Genetic Transformation of Wheat: Advances in the Transformation Method and Applications for Obtaining Lines with Improved Bread-Making Quality and Low Toxicity in Relation to Celiac Disease

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1. Introduction

Wheat is one of the most important crops and is counted among the "big three" cereal crops (rice, wheat and maize), with an annual world production of around 680 million tonnes in 2009. Wheat is also one of the main sources of calories and proteins in the human diet. However, in spite of its global importance, wheat has been one of the last crops being transformed and it was not until 1992 when Vasil et al. (1992) obtained the first fertile transgenic plant of wheat. Nowadays, wheat transformation still presents more difficulties than transformation of other cereals, such as rice and maize, with lower transformation efficiencies and greater genotype dependence (Shewry & Jones, 2005). Particle bombardment is the most widely used method for genetic transformation of wheat, presenting higher transformation efficiencies than *Agrobacterium*-mediated transformation (Lazzeri & Jones, 2009). However, particle bombardment causes physical damage to the scutellar tissues used for transformation, negatively affecting the embryogenesis, *in vitro* regeneration of the explants and therefore the transformation efficiency. Osmotic treatment is thought to offer protection to bombarded material by minimising cytoplasm leakage from target cells (Vain et al., 1993), so it is of great importance to optimise the duration and moment of application of the osmotic treatment to the explants.

Among the applications of genetic transformation, gene over-expression and post-transcriptional gene silencing (PTGS) are two strategies successfully used to enhance the wheat quality. In particular, the baking quality of wheat, largely determined by the high molecular weight glutenin subunits (HMW-GS), is one of the most important targets for genetic transformation. Transgenic wheat lines expressing additional copies of the 1Ax1, 1Dx5, 1Dy10 HMW-GS genes were obtained by particle bombardment by León et al. (2009) (Fig. 1 A). In addition, new lines combining the three transgenic events were obtained by conventional crossing (León et al., 2010) (Fig. 1 B). Therefore, a set of

transgenic wheat lines expressing one or two extra HMW-GS was generated. These lines were analysed and changes in the protein and starch composition were studied. In addition, the rheological and pasting properties of dough were substantially improved or altered by expressing those HMW-GS genes as described in León et al. (2009, 2010a, 2010b).

The transgenic line T619, which presents down-regulation of all the HMW-GS, was obtained when aimed to over-express a D hordein from *Hordeum chilense* in *Triticum aestivum* cv Perico. D hordeins from *H. chilense* have a very similar structure and amino acid sequence to the HMW-GS from wheat as reported by Pistón et al. (2007). The result was the specific down-regulation of all the HMW-GS (Fig. 1 C), probably due to a transgene-induced silencing at the transcriptional or post-transcriptional level. These silencing phenomena presumably involve homology-dependent gene silencing (Meyer & Saedler, 1996) and resemble co-suppression in which mutual inactivation of transgenes and homologous genes occurs. Similar silencing effects in the HMW-GS were previously reported by Alvarez et al. (2000) when expressing the 1Ax1 subunit transgene and over-expressing the 1Dx5 gene in wheat.

PTGS by RNA interference (RNAi) is based on sequence-dependent RNA degradation that is triggered by the formation of double-stranded RNA (dsRNA) homologous in sequence to the targeted gene (Baulcombe, 2004). In contrast to other gene silencing methods such as insertional mutagenesis or TILLING (Targeting Induced Local Lesions in Genomes) approaches, RNAi allows silencing of one gene or all members from a multigene family by targeting sequences that are specific or shared by several genes (Miki et al., 2005).

Wheat gliadins account for about 50% of total gluten proteins. Gliadins are divided into three or four groups named α/β-, γ- and ω-gliadins, based on their mobility in an acid polyacrylamide gel electrophoresis (A-PAGE) system. Gliadins are also considered the main factor triggering celiac disease (CD), a common enteropathy induced by ingestion of wheat gluten proteins and related prolamins from oat, rye, and barley in genetically susceptible individuals. When CD patients consume foods containing gluten, their immune systems react by damaging the small intestine, with severe consequences. The only available treatment for the disease is a lifelong gluten-exclusion diet. Therefore, another priority aspect regarding the improvement of wheat quality is the reduction of gluten toxicity for CD patients. Gil-Humanes et al. (2010) reported a high efficiency RNAi hairpin vector that was used in combination with genetic transformation to down-regulate the expression of genes from the three gliadin fractions at the same time in bread wheat. The RNAi approach was very effective in the shutdown of CD-related wheat gliadin T-cell epitopes. Although the suppression of gliadins had a high impact on protein fractions such as gliadins, glutenins, albumins and globulins, it did not affect significantly to the total protein content (Gil-Humanes et al., 2011).

In this work we have optimised the osmotic treatment for wheat transformation using particle bombardment. Immature scutella were exposed to 0.4 M mannitol treatment during 4h or 16h pre- or post-bombardment, and the results obtained have positively contributed to the optimization of the transformation method in wheat, increasing significantly the transformation efficiency. These osmotic treatments are now routinely used in the generation of transgenic plants at high efficiency. We also report the effects of HMW-GS over-expression and silencing, as well as the silencing of all the groups of gliadins, on the content and proportions of protein, starch and carbohydrates in transgenic wheat.

2. Materials and methods

2.1 Plant material and genetic transformation

Three cultivars of bread wheat (*T. aestivum*) were used in this study for genetic transformation: cv Bobwhite, supplied by the CIMMYT[1], was used for the osmotic treatment study and gliadin down-regulation; cv Anza was used for HMW over-expression; and cv Perico was used for HMW-GS down-regulation (Fig. 1).

Transgenic lines of *T. aestivum* cv Anza expressing one or two extra HMW-GS genes were described by León et al. (2009, 2010a, 2010b) and are: line T580, line T581 and line T590, which express the subunits 1Ax1, 1Dx5, 1Dy10, respectively (Fig. 1 A); and the lines obtained by conventional crossing of the previous lines: line T606, line T616 and line T617, which express the pairs of subunits 1Ax1+1Dx5, 1Ax1+1Dy10 and 1Dx5+1Dy10, respectively (León et al., 2010b)(Fig. 1 B). Transgenic line T619, with down-regulation of all the HMW-GS, was obtained by transformation of *T. aestivum* cv Perico with a D hordein gene from *H. chilense* (Fig. 1 C). The down-regulation of all the groups of gliadins in *T. aestivum* cv Bobwhite was reported by Gil-Humanes et al. (2010) and for the present work we have used the following lines: D793, D894, E42 and E82. All the transgenic plants were self-pollinated for two to three generations to obtain homozygous lines.

The transformation method used to produce all the lines described in this work was the following: donor plants for genetic transformation were grown in the greenhouse under controlled conditions with supplementary lights providing a day/night regime of 16/8h and 23-25/18-19°C. Sixteen days after anthesis immature caryopses were isolated and sterilised by rinsing in 70% (v/v) aqueous ethanol for 5 min and soaking for 15-20 min in a 1% (v/v) sodium hypochlorite solution. Then, caryopses were washed three times with sterile distilled water. Embryos of approximately 0.5-1.5 mm length were used, since they have been demonstrated to be the most responsive in our conditions. To avoid the precocious germination, immature scutella were isolated from the seed embryos by removing the embryo axis and placed with the scutellum exposed in the induction medium MP4 consisting in MS medium (Murashige & Skoog, 1962) supplemented with 30 gl^{-1} sucrose and 4 mgl^{-1} picloram. Explants were cultured in the dark at 25 °C for 4 days prior bombardment (see Fig. 2). Osmotic treatment is thought to offer protection to bombarded material by minimising cytoplasm leakage from target cells (Vain et al., 1993), so in the HMW-GS over-expression and gliadins silencing experiments, explants were subjected to a 4h osmotic treatment, before and/or after bombardment. In addition, in order to optimise the transformation conditions, different osmotic treatments (4h and 16h before bombardment and 4h and 16h after bombardment) were evaluated by placing the explants in medium MP40.4M consisting in MP4 solid medium supplemented with 0.4M mannitol (for more details see Table 1). In the transformation experiments for down-regulation of the HMW-GS genes, only 4h osmotic treatment post-bombardment was applied. Particle bombardment and selection of the transformed explants in the *in vitro* culture were as described by León et al. (2009). Modifications in the protocol were introduced in the experiments for optimization of the osmotic treatment as described below.

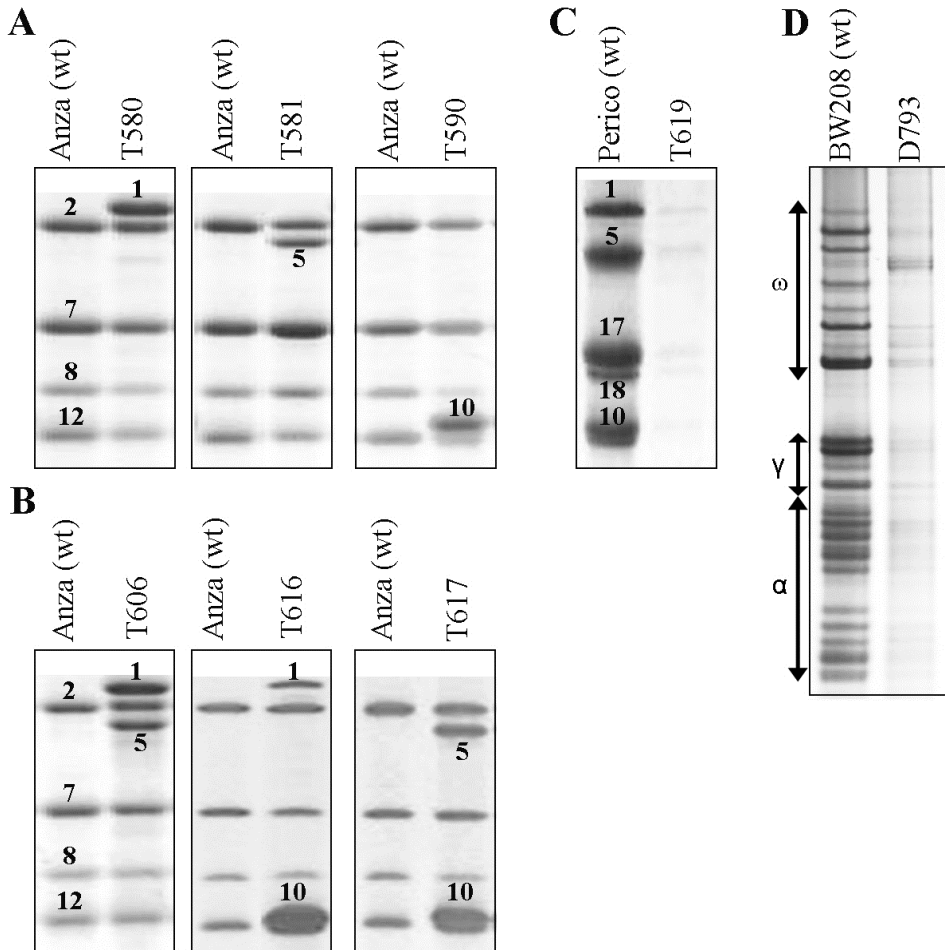---

[1] International maize and wheat improvement center

Fig. 1. SDS-PAGE gels of wild type lines and transgenic lines with over-expression of (A) one HMW-GS gene, and (B) two HMW-GS genes; and (C) down-regulation of all the HMW-GS. (D) A-PAGE of wild type and transgenic line D793 with down-regulation of all the gliadin fractions.

2.2 Osmotic treatment

Four different osmotic treatments were evaluated to increase the transformation efficiency of wheat (see Fig. 2). For each treatment, 1500 scutella distributed in 60 Petri dishes (25 scutella per dish) were isolated: 250 scutella were used as non transformed controls and did not receive the osmotic treatment (control-S); explants were transformed with the pAHC25 plasmid (Christensen & Quail, 1996) containing the *uidA* and *bar* genes, and subjected to one of the following osmotic treatments at a ratio of 250 scutella per treatment: 1) no osmotic treatment (control-B), 2) osmotic treatment of 4h prior bombardment, 3) 16h prior bombardment, 4) 4h after bombardment, and 5) 16h after bombardment (Fig. 2).
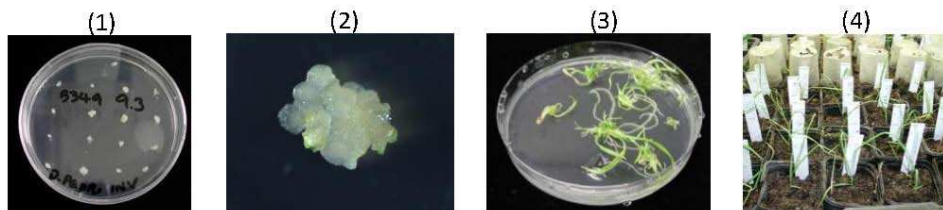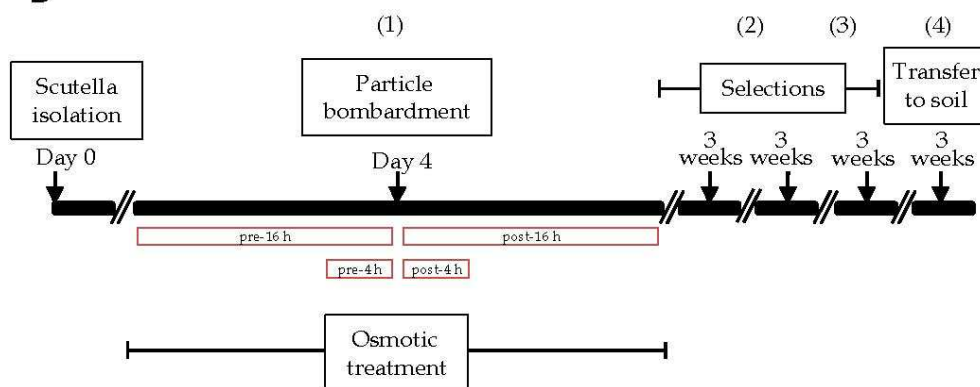
Fig. 2. Scheme of genetic transformation of wheat via particle bombardment. (A) Pictures of (1) the explants used for transformation, (2) callus formation after 3 weeks of cultivation, (3) explants after 8 weeks of cultivation and (4) plantlets after transfer to soil. (B) Time sequence for genetic transformation of wheat: scutella isolation, particle bombardment, selections and transfer to soil.

After the bombardment and the respective osmotic treatments, the explants were cultured in the MP4 induction medium for 3 weeks in the dark at 20-25ºC. Then, the percentage of embryogenesis (% of scutellum surface presenting embryogenic response) was calculated. Embryogenic calluses were transferred for shoot induction to the regeneration medium RZPPT2, consisting in RZ medium, supplemented with 2,4-dichlorophenoxyacetic acid (2,4-D), 5 mgl-1 zeatin and 2 mgl^{-1} L-phosphinothricin (L-PPT, the active ingredient of the herbicide BASTA) as described previously by Barro et al. (1998), except the untransformed explants (control-S) that were transferred to the same medium without the selective L-PPT. After 3 weeks of culture in the regeneration medium at 25 ºC in the light, the percentage of regeneration (% of explants presenting shoots) was measured, and shoots were transferred to RPPT2 (R medium supplemented with 2 mgl^{-1} L-PPT) (Barro et al. 1998), where they were cultured for another 3 weeks. Plantlets surviving were sub-cultured for another 3 weeks in RPPT2 medium, and then transferred to soil. Transgenic plants were determined by 1) the histochemical GUS assay and 2) by PCR amplification of a fragment of the *bar* gene. The

transformation efficiency of each treatment was determined as % of transgenic plants transferred to soil over the total number of scutella isolated for each treatment.

2.3 Seed composition and statistical analysis

Six lines over-expressing the HMW-GS (T580, T581, T590, T606, T616 and T617), one line with down-regulation of HMW-GS (T619) and 4 lines with down-regulation of all the groups of gliadins (D793, D894, E42 and E82) were obtained and grown as described above. Mature seeds from all the lines as well as the untransformed controls were collected and crushed into a fine powder. Three independent replicates were made for the determination of each of the following components: total protein, starch, water soluble carbohydrates, fructose, glucose, sucrose and maltose. Total protein was calculated from the Kjeldahl nitrogen content (%N x 5.7). Starch content was determined by polarimetry. Water soluble carbohydrates, as well as fructose, glucose, sucrose and maltose were quantified by HPLC with refractive index detection. Results obtained for all transgenic lines over-expressing one subunit of HMW, two subunits of HMW, and transgenic lines with down-regulation of all the gliadins, were grouped for statistical analysis and named 'HMW1', 'HMW2' and '–Gli', respectively. Line T619 with down-regulation of all the HMW-GS was named '–HMW' (Table 1).

Data were analysed with the statistical software R version 2.12.1 using the Graphical User Interface (GUI) R Commander, and the SPSS version 11.0 statistical software package (SPSS Inc., Somers, NY). Major assumptions of analysis of variance (ANOVA) were confirmed by the Kolmogorov-Smirnov's test for normal distribution and by the Levene's test for homogeneity of variances. ANOVA and two-tailed Dunnett's test for median multiple comparisons were used to analyse the results and compare between transgenic and wild type lines. P values lower than 0.05 were considered significant, and lower than 0.01 were considered highly significant.

3. Results and discussion

The effect of the different osmotic treatments (prior and after particle bombardment) on the embryogenesis as well as on the efficiencies of regeneration and transformation has been studied. The results obtained can help to improve the transformation protocols in cereals.

In addition, the application of the transformation techniques to produce over-expression and silencing of genes encoding HMW-GS and gliadins, as well as the effects on the protein, starch and carbohydrates contents are discussed below.

3.1 Transformation method improvement: osmotic treatment

The ability of the particle bombardment to consistently transform wheat has been previously reported (Lonsdale et al., 1998, Vasil et al., 1992, Witrzens et al., 1998) . However, cereal transformation is still difficult due to the number of parameters involved in the technique, and many research works have been focused on the bombardment conditions such as amount of DNA, amount and size of gold particles, acceleration pressure, bombardment distance or the osmotic condition of tissues (Altpeter et al., 1996, Becker et al., 1994, Li et al., 2003, Rasco Gaunt & Barcelo, 1998). An osmotic treatment of target tissues for stable transformation results in plasmolysis of cells and restricts damages by preventing extrusion of the protoplasm from bombarded cells (Vain et al., 1993). Osmotic treatment

both prior and after particle bombardment has been used in wheat transformation (Altpeter et al., 1996, Brinch-Pedersen et al., 2000, Jordan, 2000, Ortiz et al., 1996, Stoger et al., 1999). In this work we have studied the effect of the osmotic treatment with 0.4 M mannitol prior and after particle bombardment (4 h and 16 h) on the somatic embryogenesis, regeneration capacity and transformation efficiency (Table 1). The highest percentages of embryogenesis, 68.2% and 78.2%, were obtained with the 4 h pre-treatment (4h-pre) and 16 h post-treatment (16h-post), respectively (Table 1). No significant differences were found between these treatments and the not bombarded control (control-S), and the embryogenesis was much higher than the obtained with the bombarded control (control-B). These data indicate that particle bombardment has a negative effect on somatic embryogeneis but the osmotic treatment prevents the damages caused by the particle bombardment. The 16h pre-treatment showed the lowest level of embryogenesis, with only 38.0% of embryogenesis (Table 1).

Treatment	Embryogenesis (%)	Regeneration (%)	Plants recovered	Transgenic plants	Transformation efficiency (%)
Pre-4h	68,1 ab	78,4 ab	64	3	1,2
Post-4h	57,3 abc	49,2 b	38	17	6,8
Pre-16h	38,0 c	68,3 ab	7	2	0,8
Post-16h	78,2 a	64,1 ab	36	4	1,6
Control-B[a]	41,3 bc	63,2 ab	19	0	0
Control-S[b]	73,1 a	93,4 a	N/A	N/A	N/A

Table 1. Effect of the osmotic treatment with 0.4 M mannitol before and after particle bombardment. [a]Control-B: same conditions of bombardment and herbicide selection, but with no mannitol treatment; [b]Control-S: no bombardment, no mannitol treatment and no herbicide selection.Values within the same column followed by the same letter are not significantly different (P<0.05)

The range of regeneration of the explants subjected to the osmotic treatment was between 49.2% and 78.4% for treatments 4h-post and 4h-pre, respectively, while the control-B presented 63.2% of regeneration. The control-S (not bombarded) showed the highest level of regeneration, with 93.4%, a percentage comparable to the 76-86% reported by León et al. (2006) in not treated and not bombarded scutella. Only the 4h-pre treatment was significantly different for the regeneration, and no correlation was found between embryogenesis and regeneration of scutella (data not shown). Overall, the 4h-pre and 16h-post osmotic treatments showed high levels of embryogenesis and regeneration. Twenty-six of the 164 plants regenerated and transferred to soil were confirmed as transgenic by the histochemical assay GUS and by PCR of the *bar* gene. In spite of the high selection pressure, the number of escapes, or regenerated plants that not contained the pAHC25 plasmid, was high in all the treatments. The lowest percentage of escapes was 55.3% in the 4h-post treatment. The percentage of escapes described in previous works was also very high, ranging between 55-88% under selection with bialaphos (Altpeter et al., 1996) and around 80% under selection with L-PPT (Barro et al., 1998). The transformation efficiencies ranged between 0.8% and 6.8% in the 16h-pre and 4h-post, respectively. The 4h-post osmotic treatment also allowed recovering the highest number of positive transgenic plants with 17.

These results indicate that the post-bombardment osmotic treatment is more efficient than the pre-treatment, though long treatments, such as 16h-pre and 16h-post, drastically decreased the transformation efficiency. The transformation efficiency obtained with the 4h-post treatment (6.8%) is higher than the reported by other authors that ranged between 0.15-0.5% (Altpeter et al., 1996, Blechl & Anderson, 1996) and between 0.5-1.5% (Barro et al., 1997, Becker et al., 1994, Vasil et al., 1993). However, the transformation capacity is largely genotype-dependent and controlled by a wide number of factors as indicate the range of variation obtained in the different works.

However, high transformation efficiencies are needed for an increasingly number of applications to crops. In the case of wheat, baking quality and reducing the allergenicity of gluten for a broad sector of the population are priority goals. We have used genetic transformation protocols described in this work to over-express or silence a wide number of genes related to these two goals. The characterization of important traits of the transgenic plants produced is described below.

3.2 Grain components analysis of transgenic wheat lines with over- and down-regulated gluten proteins

Wheat flour consists mainly of starch, water and proteins. In addition, non-starch polysaccharides, in particular arabinoxylans and lipids are important minor flour constituents relevant for bread production and quality (Goesaert et al., 2005). Quantity, composition (quality), type and viscoelastic properties of wheat gluten proteins are important for bread-making (Finney & Barmore, 1948, Shewry & Halford, 2002). Wheat gluten can be divided into two protein families: the glutenins and the gliadins. The glutenins comprise high molecular weight glutenin subunits (HMW-GS) and low molecular weight glutenin subunits (LMW-GS) fractions, whereas the gliadins can be divided into three structural types: α/β-, γ- and ω-gliadins (Shewry & Halford, 2002). Although gliadins and glutenins are related with the bread-making quality, the HMW-GS are considered major determinants of quality in wheat flour (Payne, 1987, Shewry et al., 2003). In wheat grain, starch is deposited in partially crystalline granules that vary in morphology and structure between and within plant species. Starch owes much of its functionality in foods to the characteristics of the two constituent glucose polymers, amylose and amylopectin, and to the physical organization of these macromolecules into the granular structure (Annison & Topping, 1994). Changes in the above components provoked by the over-expression or silencing of glutenin and/or gliadin genes are important that they be determined and quantified, as they can largely influence the quality of the new transgenic lines of wheat.

3.2.1 HMW-GS over-expression

Table 2 shows the grain characteristics of the over-expression of HMW-GS genes on transgenic lines of bread wheat in comparison to its wild type (cv Anza). These transgenic lines contain one and two additional transgenic HMW-GS, and are named HMW1 and HMW2, respectively. The lines over-expressing one and two HMW-GS showed a significant increase of total protein content relative to its wild type. The total protein content showed no significant differences between HMW1 and HMW2 transgenic lines. Most works involving over-expression of HMW-GS in wheat seeds have not reported significant changes in total protein content between transgenic lines and their controls (León et al., 2010b, Rakszegi et al., 2005, Yue et al., 2008). Nevertheless, Rooke et al. (1999) showed that a

transgenic wheat line containing an additional gene encoding the HMW subunit 1Dx5, resulted in a slightly increase in the total grain nitrogen. Although some of the samples used in this study had much higher protein contents than those of the non-transformed, these levels were not reproducible in other greenhouse or field trials (León et al., 2010a), which could be because the total protein content is a highly environment-dependent trait.

	HMW over-expression			HMW down-regulation		Gliadins down-regulation	
	wt[a]	HMW1[b]	HMW2[c]	wt	-HMW[d]	wt	-Gli[e]
Total protein	10.4	**12.8 ****	**12.4 ***	12.1	**11.0 ***	12.8	12.6
Starch	58.0	**45.6 ****	57.7	55.8	54.7	58.7	57.6
WSC[f]	15.6	17.0	**19.5 ****	18.8	20.5	18.1	20.2
Fructose	0.37	**0.73 ****	**0.68 ****	0.49	0.64	0.40	0.39
Glucose	0.53	**0.93 ****	**0.90 ****	0.81	0.85	0.51	0.55
Sucrose	0.90	**0.27 ****	**0.22 ****	0.86	0.86	1.50	2.12
Maltose	0.76	0.33	0.38	1.07	1.19	0.95	1.02

Table 2. Seed composition of untransformed wild types and mean of transgenic lines for HMW-GS over-expression, HMW-GS down-regulation and gliadins down-regulation. All parameters are expressed as percentage (%) of the total weight. [a]wt: wild type; [b]HMW1: over-expression of one subunit of HMW; [c]HMW2: overexpression of two HMW-GS; [d]-HMW: down-regulation of all the HMW-GS; [e]-Gli: down-regulation of all the gliadins; [f]WSC: water soluble carbohidrates

Only transgenic lines HMW1 showed a high reduction of starch percentage, significantly different to the wild type and to the HMW2 transgenic lines. The low starch content of transgenic lines HMW1 could be related to the high percentage of total protein. Likewise, starch:protein ratio was significantly lower in both HMW transgenic lines (Fig. 3). It resulted in an increase of total protein in the over-expressing HMW lines and in a negative correlation between the starch and protein contents. This negative correlation was reported by other researchers (Choct et al., 1995, Kim et al., 2003, Parsaie et al., 2006, Wiseman & Inborr, 1990).
Water soluble carbohydrates (WSC) were higher in HMW2 transgenic lines, but not in HMW1 transgenic lines in comparison with the control (Table 2). On the other hand, the simple carbohydrates contents showed a significant increase in both HMW over-expression transgenic lines, excepting the maltose content, which did not show differences between wild type and transgenic lines.
The gliadin and glutenin content were measured by RP-HPLC and the ratio gliadin:glutenin (Gli:Glu) is showed in Figure 4. Both lines HMW1 and HMW2, showed a significant decrease of Gli:Glu ratio, which was mainly due to the expression of additional HMW-GS. The decrease of the Gli:Glu ratio when an additional HMW-GS is added by genetic transformation has been previously reported by other authors (León et al., 2009, Rakszegi et al., 2005).
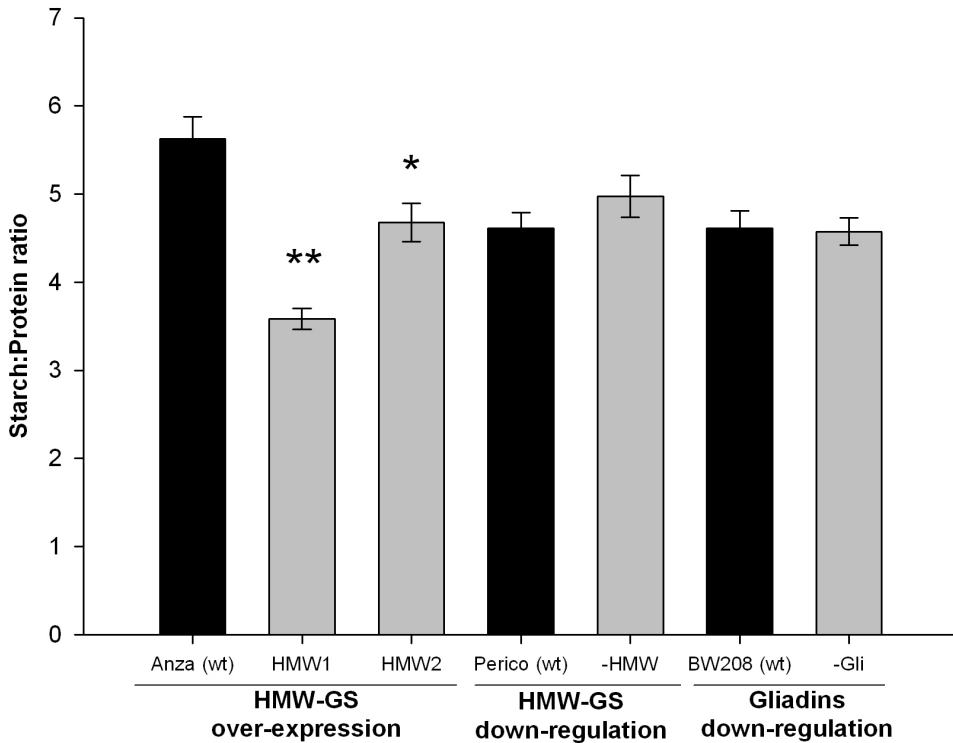
Fig. 3. Starch:protein ratio of untransformed wild types (black) and mean of transgenic lines (grey) for HMW-GS over-expression (HMW1 and HMW2), HMW-GS down-regulation (-HMW) and gliadins down-regulation (-Gli). Asterisks indicate significant differences between transgenics and the corresponding wild type line as determined by Dunnett's multiple comparisons at $P < 0.05$ (*) or $P < 0.01$ (**).
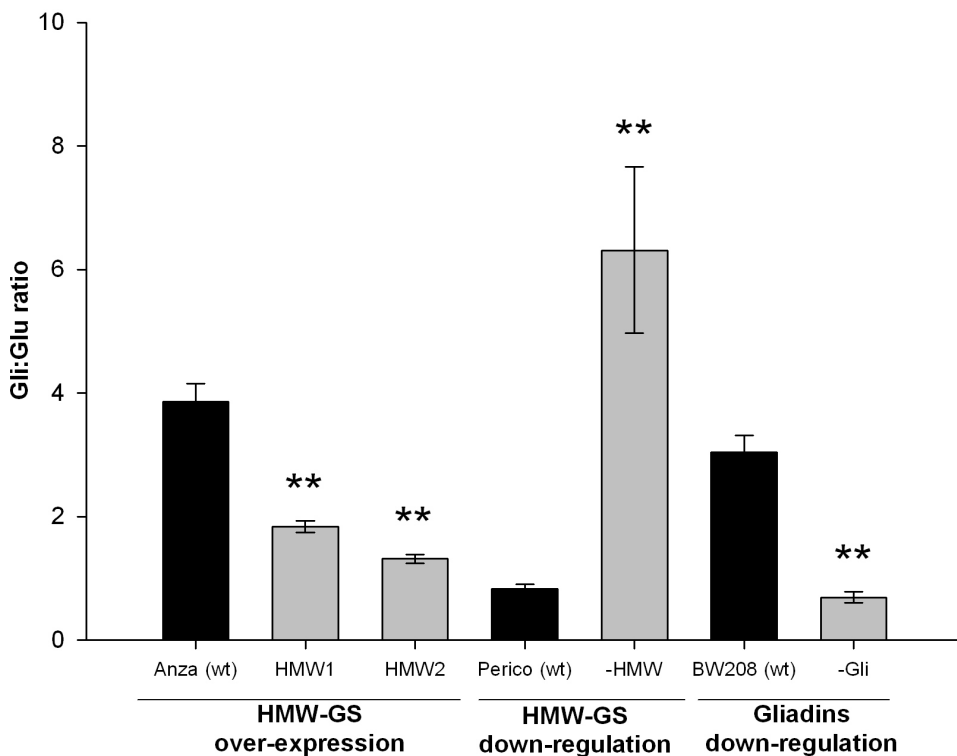
Fig. 4. Gliadins:glutenins ratio of untransformed wild types (black) and mean of transgenic lines (grey) for HMW-GS over-expression, HMW-GS down-regulation and gliadins down-regulation. Asterisks indicate significant differences between transgenics and the corresponding wild type line as determined by Dunnett's multiple comparisons at P < 0.05 (*) or P < 0.01 (**).

3.2.2 HMW-GS down-regulation

A bread wheat cultivar Perico was used to down-regulate all the HMW-GS by the transformation with a D Hordein gene from *H. chilense*. Thus, the transgenic line T619 showed a very low content of HMW-GS from the three genomes (Fig. 1 C). Grains composition analysis did not shown differences in total starch content, WSC and the simple carbohydrates between T619 and the wild type Perico. However, the total protein content was significantly lower in T619 in comparison with the wild type Perico (Table 2). This significant decrease in the protein content did not result in changes in the starch: protein ratio (Figure 3). There are no consistent data about the response of wheat grain components, and in particular the total protein content, when one or more HMW-GS genes are silenced (Alvarez et al., 2000, Yue et al., 2008). Consistently with the over-expression of HMW-GS genes, the down-regulation of HMW-GS results in a significant increase of the ratio Gli:Glu (Figure 4).

3.2.3 Gliadin down-regulation

Transgenic lines with reduction of all the gliadins had not differences in total protein, total starch, WSC and simple carbohydrates in comparison with non-transformed lines (Table 2). In addition, the ratio starch:protein did not present differences between transgenic and wild type lines (Figure 3). Gil-Humanes et al. (2010) reported no differences in total protein content between lines with reduction of gliadins and their wild types. This suggests a compensatory process that operates in the grain in response to gliadin silencing, to maintain a stable total protein content. However, the ratio Gli:Glu showed a significant decrease in the transgenic lines, overwhelmingly driven by the silencing of gliadins (Figure 4). Gil-Humanes et al. (2011) also reported increases in the amounts of glutenins, albumins and globulins in the transgenic lines with down-regulation of all the gliadins. Previously, compensatory effects were observed by Lange et al. (2007) who reported increases in the amounts of B hordeins and glutelins in transgenic lines of barley with reduced contents of C hordeins. Hansen et al. (2007) performed microarray analyses of the same lines and showed up-regulation of B and γ-hordein genes and of the gene encoding the barley prolamin-binding factor (BPBF), a transcription factor that regulates B hordein gene expression.

4. Conclusion

The genotype and quality of the explants used for transformation, and the reduction in the stress during particle bombardment with the application of the osmotic treatment, promotes an improvement in the transformation efficiency of wheat. The 4 h post-treatment produced the highest transformation efficiency and the lowest ratio of escapes, so it is highly recommended to subject the explants to this treatment in order to obtain high number of transgenic plants.

Over-expression and down-regulation of HMW-GS genes provide different changes in total protein content. It is increases with the over-expression of HMW-GS genes and decreases when they are silenced. The over-expression of HMW-GS genes causes the most severe changes in the composition of wheat grain, changing the total protein and starch contents, and the relationship between them. By contrast, the down-regulation of total gliadins does not cause major changes in the total content of protein and starch. Finally, the over-expression and down-regulation of HMW-GS and gliadin genes produce changes in the Gli:Glu ratio, which is closely related to wheat flour bread-making quality.

5. Acknowledgments

6. References

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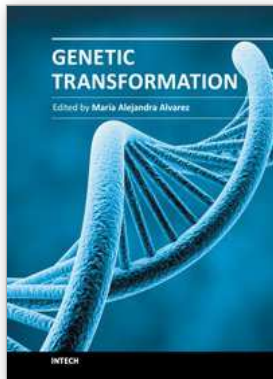
Genetic Transformation

Edited by Prof. MarÃa Alvarez

Genetic transformation of plants has revolutionized both basic and applied plant research. Plant molecular biology and physiology benefit from this power fool, as well as biotechnology. This book is a review of some of the most significant achievements that plant transformation has brought to the fields of Agrobacterium biology, crop improvement and, flower, fruit and tree amelioration. Also, it examines their impact on molecular farming, phytoremediation and RNAi tools.

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