Developing of transgenic wheat cultivars for improved disease resistance

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ABSTRACT

Wheat (Triticum aestivum L.) and (Triticum turgidum L.) are largely cultivated in the world. They are economically important because they can be grown in a wide range of climates and geographic regions. Wheat plants are exposed to a wide variety of disease pathogens, i.e., fungal, bacterial, and viral pathogens. The present work is an attempt to develop disease-resistant wheat cultivars. In this respect, immature embryo-derived calli of 2 wheat cultivars; Sids 1 (bread wheat) and Bani Suef 6 (durum wheat) were transformed by rice chitinase (cht 2), gus reporter gene, and selectable marker (bar) genes using particle bombardment technique. Transient gus expression in calli and stable gus expression in transformed nodes were observed. Transgenic calli were selected on phosphinothricin containing regeneration medium, and putative transformants were grown to maturity. 40 herbicide-resistant putative transformants were selected after leaf painting with 0.2% liberty herbicide. Presence and integration of transgenes were assessed by subjecting transgenic plants to polymerase chain reaction analysis using specific primers for gus, bar, and cht 2 genes. Transformation frequencies for cht 2 were 3.96% and 3.02% in Sids 1 and Bani Suef 6, respectively. The incorporation of rice chitinase gene in transformants was confirmed by Dot blot analyses.

1. INTRODUCTION

Wheat (Triticum aestivum L.) is the first important and strategic cereal grain crop for the majority of world’s populations. Its share of 15% proves importance of wheat production in the world economy from 1500 million hectares arable land in the world. This rate is equivalent to 225 million hectares of wheat area based on FAO figures for 2009. Wheat is an important cereal crops and an essential ingredient of the human diet indisputably in the most worldwide. Wheat provides nearly 55% of the carbohydrates and 20% of the food calories consumed globally [1]. It exceeds in acreage and production every other grain crop (including rice, maize, etc.).

Wheat plants are exposed to a wide variety of disease pathogens, i.e., fungal, bacterial, and viral pathogens. In nature, spikes, leaves, and roots of wheat plants can be infected, and that led to substantial yield loss. Chemical application is not economical and is also detrimental to the environment. Developing of genetically resistance wheat varieties is the most economical and efficient way to protect wheat from pathogens [2]. Chitinases (EC 3.2.1.14) catalyze the hydrolytic cleavage of the β-1,4-glycosidic bonds between biopolymers N-acetyl-glucosamine residues from the chitin molecule [3-6]. Chitinase proteins are known as pathogenesis-related proteins that are strongly induced when host plant cells are challenged by pathogen stress, and thus chitinases constitute an important arsenal of plants against fungal pathogens. There are some reports that chitinase activity in transgenic plants increased the inhibition of fungal growth, improving resistance against fungal attack and increase resistance to a wide range of disease pathogens [7-11].

The objective of the present study was to improve disease resistance of wheat cultivars (T. aestivum L. cv. Sids 1) and (T. turgidum ssp. Durum cv. Bani Suef 6) by introducing rice chitinase gene cht 2 which confers a wide range of disease resistance.

2. MATERIALS AND METHODS

2.1. Plant Material and Tissue Culture

Immature caryopsis of the two Egyptian wheat cultivars Sids 1 and Bani Suef 6 was collected approximately 2 weeks post-anthesis. Immature seeds were surface sterilized by soaking for 1 min in 70% (v/v) ethanol followed by 20% commercial Clorox (5.25% sodium hypochlorite) supplemented with few drops of Tween 20 and washed...
5 times with sterile ddH_2O. Immature embryos for each cultivar, scutella 1–1.25 mm in diameter, were aseptically isolated and cultured scutellum side up on callus induction medium modified for wheat cell culture [12]. Basically, it contains Murashige and Skoog (MS) salt [13], supplemented with 2 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D) as a source of auxin, 150 mg/L of L-Asparagine, 100 mg/L of myo-inositol, 20 g/L sucrose and adjusted to 5.8 pH with 1 M KOH solution and solidified by 2.5 g/L phytagel [14]. Immature embryos were maintained in the dark at 25°C for 1 week.

2.2. DNA Constructs

Two different plasmids were used for cobombardment experiments: PAHCht-2 (kindly provided by Prof. Dr. S. Muthukishnan; Department of Biochemistry, Kansas State University, Kansas, USA) contains the Cht-2 gene, which is a 1.1 Kb rice chitinase Class I under the transcriptional control of the maize ubiquitin promoter [Figure 1a] and pAB6 [Figure 1b] contains gus (1.8 Kb) gene (driven by rice Act1 intron promoter and the nopalin synthase nos terminator and the selectable marker/herbicide resistance bar (0.6 Kb phosphinothricin acetyl transferase) gene (driven by cauliflower mosaic virus 35S promoter and the nopalin synthase nos terminator) [15].

2.3. Bacterial Strain

The highly efficient competent cells of Escherichia coli (DH10β) were used for the transformation with the plasmids DNA and prepared according to the method of Ausubel et al. [16].

2.4. Plasmid Transformation into E. coli Competent Cells

Calcium chloride treatment of E. coli (DH10β) produces competent cells that will transform by the pAB6 plasmid or pAHCht-2 plasmid using heat shock step according to Tu et al. [17].

2.5. Plasmid Purification

Bacteria harboring the pAB6 plasmid or pAHCht-2 plasmid were grown in liquid Luria broth medium (pH 7.4) at 37°C in a growth shaker incubator. Megaprep purification of DNA plasmid was done using Wizard™ Megapreps DNA Purification System (Promega, USA).

2.6. Preparation and Coating of Gold Particles with Plasmid DNA

Microcarriers (1 µm gold particles) were prepared and coated with plasmid DNA according to the protocol by Sanford et al. [18].

2.7. Wheat Transformation

The transformation procedure was performed as modified by Fahmy et al. [12]. A 1:1 ratio of pAB6 and pAHCht-2 were cotransformed into Sids1 and Bani Suef 6 calli. Plant transformation was carried out by particle bombardment using the Biolistic® PDS-1000/He particle gun device (Bio-Rad, USA). 1-week-old calli were transferred to a modified callus induction medium (supplemented with 0.2 M mannitol and 0.2 M sorbitol) for 4 hours followed by bombardment with 0.6 µ gold particles coated with plasmid DNA. The distance between the particle holder and target was 6 cm, and helium pressure was 1100 psi. Calli were remained for additional 16 h on the same osmotic treatment and then transferred to recovery medium for 5 days. Calli were then assayed by histochemical GUS activity assay. The remaining calli were transferred to selective medium supplemented with 3 mg/L PPT. After 4 weeks the survived embryogenic calli were placed on regeneration medium supplemented with 1 mg/L PPT and 1.5 mg/L thidiazuron (TDZ) for 4 weeks under light (16 h) and temperature 25°C and finally the green shoots were placed on rooting medium (half strength MS medium).

2.8. Acclimatization

After development of a root system, regenerated putatively transgenic plants were then transferred to soil mixture; peat moss:sand:clay with a ratio (1:1:1); respectively, in small pots and covered with plastic pages, and then placed in a controlled growth chamber at 25°C for 3 weeks, they were transferred to big pots and grown to maturity under greenhouse conditions.

Figure 1: Schematic representation of plasmids pAHCht-2 (a) and pAB6 (b) used for cobombardment.
2.9. Assay of β-glucuronidase (gus) Activity
GUS assay was carried out as described by Jefferson et al. [19]. Calli were incubated in X-Glue solution containing 1 mM (5-bromo-4-chloro-3-indolyl-β-D-glucuronic acid), 0.1% (v/v) Triton X-100, 20% methanol and 100 mM sodium phosphate buffer (pH 7.0), and 0.5 mM potassium ferricyanide. To compare transient and stable gus transformation, GUS analysis was done.

2.10. Transient gus Expression
For observing transient gus expression, bombardment calli after 2 days on induction medium were dipped in GUS staining solution and were incubated at 37°C for 2–3 days and then callus expressing gus photographed under the binocular stereomicroscope.

2.11. Stable gus Expression
For observing stable gus expression, regenerated plants were assayed by dipping transformed plantlets into GUS staining solution. The reaction mixture was incubated at 37°C for 2–3 days, chlorophyll was extracted from the tissue by incubation in 70% ethanol followed by 100% ethanol and regenerated plants expressing gus were photographed under the binocular stereomicroscope.

2.12. Assay of bar Expression Analysis
Leaf painting assay was used to study the integration and expression of the bar gene in T0 plants according to Cho et al. [20]. Liberty solution (0.2%), containing 0.1% (v/v) Tween-20, was applied to leaf sections using a cotton swab. Resistance to the herbicide solution was examined after 7 days of application by observing leaf necrosis.

2.13. Polymerase chain reaction (PCR) analysis
Total genomic DNA was isolated from wheat leaves using a cetyltrimethylammonium bromide extraction method [21]. The PCR analysis was used to confirm the presence or the absence of the three transgenes (cht 2, bar, and gus) in the transformed plants. The specific primers used to amplify of cht 2 gene were 5'TAAGGATGTCGACCGAGAGG'3 and 5'CGTACGTCCTCATACCTGCTCCCGG'3. The forward and reverse primers employed for detection of bar gene were 5’CAG ATC TCG GTG ACG GCC AGG C3’ and 5’ CCG TAC CGA GCC GGA AC -3’; and for gus gene were 5’AGT GTA CGT ATC ACC GTG TGT GTG AAC 3’ and 5’AGT GTA CGT ATC ACC GT ACC TTG TGT GAA C3’. The PCR program profile for three genes was done as follow: Initial denaturation at 94°C for 5 min, followed by 35 cycles at 94°C for 30 sec, annealing for 30 sec and 72°C for 1 min and finally, an additional elongation step was performed for 5 min at 72°C. The annealing temperature for the amplification of cht 2, bar, and gus genes was 61.3°C, 58°C, and 62°C, respectively. The PCR reaction mixture contained 50 ng of template DNA, 0.5 μM of each primer, 10 mM of deoxynucleotides, 2.5 mM of MgCl₂, PCR buffer, and Taq polymerase in a volume of 25 μl. The amplified products were electrophoretically resolved on a 1% agarose gel in Tris-acetate EDTA buffer.

2.14. Dot Blot Hybridization Analysis
Dot blot analysis was performed to confirm stable integration of the cht-2 gene. Total genomic DNA was isolated from wheat leaves of putative transgenic of both cultivars that showed the presence of 1.1 Kb chitinase amplification products in PCR analysis and from non-transgenic plants (negative control) and pAHCht-2 (positive control), they were denatured and neutralized with 0.4 M NaOH, 10 mM EDTA, incubated at 96°C for 10 min, then rapidly cooled in ice. Using a dot blot manifold, samples were spotted onto pre-soaked nitrocellulose membrane cover two pieces of Whatman paper. Membrane was cross-linked under UV light. Hybridization was performed at 45°C for overnight in a buffer containing 5× Denhardt’s solution, 6× sodium chloride-sodium citrate (SSC), 0.5% sodium dodecyl sulfate (SDS), and 50% (v/v) deionized formamide and followed by the addition of pAHCht-2 as a probe. Membrane was washed twice at room temperature in 2× SSC/0.1% SDS for 5 min followed by two washes in 0.1× SSC/0.1% SDS for 20 min at 70°C. Direct procedure and detection system were carried out by Biotin Chromogenic Detection Kit (Thermo Scientific). Blot was washed and product detection conducted by the addition of 5-bromo-4-chloro-3-indolyphosphate p-toluidine salt/nitro-blue tetrazolium chloride solution, then the blot was exposed to photography.

3. RESULTS
In the present study, the particle bombardment approach was used in the wheat transformation to produce transgenic plants, harboring cht 2 and bar genes to enhance disease resistance against fungal diseases and herbicides.

3.1. Callus Induction and Bombardment
Immature embryos of bread wheat cultivar (Sids 1) and durum wheat cultivar (Bani Suef 6) were used as explants for wheat transformation [Figures 2a and 3a]. In total, 500 immature embryos from each cultivar were cultured on callus induction medium [Tables 1 and 2]. Immature embryos from the two cultivars were cultured on callus induction medium containing 2 mg/L auxin (2,4-D) and incubated at 25°C in the dark for one week. Callus initiation was observed after 72 h for both wheat cultivars. After 1 week from incubation, cultured immature embryos from wheat cultivars were induced and formed callus as shown in Figures 2b and 3b. Callus induction rate (%) was calculated as number of explants forming callus/cultured immature embryos × 100. The callus induction rate was higher in case of bread wheat cultivar Sids 1 (75.6%) compared to 72.8 % callus induction rate in durum wheat cultivar Bani Suef 6.

Immature embryo-derived calli from both wheat cultivars were transferred and arranged in the center of a Petri dish containing osmotic medium and then cotransformed with gold particles coated with pAHCht-2 and pAB6 plasmids. Bombarded calli from both wheat cultivars were remained on osmotic medium for 16 h and then transferred into induction medium (recovery) for 5 days as shown in Figures 2d and 3d.

3.2. Callus Selection
After 5 days from bombardment, bombarded calli from both wheat cultivars were transferred to selection medium supplemented with 3 mg/L PPT. The number of survived calli after the first selection phase for resistant calli, which showed active proliferation, was 262 for cv. Sids 1 and 181 for cv. Bani Suef 6 that represented 69.3% and 49.7% for resistant calli, which showed active proliferation, was 262 for cv. Sids 1 and 181 for cv. Bani Suef 6 that represented 69.3% and 49.7% respectively.
3.3. Regeneration of Transgenic Wheat Plants

Survived calli from the two wheat cultivars were transferred onto regeneration medium containing 1.5 mg/L TDZ plus 1 mg/L PPT. After 4 weeks, the percentage of regeneration was calculated as number of callus lines with green plantlets/number of calli transferred for regeneration × 100. The percentage of regeneration was 63.15% for Sids 1 and 51.96% for Bani Suef 6 [Tables 1 and 2, Figures 2f and 3f].

The regenerated plantlets from three wheat cultivars were transferred to rooting medium [Figures 2g and 3g], and healthy plantlets were transferred to small pots and kept in growth chamber [Figures 2h and 3h], then transferred to big pots and placed on the greenhouse for acclimation [Figures 2i and 3i]. Results from Tables 1 and 2 showed that the transformation efficiency was 5.29% for cv. Sids 1 and 4.67% for cv. Bani Suef 6.

3.4. GUS Expression Analysis

Results from Figures 4a and c showed that the non-transformed calli for each wheat cultivars did not change in color, whereas transformed calli exhibited the characteristic blue color that indicates integration and expression of gus gene [Figure 4b and d].

3.5. Bar Expression Analysis

All acclimatized plants in both bread wheat cultivar (Sids 1) and durum wheat (cv. Bani Suef 6) showed a high level of resistance to the herbicide most likely because they carried two copies of the bar gene, one from each transformation plasmid. Non-transformed plants showed yellowing in the applied area after 3 days from Liberty application in both wheat cultivars [Figure 5a and c]. While transgenic plants of wheat cultivars stayed healthy and green [Figure 5b and d].
3.6. Screening of Transgenic Plants for the Transgenes

The leaf genomic DNAs extracted from all the independent putative transformants in both bread wheat cultivar (Sids 1) and durum wheat cultivar (cv. Bani Suef 6) and tested by PCR amplification using primers specific to cht-2, bar, and gus genes.

PCR results revealed products of the expected sizes for all transgenes: 1100 bp for cht-2 gene, 443 bp for bar gene, and 1050 bp for gus gene [Figures 6 and 7].

Transformation frequency for cht-2, bar, and gus genes recorded higher values in case of cv. Sids 1 calculated by 3.96%, 5.29%, and 5.02%, respectively, compared to 3.02%, 4.67%, and 3.57%, respectively, in durum wheat cultivar Bani Suef 6.

3.7. Dot Blot Analysis

The stable integration of cht-2 gene into the genome of the PCR positive transformants in bread wheat cultivar and durum wheat cultivar was also confirmed by cht-2 Dot blot assay [Figure 8]. Genomic DNA isolated from PCR-positive plants of both wheat cultivars was denatured, neutralized, and then subjected to Dot blotting using the probe for chitinase gene; non-transformed plant was used as negative control. The obtained results are similar to PCR analysis results, 15 transformed plants in the case of cv. Sids 1 and 11 transformed plants in the case of cv. Bani Suef 6 were positive for PCR analysis and Dot blot.

4. DISCUSSION

Although wheat represents the major food for wide world, it was the latest of cereals that transformed. Genetic transformation of wheat...
### Table 1: Transformation characteristics of bread wheat cultivar Sids 1

| Experiment | Number of explants (immature embryos) | Number of induced calli | Number of surviving calli on selection medium I | Number of surviving calli on selection medium II | Number of callus lines with green plantlets | Number of regenerated shoots | Number of acclimatized plantlets | Number of positive plants for PCR cht-2 | Transformation frequencies % |
|------------|--------------------------------------|-------------------------|-----------------------------------------------|-----------------------------------------------|-------------------------------------------|-------------------------------|-------------------------------------|-----------------------------|-------------------------------|
| I          | 100                                  | 68                      | 49                                            | 21                                            | 12                                       | 23                           | 5                                   | 4                          | 5                             | 7.35                          |
| J          | 100                                  | 71                      | 48                                            | 22                                            | 10                                       | 21                           | 4                                   | 3                          | 4                             | 5.63                          |
| K          | 100                                  | 78                      | 57                                            | 25                                            | 16                                       | 34                           | 6                                   | 3                          | 5                             | 6.41                          |
| L          | 100                                  | 76                      | 50                                            | 21                                            | 14                                       | 26                           | 3                                   | 2                          | 2                             | 2.63                          |
| M          | 100                                  | 85                      | 58                                            | 25                                            | 20                                       | 35                           | 5                                   | 3                          | 4                             | 4.70                          |
| Total      | 500                                  | 378                     | 262                                           | 114                                           | 72                                       | 139                          | 23                                  | 15                         | 20                            | 5.29                          |

Transformation frequencies % = (Number of positive plants for PCR / Number of bombarded calli) x 100. PCR: Polymerase chain reaction.

### Table 2: Transformation characteristics of durum wheat cultivar Bani Suef 6

| Experiment | Number of explants (immature embryos) | Number of induced calli | Number of surviving calli on selection medium I | Number of surviving calli on selection medium II | Number of callus lines with green plantlets | Number of regenerated shoots | Number of acclimatized plantlets | Number of positive plants for PCR cht-2 | Transformation frequencies % |
|------------|--------------------------------------|-------------------------|-----------------------------------------------|-----------------------------------------------|-------------------------------------------|-------------------------------|-------------------------------------|-----------------------------|-------------------------------|
| N          | 100                                  | 80                      | 35                                            | 19                                            | 8                                       | 29                           | 5                                   | 3                          | 5                             | 4                             | 6.25                          |
| O          | 100                                  | 67                      | 29                                            | 17                                            | 9                                       | 21                           | 3                                   | 2                          | 2                             | 2                             | 2.98                          |
| P          | 100                                  | 89                      | 45                                            | 27                                            | 16                                       | 37                           | 4                                   | 3                          | 4                             | 4                             | 4.49                          |
| Q          | 100                                  | 65                      | 35                                            | 18                                            | 11                                       | 21                           | 3                                   | 1                          | 3                             | 2                             | 4.61                          |
| R          | 100                                  | 63                      | 37                                            | 21                                            | 9                                       | 18                           | 3                                   | 2                          | 3                             | 1                             | 4.76                          |
| Total      | 500                                  | 364                     | 181                                           | 102                                           | 53                                       | 126                          | 18                                  | 11                         | 17                            | 13                            | 4.67                          |

Transformation frequencies % = (Number of positive plants for PCR / Number of bombarded calli) x 100. PCR: Polymerase chain reaction.
was low in successful because of many technical difficulties such as reduction of transformation frequencies, shortage of suitable explant source (immature embryos), which is not available throughout the year. Vasil et al. [22] reported the first fertile transgenic wheat using immature embryos. There are numerous reports employing immature and mature embryo, embryo derived-calli and scutellar tissue as the explants for transformation by biolistic gun [13,23-29].

In Egypt, bread wheat (*T. aestivum* L.) and durum wheat (*T. turgidum* ssp. Durum) are largely cultivated. The grain used for the human alimentation and animal feeds. In this study, rice chitinase gene was introduced into Egyptian wheat to improve pathogen resistance. Two Egyptian wheat cultivars have been used, bread wheat cultivar (Sids 1) and durum wheat cultivar (Bani Suef 6). The chitinase gene has been used for defending crop plants from fungal diseases. The advantages of using chitinases to protect plant are these fungicidal enzymes are part of the plant defense system and

**Figure 4:** Expression of *gus* gene in embryogenic callus derived from scutellum tissues of bread wheat plants (cv. Sids 1) and durum wheat (cv. Bani Suef 6) after 48 from bombardment with pAHCht-2 plasmid and pAB6 plasmid. (a) Non-transformed callus; (b) transformed callus of cv. Sids 1 (c) non-transformed callus; and (d) transformed callus of cv. Bani Suef 6.

**Figure 5:** Identification of the herbicide resistance of the leaves of transgenic wheat plants using 0.2% Liberty herbicide solution. (a and c) Non-transgenic leaves are showing necrosis; (b and d) transgenic leaves showing resistance to herbicide in both wheat cultivars.

**Figure 6:** Polymerase chain reaction (PCR) screening analysis of genomic DNA from leaf tissues of *T. aestivum* bread wheat plants cv. Sids 1. Lane (M) is DNA marker (100 bp ladders), (−Ve) genomic DNA from non-transgenic plant as negative control, (+Ve) plasmid pAHCht-2 in a and plasmid pAB6 in b and c. Lanes I-1- M-5: Genomic DNA from leaf tissues of 23 *T. aestivum* transgenic plants of cv. Giza 164 was used in PCR reaction. (a) Amplification product of *Cht-2* gene (1100 bp). (b) Amplification product of *bar* gene (443 bp). (c) Amplification product of *gus* gene (1050 bp).

**Figure 7:** Polymerase chain reaction (PCR) screening analysis of genomic DNA from leaf tissues of *T. turgidum* durum wheat plants cv. Bani Suef 6. Lane (M) is DNA marker (100 bp ladder). (−Ve) Genomic DNA from non-transgenic plant as a negative control, (+Ve) plasmid pAHCht-2 in a and plasmid pAB6 in b and c. Lanes N-1- R-3: Genomic DNA from leaf tissues of 18 *T. turgidum* transgenic plants of cv. Giza 164 was used in PCR reaction. (a) Amplification product of *Cht-2* gene (1100 bp). (b) Amplification product of *bar* gene (443 bp). (c) Amplification product of *gus* gene (1050 bp).
not harmful to plants, as the substrate chitin is not found in plants. It has been proposed that overexpression of a chitinase transgene protein may function to provide fungal pathogen resistance on 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 [30,31].

In the present study, the bread wheat cultivar (Sids 1) recorded higher regeneration frequency reached to 63.15% compared with durum wheat cultivar (Bani Suef 6) which recorded only 51.96% as shown in Tables 1 and 2. The use of TDZ resulted in higher regeneration frequencies in wheat plants as reported by several authors [32-34]. In monocotyledons species, several authors reported that TDZ induces multiple shoot formation. In this respect, Shan et al. [32] demonstrated that the regeneration percentage of barley and wheat was higher on medium containing TDZ than other plant growth regulators. This indicates TDZ is an efficient regulator for in vitro regeneration of barley and wheat.

The transformation frequency in wheat plants in literature was low and depended significantly on the genotype. In this respect, transformation frequencies in previous reports were 2.4% by Burdon et al. [35]; 0.6–3.1% by Tosi et al. [36]; 1.4–3% by Mackintosh et al. [37]; and 1.8–2.7 by Fahmy et al. [29]. In the present work, transformation frequency was 5.29% for cv. Sids 1 and 4.67% for cv. Bani Suef 6 [Tables 1 and 2]. Several studies have been reported that the variability of transformation frequencies was more due to the genotypic and physiological status of the donor plants that due to the efficiency of the biolistic procedure [38].

PCR method is used to confirm integration of the transgenes in putative of wheat cultivars as shown in Figures 6 and 7. In this study, cht-2 gene introduced into two Egyptian wheat cultivars by gene gun bombardment, and conducted the fast DNA molecular identification by dot blot hybridization and PCR. These methods efficiently and conveniently identify transgenic plants, but it was still necessary to conduct further identification by Southern blotting hybridization to evaluate the performance of transformed exogenous genes.

To confirm the presence of cht-2 in the putative plants T<sub>2</sub>, all the independent putative transforming wheat cultivars were analyzed by PCR amplification using primers specific to cht-2 gene. PCR analysis amplified the expected size for cht-2 (1100 pb). As shown in Tables 1 and 2 65% and 61% of cv. Sids 1 and cv. Bani Suef 6, respectively, exhibited the expected size. DNA Dot blot was performed, and positive signal after hybridization of the genomic DNA with the probe was detected in putative plants of three wheat cultivars as shown in Figure 8. This method provides advantages of rapid, easy, and convenient analysis [39,40].

In addition, the integration of marker genes (gus and bar) was confirmed in the genome of the putative plants of three wheat cultivars by PCR technique. PCR results revealed products of the expected sizes, 1050 bp for gus gene and 444 bp for bar gene as shown in Figures 6 and 7. Expression of marker genes was examined histochemically for gus activity and by leaf painting assay for bar.

Plants that survived after selection, but revealed negative PCR for the presence of transgene (bar) were considered escapes. The number of 139 and 126 plantlets survived after the two selection stages for cv. Sids 1 and Bani Suef 6, respectively. Only 23 and 18 plants grow to maturity, and 23 and 17 were PCR positive for the selection marker gene bar for cv. Sids 1 and Bani Suef 6, respectively. This study recorded 14.38% and 13.49% escapes for cv. Sids 1 and Bani Suef 6, respectively. Wheat transformation suffers high escape frequencies (50–95%) as reported by several workers [23,29,41-44].

5. CONCLUSION

We successfully transferred rice chitinase (cht 2) gene, and bar gene into an immature embryo-derived calli of two Egyptian wheat cultivars by biolistic device to enhance resistance against pathogens and herbicide. This genetic engineering approach may be improved wheat quality and yield. Transformation frequencies were higher in bread wheat cultivar than in durum wheat cultivar. At the moment, these data are only valid for the wheat cultivars used. If nothing more, they indicate that it seems advantageous in the genetic transformation to carefully choose the cultivars with best transformation frequency.

ACKNOWLEDGMENTS

The authors thank appreciably Prof. Dr. S. Muthukishnan (Department of Biochemistry, Kansas State University, Kansas, USA) for generously providing rice chitinase gene.

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Figure 8: Dot blot analysis of transgenic wheat (a) cv. Sids 1 plants; Dot (+Ve) is pAHCht-2 plasmid (positive control), Dot (−Ve) is non-transformed wheat cv. Sids 1 (negative control), and Dots (1-1- M-5) are the 15 wheat Cht-2 transgenic plants and (b) cv. Bani Suef 6 plants; Dot (+Ve) is pAHCht-2 plasmid (positive control), Dot (−Ve) is non-transformed wheat cv. Bani Suef 6 (negative control) and Dots (N-2- R-3) are the 11 wheat Cht-2 transgenic plants.
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How to cite this article:
Fahmy AH, Hassanein RA, Hashem HA, Ibrahim AS, El Shihy OM, Qaid EA. Developing of transgenic wheat cultivars for improved disease resistance. J App Biol Biotech. 2018;6(2):31-40. DOI: 10.7324/JABB.2018.60206