

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

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## 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  $\times$  *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; Martín *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 (Martín *et al.*, 1998), such as resistance to septoria tritici, abiotic stress tolerance or endosperm storage proteins (Martín *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 (Rodríguez-Suárez *et al.*, 2010). Similarly, the development of *H. chilense* durum

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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<sup>chs</sup> 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<sup>chs</sup> 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<sup>chl</sup> and that the restorer gene is located on the retained segment from the 1H<sup>chl</sup> (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 (Volvevich and Bulovich, 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.

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