RESUMEN DE LA MEMORIA - SUMMARY

Antecedents

Transformation of cereal crops is a powerful research tool that facilitates the discovery of genes and their functions, and is rapidly becoming a key element in varietal improvement (Jones et al., 2005).

The first fertile transgenic plants was produced more than a decade ago using particle bombardment (Vasil et al., 1992, 1993; Weeks et al., 1993; Becker et al., 1994; Nehra et al., 1994; Zimny et al., 1995). However, genetic transformation of recalcitrant species such as wheat (*Triticum aestivum* L.) and triticale (*xTriticosecale* Wittmack) was not achieved until more recently.

Triticale is a 'man-made' cereal produced by hybridising wheat (*Triticum aestivum* or *T. turgidum*) and rye (*Secale cereale*) prior to chromosome doubling. It has a high protein content, is tolerant to adverse environmental conditions (dry and cold environment and acid soils), and is resistant to a range of diseases and pests; it is therefore, a good alternative to wheat. However, the cultivated surface of the world presently under triticale is 3.04 million hectares (FAO,2005) which is very little when compared to wheat which is grown on more than 200 million hectares (over 17 % of the world´s cultivable land).

A key target for the application of transgene technology is the grain quality enhancement. This can be achieved by increasing the content of high molecular weight (HMW) glutenins that are associated with superior bread making. The development of a transformation protocol for wheat and triticale by particle bombardment or *Agrobacterium* mediated systems requires identification and optimisation of factors that affect DNA delivery into tissue from which whole plants can be regenerated.

The most commonly used explant for wheat and triticale transformation is the immature scutellum, a specialised tissue that forms part of the seed embryo. It is amenable to both biolistics and *Agrobacterium*-mediated DNA delivery methods and can be readily induced to form embryogenic callus (Jones, 2005). *Agrobacterium*-based transformation is perceived to exhibit several advantages over other forms of direct transformation including the ability to transfer large segments of DNA with

minimal rearrangement, the precise insertion of fewer copies of transgenes with lower cost than other methods (Amoah *et al*., 2001).

Our research group has successfully developed *in vitro* culture technics in anthers to obtain double haploid lines by androgenesis and a protocol for development of plantles from *in vitro* culture of immature embryos. The aim of this work was to investigate the physical and biological factors affecting DNA transfer by biolistic and *Agrobacterium*-mediated transformation of wheat and triticale in order to introduce genes that result in nutritional improvement of grain.

Methodology

We used two different explants for triticale transformation: haploid embryo-like structures (ELS) obtained by *in vitro* androgenesis in two lines of triticale 'ATOPE-22' and 'ATOPE-6' proceeding from the intervarietal hybrid 'Torote'×'Presto' (González y col., 1997, 2000) and immature zygotic embryos of 'ATOPE-22' line. Wheat transformation was done with immature zygotic embryos of the varieties 'Anza' and 'Craklin'.

The cassette containing the promoter CaMV 35S+ *uidA*+terminator 35S was extracted from the plasmid pJIT61 (John Innes Centre, Norwich) and cloned in pBluescript IISK. The resultant 5482 bp plasmid was named pAHGUS and was used for transitory expression assays. In addition, we constructed the plasmids pAHDx5-BAR, pAHDy10 and pAHBx7 which carried some HMW-glutenin genes: *Glu-D1x5*, *Glu-D1y10* and *Glu-Bx7* and the selection marker *bar* gene (bialaphos resitance gene) for introduction into wheat and triticale using the biolistic approach.

Particle bombardment experiments were applied following the method of Rubio *et al* (2004), using an equipment Biolistic® PDS-1000/He (BioRad). *Agrobacterium*-mediated transformations were carried out using *A. tumefaciens* strain AGL1 (Lazo et al., 1991) harbouring the binary vectors pAL154/pAL156 (Hellens et al., 2000) following the method reported by Wu et al., (2003). The embryos were kept on induction medium containing timentin for 2 days and the selection was carried out in regeneration medium with 4mg/l of phosphinotricine.

In transitory expression assays all of embryos were incubated at 37ºC overnight in GUS substrate mixture as described by Jefferson (1987). Statistical analyses were performed using the Statgraphics Plus (version 7.1) software package.

Conclusions

-The vector pAHGUS got better results than the original pJIT61 after transitory expression experiments of gene *uid*A.

-The variation of the preculture time in ELS or immature zygotic embryos of triticale to transform by the biolistic method, does not affect significantly the transitory expression. The analysis of other parameters intervening in particle bombardment demonstrated that the helium pressure has more influence in the number of foci than the shooting distance, the best combination being 1100 psi and 6 cm.

-The distribution of foci was different using both methods of transformation. In *Agrobacterium*-mediated transformation the foci appear as confluent spots instead of points when the time in contact between embryos and bacteria was more than two or three hours in the inculation and more than three days about co-culture.

-The application of *Agrobacterium*-mediated transformation in ELS did not gave good results, probably due to the influence of hormones like 2,4D in the medium where the embryos were developed, what could alter the conditions of the bacterial infection. Regarding immature zygotic embryos of triticale and wheat, the best condition was a time of preculture of 0.5 hours. No significative differences were observed about of the different times of inoculation.

-The best results were got with immature zygotic embryos whose size was 0.8-1.5 mm in triticale and wheat so much biolistic as *Agrobacterium*-mediated transformation.

-The results about *Agrobacterium*-mediated transformation prove the possibility of apply this biotechnology not only in triticale but also to transform the commercial varieties of wheat tested in the present work. This result completes the previous studies using biolistic and model genotypes.

- We obtained some transgenic T0 plants of the wheat variety ‘Craklin’ using *Agrobacterium*-mediated transformation, incorporating the genes *uid*A and *bar* and

T0 plants of the variety ‘Anza’ using biolistic, carrying *Glu-D1x5, Glu-D1y10* and *bar* gene. T1 plants were also obtained in ‘Craklin’. We discuss that one of the big problems in getting results for both methods is the high number of scapes. It is estimated that the modification of wheat, by biolistic and *Agrobacterium*, needs 12-16 weeks since original explants until the transfer of the young plants to soil. However, the transformation trough *Agrobacterium* looks like recommendable about its low cost comparing with particle bombardment.

- The success of the transformation is very dependent of genotype and *in vitro* culture. Despite the varieties of wheat ‘Craklin’ and ‘Anza’ can not be considered good ones to *in vitro* culture, in a global regeneration-transformation process, they offer possibilities of transformation inside the range of the model varieties used previously in other experiments.