Integration and expression of the high-molecular-weight glutenin subunit Dy10 gene into Egyptian wheat

(Received: 15.10.2006; Accepted: 28.10.2006)

Ashraf H. Fahmy*, **; Khaled S. Abdalla* and Mohamed Abdallah*
*Agricultural Genetic Engineering Research Institute,
9 Gamaa St. Giza, Egypt.

ABSTRACT

To investigate the possibility of manipulating gluten dough strength and elasticity by increasing the high molecular weight glutenin subunits (HMW-GS), bread wheat cv. Giza 164 was transformed with HMW-Dy10 subunit gene. Immature embryo-derived calli were co-transformed with a plasmid (pK-Dy10) harboring HMW-gene (Dy10) driven by its own promoter and pACH25 plasmid containing the scorable gus gene and the selectable bar gene. Integration of the three transgenes had been confirmed in the genome of transgenic T_0 plants by PCR analysis. Expression of gus gene was detected in transformed plants by histochemical staining and the expression of bar gene was detected using leaf painting assay. Grains of transgenic and non-transgenic (control) wheat plants were analyzed to estimate the level of glutenin protein using HPLC and it revealed higher levels of glutenin in transgenic grains comparing with control.

Keywords: *Wheat, gluten, plant transformation, gus, bar, PCR, HPLC.*

* The authors contributed equally to this work, ** Corresponding author.

INTRODUCTION

Wheat is one of the most abundant sources of energy and nourishment for mankind. It is a member of the family *Poaceae* which includes the major cereal crops of the world such as maize, wheat, barley and rice. Ninety-five percent of wheat grown today is of the hexaploid type; used for the preparation of bread and other baked products. Nearly all of the remaining 5% durum wheat (tetraploid) which is mainly used for making pasta, macaroni and biscuits (Patnaik and khurana, 2001). Breadmaking is one of humankind's oldest technologies, being established some 4,000 years ago. The ability to make bread depends largely on the visco-elastic properties of wheat dough. These allow the entrapment of carbon dioxide released by the yeast, giving rise to a light porous structure. Wheat's unique bread-making properties come from complex proteins called gluten, found in no other cereal grain. One group of gluten proteins, the high molecular weight (HMW) subunits, are largely responsible for gluten elasticity, and variation in their amount and composition is associated with differences in elasticity (and hence quality) between various types of wheat. These proteins form elastomeric polymers stabilized by inter-chain disulphide bonds, and detailed studies of their structures have led to models for the mechanism of elasticity (Shewry *et al*., 1995).

One of the first targets for the genetic engineering of wheat has been the enhancement of its flour utilization properties.

Among these properties is dough strength, which depends, in large part, on seed storage protein composition and quantity, particularly HMW glutenin subunits (Blechl and Anderson, 1996). Progress in biotechnology of wheat, including genetic transformation and expression of HMW glutenin subunit genes, now make it possible to engineer the gluten proteins and proved that functional properties of wheat could be improved by gene transfer technology (Altpeter *et al*., 1996; Blechl and Anderson 1996; Alvarez *et al.,* 2000 and Pastori *et al.,* 2000). This study was conducted to transform HMW glutenin Dy10 subunit gene into Egyptian wheat to improve the flour quality.

MATERIALS AND METHODS

Wheat transformation

Immature caryopsis of the Egyptian wheat cultivar Giza 164 were collected approximately two weeks postanthesis. Grains were surface sterilized with 20% commercial Clorox (5.25% sodium hypochlorite) supplemented with few drops of Tween 20 for 15 minutes, and then washed five times with sterile distilled water. Immature embryos were aseptically isolated and cultured with the scutellum side up onto callus induction medium for one week before bombardment. Embryo-derived calli were co-transformed with two plasmids using biolistic bombardment, Biolistic® PDS-1000/H device (Bio-Rad, USA) according to Fahmy *et al.* (2006). The first plasmid pAHC25 is harboring *gus* and *bar* genes and both genes are driven by *ubi* promoter, the second one is pK-Dy10 which is BlueScript KS plasmid harboring HMW-GS Dy10 gene driven by its own promoter (Vasil *et al.,* 1993). Regenerated putatively transgenic wheat plants were transferred into soil, then incubated in control growth chamber (Conviron®) for acclimation and analysis until setting seeds.

DNA analysis

Putative transgenic plants were subjected to molecular analysis to confirm the integration of the foreign genes (*gus*, *bar* and Dy10) into plant genome by PCR analysis. Total genomic DNA was isolated from leaf material using DNeasy Plant Mini Kit (QIAGEN, Germany) and 50 ng DNA were used as a template in the PCR analysis. Specific oligonucleotide primers for *gus, bar* and Dy10 were used (Table 1). For *gus* gene the reaction mixture was denatured at 94°C for 3 min and subjected to 35 cycles. Each cycle consisted of denaturation at 94°C for 30 sec, annealing at 62°C for 30 sec and extension at 72°C for 2 min. After the final cycles the extension was allowed to continue for additional 7 min at temperature 72°C. For *bar* and Dy10 genes, the cycles condition were the same as described above except the annealing temperatures were 57°C and 55 °C for *bar* and Dy10, respectively.

Table (1): Primer sequences used in the PCR analysis.

Name	Primer	Expected Size (pb)
gus F	5`-AGTGTACGTATCACCGTTTGTGTGAAC3`	1050
gus R	5`-ATCGCCGCTTTGGACATACCATCCGTA-3`	
barF	5`-GTCTGCACCATCGTCAACC-3`	443
barR	5`-GAAGTCCAGCTGCCAGAAAC-3	
KS	5' -CCGGCCCTGACTCCTAATACACAT-3'	915
Dy10R1	5'-TCGAGGTCGACGGTAATC'-3	

HPLC analysis

Gluten was determined in the grains of the transgenic wheat plants (T_0) by HPLC method according to Wieser *et al.* (1998). The extracts were analyzed using HPLC apparatus (Hewlett-Pakard 1100). The column used was C8 reverse phase column (4.6 mm × 25 cm with 5 μm packing). The mobile phase used was acetonitrile gradient from 50 to 100% at a flow rate of 1.5 ml/min.

Expression of marker genes

Expression of *gus* and *bar* genes was studied in T_0 plants. Histochemical staining analysis was conducted to study the expression of *gus* gene according to Jefferson (1987), while leaf painting assay was conducted to study the expression of *bar* gene according to He *et al.* (1999).

RESULTS AND DISCUSSION

The wheat HMW glutenins have a large impact on the physical properties of flour made from wheat (Blechl and Anderson, 1996). The quality of wheat cultivars depends on the number and composition of the HMW-GS present (Altpeter *et al.,* 1996). Genetic modification of wheat seed storage proteins is important for the impact of food products specially bread that have a socio-economical and political effects in Egypt. Some groups transforming HMW-GS genes into wheat to improve its flour quality for food industry. In addition to that purpose, our group has additional insight which is transferring such genes into wheat, maize and barley to produce transgenic plants from these crops expressing reasonable gluten content in their grains. Such strategy could help to increase the amount of maize and/or barley flour added to wheat flour in bread industry and hence reduces the amount of imported wheat. To achieve this goal, we started to transfer one of HMW-GS genes (Dy10) into maize (Abdallah *et al.*, 2004) and barley (unpublished data). In this study we report the transformation of Egyptian wheat with Dy10 gene.

The last two decades have witnessed the widespread use of varied approaches for introduction of exogenous DNA into wheat. Wheat improvement by genetic engineering requires the delivery, integration and expression of defined foreign genes into suitable regenerable explant (Patnaik and Khurana, 2001). Therefore, we used Biolestic bombardment to transfer Dy10 gene into the wheat variety Giza 164. Immature embryo-derived calli were co-transformed with plasmid containing Dy10 gene and another plasmid containing *gus* and *bar* genes. Four putative transgenic wheat plants were regenerated from the transformation experiment. These plants were subjected to molecular analysis to confirm the integration and the expression of the transgenes.

Table (2): Analysis of transgenic wheat plants.

Plant no.	PCR analysis *uidA* gene	*bar* gene	HMW subunit	GUS *stain*	*Leaf painting*	Fertility	gluten content μg/g
control	−	−	−	−	−	+	915
1	+	+	+	+	+	+	44617.5
2	+	+	+	+	+	+	4000.6
3	+	+	+	+	+	+	2742
4	+	+	+	+	+	+	1443.3

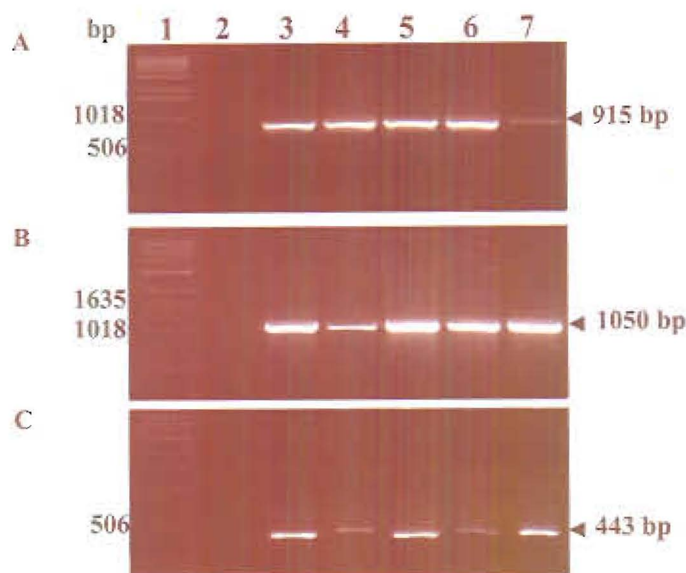

Fig.(1):PCR analysis of transgenic wheat plants. A: Amplified PCR products for Dy10 gene (915 bp). B: Amplified PCR products for gus gene (1050 bp). C: Amplified PCR products for bar gene (443 bp). Lane 1: DNA marker (1 kb ladder), lane 2: negative control, lane 3: positive control (plasmid), lanes 4-7: transgenic plants.

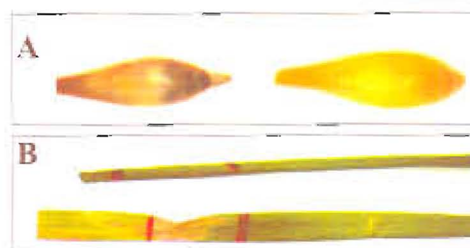

Fig. (2): Expression of marker genes. (A) GUS expression in kernel of transgenic plant (left) and no expression in non-transgenic plant (right). (B) leaf of transgenic plant showing resistance to herbicide (top) and leaf of non-transgenic plant showing necrosis (bottom).

Our initial attempt to detect the integration of the transgenes in the genome of the transgenic plants was performed using PCR analysis. DNA was isolated from putative transgenic and non-transgenic plants (control) then subjected to PCR analysis using specific primers for each transgene (Table 1). The primers of marker genes are routinely used to detect both genes in transgenic plants (He *et al.*, 1999; Abdallah *et al.*, 2004 and Fahmy *et al.* 2006).

PCR results revealed products of the expected sizes, 1050 bp for *gus* gene and 443 bp for *bar* gene as shown in Fig. (1). the data confirmed the integration of both marker genes in the genome of the four wheat plants (Table 2). Interestingly, no escapes were developed indicating that the selection system used in this study is efficient. A similar finding was obtained previously when the original protocol was published (Fahmy *et al.*, 2006). To our knowledge PCR analysis has not been used to detect transgenic HMW glutenin genes in transgenic bread-making wheat varieties because those varieties already have endogenous copies of HMW glutenin genes. In our lab, we designed specific primers (Table 1) that can distinguish between the transgene (Dy10) and the endogenous copy (Abdalla, 2007, in press). These primers worked efficiently (Fig. 1) where they amplified 912 bp from putatively transgenic wheat plants while no amplification was detected from the endogenous copy of the Dy10 gene. The data confirmed the integration of Dy10 transgene in the genome of the four wheat plants.

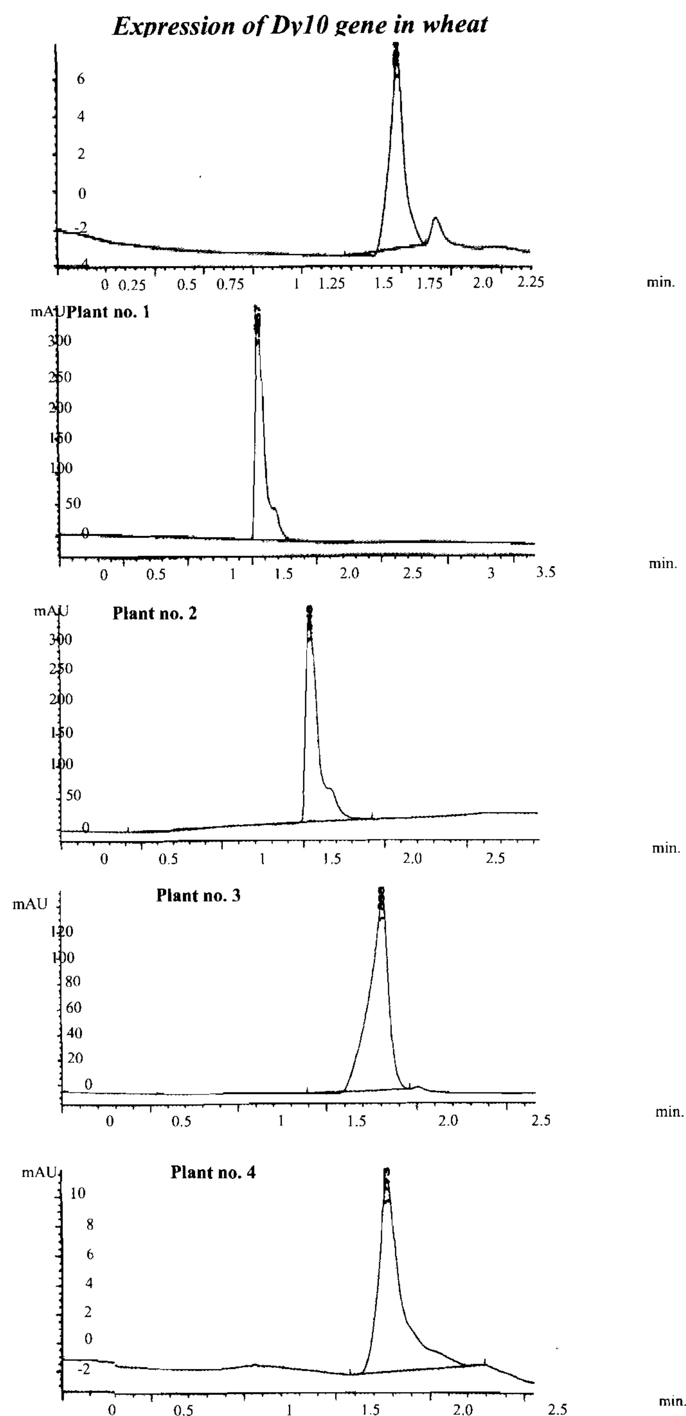

Fig. (3): ***P-HPLC results for glutenin extract from non- transgenic (control) and four transgenic wheat plants.***

Expression of marker genes was examined histochemically for *gus* activity and by leaf painting assay for *bar* (Fig. 2 and Table 2). The four plants were positive for both marker genes. It is worth to mention that the integration and expression of the transgenes did not affect the fertility of the transgenic plants where all of them set seeds (Table 1).

HPLC has frequently been used to relate the quantity of gluten protein fractions for

flour properties (Jia *et al.*, 1996 and Wieser *et al.,* 1998). Therefore, we used HPLC to quantify the amount of gluten protein in the kernels of transgenic wheat plants. The charts of HPLC analysis are shown in Fig. (3). The results of HPLC analysis were 951 µg/g for control (non-transformed), 4617.5 µg/g for plant number 1, 4000.6 µg/g for plant number 2, 2742 µg/g for plant number 3 and 1443.3 µg/g for plant number 4 (Table 1). These results indicate that the four transgenic wheat plants showed over-expression of glutenin protein comparing with the control. This indicates that the transgene was stably integrated in the genome of transgenic plants and successfully expressed in the kernels. However, the level of expression varied among the four plants. The variation of expression is often observed with a population of transgenic plants transferred with the same construct and under identical transformation conditions. This intertransformation variation highly complicates phenotype analysis and production of commercial crops with stable and predictable transgene performance. The reason of such variation could be the influence of the location of the transgene in the genome of transformants (Odell *et al.*, 1985; Keinonen-Mettala *et al.* 1998). In addition, the variation could be resulted from the copy number of the transgene inserted in the genome of transgenic plants (Blechl and Anderson, 1996).

The over-expression of glutenin in transgenic plants ranged from 1.6 fold (plant number 4) to 5.0 fold (plant number 1) comparing with the control. Similar finding was reported when the HMW-Dx5 subunit transformed into wheat and resulted in a four-fold increase in gluten content of the kernels (Rooke *et al.*, 1999). Other findings indicated an increase in gluten content reaching up to 1.5 fold (Blechl and Anderson, 1996). These differences are expected due to the use of different HMW-GS genes where we used Dy10 gene in this study, Rooke *et al.*, 1999 used Dx5 gene while Blechl and Anderson, 1996 used both genes fused together.

In summary, transgenic wheat plants from the Egyptian variety Giza 164 with HMW-Dy10 gene were obtained in this study. The plants over-expressed seed storage glutenin protein. These results demonstrate the feasibility of modifying the technological properties of wheat flour and hence can help to improve bread industry.

ACKNOWLEDGMENT

The authors are grateful to Dr. Olin Anderson, UDSA, Albany, CA, USA for providing Dy10 construct used in this study.

REFERENCES

Abdalla, K.S. (2007). A simple and reliable PCR-based method for detection and screening of transgenic plants transformed with the same endogenous gene. Arab J. Biotech. (In Press).

Abdallah, M.; Fahmy, A.H.; Abdalla, K.S. and Maaty, W. (2004). Transformation of a high-molecular-weight (HMW) glutenin subunit Dy10 gene into maize. Arab J. of Biotech., 7(2): 156-172.

Altpeter, F.; Vasil, V.; Srivastava, V. and Vasil, I. K. (1996). Integration and expression of the high-molecular-weight glutenin subunit 1Ax1 gene into wheat. Nature Biotechnology, 14(9): 1155-1159.

Alvarez, M. L.; Guelman, S.; Halford, N. G.; Lustig, S.; Reggiardo, M. I.; Ryabushkina, N.; Shewry, P.; Stein, J. and Vallejos, R. H. (2000). Silencing of HMW glutenins in transgenic wheat expressing extra HMW subunits. Theoretical and Applied Genetics, 100(2): 319-327.

Blechl, A. E. and Anderson, O. D. (1996). Expression of a novel high-molecular-weight

glutenin subunit gene in transgenic wheat. Nature Biotechnology, 14(7): 875-879

Fahmy, A.H.; El-Shihy, O. M.; El-Shafey, Y. H. and Madkour, M. A. (2006). Genetic Transformation of Egyptian Wheat Cultivars (*Triticum aestivum* L.) *Via* Biolistic Bombardment Using Different Constructs. American-Eurasian J. Agric. & Environ, 160(8): 931-94.

He, G.Y.; Rooke, L.; Steele, S.; Bekes, F.; Gras, P.; Tatham, A.S.; Fido, R.; Barcelo, P.; Shewry, P.R. and Lazzeri, P.A. (1999). Transformation of pasta wheat (*Triticum turgidum* L. var. *durum*) with high-molecular-weight glutenin subunit genes and modification of dough functionality. Molecular Breeding, 5(4): 377-386.

Jefferson, R.A. (1987). Assaying chimeric genes in plants. The GUS gene fusion system. Plant Molecular Biology Reporter, 5:387-405.

Jia, Y.Q.; Febre, J.L. and Aussenac, T. (1996). Effects of growing location on response of protein polymerization to increased nitrogen fertilization for the common wheat Soisson: relationship with some aspects the of breadmaking quality. Cereal Chemistry, 73: 526-532.

Keinonen, M.K.; Papinen, A. and Weissenberg, K (1998). Comparison of the efficiency of some promoters in silver birch (*Betula pendula*). Plant Cell Reports, 17(5): 356-361.

Odell, J.T.; Nagy. F. and Chua, N.H. (1985). Identification of sequences required for activity of the cauliflower mosaic virus 35S promoter. Nature, 313: 810-812.

Pastori,G.M.; Steele, S.H.; Jones, H.D.; Shewry, P.R.; Gooding, M.J.; Barton, S.A. and Smith, G.P. (2001). Improving the breadmaking quality of elite wheat varieties by genetic engineering. Wheat Quality. Meeting organized by the Association of Applied Biologists, Reading, UK, 17-19 September 2001. Aspects of Applied Biology, 64: 61-62.

Patnaik, D. and Khurana, P. (2001). Wheat biotechnology: A minireview. Electronic Journal of Biotechnology, 4(2): 94-100.

Rooke, L.; Barro, F.; Tatham, A.S.; Fido, R.; Steele, S.; Bekes, F.; Gras, P.; Martin, A.; Lazzeri, P.A.; Shewry, P.R. and Barcelo, P. (1999). Altered functional properties of tritordeum by transformation with HMW glutenin subunit genes. Theoretical and Applied Genetics, 99(5): 851-858.

Shewry, P. R; Tatham,A.S.;Barro,p.; and Lazzeri,P.(1995). Biotechnology of bread making: unraveling and manipulating the multi- protein gluten complex. Biotechnology ,13:1185-1190.

Vasil, V.; Srivastava, V.; Castello, A.M.; Fromm, M.E. and Vasil, I.K. (1993). Rapid production of transgenic wheat plants by direct bombardment of cultured immature embryos. Bio/Technology, 11: 1553-1558.

Wieser, H., Ante, S. and Seilmeier, W. (1998). Quantitative determination of gluten protein types in wheat flour by reversed-phase High-Performance Liquid Chromatography. Cereal Chemistry, 75(5): 644-650.

الملخص العربي

انتاج نباتات قمح محورة التركيب الوراثى بجين Dy10 ذى الوزن الجزيئ العالى و دراسة التعبير الجينى لجين التحور

أشرف حسين – خالد صبرى – محمد عبدالله

معهد بحوث الهندسة الوراثية الزراعية – مركز البحوث الزراعية ٩ ش الجامعة – الجيزة ١٢٦١٩- جمهورية مصر العربية

لدراسة امكانية تحسين صفات عجين القمح المصرى تم ادخال جين Dy10 ذى الوزن الجزيئى العالى لصنف القمح المصرى جيزة ١٦٤، و قد تم استخدام جهاز الدفع الجينى لنقل جين Dy10 المحمول فى بلازميد pK-Dy10 مع بلازميد pAHC25 الذى يحتوى على جين *bar* الانتخابى و جين *gus* القياسى الى نسيج الكالوس الناتج من الأجنة غير الناضجة. و قد تم الحصول على نباتات من تلك التجارب تم اختبارها على المستوى الجزيئى للتحقق من أنها تحتوى على جينات التحور الثلاثة ضمن محتواها الوراثى، و قد أكدت نتائج تلك التحليلات على وجود هذه الجينات ضمن المحتوى الوراثى لتلك النباتات. هذا و قد استخدم جهاز HPLC لتقدير المحتوى الجلوتينى فى حبوب تلك النباتات و أظهرت نتائج تلك التحليلات زيادة المحتوى الجلوتينى للنباتات المحورة وراثيا عن تلك غير المحورة وراثيا (الشواهد).