J. Crop Prot. 2013, 2 (3): 297-304

A modified method for transformation of *Fusarium graminearum*

Sohrab Moradi[1, 2], Forough Sanjarian[1*], Naser Safaie[3], Amir Mousavi[1] and Gholam Reza Bakhshi Khaniki[2]

1. Plant Biotechnology Department, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran.
2. Faculty of Agricultural Sciences , Payame Noor University, Tehran, Iran.
3. Plant Pathology Department, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran.

Abstract: One of the important technical obstacles in the study of many filamentous fungi is the development of efficient transformation system. Transformation of filamentous fungi is difficult because they have a cell wall and for most frequently used approaches preparing of protoplast is necessary. Protoplast preparation is batch-dependent and often frustrating work. In this study, a modified method was introduced for gene transfer to the plant pathogenic fungus, *Fusarium graminearum*, the major causal agent of Fusarium Head Blight disease in small grains. This protocol was based on protoplast-PEG method. Age of mycelia, enzymes exposure time and mycelium/enzyme ratio were optimized for the purpose of protoplast preparation. The outcome showed that the best result for protoplast preparation was obtained when 1.5×10^5 spores were let germinate for 6 h then exposed to 10 ml of enzyme solution for 3 h. The effect of other parameters that might enhance transformation yields including PEG concentration, DNA quantity and number of protoplasts was also examined. The most efficient condition for transformation involved the use of 10^6 protoplasts, 20 µg DNA and 30% PEG (w/v). In the course of this study, a simple and appropriate modified protocol for transformation of *F. graminearum* was established. The method introduced is also more economical and faster than other current methods.

Keywords: Filamentous fungi, *Fusarim graminearum*, PEG, Protoplast, Transformation

Introduction

Fungi have had numerous traditional industrial uses in beer, bread and cheese making since centuries ago. Nowadays their application has expanded with the synthesis of priceless secondary metabolites such as pharmaceuticals and industrial enzymes (Moore, 2007). Among these organisms, filamentous fungi are a large group of heterogeneous and heterotrophic organisms that have a deep impact on mankind's activities. Although plant pathogenic fungi cause considerable economic losses, they have important applications in industry that benefit humanity. Filamentous fungi can grow on simple and reasonably priced media. They also are capable of producing large amounts of commercially attractive metabolites. Accordingly, fungi are interesting production organisms in biotechnology (Meyer, 2008).

Fusarium graminearum Schwab. (Tel.: *Gibberella zeae* Schw.) is a filamentous fungus which is the main agent of the destructive disease, Fusarium Head Blight (FHB), in cereals such as wheat, maize, barley and rice (McMullen *et al.*, 1997; Goswami and Kistler, 2004; Becher *et al.*, 2010). FHB causes severe

Handling Editor: Dr. Vahe Minassian

[*] **Corresponding author**, e-mail: fsanjarian@nigeb.ac.ir
Received: 11 August 2012; Accepted: 4 March 2013

decrease in crop yield and the resulting harvested grains carry the residual mycotoxins, such as nivalenol, deoxynivalenol and the estrogenic toxin, zearalenone (Bennett and Klich, 2003; Desjardins and Proctor, 2007). Being associated with a lot of health hazards, mycotoxin- contaminated foods and feeds are one of the biggest problems the food industries are faced with(Gilbert and Tekauz, 1995; Proctor *et al*., 2002).

Transformation is an effective strong tool to study gene function in microorganisms since it allows the alteration and monitoring of particular genes within living cells (Mullins and Kang, 2001). Protoplast-PEG mediated transformation is a common method with regards to the transformation of fungi. The cell wall is a major barrier during the transformation of filamentous fungi. Hence protoplast preparation and their regeneration after transformation are critical stages in the transformation process. The goal of this work was to introduce an alternative and effective protocol for protoplast-PEG transformation of *F. graminearum*.

Materials and Methods

Fungal strain: The Fg99, wild type strain of *F. graminearum*, was used throughout this study. This strain was previously isolated from naturally infected wheat and has been investigated in field trials. Three media were used for preparation of conidia: Salt Nutrient broth (SNB), Mung bean and Carnation leaf (CL) broth. A plug of *F. graminearum* from a Potato Dextrose Agar (PDA) plate was added to 200 ml of each media and grown by shaking at 28 ºC for four days with a 12 h photoperiod. The culture was subsequently filtered and centrifuged to gather macroconidia.

Plasmid: The shuttle vector pDL2 (kindly donated by Dr. Mehrabi) was used in this study that contains an enhanced green fluorescent protein (*egfp)* gene with an upstream regulatory sequence RP27 and its terminator. This vector also carries a gene for hygromycin B phosphotransferase (*hph*) for selection in Eukaryotic cells and an ampicillin resistance gene (*amp*) derived from pBR322 for selection in *E. coli* DH5α.

Protoplasting: 1 ml of the macroconidial filtrate (containing approximately 10^5 macroconidia) was used to inoculate 250 ml Erlenmeyer flask containing 100 ml of YEPD medium (W/V; 1% Pepton, 0.3% yeast extract, 2% dextrose). The cultures were incubated for 6, 8, 12 and14 h at 28 ºC with shaking at 170 rpm. Germlings were collected by filtration using sterile filter paper and a Buchner funnel, and re-suspended in 10 ml volumes of protoplasting solution [1.2 M KCl containing 25 mg/ml Driselase (Crude powder containing laminarinase, xylanase and cellulose, EC 3.2.1.8), 5 mg/ ml lysing enzyme from *Trichoderma harizianum* (Contains β-glucanase, cellulase, protease, and chitinase activities) and 100 µg/ml of Chitinase (2.4 Umg^{-1}, EC.3.2.1.14) sterile- filtered]. All enzymes were prepared from Sigma- Aldrich Company. The resulting suspension was incubated at 30 ºC for 1, 2, 3 and 4 h with gentle shaking. Protoplasts were collected at 3500 rpm for 5 min at 4 ºC, washed three times in STC (1.2 M Sorbitol, 10 mM Tris-HCl pH 8, and 50 mM $CaCl_2$) and subsequently re-suspended in 1ml of STC.

Protoplast-PEG transformation: 50 µl Polyethylene glycol (PEG) solution containing 10 mM Tris-HCl, 50 mM $CaCl_2$ different percents of PEG [30, 40, 50 and 60% (W/V)], and 5, 10, 15 and 20 µg of plasmid DNA (up to 5 µl) were added to 200 µl of protoplast and mixed gently for 20 min at room temperature; after mixing 2 ml of PEG was added to the solution. Subsequently 4 ml of STC buffer was added to the mixture for preparing protoplast mixture. For each plate 13 ml of melted (50 ºC) regeneration medium containing (W/V): yeast extracts 0.1%, casein hydrolysate 0.1%, sucrose 27.5% and agarose 1% were sufficient. After adding 1 ml of the protoplast mixture to this amount of regenerating media, it was spread onto the plates and then incubated at room temperature for 16-18 h. Subsequently, 10 ml of melted regeneration medium, containing 150 μg/ml hygromycinB (Sigma), for selecting transformants, was spread on the top of the plates. Plates were then incubated for 4-7 days in the dark at 28 ºC for emerging transformants. Transformants were purified by two consecutive transfers from the edges of colonies grown on plates containing 100 μg/ml hygromaycin B.

Genomic DNA extraction from *F. graminearum*: DNA was extracted from the mycelium of six selected purified transformants grown in PDA medium containing hygromycin by using the modified CTAB method (Brandfass and Karlovsky, 2006). Mycelia were scraped from the surface of the plate and then dried and ground with a tissue lizer [UTRA-TURRAX T8 (IKA^{TM})]. The powder was mixed with 1.4 ml of CTAB buffer (containing 10 mm Tris-HCl (pH 8), 20 mm EDTA, 20 mm CTAB, 0.8 M NaCl, 0.13 M sorbitol and 1% (w/v) PVP). The resulting mixture was incubated at 42 ºC for 10 min followed by 65 ºC for 10 min. DNA was extracted by adding 800 μl of chloroform/isoamyl alcohol (24:1 V/V) and centrifuged at 8000 rpm for 10 min. DNA was precipitated by adding 500 μl of isopropanol and after centrifugation, re-suspended in sterile deionized water.

The sequences of the PCR primers were as follows: for *hph* gene amplification (HphFw: AGCCTGAACTCACCGCGAC, HphRe: CTATTCCTTTGCCCTCGGAC) and for *egfp* gene (GfhFw: TTCAAGGACGACGGCAACTACAA, GfhRe: GTCACGAACTCCAGCAGGACCAT).

Cycling condition of amplification reaction was: 94 ºC for 5 min, followed by 30 cycles of 94 ºC for 1min, 59 ºC (for *hph* gene) or 57 ºC (for *egfp* gene) for 30 s, and 72 ºC for 30 s, with a final extension at 72 ºC for 5 min.

RNA extraction from putative transformants: RNA extraction from positive genomic polymerase chain reaction transformants was carried out using RNXplus kit (Cinagen, Iran). Total RNA was converted to single-strand cDNA by applying *egfp* reverse primer then amplified via PCR reaction as described for genomic DNA amplification.

Fluorescent microscopy: microscopic images were acquired using a Fluorescence Axiophot microscope (Zeiss, Germany) with a 40X objective and a GFP filter cube. Bright-field images were used to focus on the mycelia.

Results

As illustrated in Fig. 1 there was a significant difference between the tested media for production of conidia and the best medium was Carnation leaf medium.

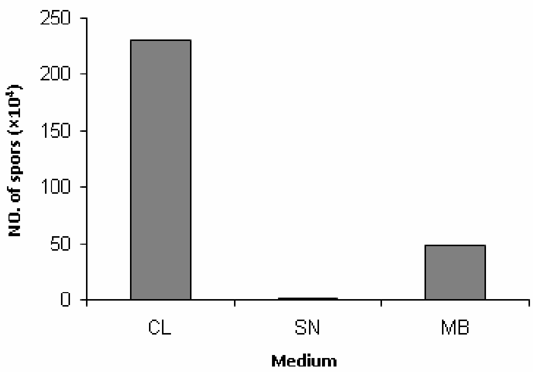

Figure 1 Comparison of conidia production in different media after 4 days.

Important factors involved in protoplast preparation are the age of mycelium, time of exposure to enzyme and the ratio of mycelium to enzyme. Mycelia in different times after culturing (6, 8, 12 and 14) were examined for protoplast preparation. Various enzyme solution exposure times (1, 2, 3 and 4 h) also were studied. The best condition for protoplast production was obtained when spores were left to germinate for 6 h followed by exposing them to 10ml of enzyme solution for 3 h. About 3.4 × 10^5protoplasts were obtained in this way.

The number of the protoplasts, PEG concentration and the quantity of DNA were examined as important factors in fungus transformation. There is a direct relationship between the number of protoplasts and the obtained transformants. Increasing the number of protoplasts resulted in increased efficiency of the transformation and the best results were obtained using 10^6 protoplasts (Fig 2.).

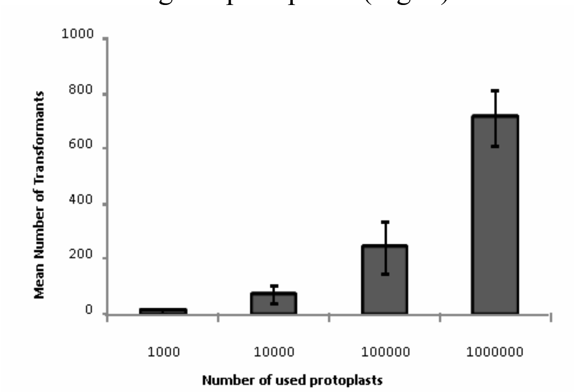

Figure 2 Effect of the number of used protoplasts on the obtained transformants number. Data represent average of three replications with their SD.

PEG concentration was examined at four levels: 30%, 40%, 50% and 60%. The results showed that there was no significant difference between 30% and 40%, nor between 50% and 60% concentrations of PEG. However, 30% PEG worked better than all others (Fig 3.).

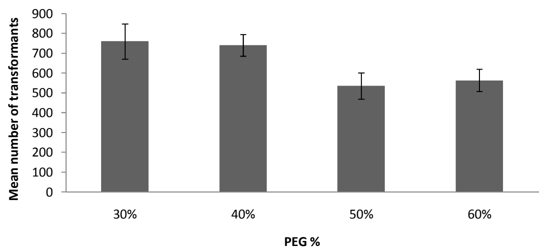

Figure 3 Effect of PEG concentration on number of obtained transformants. Data represent average of 3 replications with their SD.

Another crucial factor considered during fungus transformation is the quantity of plasmid DNA. The results denoted a direct correlation between the amount of DNA and the number of transformants (Fig 4.).

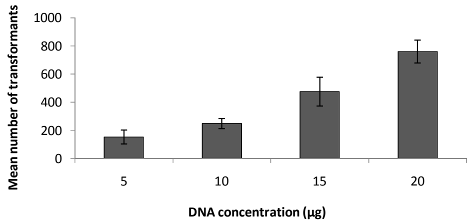

Figure 4 Effect of DNA quantity on the number of transformants. Data represent average of 3 replications with their SD.

The resistance of transformants to hygromycin B greatly increased over the original strain. Six obtained transformants which had grown on plates containing hygromycin were randomly selected for molecular analysis. For this purpose, PCR amplification of *hph* and *egfp* genes and transcription of *egfp* gene of the transformants were examined. In the PCR analysis in which HphFw and HphRe were employed, the resulting 1000 bp fragment comfirmed that the *hph* gene was amplified (Fig 5.).

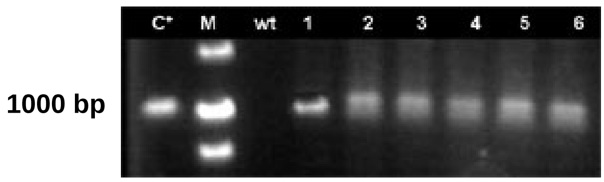

Figure 5 Amplification of 1000 bp fragment from *hph* gene by using *hph* primer pairs and genomic DNA of selected purified transformants. C^+: pDL2 as template DNA. M: 1kb molecular weight marker (Fermentas). WT: non transformed *Fusarium graminearum*. 1-6: independent transformants of *F. graminearum*.

PCR amplification showed that *gfp* gene has been transferred into *F. graminearum* (Fig 6). The expression of *egfp* gene was confirmed by RT-PCR in transformants (Fig 7).

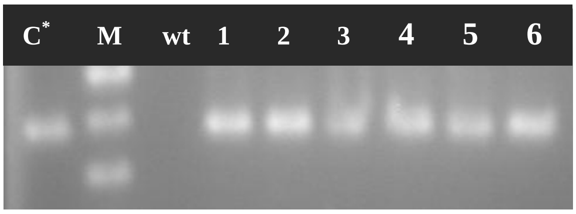

Figure 6 Amplification of approximately 400 bp fragment from *egfp* gene using *egfp* primer pairs and genomic DNA of selected purified transformants. C^+: pDL2 as template DNA. M: 100 bp molecular weight marker (Fermentas). WT: non transformed *Fusarium graminearum*. 1-6: independent transformants of *F. graminearum*.

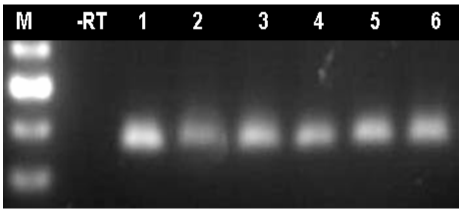

Figure 7 RT-PCR analysis of six independent *Fusarium graminearum* transformants. An approximately 400 bp product represents the transcript of *egfp* gene in selected purified transformants (1-6), no product in RNA sample without reverse transcriptase (–RT). M: 100bp molecular weight marker (Fermentas).

Green Fluorescent Protein assay of transformed mycelium was done by using Fluorescence Axiophot microscope. The flurescence of hyphae under UV illumination indicates that GFP was produced (Fig 8).

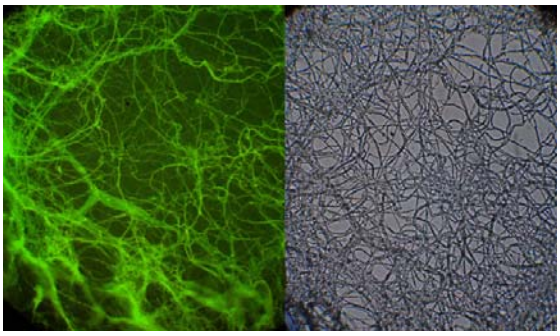

Figure 8 The hyphae of *F. graminearum* transformant. Left: Fluorescent Microscopy, Right: Direct illumination.

The most efficient transformation was carried out by mixing 20 µg DNA with 30% PEG solution, then adding to 10^6 protoplast.

Discussion

Development of efficient strains for production of desirable metabolites through the molecular methods is very important in fungal biotechnology. Transformation improves gene regulation for producing industrial materials and components from the fungi (Meyer, 2008). Moreover it can aid gene identification and study of their functions in plant pathogens, and is also an effective approach for study of plant-fungus interactions which can lead to combating fungal diseases.

The first successful transformation via the protoplast method was reported in *Saccharomyces cerevisiae* in 1975 (Hinnen *et al*., 1978). Transformation of filamentous fungi requires young mycelium from germinated spores. Induction of sporulation is difficult in some fungi including *F. graminearum*. A variety of media have been studied for this purpose, among which are CMC (Lee *et al*., 2002), V8 juice (Proctor *et al*., 1997), SNA (Jenczminoka *et al*., 2003), Mung bean (Dufresene *et al*., 2008; Okubara *et al*., 2003) and CL (Argyris *et al*., 2003). We examined three of these media and chose the most effective one for this study. Several other media such as Littman oxgall (Watson *et al*,. 2008) GYEP (Mc Cormick *et al*., 1999) and YPG (Lee *et al*., 2002) have also been suggested for spore germination. Here YEPD medium was used for this purpose. This medium is the most available and simplest among those recommended.

Many factors affect the quality and quantity of the protoplast including the appropriate enzymes, mycelium age, the temperature and time of the cell wall digestion. In this study, some of these factors were examined and a modified protocol for *Fusarium graminearum* transformation was established. Phase contrast microscopy showed that cultured spores started to germinate after 5 h and that germination was complete in 6 h. Mycelia started branching after 8 h and cell walls started to become thick (data not shown). Hence the best mycelium age for preparing protoplast was determined to be 6 h old culture of germinated spores. There are some reports that the mycelium at the ages of 8-9 h (Proctor *et al.*, 1997), 12 h (Lee *et al.*, 2002) or 10-18h (Watson *et al.*, 2008) have been used. Our observation showed that younger mycelia are much more prone to lysing enzymes and produce more protoplasts with high viability. In this study 250 mg Driselase, 50 mg lysing enzyme and 1 mg of Chitinase were used for preparation of protoplasts and resulted in 100% protoplast formation. This amount is half of that recommended by other researchers (Watson *et al.*, 2008; Jansen *et al.*, 2005; McCormick *et al.*, 1999). There is no exact time reported for exposure of mycelia to enzymes in most of the literature. In this research it was found that 3 h is enough for enzyme exposure. Proctor *et al.*, recommended 30-90 min exposure to enzyme, but the concentration of enzymes was two folds for of Driselase and lysing enzyme and five folds for Chitinase (Proctor *et al.*, 1997). Some osmolites such as NH_4Cl, NaCl, $MgSO_4$, glucose, sucrose and sorbitol have been recommended for keeping osmotic balance in protoplasts (Jansen *et al.*, 2005; McCormick *et al.*, 1999; Wiebe *et al.*, 1997). In this work, sorbitol (1.2 M) was used during the regeneration step and highly concentrated sucrose was used in the selection of transformants. Langin *et al.*, (1990) have shown that in *F. oxysporum*, there is a direct correlation between PEG concentration and the number of transformants but the results of this research have shown that 30% (w/v) of PEG is sufficiently efficient and that higher concentrations do not affect the rate of transformation.

In summary we have developed a rapid, reliable and low cost alternative protocol for transformation of *F. graminearum* which facilitates work with this microorganism in research.

Acknowledgment

This research was supported by the National Institute of Genetic Engineering and Biotechnology (project No. 333) and was a part of Msc. thesis of the first author. The authors wish to thank Dr. Shariati for critically reading and editing the manuscript.

References

Argyis, J., van Sanford, D. and Tekrony, D. 2003. *Fusarium graminearum* infection during wheat seed development and its effect on seed quality. Crop Science, 43: 1782-1789

Becher, R., Hettwer, U., Karlovsky, P., Deising, H. B. and Wirsel S. G. R. 2010. Adaptation of *Fusarium graminearum* to tebuconazole yielded descendants diverging for levels of fitness, fungicide resistance, virulence, and mycotoxin production. Phytopathology, 100: 444-453.

Bennett, J. W. and Klich, M. 2003. Mycotoxins. Clinical Microbiology Review. 16: 497-516.

Brandfass, C. H. and Karlovsky, P. 2006. Simultaneous detection of *Fusarium culmorum* and *F. graminearum* in plant material by duplex PCR with melting curve analysis. BMC Microbiology, 6-4 doi: 10.1186/1471-2180-6-4

Desjardins, A. E. and Proctor, R. H. 2007. Molecular biology of *Fusarium* mycotoxins. International Journal Food Microbiology, 119: 47-50.

Dufresne, M., van der Lee, T., Ben M'barek, S., Xu, X., Zhang, X., Liu, T., Waalwijk, C., Zhang, W., Kema, G. H. and Daboussi, M. J. 2008. Transposon-tagging identifies novel pathogenicity genes in *Fusarium graminearum*. Fungal Genetic Biology, 45 (12): 1552-61.

Gilbert, J. and Tekauz, A. 1995. Effects of Fusarium head blight and seed treatment on germination, emergence, and seedling vigor of spring wheat. Canadian Journal of Plant Pathology, 3: 252-259.

Griffen, A. M., Wiebe, M. G., Robsonand, G D. and Trinci, A. P. J. 1997. Extracellular proteases produced by the Quorn@myco-protein fungus *Fusarium graminearum* in batch and chernostat culture. Microbiology, 143: 3007- 3013.

Goswami, R. S. and Kistler, H. C. 2004. Heading for disaster: *Fusarium graminearum* on cereal crops. Molecular Plant Pathology, 5: 515-525.

Hinnen, A., Hicks, J. B., and Fink, G. R.1978. Transformation of yeast. PNAS, 75: 1929-1923.

Jansen, C., Wettestein, D. V., Schafer, W., Kogel, K. H., Felk, A., and Maiert, F. J. 2005. Infection pattern in barley and wheat spikes inoculated with wild-type and trichodiene synthase gene disrupted *Fusarium graminearum*. PNAS, 12: 16892-97.

Jenczmionka, N. J., Maier, F. J., Lösch, A. P.and Schäfer, W. 2003. Mating, conidiation and pathogenicity of Fusarium graminearum, the main causal agent of the head-blight disease of wheat, are regulated by the MAP kinase gpmk1. Current Genetic. 43 (2): 87-95

Langin, T., Daboussi, M. J., Gerlinger, C. and Brygoo, Y. 1990. Influence of biological parameters and gene transfer technique on transformation of *Fusarium oxysporum*. Current Genetic, 17: 313-319.

Lee, T., Han, Y. K., Kim, K. H., Yun, S. H. and Lee, Y. W. 2002. *Tri3* and *Tri7* determine Deoxynivalenol- and Nivalenol-producing chemotypes of *Gibberella zeae*. Applied and Environmental Microbiology, 68: 2148-2154.

McCormick, S. P., Alexander, N. J., Trapp, S. E. and Hohn, T. M. 1999. Disruption of *TRI101*, the gene encoding trichothecene 3-O-acetyltransferase, from *Fusarium sporotrichoides*. Applied and Environmental Microbiology, 65: 5252-56.

McMullen, M., Jones, R. K. and Gallenberg, D. 1997. Scab of wheat and barley: A re-emerging disease of devastating impact. Plant Disease. 81: 1340-1348.

Meyer. V. 2008. Genetic engineering of filamentous fungi – Progress, obstacles and future trends. Biotechnology Advances, 26: 177-185.

Moore, M. M. 2007. Genetic engineering of fungal cells. In Biotechnology Vol. III. (Ed. H. W. Doelle and E. J. Dasilva), EOLSS. Ontario, Canada. pp. 36-63.

Mullins, E. D. and Kang, S. 2001. Transformation: a tool for studying fungal pathogens of plants. CMLS, 58: 2043-2052.

Proctor, R. H., Desjardins, A. E., McCormick, S. P., Plattner, R. D., Alexander, N. J. and Brown, D. W. 2002. Genetic analysis of the role of trichothecene and fumonisin mycotoxins in the virulence of Fusarium. European journal of Plant Pathology, 108: 691-698.

Okubara, P.A., Blechl, A. E., McCormick, S. P., Alexander, N. J., Dill-Macky, R.and Hohn, T. M. 2002. Engineering deoxynivalenol metabolism in wheat through the expression of a fungal trichothecene acetyltransferase gene. Theoretical and Applied Genetic. 106 (1): 74-83

Proctor, R. H., Hohn, T. M.and McCormick, S. P.1997. Restoration of wild-type virulence to Tri5 disruption mutants of *Gibberella zeae* via gene reversion and mutant complementation. Microbiology. 143:2583-91.

Watson, R. J., Burchat, S. and Bosley, J. 2008. A model for integration of DNA into the genome during transformation of *Fusarium graminearum*. Fungal Genetic and Biology, 45: 1348-63.

Wiebe, M. G., Novakova, M., Miller, L., Blakebrough, M. L., Robson, G. D., Puntand, P. J. and Trinci, A. P. J. 1997. Protoplast production and transformation of morphological mutants of the Quorn® myco-protein fungus, *Fusarium graminearum* A3/5, using the hygromycin B resistance plasmid pAN7–1. Mycological Research, 101: 871-877.

روش اصلاح شده تراریختی قارچ فوزاریوم گرامیناروم

سهراب مرادی[۱،۲]، فروغ سنجریان[۱*]، ناصر صفایی[۳]، امیر موسوی[۱] و غلامرضا بخشی خانیکی[۲]

۱- گروه بیوتکنولوژی گیاهی، پژوهشگاه ملی مهندسی ژنتیک و زیست فناوری، تهران، ایران
۲- دانشکده علوم کشاورزی، دانشگاه پیام نور مرکز تهران، تهران، ایران
۳- گروه بیماریهای گیاهی، دانشکده کشاورزی، دانشگاه تربیت مدرس، تهران، ایران
* پست الکترونیکی نویسنده مسئول مکاتبه: fsanjarian@nigeb.ac.ir
دریافت: ۲۱ مرداد ۱۳۹۱؛ پذیرش: ۱۴ اسفند ۱۳۹۱

چکیده: یکی از مهم‌ترین موانع تکنیکی در مطالعه خیلی از قارچهای رشته‌ای، توسعه روشی کارا برای تراریختی آنهاست. به‌دلیل وجود دیواره سلولی در قارچهای رشته‌ای، تراریخت کردن آن‌ها دشوار است و در بیشتر روش‌های به‌کار رفته در تراریختی، تهیه پروتوپلاست ضروری است. تهیه پروتوپلاست به عوامل متعددی بستگی دارد و اغلب ناامیدکننده است. در این مطالعه، روشی اصلاح شده برای تراریختی قارچ بیماریزای گیاهی فوزاریوم گرامیناروم، عامل بیماری بلایت فوزاریومی در غلات دانه‌ریز، معرفی شده است. اساس روش به‌کار رفته، تراریختی با استفاده از Protoplast/PEG بود. برای تهیه پروتوپلاست سه عامل مهم، سن میسلیوم‌ها، زمان در معرض قرار دادن آنزیمی و نسبت میسلیوم به آنزیم، بهینه‌سازی شد. نتایج نشان داد که بهترین شرایط تولید پروتوپلاست زمانی حاصل می‌شود که $۱/۵ \times ۱۰^{۵}$ عدد اسپور پس از ۶ ساعت از جوانه‌زنی، به‌مدت ۳ ساعت در معرض ۱۰ میلی‌لیتر آنزیم قرار گیرند. عواملی دیگر مانند غلظت PEG، مقدار DNA و تعداد پروتوپلاست که ممکن است بازده تراریختی را افزایش دهند، بررسی شدند. بیشترین بازدهی تراریختی با استفاده از $۱۰^{۶}$ عدد پروتوپلاست، ۲۰میکروگرم DNA و ٪۳۰(W/V) PEG حاصل شد. در این مطالعه، روشی اصلاح شده، ساده و مناسب برای تراریختی کردن قارچ فوزاریوم گرامیناروم معرفی شد. این روش، از نظر هزینه مقرون به صرفه‌تر و همچنین سریع‌تر از روش‌های دیگر حال حاضر است.

کلمات کلیدی: قارچ رشته‌ای، فوزاریوم گرامیناروم، PEG، پروتوپلاست، تراریختی