Development and cytogenetic characterisation of non-brittle rachis tritordeum lines.

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ABSTRACT

\(\times\)Tritordeum (Ascherson et Graebner, an amphiploid between *Triticum turgidum* conv. durum and *Hordeum chilense*), and a set of chromosome substitution lines of tritordeum, where 2H\textsuperscript{ch} or 3H\textsuperscript{ch} *H. chilense* chromosomes were replaced with 2D chromosome of *T. aestivum* or 3H\textsuperscript{v} chromosome of *H. vulgare*, were used to assess the effect of the specific chromosome on brittle rachis in tritordeum. \(\times\)Tritordeum has brittle rachis. In contrast, the 2D(2H\textsuperscript{ch}) and 3H\textsuperscript{v}(3H\textsuperscript{ch}) substitution lines showed non-brittle rachis, and they also have tenacious glumes and compact spikes which are excellent characters to improve the tritordeum threshing. We have also developed different combinations between 2D and 3H\textsuperscript{v} translocation lines in tritordeum. We present in this paper the identification and characterisation of all these introgression lines in tritordeum by fluorescent *in situ* hybridisation.

Key words

*in situ* hybridisation, rachis tenacity, *tritordeum*, wheat
INTRODUCTION

Since the beginning of the last century, plant breeders have been interested in the production of amphiploids between wheat and barley (Farrer 1904). Martín and Chapman (1997) got the first fertile amphiploid between a *Hordeum* species (*Hordeum chilense* Roem. et Schult.) and the cultivated wheat (*Triticum aestivum*). The amphiploid between *H. chilense* and *Triticum turgidum* conv. *durum* has also been obtained and named ×*Tritordeum*. Tetra, hexa and octoploid amphiploids have been synthesised from crosses between *H. chilense* and diploid, tetraploid and hexaploid wheats, respectively, to increase the variability of the tritordeum. Hexaploid tritordeum (AABBH<sup>ch</sup>H<sup>ch</sup>, 2n = 6x = 42) has some favourable agronomic traits including good seed size and high grain protein content which indicate the potential of this amphiploid as a possible new crop (Martín and Cubero 1981, Martín *et al.* 1996). After several years of breeding, its potential yield has been confirmed (Martín *et al.* 1999). Tritordeum can also be used as animal feed and tritordeum flour has good characteristics to be used in bread or biscuit instead of wheat flour (Álvarez *et al.* 1992). Tritordeum is also very important as a bridge species to introgress the genetic variability of *H. chilense* into wheat and to detect agronomic characters of interest that appear from the interaction of *H. chilense* and wheat.

Early studies in plant chromosomes showed that whole chromosomes carried similar group of genes in different species (Darlington and LaCour 1942, Sears 1941). Today molecular markers show that gene orders are conserved over substantial evolutionary distances (Ahn *et al.* 1993, Devos and Gale 1993, 1997), with the number of chromosomal and genetic differences between species generally increasing with evolutionary distance (Bennetzen *et al.* 1998, Tikhonov *et al.* 1999).

Barley (*Hordeum vulgare* L.) carries potentially useful genes for tritordeum improvement and *H. vulgare* substitution lines provide excellent material for the study of alien gene expression in tritordeum background. For example, cultivated barley (*H. vulgare* ssp. *vulgare*) has non-brittle rachis. Hexaploid tritordeum has brittle rachis, which makes impossible to thresh it in a mechanical harvester. The concept of rachis fragility can be simply defined as a spike having a rachis which can be easily broken. The genetics of brittle rachis has been extensively studied. Non-brittle rachis was a key character in the evolution of cultivated barley (Harlan 1968, Bothmer and Jacobsen 1985). The brittle rachis is formed by complementary genes at two tightly linked loci, *btr1* and *btr2*, located on *H. vulgare* 3<sup>HS</sup> chromosome arm (Takahashi and Hayashi 1964, Franckowiak and Konishi 1997a, b). Position of non-brittle rachis loci *btr1* and
btr2 on the short arm of chromosome 3 has been recently mapped using molecular markers (Komatsuda and Mano 2002, Kandemir et al. 2004, Komatsuda et al. 2004). Wild barley (H. vulgare ssp. spontaneum) has brittle rachis as a consequence of possessing the dominant forms of both genes (Btr1Btr1 Btr2Btr2) (Takahashi, 1964). Cultivated barley has a mutation in one of these loci: most Occidental cultivars are recessive homozygous at the btr1 locus (btr1btr1Btr2Btr2), whereas most Oriental cultivars are recessive homozygous at the btr2 locus (Btr1Btr1btr2btr2) resultanting both resulting in a non-brittle rachis (Takahashi 1955, 1963, Takahashi et al. 1983).

In situ hybridisation (ISH) techniques applied to plant mitotic chromosomes effectively combine specificity and resolution for screening a high number of plants in an early developmental stage. Genomic in situ hybridisation (GISH) and fluorescent in situ hybridisation (FISH) are well-established and highly efficient techniques for cytogenetical identification. The physical size of introgressed segments can be assessed by in situ hybridisation, which readily distinguishes H. chilense and H. vulgare chromosomes from those of wheat (Cabrera et al. 1995, Prieto et al. 2001).

The genetic background of tritordeum has been previously expanded by the synthesis of new amphiploids using additional accessions of H. chilense collected in Chile and Argentina (Tobes et al. 1995, Giménez et al. 1997). In the present report, 3H substitution lines in hexaploid tritordeum have been developed and analysed by in situ hybridisation with the aim of introgressing the non-brittle rachis character into tritordeum.
MATERIAL AND METHODS

Plant material.
Advanced lines of the cross between disomic chromosome addition line for chromosome 3H\(^v\) of *Hordeum vulgare* in *T. aestivum* cv. *Chinese Spring* (CS) (AABBDD\(^+\) pair 3H\(^v\)) (Islam *et al.* 1975) with hexaploid tritordeum (AABBH\(^c\)H\(^b\)) have been analysed. *H. vulgare* addition line was kindly supplied by Dr. Islam (Waite Agricultural Research Institute, Glen Osmond, South Australia).

Cytogenetic analysis.
Root tips were collected from germinating seeds and were pre-treated for 3 hours in a 0.05% colchicine solution at 25ºC and fixed in 100% ethanol-acetic acid, 3:1 (v/v) for at least a week at room temperature. Preparations were as described by Prieto *et al.* (2001). GAA-satellite sequence (Pedersen *et al.* 1996) and pAs1 probe (Rayburn and Gill, 1986) were used to identify substituted chromosomes and chromosomes involved in translocations. The barley clone pHvG38 containing the GAA-satellite sequence was kindly provided by Dr. Rasmussen from the Risø National Laboratory, Roskilde (Denmark) and the pAs1 probe isolated from *T. tauschii* was kindly provided by the Wheat Genetics Resource Centre, University of Kansas, USA. The GAA-satellite sequence and the pAs1 probes were labelled by nick translation with biotin-11-dUTP (Roche Corporate, Basel, Switzerland) and digoxigenin-11-dUTP (Roche Corporate, Basel, Switzerland), respectively, and mixed to a final concentration of 5 ng \(\mu\)l\(^{-1}\) in the hybridisation solution.
After examination of nuclei hybridised with the repetitive probes, preparations were reprobed using total genomic DNA of *H. chilense* and *H. vulgare* as probes. Total genomic *H. chilense* and *H. vulgare* DNA were also labelled by nick translation with digoxigenin-11-dUTP and biotin-11-dUTP, respectively. Both probes were mixed to a final concentration of 5 ng \(\mu\)l\(^{-1}\) in the hybridisation mixture. The *in situ* protocol was performed according to Cabrera *et al.* (2001).
Digoxigenin-labelled and biotin-labelled probes were detected with antidigoxigenin-FITC (Roche Corporate) and streptavidin-Cy3 conjugate (Sigma, St. Louis, MO, USA), respectively. Chromosomes were counterstained with DAPI (4’,6-diamidino-2-phenylindole) and mounted in Vectashield. Signals were visualized using a Leica epifluorescence microscope. Images were captured with a SPOT CCD camera using the appropriate SPOT 2.1 software (Diagnostics Instruments, Inc., Sterling Heights, Michigan, USA) and processed with PhotoShop 4.0 software (Adobe Systems Inc., San
Jose, California, USA). Images were printed on a Hewlett Packard Deskjet HP 840C Colour Printer.
RESULTS AND DISCUSSION

Crosses between disomic chromosome addition line for chromosome 3H\(^v\) of barley (\(H. vulgare\)) in \(Triticum aestivum\) conv. Chinese Spring (CS) (AABBDD + pair 3H\(^v\), Islam, 1975) and hexaploid tritordeum (2n = 6x = 42, AABBH\(^{ch}\)H\(^{ch}\)) were developed with the aim to introgress the non-brittle rachis character (located on chromosome 3H\(^v\)) into tritordeum. Initial backcrosses with the hexaploid tritordeum were developed to reduce the number of D chromosomes and to increase the number of H. chilense chromosomes in the tritordeum background. After several backcrossing and selfing generations we have obtained more than one hundred plants and analysed the 70% of the plants obtained. More than the 50% of the plants obtained were fertile (Table 1).

Fluorescent \textit{in situ} hybridisation using genomic \(H. vulgare\) and \(H. chilense\) DNA as probes was used to identify and select \(H. vulgare\) introgressions along these several plant generations and to analyse the tritordeum lines obtained. The use of total genomic DNA as species-specific probes resulted in an efficient procedure to distinguish between different genomes in the material analysed. Previous workers have successfully applied fluorescence in situ hybridization, including the multi-colour type, for wheat and its relatives (Schwarzacher \textit{et al.} 1992, Prieto \textit{et al.} 2001). In these experiments, total genomic DNA from \(H. chilense\) and \(H. vulgare\) were used as probe for DNA:DNA \textit{in situ} hybridization. In all our samples analysed the quality of \textit{in situ} hybridization was highly satisfactory and clearly differentiated between wheat, \(H. vulgare\) and \(H. chilense\) genomes without the need of blocking DNA.

Table 1 shows the number of plants with 3H\(^v\) chromosome substitution and the number of plants with intergenomic translocations. GISH (genomic \textit{in situ} hybridisation) results show different chromosome combinations between \(H. vulgare\) and \(H. chilense\) chromosomes (Table 1, Figure 1) and it has been especially useful in the breeding program of the tritordeum because we had to analyse a very high number of tritordeum lines. GISH technique allowed detecting \(H. vulgare\) introgressions not only in somatic metaphase but also in somatic interphase (Figure 1).

We have detected more than 25% monosomic or disomic substitution lines for the chromosome 3H\(^v\) substituted. We have also found a high number (63%) of intergenomic translocations by FISH analysis using total genomic DNA from both \(H. chilense\) and \(H. vulgare\) (Table 1). Translocations involving wheat - 3H\(^v\), wheat - \(H. chilense\) and \(H. chilense\) - 3H\(^v\) have been detected (Table 1). Translocations between \(H. chilense\) and wheat are the most frequent translocations. FISH analysis using pAs1 or GAA-satellite probes prior to GISH in the same metaphases makes it possible to determine the exact
chromosomal composition of every tritordeum line and which arm of the 3HV, H. chilense or wheat chromosomes are involved in translocations (Figure 1). A 3HV chromosome introgression was identified in tritordeum lines with non-brittle rachis. Plants with non-brittle rachis had a 3HV monosomic or disomic introgression, 3HV telocentric chromosome or as a 3HV translocation chromosome (Table 2).

We have also obtained several substitution lines in tritordeum with different combinations involving chromosome 2D of T. aestivum and chromosome 3HV of H. vulgare (Tables 1 and 2).

The 3HV(3Hch) and 2D(2Hch) double substitution line has been developed. This double chromosome substitution is isomic for both chromosomes substituted and it has been fixed along several self-pollinated generation in more than 15 tritordeum lines. All these lines presented tough rachis, tenacious glumes and compact spike, characters controlled by genes located on 3HS, 2DS and 2DL chromosome arms, respectively (Takahashi and Hayashi 1964, Franckowiak and Konishi 1997a, b, Jantasuriyarat et al., 2004). These characters are extremely important to get the tritordeum as a new cultivated cereal. The progeny of the 2D(2Hch) and 3HV(3Hch) lines is also homogenous and fertile. We don’t expect meiotic recombination because no meiotic pairing has been detected between H. chilense, wheat and H. vulgare chromosomes (Martín and Sánchez-Monge, 1980; Martín et al. 1995). Cytogenetic analysis of 2D-3HV double substitution line is shown in Figure 1.

The 2D(2Hch) chromosome substitution occurs spontaneously in the progeny of the crosses between the 3HV addition line in T. aestivum with hexaploid tritordeum. We have fixed the 2D(2Hch) chromosome substitution in more than 20 tritordeum lines. All these plants were fertile and presented tough rachis. They also present tenacious glumes and compact spike, character located on chromosome 2DS and 2DL by Tg and C genes, respectively. It has been recently described that coincident QTL on the short arm of 2D, probably representing the effect of Tg, explained 44% of the variation in threshability, 17% of the variation in glume tenacity, and 42% of the variation in rachis fragility (Jantasuriyarat et al., 2004).

Translocations occur spontaneously in the descendent of the crosses between H. vulgare addition lines in T. aestivum with the hexaploid tritordeum (Prieto et al. 2001). 3HV-Hch translocation lines have been also developed with the chromosome 2D introgression and without it. These lines also show non-brittle rachis. Most of the translocation lines obtained in the present work were fertile and could be also useful for introgression into wheat from both H. vulgare and H. chilense genomes.
Barley genes could be introgressed into tritordeum by genetic transformation. This technique was successfully developed in tritordeum (Barceló, 1994) and transgenic plants expressing genes for wheat storage proteins have been developed (Barro, 2002). Unfortunately we don’t have the cloned barley genes related with the rachis brittleness ($btr1$ and $btr2$). We haven’t found tough or non-brittle rachis tritordeum line or it hasn’t been obtained by mutagenesis in $H.\ chilense$ or tritordeum either. In contrast, the first genetic map of $H.\ chilense$ has been developed and the overall structure of the $H.\ chilense$ linkage groups is similar to that of the B and D genomes of wheat and the $H^v$ genome of barley (Hernandez et al. 2001). Based on this, $H.\ chilense$ must have at least one homeologous of the $btr\ H.\ vulgare$ genes on chromosome 3$H^{ch}$. The material developed in the present work offers the opportunity to obtain non-brittle rachis tritordeum lines by the introgression of the 3$H^v$ $H.\ vulgare$ chromosome in tritordeum background.
ACKNOWLEDGEMENTS

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REFERENCES


Table 1. Progeny of the cross between disomic chromosome addition line for chromosome 3H⁺ of barley (H. vulgare) in Triticum aestivum conv. Chinese Spring (CS) with hexaploid tritordeum.

<table>
<thead>
<tr>
<th>Tritorium line</th>
<th>Chromosomal composition</th>
<th>Rachis</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>HT623 (n=42)</td>
<td>28 wheat +14 H⁺</td>
<td>brittle</td>
<td>no</td>
</tr>
<tr>
<td>2D(2H⁺) and 3H⁺(3H⁺) substitution line (n=42)</td>
<td>30 wheat +10 H⁺ +2 H⁻</td>
<td>non-brittle</td>
<td>yes</td>
</tr>
<tr>
<td>2D(2H⁺) and 3H⁺L substitution line (n=40 + 2 telos)</td>
<td>30 wheat +10 H⁺ +2 telos 3H⁺L</td>
<td>non-brittle</td>
<td></td>
</tr>
<tr>
<td>3H⁺S-H⁺ and 3H⁺L-H⁺ translocation line (n=42)</td>
<td>2 wheat +10 H⁺ +2 translocations D-H⁺ +1 translocation 3H⁺S-H⁺ +1 translocation 3H⁺L-H⁺</td>
<td>non-brittle</td>
<td>no</td>
</tr>
<tr>
<td>2DL-2H⁺S and 3H⁺-3H⁺ translocation line (n=42)</td>
<td>28 wheat +10 H⁺ +2 translocations 2DL-2H⁺S + 2 translocations 3H⁺S-3H⁺L</td>
<td>non-brittle</td>
<td>yes</td>
</tr>
</tbody>
</table>

Table 2. Effect of the 3H⁺ and 2D chromosome on the Rachis Brittleness.
**Figura 1.** GISH using both *H. chilense* (detected with fluorescein, green) and *H. vulgare* (detected with rhodamine, red) genomic DNA probes in interphase cells of tritordeum lines with a) two *H. vulgare* chromosomes, b) no *H. vulgare* introgression, c) one *H. vulgare* chromosome and d) two *H. vulgare* telocentric chromosomes. Cytological analysis using *H. chilense* (detected with fluorescein, green) and *H. vulgare* (detected with rhodamine, red) genomic DNA in metaphase cells of tritordeum e) HT623, g) 2D(2Hch) and 3Hv(3Hch) substitution line, i) 3HvL telocentric substitution line, k) 2DL-2HchS and 3Hv-3Hch translocation line; (f), (h), (j) and (l) double FISH signals with the pAs1 (green) and GAA-satellite (red) sequences hybridized to the same metaphase as in (e), (g), (i) and (k), respectively. Bar = 10 µm.