethowan R, Rajaram S, Pfeiffer W, Zhang P, Dreisigacker S and Ginkel M (2006) Bringing wild relatives back into the family: recovering genetic diversity in CIMMYT improved wheat germplasm. *Euphytica* 149: 289–301.

Zhang A, Yu F and Zhang F (2003) Alien cytoplasm effects on phytosiderophore release in two spring wheats (*Triticum aestivum* L.). *Genetic Resources and Crop Evolution* 50: 767–772.