Study of the Homologous Recombination Genetic System to Improve Genetic Transformation of Wheat

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Abstract: The major aim of plant biotechnology is to develop new materials and to improve the existing cultivars. Genetic transformation is being used for this purpose, but most genes are integrated in a random manner into the genome, producing undesirable effects. For this reason, it is important to develop a technique to insert the transgene in a convenient manner at a specific position of the genome. This process is known as "gene targeting" and is produced by homologous recombination (HR). However, in spite of their importance, little is known about the genetic system of HR in crops of economic interest like wheat. This paper reports the isolation and molecular characterization of the gene *Mre11*. The analysis has been developed from genomic and cDNA sequences of the orthologous genes existing in each diploid genome of wheat. Phylogenetic analysis showed a close relationship between the gene *Mre11* of wheat and rice. Expression analysis carried out using quantitative PCR demonstrated different expression between diploids and polyploids, suggesting the existence of mechanisms of gene silencing.

Keywords: homologous recombination; gene targeting; *Mre11* gene.

The major goal of plant biotechnology is to develop new and improve existing elite cultivars. To reach this goal, it will be necessary to both improve existing and develop novel strategies for genomic manipulation in plants. The *in situ* modification of a resident gene or the insertion of a transgen in a controlled manner at a specific genomic position via homologous recombination is generally known as "gene targeting" (GT). Homologous recombination (HR) is the main mode of DNA integration in bacteria and lower eukaryotes. In higher eukaryotes, including plants, DNA integrates in the genome mainly by illegitimate recombination in a sequence-independent way. In plants, the targeting frequencies are very low, of one target in about 1000 to 100 000 transformation events (Hohn & Puchta 1999).

An important aspect in the study of HR is the analysis of genetic systems that take part in the process. Different groups have recently isolated genes involved in DNA integration in humans and yeast. The gene *Mre11* is a key actor in different recombination reactions, namely in meiotic and illegitimate recombination. It acts in a complex named MRN with two other proteins, Rad50 and NBS (D'Amours & Jackson 2002). The fact that *Arabidopsis* contains a single homologue of *Mre11* strongly suggests that the major recombination pathways are conserved between eukaryotes. This kind of study has been carried out in model species but little is known about HR in crops of economic interest like wheat. This work presents the molecular characterization and the expression analysis of the gene *Mre11* involved in the HR process.

MATERIALS AND METHODS

The plant material used is representative of three genomes of wheat: *Triticum monococcum* (genome A), *Aegilops tauschii* (genome D), *Triticum turgidum*

Table 1. Size of DNA and cDNA of the gene *Mre11* of the three genomes of wheat, number of introns and exons and size of putative protein

Species	Genome	Genomic DNA (bp)	cDNA + UTR (bp)	Introns/Exons	Putative protein (amino acids)
T. monococcum	A	4678	2457	22–21	699
T. turgidum cv. Vitron	A	4662	2510	22–21	699
T. turgidum cv. Vitron	B	4719	2440	22–21	699
Ae. tauschii	D	4766	2456	22–21	699

cultivar Vitron (genomes A and B) and *Triticum aestivum* cultivar Chinese spring (genomes A, B, and D).

The amplification of the gene *Mre11* was performed using primers designed based on published sequences of rice and *Arabidopsis thaliana* and also from EST database of wheat, barley, and rye. DNA purification and Southern blot were performed following standard protocols. RNA was purified using Tripure reagent (Roche). Turbo DNA-free Kit (Ambion) was used to remove contaminating DNA. cDNA was synthesized from RNA using Transcriptor reverse transcriptase (Roche). RACE experiments were conducted using the RACE kit from Roche. Quantitative PCR was performed on the ABI Prism 7000 SDS (Applied Biosystems) using the parameters recommended by the manufacturer. Each PCR reaction was performed in triplicate.18S rRNA was used as housekeeping.

Nucleotide sequences were aligned using the Clustal W 1.5; PHYLIP was used to construct phylogenetic trees.

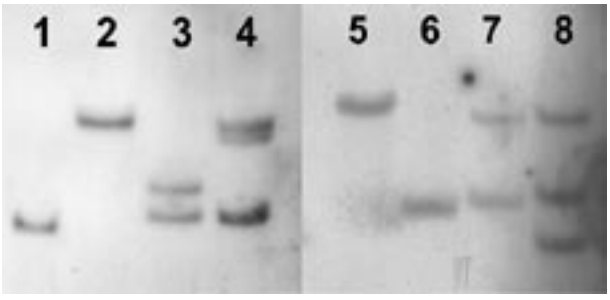

Figure 1. Southern blot analysis of number of copies of gene *Mre11* in the species studied. DNA from *T. monococcum* (genome A), *Ae. tauschii* (genome D), *T. turgidum* cultivar Vitron (genomes A and B) and *T. aestivum* cultivar Chinese spring (genomes A, B y D) was cut with *Hind*III and *Eco*RI and hybridised with a probe *Mre11*. Lanes 1–4 DNA cut with *Eco*RI (in the same order as above) and lanes 5-8 DNA cut with *Hind*III

RESULTS AND DISCUSSION

Amplification of fragments performed from genomic DNA covered the entire coding region of the gene for each of the three genomes of wheat: genome A from *Triticum monococcum* and *T. turgidum*, genome B from *T. turgidum* and genome D from *Aegilops tauschii*. General characteristics of these sequences are shown in Table 1. Although some differences were found between genomes (mainly due to insertions/deletions in the introns), these genes code for proteins of the same size. The southern blot analysis performed revealed that this gene presented a unique copy for each genome of wheat (Figure 1) and showed the same domains as those described earlier for human gene *Mre11* (Petrini *et al*. 1995) (Figure 3).

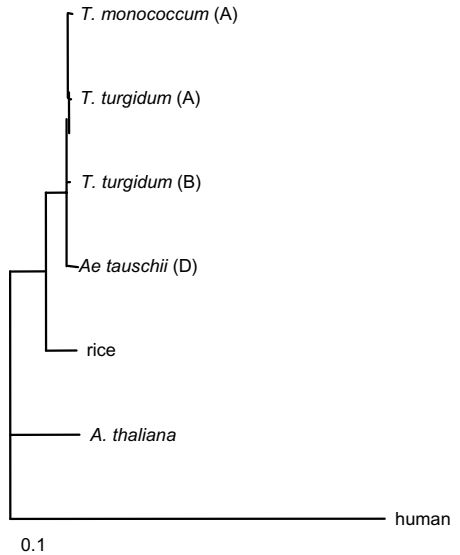

Figure 2. Dendrogram obtained from the alignment of the sequences characterised in four species of wheat and those previously published from rice, *A. thaliana* and human

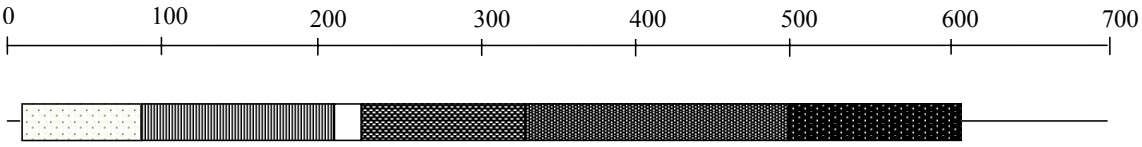

Figure 3. The domains of *Mre11*: these domains catalyse different enzymatic activities like endonuclease, 3′- and 5′-exonuclease, phosphoesterase and manganese hydrolase

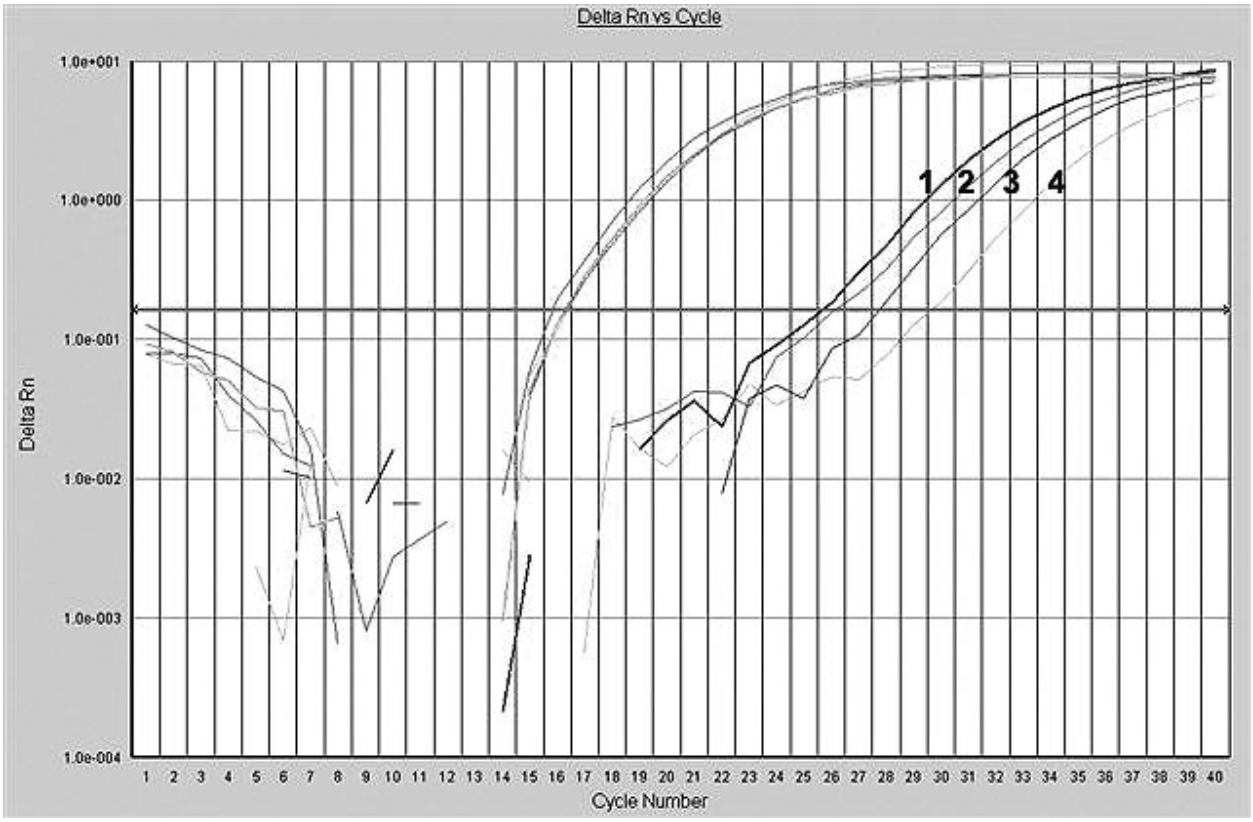

Figure 4. Q-PCR analysis of expression of the gene *Mre11* performed. The expression of 18S rRNA gene used as housekeeping was similar for all the samples (first group of curves around 14-15 cycles) whereas expression of *Mre11* was different. The results showed a higher expression of hexaploid wheat (1) followed by the diploid *T. monococcum* (2). Less expression was found in the tetraploid (3) and the other diploid specie *Ae. tauschii* (4)

The analysis of phylogenetic relationships performed between the sequences characterised and others previously published pointed out a high degree of conservation of this gene among the genomes of wheat and a close relation with the gene *Mre11* of rice (Figure 2).

It has been reported that genes related to DNA repair are usually silenced in polyploid species or even lost during evolution (Blanc & Wolfe 2004). Analyses of expression were carried out to determine if different expression of gene *Mre11* occurs in wheat genomes. Results of quantitative PCR showed a clear difference in the expression of this gene (Figure 4) indicating regulation of expression in wheat species.

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