GENETIC TRANSFORMATION OF EGYPTIAN WHEAT (*Triticum aestivum* L.) WITH CHITINASE GENE *VIA* MICROPROJECTILE BOMBARDMENT

A. H. FAHMY[1], K. EL MANGOURY[1], WALAA ABOU EL-WAFA[2], HODA M. S. BARAKAT[2], SOHEIR EL-SAEED EL-KHODARY[2] AND SUBBARATNAM MUTHUKRISHNAN[3]

1. *Agriculture Genetic Engineering Research Institute, Agricultural Research Center, Egypt*
2. *Plant Science Department, Faculty of Science, Ain Shams University, Egypt*
3. *Department of Biochemistry, Kansas State University, Manhattan, USA*

To introduce foreign genes, efficient protocols for embryogenic callus induction, transformation and selection and transgenic plants regeneration are considered necessary. In cereals, biolistic procedures have been used extensively in transformation (Cho *et al.*, 2004). In wheat, poor tissue culture performance which limits the number of wheat genotypes that can be stably transformed has been the major hurdle (Varshney and Altpeter, 2002). Fahmy and El-Shihy (2006) developed an efficient protocol for regeneration of plants from long-term cultured immature embryo callus onto medium containing 2.0 mg/l 2, 4-D. Monostori *et al*. (2008) and Yu *et al*. (2008) suggested that regeneration system is an effective significant step in wheat genetic transformation. Several important reports have pointed to Murashige and Skoog's medium supplemented with 2,4-D as the ideal medium for embryogenic callus induction proliferation in wheat (Raja *et al.*, 2008; Mahmood *et al.*, 2009; Munazir *et al.*, 2010) and that in comparing TDZ with other common growth regulators used in wheat regeneration. Moreover, it was suggested that TDZ was the best for *in vitro* regeneration (Fahmy *et al.*, 2006; Chauhan *et al.*, 2007; Biesaga *et al.*, 2010). The last two decades witnessed the widespread use of biolistic bombardment for introduction of exogenous DNA into plant. The biolistic approach has been most successful in delivering foreign genes into wheat. Gene introduction into crop plants *via* genetic transformation is a better alternative to traditional breeding (Rashid *et al.*, 2011). In the two past decades, the importance and steady use of *gus* gene as scrollable marker appeared as a necessary and many investigators accustomed to use in different co-bombardment procedures such as Yao *et al.* (2007), Fahmy *et al.* (2007), He *et al.* (2010) and Shi *et al.* (2011), since it produces a visible effect due to their activity in the transformed cells and the availability of a simple histochemical detection procedure. Also, the abundant utility of *bar* selectable gene in transformation experiments is not

less than *gus* scrollable gene. In the early trials, Chen *et al.* (1999) introduced chitinase gene (*chi*) into wheat cultivar Bobwhite with pAHG11 (harboring *chi* and *bar*) so as to use bar gene as a selectable marker, also, many researchers used the same approach such as Varshney and Altpeter (2002) and Wada *et al.* (2009). Transformation of a number of disease-resistance genes in transgenic plants has been reported. Chitin, an unbranched homopolymer of 1, 4- β-linked *N*-acetyl-D-glucosamine (GlcNAc), is widely distributed in nature. It is believed to be the second most abundant and renewable polymer on earth, next to cellulose. Chitinases are chitin degrading enzymes, and hydrolyze the β-(1, 4) linkages of chitin (Li 2006). The enzymes occur in a broad range of organisms including viruses, bacteria, fungi, insects, plant, and animals. Plant chitinases are participatory in defense and development (Graham and Sticklen, 1994). Moreover, chitinases have shown an immense potential application in agricultural, biological and environmental fields (Li, 2006). It has been proposed that chitinase transgene protein may function to provide fungal pathogen resistance at both direct and indirect levels. On the direct level it degrades chitin of growing hyphae, whereas, on the indirect level it results in the release of chitin oligomers which can act as elicitors of plant defense mechanisms (Collinge *et al.,* 1993). Several authors indicated to the important role of chitinase gene in producing antifungal activity in plants, Furthermore, chitinase gene was introduced in numerous crops as a disease resistance gene as in rice by Nishizawa *et al.* (1999), Kim *et al.* (2003), Sridevi *et al.* (2003), Datta *et al.* (2004) and Nandakumar *et al.* (2007), and in sorghum by Krishnaveni *et al.* (2001), and also in barley by Tobias *et al.* (2007) and in sugarcane by Khamrit *et al.* (2012). Also, Shin *et al.* (2008) developed wheat cultivar cv. Bobwhite by biolistic bombardment of chitinase gene and produced seven transgenic plants. Recently, Huang *et al.* (2013) produced transgenic wheat developed by chitinase gene *via* particle bombardment of immature embryos and affirmed that polymerase chain reaction analysis indicated that chitinase gene was transferred into 17 transformants with transformation frequency of 1.8%. Introduction of disease resistance genes into wheat genome are considered necessary, since wheat production in Egypt is decreased drastically due to infection with wide range of fungal pathogens. This goal could be attained *via* gene transfer technology. The aim of the present investigation is to develop Egyptian wheat (*Triticum aestivum* L.) cultivar cv. Giza 164 with improved disease resistance by introduction of chitinase gene which has a role in plant defense *via* catalyzing the degradation of chitin, which is the major constituent of many fungal cell wall using modern biotechnology tools.

MATERIALS AND METHODS

Sterilization and isolation of explants

Egyptian wheat cultivars Giza 164 seeds were obtained from the Department of Wheat, Field Crops Institute, Agricultural Research Center (ARC), Ministry of

Agriculture and Land Reclamation, Egypt. Wheat spikes were collected from field grown plants approximately 10-12 days post anthesis. Immature caryopsis were removed from spikelets under aseptic condition in laminar flow hood (NUAIRE™, USA) where grains were surface sterilized with 20% (v/v) commercial Clorox® (5.25% Sodium hypochlorite) supplemented with few drops of Tween 20, followed by soaking for 2 min in sterile DD H_2O, then rinsed five times in sterile DD H_2O. Semi-transluscent immature embryos 1-1.25 mm in size were aseptically dissected under a stereo binocular microscope.

Culturing of explants

Immature embryos were cultured with scutellum side up onto callus induction medium (CIM) (Weeks *et al.*, 1993) basically contains Murashige and Skoog salts (Murashige and Skoog, 1962) supplemented with 0.15 g of L-Asparagine, 0.1 g of myo-inositol, 20 g sucrose, 2.5 g Phytagel as a solidifying agent and 2.0 mg/l 2,4-Dichlorophenoxyacetic acid (2,4-D) as an auxin source, then incubated at dark for five days from culturing before bombardment in controlled growth chamber (Shel-Lab, USA) at 25°C.

Biolistic gene transformation

After one week from culturing, twenty five immature embryo-derived calli were then transferred to CIMS osmotic medium (CIM medium supplemented with 0.4 M sorbitol) for four hours before bombardment. Twenty five immature embryo-derived calli were placed in the center of a Petri dish (15x100 mm) and co-bombarded using the helium driven Biolistic Delivery system (Model PDS-1000/He, Bio-Rad) under a vacum of 25 in. Hg Vac with l.0 µ Golden microcarriers (BIO-RAD) coated with the two plasmids; the first plasmid is pAHC25 (10.0 kb), containing *gus (uidA)* (4.181 kb), (Jefferson *et al.*, 1987) and *bar* (2.871 kb) cassettes (Thompson *et al.*, 1987) both under control of *Ubil* promoter (Christensen *et al.*, 1992) (Fig. 1A) and the second plasmid is pAHC20Ubi383 containing chitinase gene, fragment containing the cauliflower mosaic virus transcription terminator (CaMV polyA) and the bar gene under the control of the maize ubiquitin promoter-intron (Fig. 1B). Target immature embryo-derived calli were bombarded at a distances of 6 cm from stopping screen with rupture disc strength of 1100 psi. After bombardment, calli were kept for additional 16 hr on the same osmotic medium (CIMS). Then, calli were transferred to CIM medium once more to recover for additional five days.

Histochemical analysis

After seven days from bombardment, histochemical staining analysis was conducted to study the expression of *gus* gene according to Jefferson (1987), where calli tissues samples were assayed for *gus* activity in *gus* buffer (Daniell *et al.*, 1991). Then Petri dishes were incubated at 37°C overnight. Transient expression was photographed under the microscope.

Selection and regeneration of transformed calli

After five days of recovery period, calli were then transferred to CIMB selection medium (CIM medium with 3 mg/l bialaphos) for two subcultures every three weeks. After the six weeks, the survived embryogenic calli were placed on MSRB regeneration selection medium (Murashige and Skoog's medium supplemented with 3 mg/l bialaphos and 0.125 mg/1 TDZ) for two weeks at growth conditions of 25°C, 25-50 µl E/m^2 light intensity and 16 hr photoperiod. Finally, the green regenerated shoots produced were then transferred onto FMSB rooting selection medium (free Murashige and Skoog medium containing 1 mg/l bialaphos) for two weeks. Then, healthy rooted plantlets were established under the FMSB rooting selection medium, and were then transferred into soil pots, then incubated in controlled growth chamber (Conviron®) for acclimation and subsequently transferred to greenhouse until seed setting.

Leaf painting assay

In order to examine the expression of the *bar* gene in plants, a freshly prepared aqueous solution of 0.2% glufosinate-ammonium herbicide was applied on the mid-lamina portion (about 2.5 cm long) of the second/third youngest leaf using a cotton plug swap according to Schroeder *et al.* (1993).

DNA isolation and analysis

Putative transgenic plants were subjected to molecular analysis to confirm the integration of the foreign genes (*gus*, *bar* and *chi*) into plant genome by PCR analysis. Total genomic DNA of putative transgenic plants and controls (non-transgenic) were isolated using DNeasy Plant Mini Kit (Qiagen, Germany).

Polymerase Chain Reaction (PCR)

DNA was subjected to PCR analysis for *gus*, *bar* and *chi* genes. Specific oligonucleotide primers for *gus*, *bar* and *chi* were used (Table 1). DNA amplifications were performed in a thermal cycler using initial denaturation at 94°C for 5 min, followed by 30 cycles of 1 min at 94°C, 1 min at 58°C (for *gus* gene), 62°C (for *bar* gene), 60°C (for *chi* gene) and 2 min at 72°C. One additional complete extension cycle was performed for 10 min at 72°C. At the end of the cycles, 5 µ1 of the reaction products was mixed with 8 µ1 loading buffer and electrophoresed on 1% agarose gel at 80 Volt.

Dot blot hybridization analysis

Dot-Blot hybridization analysis was conducted to confirm the integration of chitinase gene in the transgenic plants. DNA from transgenic and non-transgenic plants (negative control) as well as the transformation vector pAHC20Ubi383 (positive control) were used in this experiment. DNAs were de-naturated by heating at 95°C for 10 min., and then were directly spotted onto nitrocellulose membrane followed by a fixation step by exposure to UV waves for 1 min. The plasmid pAHC20Ubi383 was used as probe. Labeling and detection procedure was ac-

complished using DNA Labeling and Biotin Chromogenic Detection Kit (Thermo Scientific) by random primed DNA labeling with digoxigenin-dUTP, alkali label and detection of hybrids by enzyme immunoassay according to the kit instruction manual.

RESULTS AND DISCUSSION

In our present research, immature embryo derived calli were co-transformed with the two previously mentioned plasmids pAHC25 and pAHC20Ubi383. Embryo-derived calli (625 calli) were subjected to the co-transformation process *via* microprojectile bombardment at the condition previously mentioned before. Following bombardment, histochemical staining analysis for bombarded callus was performed in a separate experiment to study the *gus* gene expression. Consequently, *gus* gene expression was photographed under the microscope (Fig. 3a). After a recovery period of five days from bombardment, calli were subsequently transferred to selection medium (CIMB medium) for two subcultures every three weeks. Table (2) and Fig. (2) demonstrated that the total number of survived callus achieved after this selection phase onto CIMB medium was 175 calli. After six weeks, the 175 calli survived on CIMB selection medium were then placed onto regeneration selection medium (MSRB) for two more weeks, where, 71 calli out of the 175 survived calli on CIMB selection medium regenerated shoots. Subsequently, regenerated shoots were then transferred onto FMSB rooting selection medium for two more weeks. After the two weeks, a total number of 111 healthy rooted plantlets were successfully established under the FMSB rooting selection medium, and were then transferred into soil pots for acclimatization in the control growth chamber (Conviron®). Subsequently, a total number of 55 plants succeeded in acclimatization and reached seed setting in biocontainment greenhouse. Thereafter, leaf painting assay was conducted to examine the expression of the *bar* gene in plants as previously mentioned (Fig. 3b). Putative transgenic plants were subjected to molecular analysis to verify the integration of the foreign genes (*gus*, *bar* and *chi*) into plant genome by PCR analysis. Total genomic DNA of putative transgenic plants and control (non-transgenic) were isolated using DNeasy Plant Mini Kit (Qiagen, Germany). DNA was then subjected to PCR analysis for *gus*, *bar* and *chi* genes. The PCR products were separated on 1% agarose gel. PCR results revealed products of the expected sizes for all transgenes; 523 bp for *chi* gene, 1050 bp for *gus* gene and 443 bp for *bar* gene as shown in Fig. (4 A, B and C). Also, PCR analysis affirmed that total number of 15, 17 and 11 plant's transgene insertion were positive for *gus*, *bar* and *chi* gene, respectively. In addition, Dot-Blot hybridization analysis was used to confirm the integration of the *chi* gene. Data confirmed the integration of *chi* gene in all eleven positive PCR plants (Fig. 5). Results of PCR and Dot-blot analysis pointed to that the frequency of chitinase gene transformation process scored 1.8% (Table 1). All produced

plants were fertile and set seeds (Fig. 2), which indicate that transgenes insertion did not affect the fertility of plants or seed setting. Also, Nandakumar *et al.* (2007) transformed four rice genotypes of with chitinase, and its integration was confirmed through polymerase chain reaction (PCR) with transformation efficiency ranged from 0.9 to 5.2%. Lately, Huang *et al.* (2013) produced transgenic wheat developed by chitinase gene *via* particle bombardment of immature embryos and affirmed that polymerase chain reaction analysis indicated that chitinase gene was transferred into 17 transformants with a transformation efficiency of 1.8%.

Wheat production in Egypt decreased drastically due to infection with wide range of pests and pathogens (e.g., rusts, sheath blight, scab and insects). Our gene of interest chitinase is an antifungal protein as classified by Wani (2010). Chitinase, limit fungal growth by degrading the major structural polysaccharide of fungal cell walls (Leah *et al.*, 1991). Therefore, production of transgenic Egyptian wheat plants with improved disease resistance is a precious component for a disease management program, in this respect; it is desirable to transform wheat with genes that are capable for conferring broad and durable fungal resistance. Here, we transformed the Egyptian wheat cultivar cv. Giza 164 with *chi* gene, which plays an important role in improving wheat fungal resistance *via* catalyzing the degradation of cell wall chitin of many fungi. Thus, the objectives of the present investigation directly is the development of Egyptian wheat with improved disease resistance *via* chitinase gene introduction, and indirectly is increasing wheat production, and therefore, decreasing equivalently wheat importation, and accordingly saving a huge percentage from the Egyptian foreign currency.

SUMMARY

Plant diseases are caused by a variety of plant pathogens including fungi, and their management requires the use of techniques like transgenic technology and genetics. The chitinase gene, known to have a vital role in fungal disease defense, was introduced into the Egyptian wheat cv. Giza 164 *via* biolistic bombardment. Immature embryo derived calli were subcultured on CIMB medium containing bialaphos. After six weeks, the survived embryogenic calli were placed on MSRB regeneration selection medium. Finally, the green regenerated shoots produced were then transferred onto FMSB rooting selection medium. After the two weeks, healthy rooted plantlets were established under the rooting selection medium, and were then transferred into soil pots, then incubated in control growth chamber for acclimatization and subsequently transferred to greenhouse until seed setting. Integration of the transgene with a transformation frequency of 1.8% was confirmed by PCR and dot-blot analyses.

ACKNOWLEDGMENT

This research was supported by The US - Egypt Science and Technology (S&T) Joint, Contract/Agreement No.:

301. We sincerely thank Biochemistry Department, Kansas State University, for giving us opportunity to work at their Labs providing us with the inputs, facilities and motivation for this study.

REFERENCES

Biesaga, J., J. Koscielniak and A. Janeczko (2010). The impact of zearalenone and thidiazuron on indire plant regeneration of oilseed rape and wheat. Acta Physiologiae Plantarum, 32: 1047-1053.

Chauhan, H., S. A. Desai and P. Khurana (2007). Comparative analysis of the differential regeneration response of various genotypes of *Triticum aestivum*, *Triticum durum* and *Triticum dicoccum*. Plant Cell Tissue and Organ Culture, 91: 191-199.

Chen, W. P., P. D. Chen, D. J. Liu, R. Kynast, B. Friebe, R. Velazhahan, S. Muthukrishnan and B. S. Gill (1999). Development of wheat scab symptoms is delayed in transgenic wheat plants that constitutively express a rice thaumatin-like protein gene. Theor. Appl. Genet., 99: 755-760.

Cho, J., H. Hur-HanSun and L. JangYong (2004). Screening of Korean rice cultivars for their shoot regeneration ability *in vitro* and the effect of maltose on shoot regeneration. Korean Journal of Breeding, 3: 229-236.

Collinge, D. B., K. M. Kragh, J. D. Mikkelsen, K. K. Nielsen, U. Rasmussen and K. Vad (1993). Plant chitinases. Plant J., 3: 31-40.

Daniell, H., M. Krishnan and B. F. Mcfadden (1991). Transient expression of β-glucurobidase in different cellular compartments following Biolistic delivery of foreign DNA into wheat leaves and calli. Plant Cell Report, 9: 615-619.

Datta, K., M. Rai, N. Baisakh, N. Oliva, M. T. Khin, J. M. Tu and S. K. Datta (2004). Transgenesis-breeding for multiple plant protection in rice. Philippine Journal of Crop Science, 29: 33-43.

Fahmy, A. H. and O. M. El-Shihy (2006). Improvement of plant regeneration from long-term callus cultures of two Egyptian wheat cultivars. World Journal of Agricultural Sciences, 2: 274-281.

Fahmy, A. H., K. S. Abdalla and M. Abdallah (2007). Integration and expression of the high-molecular-weight glutenin subunit Dy10 gene into Egyptian wheat. Arab Journal of Biotechnology, 10: 49-56.

Fahmy, A. H., Y. H. El-Shafy, O. M. El-Shihy and M. A. Madkour (2006). Highly efficient regeneration *via* somatic embryogenesis from immature embryos of Egyptian wheat cultivars (*Triticum aestivum* L.) using different growth

regulators. World Journal of Agricultural Sciences, 2: 282-289.

Graham, L. S. and M. B. Sticklen (1994). Plant chitinases. Can. J. Bot., 72: 1057-1083.

He, Y., H. D. Jones, S. Chen, X. M. Chen, D. W. Wang, K. X. Li, D. S. Wang and L. Q. Xia (2010). *Agrobacterium*-mediated transformation of durum wheat (*Triticum turgidum* L. var. durum cv Stewart) with improved efficiency. Journal of Experimental Botany, 61: 1567-1581.

Huang, X., W. Jain, D. Zhen, Z. Chen, L. Lan and X. Zinqin (2013). Enhanced resistance to stripe rust disease in transgenic wheat expressing the rice chitinase gene *RC24*. Transgenic Research, 22: 939-947

Jefferson, R. A., T. A. Kavanagh and M. W. Bevan (1987). GUS fusion: beta-glucuronidase as a sensitive and versatile gene fusion marker in higher plants. EMBO Journal, 6: 3901-3907.

Khamrit, R., J. Prasit and B. Sumontip (2012). Callus induction, regeneration and transformation of sugarcane (*Saccharum officinarum* L.) with chitinase gene using particle bombardment. African Journal of Biotechnology, 11: 6612-6618.

Kim, J. K., I. C. Jang, R. Wu, W. N. Zuo, R. S. Boston, Y. H. Lee, I. P. Ahn and B. H. Nahm (2003). Co-expression of a modified maize ribosome-inactivating protein and a rice basic chitinase gene in transgenic rice plants confers enhanced resistance to sheath blight. Transgenic Research, 12: 475-484.

Krishnaveni, S., J. M. Jeoung, S. Muthukrishnan and G. H. Liang (2001). Transgenic sorghum plants constitutively expressing a rice chitinase gene show improved resistance to stalk rot. Journal of Genetics & Breeding, 55: 151-158.

Leah, R., H. Tommerup, I. Svendsen, and J. Mundy (1991). Biochemical and molecular characterization of three barley seed proteins with antifungal properties. J. Biol. Chem., 266: 1564-1573.

Li, Duo-Chuan (2006). Review of fungal chitinases. Mycopathologia, 161: 345-360.

Mahmood, I., Z. Ahmed, M. Munir, S. I. Malik and K. Shahzad (2009). Effect of sorbitol on callus induction and plant regeneration in wheat. African Journal of Biotechnology, 8: 6529-6535.

Monostori, T., E. Rozik, T. G. Bus and L. Tanacs (2008). The use of field grown plant material in somatic

tissue cultures of spring wheat genotypes. Cereal Research Communications, 36: 1135-1138.

Munazir, M., R. Qureshi, G. M. Ali, U. Rashid, S. Noor, K. Mehmood, S. Ali and M. Arshad (2010). Primary callus induction, somatic embryogenesis and regeneration studies in selected elite wheat varieties from Pakistan. Pakistan Journal of Botany, 42: 3957-3965.

Murashige, T. and F. Skoog (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum, 15: 473-497.

Nandakumar, R., S. Babu, K. Kalpana, T. Raguchander, P. Balasu-bramanian and R. Samiyappan (2007). *Agrobacterium*-mediated trans-formation of indica rice with chitinase gene for enhanced sheath blight resistance. Biologia Plantarum, 51: 142-148.

Nishizawa, Y., Z. Nishio, K. Nakazono, M. Soma, E. Nakajima, M. Ugaki and T. Hibi (1999). Enhanced resistance to blast (*Magnaporthe grisea*) in transgenic *japonica* rice by constitutive expression of rice chitinase. Theor. Appl. Genet., 99: 383-390.

Raja, N. I., H. Rashid, A. Bano and Z. Chaudhry (2008). Effect of growth regulators on enhanced plant regeneration from embryo derived calli of wheat (*Triticum aestivum* L.). Pakistan Journal of Agricultural Research, 21: 65-71.

Rashid, H., Z. Chaudhry and M. H. Khan (2011). Effect of explant plant source and acetosyringone concentration on transformation efficiency of wheat cultivars. African Journal of Biotechnology, 10: 8737-8740.

Schroeder, H. E., A. H. Schotz, T. Wardley-Richardson, D. Spencer, and T. J. V. Higgins (1993). Transformation and regeneration of two cultivars of pea (*Pisum sativum* L.). Plant Physiol., 101: 751-757.

Shi, Z. Y., G. X .Yin, L. P. Du, H. J. Xu, and X. G. Ye (2011). Plant regeneration and *Agrobacterium*-mediated transformation using large immature embryos of wheat. Scientia Agricultura Sinica, 44: 225-232.

Shin, S. H., C. A. Mackintosh, J. Lewis, S. J. Heinen, L. Radmer, R. Dill-Macky, G. D. Baldridge, R. J. Zeyen and G. J. Muehlbauer (2008). Transgenic wheat ex-pressing a barley class II chitinase gene has enhanced resistance against *Fusarium graminearum*. Journal of Experimental Botany, 59: 2371-2378.

Sridevi, G., N. Sabapathi, P. Meena, R. Nandakumar, R. Samiyappan, S. Muthukrishnan and K. Veluthambi (2003). Transgenic *indica* rice variety Pusa Basmati 1 constitutively expressing a rice chitinase gene exhibits enhanced resistance to *Rhizoctonia solani*. Journal of Plant Biochemistry and Biotechnology, 12: 93-101.

Thompson, C. J., N. R. Movva, R. Tizard, R. Crameri, J. E. Davies, M. Louwereys and J. Botterman (1987). Characterization of the herbicide resistance gene *bar* from *streptomyces hygroscopicus*. The EMBO Journal, 6: 2519-2523.

Tobias, D. J., M. Manoharan, C. Pritsch and L. S. Dahleen (2007). Co-bombardment, integration and expression of rice chitinase and thaumatin-like protein genes in barley (*Hordeum vulgare* cv. Conlon). Plant Cell Rep., 26: 631-639.

Varshney, A. and F. Altpeter (2002). Stable transformation and tissue culture response in current European winter wheats (*Triticum aestivum* L.). Molecular Breeding, 8: 295-309.

Wada, Y., N. A. Carsono, L. Tong and T. Yoshida (2009). Genetic transformation of a high molecular weight glutenin (Glu-1Dx5) to rice cv. Fatmawati. Plant Production Science, 12: 341-344.

Wani, S. H. (2010). Inducing fungus-resistance into plants through biotechnology. Not. Sci. Biol., 2: 14-21

Weeks, J. T., O. D. Anderson and A. E. Blechl (1993). Rapid production of multiple independent lines of fertile transgenic wheat (*Triticum aestivum*). Plant Physiology, 102: 1077-1084.

Yao, Q., L. Cong, G. Y. He, J. L. Chang, K. X. Li and G. X. Yang (2007). Optimization of wheat co-transformation procedure with gene cassettes resulted in an improvement in transformation frequency. Molecular Biology Reports, 34: 61-67.

Yu, Y., J. Wang, M. L. Zhu and Z. M. Wei (2008). Optimization of mature embryo-based high frequency callus induction and plant regeneration from elite wheat cultivars grown in China. Plant Breeding, 127: 249-255.

Table (1): Specific PCR primers for *gus*, *bar and chi* genes.

Gene	Sequence	Fragment size (bp)
Gus R	5`-AGTGTACGTATCACCGTTTGTGTGAAC-3`	1050
Gus F	5`-ATCGCCGCTTTGGACATACCATCCGTA-3`	
Bar R	5`-CAGATCTCGGTGACGGGCAGGC-3`	443
Bar F	5`-CCGTACCGAGCCGCAGGAAC-3`	
Chi R	5`-GTTATTGCGGGACCGATGACAG-3`	523
Chi F	5`-CAGAACCAGAACGCCGCCTTGAAC-3`	

Table (1): Transformation characteristics of Egyptian wheat cv. Giza 164 cultivar representing numbers of bombarded callus, survived callus on selection, shooted callus on selection, regenerated plantlets on selection, acclimatized plants, Gus gene positive PCR plants, *bar* gene positive PCR plants, *chi* gene positive PCR plants and *chi* gene Dot Blot positive plants.

Shoot number	Number of bombarded callus	Number of survived callus on selection	Number of shooted callus	Number of regenerated plantlets	Number of acclimatized plants	Gus gene +ve PCR plants	*bar* gene +ve PCR plants	*chi* gene +ve PCR plants	*chi* gene Dot Blot +ve plants
1	25	4	1	2	1	0	0	0	0
2	25	8	4	6	4	1	2	0	0
3	25	9	3	3	1	0	0	0	0
4	25	6	3	5	2	1	1	0	0
5	25	7	1	1	0	0	0	0	0
6	25	6	4	7	3	1	0	1	1
7	25	5	1	2	2	1	0	0	0
8	25	7	5	6	3	0	1	1	1
9	25	7	4	7	4	1	2	2	2
10	25	9	5	9	3	1	1	1	1
11	25	6	3	3	1	0	0	0	0
12	25	5	2	4	3	1	1	1	1
13	25	8	5	8	3	1	2	1	1
14	25	5	0	0	0	0	0	0	0
15	25	7	3	4	1	1	0	1	1
16	25	10	5	9	5	2	2	1	1
17	25	4	1	3	1	0	1	0	0
18	25	8	2	5	3	1	0	0	0
19	25	9	3	4	3	0	1	0	0
20	25	6	0	0	0	0	0	0	0
21	25	8	3	3	1	0	0	0	0
22	25	8	5	7	5	2	1	1	1
23	25	9	4	8	4	1	1	1	1
24	25	6	1	2	0	0	0	0	0
25	25	8	3	3	2	0	1	0	0
Sum	625	175	71	111	55	15	17	11	11
Transformation Efficiency %	-	-	-	-	-	2.4	2.7	1.8	1.8

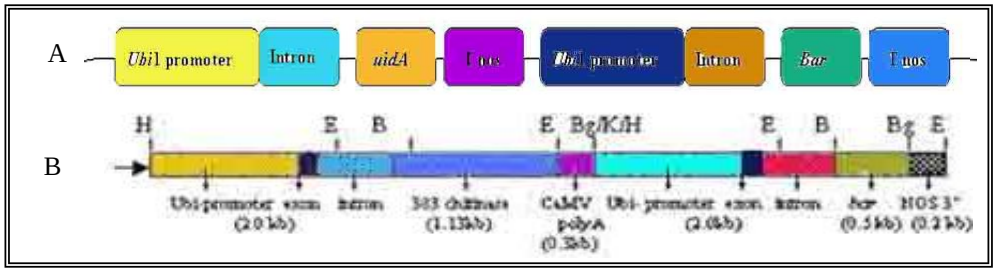

Fig. (1): Schematic representation of the two plasmids (A) pAHC25 plasmid and (B) pAHC20Ubi383 plasmid.

Fig. (2): Production stages of chitinase gene transfer into wheat cultivar cv. Giza 164 plants. (A) immature embryos cultured on CIM medium, (B) induced callus on CIM medium, (C) induced callus on CIMS medium, (D) bombarded callus on CIM recovery medium, (E) non-transformed calli on CIMB selection medium, (F) Bialaphos tolerant calli (putative transgenic) on MSRB regeneration selection medium showing putative transgenic shoots, (G) putative transgenic plantlets on FMSB selection rooting medium, (H) putative acclimatized transgenic plants incubated in control growth chamber (Conviron®), (I) fertile transgenic plants grown onto soil pot in bio-containment greenhouse and (J) spike showing seeds filling.

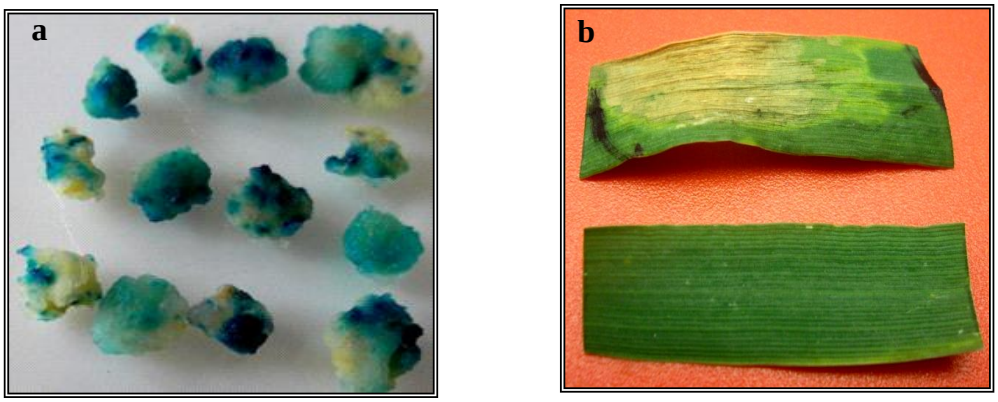

Fig. (3): (a) transient expression of the marker gene (*gus*) in transgenic wheat callus, (b) leaf painting assay examining the *bar* gene expression in transgenic plants: the upper leaf from un-transformed plant (control) showing leaf necrosis and the lower leaf from transgenic plant showing leaf resistance to 0.2% glufosinate-ammonium herbicide.

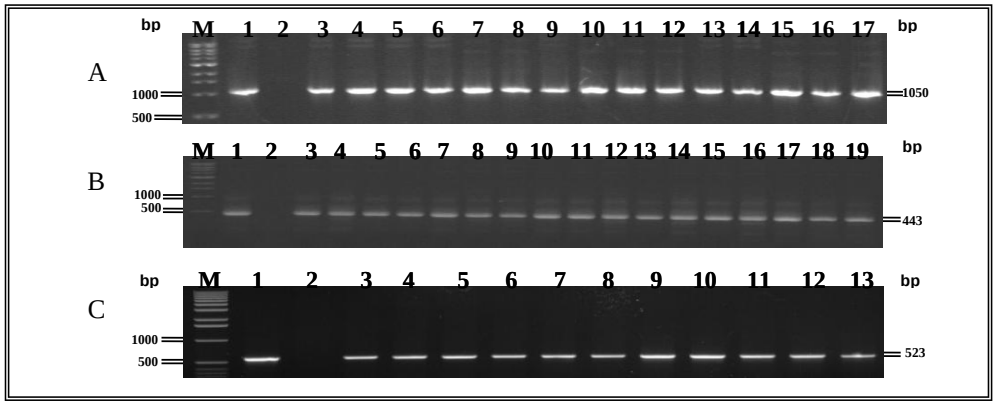

Fig. (4): PCR analysis of T0 plants. (A) Amplification product of *gus* gene (1050 bp). (B) Amplification product of *bar* gene (433 bp). (C) Amplification product of *chi* gene (523 bp). Lane M is DNA marker (1 kb ladder). Lane 1: positive control (plasmid), Lane 2 is non-transformed wheat cv. G164 (negative control). Other Lanes are the transgenic wheat plants.

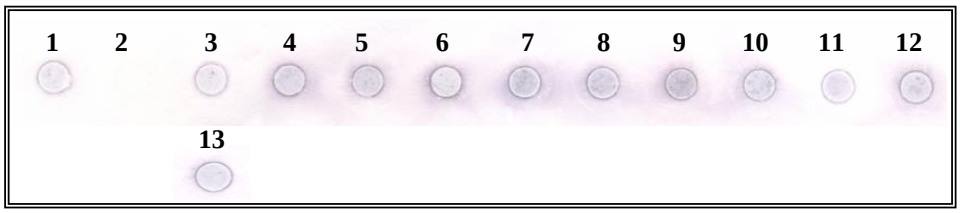

Fig. (5): Dot blot analysis of transgenic wheat cv. G164 plants; Dot 1 is pAHC20Ubi383 plasmid (positive control), Dot 2 is non-transformed wheat cv. G164 (negative control) and Dots 3-13 are the 11wheat chitinase transgenic plants.