SUMMARY

ANTECEDENTS

A major focus of plant biotechnology over the last years is the development of improved tools for the genetic modification of crop plants. The ability to modify a resident gene *in situ* or to integrate a transgene at a specific genomic position in a controlled way is a central issue in this context. This process is known as “gene targeting”.

There are two possible ways for the integration of DNA molecules into genomes: non-homologous end-joining (NHEJ) or homologous recombination (HR). NHEJ joins sequences at the broken ends, which have little or no homology, in a non-conservative manner, and some genetic information is lost. HR, on the other hand, requires extensive tracts of sequence homology and is basically error-free. HR is the primary double-strand breaks (DSB) repair pathway in yeast and prokaryotes; but the targeted integration of a transgene into the endogenous homologous sequence in higher plants has been regarded to be in the order of 10^{-4} to 10^{-6} compared with random integration.

A variety of approaches have been tried in higher plants to overcome the barrier imposed by illegitimate recombination. One of the most effective means for enhancing the frequency of homologous recombination is to create a chromosome break at the target site. The break stimulates the cells DNA repair system, and in the presence of a homologous template repair proceeds through homologous recombination.

The processes of DNA repair and recombination have been studied for many years, and have revealed the molecular mechanisms underlying these processes showing on the one hand clear conservation of these processes in evolution, and on the other hand clear differences in their regulation among different organisms. The MRN (*Mre11*, *Rad50* and *Nbs1*) complex is a highly conserved protein complex and is a key player in the cellular response to DSBs and is involved in virtually all aspects of DNA end metabolism, including DSB detection, DSB processing, homologous recombination and meiosis, NHEJ, telomere maintenance and the DSB-activated cell cycle checkpoint response. Homologous of *Mre11* and *Rad50* are found in all kingdoms of life and are essential for genome integrity. Eukaryotic MRN contains a third component, *Nbs1* (or yeast *Xrs2*), that links the MRN complex to damage-induced cell cycle checkpoints.

METHODOLOGY

In the present work it has been addressed the study of different aspects of the homologous recombination processes in two high agronomic interest cereals, wheat and barley: on the one hand the mechanisms of homologous recombination in these cereals by means of DSBs induction; and on the other hand the complex MRN as a genetic system of great importance in all the processes of recombination.

In regard to the study of the recombination mechanisms, it has been tried to move to cereals a system already employed in model plants as *Arabidopsis* and tobacco. This system induces the formation of DSBs on target sequences on the plants genome and, at the same time, provides target homologous sequences in order to be used as templates. The efficiency of homologous recombination pathway in somatic plant cells is determined by monitoring the restoration of the GUS marker gene. The starting point for these assays is obtaining transgenic plants that contain the reporter constructions. It has been obtained so far transgenic barley and wheat plants via *Agrobacterium*-mediated transformation of immature embryos.

Regarding the study of MRN complex, it has been achieved a molecular characterization of *Mre11*, *Rad50* and *Nbs1* genes in all three genomes of hexaploid wheat, A, B and D, making use of the diploid species *Triticum monococcum* (genome A) and *Aegilops tauschii* (genome D), the tetraploid *T. turgidum* (genomes A and B), and the hexaploid *T. aestivum* (genomes A, B and D). Genomic sequences and the cDNA corresponding to the processed mRNA was isolated and sequenced. Southern blotting was performed to determine the genes copy numbers, and the localization of *Rad50* loci in the wheat chromosomes was carried out using FISH techniques. The expression rate was determined by real-time PCR in all the species analysed, and SSCP technique was modified by introducing fluorescent labeling to the procedure in order to analyse the expression of the different homeologous genes of polyploid species. Finally, two-hybrid assays were performed for detecting interactions between the different proteins to constitute the MRN complex.

CONCLUSIONS

1. We have designed an efficient technology for *Agrobacterium*-mediated transformation of immature embryos of barley. With this technique we have

obtained transgenic plants carrying the constructions pAR-ISceI, pIU.GUS and pDGU.US.

2. The molecular characterization of the *Mre11*, *Rad50* and *Nbs1* genes in wheat has demonstrated that there is only one copy of these genes per genome in wheat species analysed. The genes characterized showed strong conservation of sequences among homeologous genes, which indicates the important functional role of the MRN complex in the maintenance of the genome integrity.

3. The locus *Rad50* was located in the short arm of the chromosomes of the homology group 5 of wheat by means of FISH. This location has been confirmed by PCR analysis using two aneuploid lines.

4. The study of the expression of these genes by quantitative PCR has showed similar levels of global expression of the *Mre11*, *Rad50* and *Nbs1* genes in the diploid and tetraploid species. The hexaploid species *Triticum aestivum* presents a higher level of expression, what could be related to the major need of control of the recombination processes.

5. The expression of the *Nbs1* genes in meiosis in wheat is much higher than *Mre11* and *Rad50*, being similar in these last two cases. This increase in the expression can be related to the function of NBS1, taking part in an important cell-cycle checkpoint response pathway, as well as in the location of MRE11 and RAD50 in the break points.

6. The SSCP analysis has revealed that the relative expression of the homeologous *Mre11B* and *Nbs1B* is reduced in the polyploids species of wheat, which supposes a regulation of these genes expression after the hybridization processes that originated the tetraploid species *T. turgidum*.

7. The formation of the MRN complex implies the interaction between MRE11 to form homodimers, as well as the interaction of this one with RAD50 to give place to the M_2R_2 complex. By means of two-hybrid analysis it has seen that these interactions are independent from the coding genome. No interaction has been detected for the formation of homodimers RAD50-RAD50 neither NBS1-NBS1, as well as interactions MRE11-NBS1 not NBS1-RAD50.