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Analysis of putative sclerotia maturation-related gene expression in *Rhizoctonia solani* AG1-IA

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Abstract: *Rhizoctonia solani* AG1-IA (*R. solani* AG1-IA) is a major soil-borne fungal pathogen of maize that causes significant yield losses in all maize-growing regions worldwide. The sclerotium produced by *R. solani* AG1-IA can overwinter in grass roots or diseased plants and infect crops the following year. The molecular mechanism underlying sclerotium formation in *R. solani* is poorly understood. In this study, we constructed the cDNA library of the *R. solani* AG1-IA pathogenic strain WF-9, from which 329 high-quality expressed sequence tags (ESTs) were obtained. Of the 250 clustered unigenes, 12 genes were selected for further expression analysis during the three stages of sclerotial growth (mycelium, initiation of sclerotium, and maturation of sclerotium). The results of expression analysis support the previously suggested roles of chitin synthase D and exo-beta-1,3-glucanase in facilitating sclerotial growth through preservation of water content and energy. In addition, cytochrome P450, NADPH oxidase, catalase (CAT), acyl-CoA oxidase 1 (ACOX1), mitogen-activated protein kinase (MAPK), mitogen-activated protein kinase HOG1 (HOG 1), and the G-protein α subunit play important roles in balancing reactive oxygen species (ROS) levels during sclerotial development. The findings of this study can help understand the molecular mechanism of sclerotial development further.

Key words: *Rhizoctonia solani* AG1-IA; expressed sequence tags; qRT-PCR; sclerotial formation; sheath blight of maize; cDNA library

INTRODUCTION

Rhizoctonia solani (*R. solani* Kuhn) is a soil-borne fungus belonging to the Basidiomycete group that can infect hundreds of crops, including maize, potato, rice, and soybean, leading to severe crop losses [1]. It has been classified into 14 anastomosis groups (AG-1 to AG-13 and AG-BI) [2], with three intraspecific groups (ISG) forming the *R. solani* AG1 subgroup. *R. solani* AG1 subgroup is known to cause many diseases such as sheath blight and aerial blight [3]. Sheath blight of maize is one of the most serious and widespread crop diseases worldwide. In recent years, the increase in the planting density of maize and in the amount of nitrogen fertilizers used has made the microclimate conditions conducive to the frequent occurrence of sheath blight [4,5]. Sheath blight shows an annual aggravating trend and has become the major disease that restricts the continuous increase in maize yield [6].

Pathogenic differentiation is prevalent in plant pathogenic fungi. The study of pathogenic differentiation plays a significant role in the research of pathogenic fungi, including composition, diversity and genetic differentiation, resistance identification, breeding of disease-resistant varieties and rational distribution of varieties. Previous studies have reported differences in composition of fusion groups of *R. solani* Kuha in different regions and pathogenic differences between different fusion groups and different strains of the same fusion group [7,8]. The pathogenicity of mature *R. solani* AG1-IA and selected disease spot height and average daily expansion rate have been determined as statistical standards; the results showed that each strain had evident differences in expansion height, disease spot height and daily average rate of expansion, indicating the existence of pathogenic differences.

Sclerotium is a structure formed from aggregated mycelia. In 1954, Townsend and Willetts [9] divided the development of sclerotia into three stages: initiation, development, and maturation. The initiation stage is marked by the appearance of sclerotium with white aerial mycelia entangled around the edges of the culture medium. In the development stage, the sclerotium is further entangled and an increase in size can be observed along with a clear or tan-colored liquid secretion on the surface. The maturation phase involves surface delineation and melanin deposition in peripheral rind cells, followed by internal consolidation [9].

Kwon [10] identified 55 proteins that were differentially expressed during sclerotial maturation using matrix-assisted laser desorption/ionization (MALDI)-time of flight (TOF) mass spectrometry (MS). These proteins can be categorized into 10 categories based on their functions, including carbon metabolism, cell defense, and amino acid metabolism. Among them, the expression of two vacuole function-related proteins was confirmed to be highly increased, as determined by quantitative real-time reverse transcription-polymerase chain reaction (qRT-PCR). The proteins were found to be closely related to sclerotial growth [10]. The disruption of Rga1 (G-protein α subunit) led to decreased vegetative growth and pathogenicity. This demonstrated that Rga1 plays a key role in the growth of sclerotium [11]. NADPH oxidase 1 (NOX 1) is thought to be involved in superoxide anion generation, fungal growth, and cell differentiation [12]. Nox 1 silencing in *Sclerotinia sclerotiorum* and loss of ROS in the mutant suppressed sclerotium formation [13].

A cDNA library provides the foundation for studying the gene expression of a specific organ, specific tissue, and specific growing period [14-16]; a full-length cDNA library is an important resource to identify, screen, and analyze genes and to obtain full-length genes [17-19]. To date, few studies have investigated the response of sclerotial formation of *R. solani* AG1-IA in maize. We established the cDNA library of *R. solani* AG1-IA to obtain additional molecular information about it and to better understand gene expression during sclerotium growth. The findings of this study may be useful in developing new methods of disease control and prevention.

MATERIALS AND METHODS

Pathogenicity determination

An appropriate amount of hypha was selected from the storage tube of *R. solani* AG1-IA, transplanted onto potato dextrose agar (PDA) and incubated at 25°C for three days for activation of culture. The activated strain was again transplanted onto the surface of PDA and incubated at 25°C for three days. A mycelial plug (5 mm) was excised from the periphery of the resulting colony after 4 days and prepared for inoculation [20].

The maize varieties were Liaoning single 565 (Liaoning Academy of Agricultural Sciences) and East Asia single 5 (Corn Breeding Office of Liaoning Province East Asian Seed Science Institute). The experiment was carried out at the seedling stage; the inoculation method in the greenhouse at 25°C was used, and the inoculation was performed on the leaf of maize. The prepared mycelial plug was inoculated into the first leaf sheath of maize seedlings, which includes two corn varieties, and each one was inoculated into 10 plants. The soil surface was kept wet by spray moisturizing daily. Corn growth and disease development were observed daily, and disease investigation was pursued 15 days later using the formula:

$$\text{disease index} = \Sigma (\text{disease grade} \times \text{strains}) / (\text{maximum disease series} \times \text{total surveyed strains}) \times 100.$$

RNA extraction and cDNA synthesis

RNA was extracted from the three developmental stages of sclerotium (designated mycelium, initiation of sclerotium, and maturation of sclerotium) using the TaKaRa MiniBEST Universal RNA Extraction Kit (TaKaRa, Shiga Pref, Japan). RNA degradation and contamination were monitored on 1% agarose gels. RNA purity was measured by a NanoPhotometer® spectrophotometer (IMPLEN, CA, USA), and RNA concentration was measured by a Qubit® RNA Assay Kit in a Qubit® 2.0 Fluorometer (Life Technologies, CA, USA). RNA integrity was assessed using an RNA Nano 6000 Assay Kit of the Bioanalyzer 2100 system (Agilent Technologies, CA, USA). PrimeScript™ RT reagent Kit with gDNA Eraser (TaKaRa, Shiga Pref, Japan) was used to synthesize the cDNA.

Construction of *R. solani* AGI-IA cDNA library

The sclerotium initiation stage was used to construct a cDNA library. The double-stranded cDNA was purified by the QIAquick PCR purification kit (QIAGEN, Germany), and the cDNA was fractionated by CHROMASPIN + TE-1000 column and 500-3000-bp fragments were recovered. The cDNA was ligated with the pSMART2IFD linearized vector provided with the kit [18] and incubated in a PCR TProfessional Thermocycler (Biometra, Germany) at 50°C for 15 min. Then, the cDNA was transferred to ice and 2 μL of the ligated product was electroporated into DH 5α competent cells and cultured at 37°C for 1 h. Next, lysogeny broth (LB) culture medium (prewarmed to 37°C) was added. The transformation product (5 μL) was added to 95 μL of LB liquid medium, and 40 μL of 100 mmol•L^{-1} isopropyl β-D-1-thiogalactopyranoside (IPTG) and 40 μL of 20 mg•mL^{-1} X-Gal were uniformly applied on LB agar plates (with 100 μg•mL^{-1} ampicillin). The mixed bacterial solution was coated evenly on the culture plate and then placed upside-down in a 37°C incubator for a 12-h culture. The growth of the *R. solani* AG1-IA isolated colony was checked and the recombination rate (proportion of leukoplakia) was calculated [21].

Processing of R. solani AG1-IA EST library

Four hundred clones were randomly picked from the cDNA library plates and sent to BGI China for 5′ sequencing. The 3730xl DNA analyzer (Applied Biosystems, USA) was used for sequencing. The Phred package (http://www.phrap.org/consed/consed.html) was used to convert the sequencing peak figure files into sequence and quality files. It removes unloaded sequences, low-quality sequences (Q-value<13) (low-quality bases of head and tail), vector sequences, and sequences whose length was less than 100 bp. CAP3 [22] (http://seq.cs.iastate.edu/cap3.html) was used to splice the obtained EST sequences to generate contigs and singletons [23], and statistical data were produced. For annotations, all genes were searched against the Nr database using BLASTx, with 10^{-5} as the E-value cut-off point. The sequences with the highest similarities were retrieved from the NCBI web server (https://blast.ncbi.nlm.nih.gov), and the functions of the genes were retrieved from the Clusters of Orthologous Group (COG) database [24,25].

Candidate gene expression analysis by qRT-PCR

The primer sequences and related information are shown in Table S1. According to the SYBR Premix Ex Taq Kit (TaKaRa, Shiga Pref, Japan) protocol, the reactions were run on an CFX-96 (BioRad, Hercules, CA, USA) using a 20-μL reaction system. The reaction conditions were as follows: initial denaturation at 95°C for 3 min, followed by 39 cycles of denaturation at 95°C for 30 s and annealing for 30 s at 60°C. All experiments were performed in triplicate and two repeated tests. The relative expression levels were analyzed and normalized to 18S rRNA transcript levels using the CFX_Manager analysis software. Mapping was performed by SigmaPlot 12.5.

RESULTS

Virulence of strains

We measured the isolates of *R. solani* AG1-IA in the anastomosis group, and the results showed that saprophytic colonization and pathogenicity between the isolates were significantly different. Two corn cultivars developed typical symptoms of sheath blight upon infection with *R. solani* AG1-IA. The tested isolates of *R. solani* AG1-IA showed differences in pathogenicity (Table 1), with isolate WF-9 displaying the strongest pathogenicity and isolate DR-28 displaying the weakest pathogenicity (an average disease index of 79.69 and 23.44, respectively).

Gene annotation

The sequencing of 400 randomly selected clones from the cDNA library of the *R. solani* AG1-IA WF-9 isolate (initiation of sclerotium) resulted in 329 high-quality ESTs. The high-quality ESTs obtained were clustered and spliced using CAP3, which resulted in 250 non-repeated sequences (unigene), including 36 contigs and 214 singletons. A comparison of sequences showed that 90.8% of ESTs were homologous to known genes and proteins at various levels (Table S2). By screening the library, we identified pathogenic genes, namely, the G-protein α subunit, G-protein β subunit, mitogen-activated protein kinase (MAPK), mitogen-activated protein kinase kinase (MAPKK),

Table 1. Result of the pathogenicity test of different isolates on two corn cultivars.

Isolates	Maize Cultivars (Disease Index)			Isolates	Maize Cultivars (Disease Index)		
	Liaoning single 565	East Asia single 5	Average		Liaoning single 565	East Asia single 5	Average
JR-80	78.13	71.88	75.01	JR-42	37.50	46.88	42.19
TR-37	68.75	62.50	65.63	DR-78	56.25	53.13	54.69
JR-89	46.88	50.00	48.44	DR-28	25.00	21.88	23.44
DR-84	34.38	43.75	39.07	JR-87	40.63	53.13	46.88
WF-10	59.38	53.13	56.26	SY-69	75.00	71.88	73.44
JR-90	31.25	37.50	34.38	DR-31	31.25	28.13	29.69
WF-9	81.25	78.13	79.69	DR-24	62.50	59.38	60.94
SY-39	65.63	53.13	59.38	TR-43	46.88	53.13	50.01
DR-35	78.13	62.50	70.32	SY-67	50.00	50.00	50.00
DR-30	75.00	65.63	70.32	SY-72	37.50	43.75	40.53
WF-8	56.25	62.50	59.38	TR-54	56.25	43.75	50.00

JR-80, TR-37, JR-89, etc. is the number of isolates; Liaoning single 565 and East Asia single 5 are maize varieties; disease index = Σ (disease grade × strains)/(maximum disease series × total surveyed strains) ×100; disease grading was as follows: grade 0 – no disease in whole plant; grade 1 – the 4th leaf sheath and above leaf sheath under ear; grade 2 – 3rd leaf sheath and above leaf sheath under ear; grade 3 – 2nd leaf sheath and above leaf sheath under ear; grade 4 – the 1st leaf sheath under the ear and above the leaf sheath disease; grade 5 – ear and above leaf sheath disease.

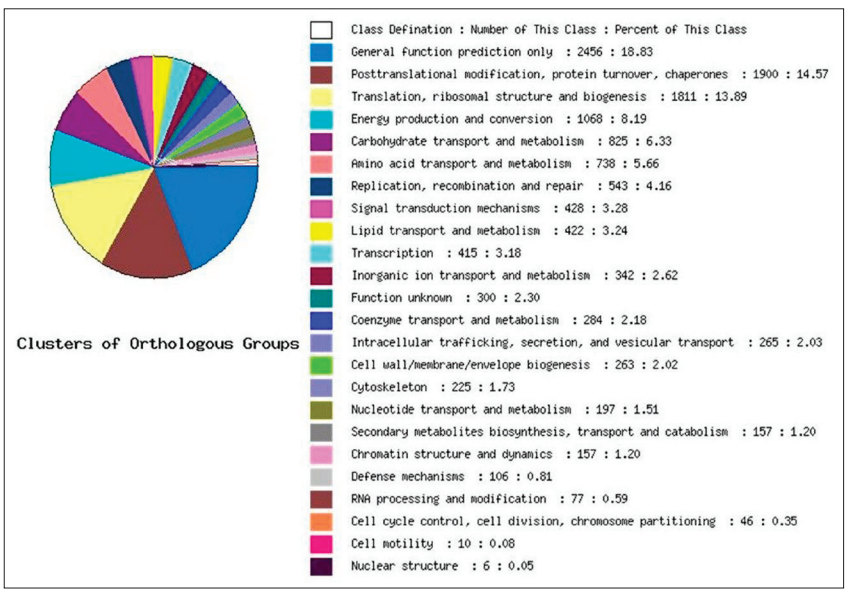

Fig. 1. Functional classification and identification of characteristics of *Rhizoctonia solani* AG1-IA genes according to the COG database. Different colors represent different class definitions. For each class definition, the first number indicates the number of genes in the class, and the second number indicates the percentage of genes in the class.

chitin synthase D, cellulase, protein phosphatase, and cell wall-related hydrolase. In addition, we identified genes with a higher frequency of expression, namely, heat shock protein, ribosomal protein, aminopeptidase, cytochrome P450, mannoprotein, elongation factor, ATP synthase, and glycosyl hydrolase family proteins.

Functional annotation and classification

All the functions of ESTs were annotated (NCBI database search using BLASTX). The functions included transcription, signal transduction, metabolism, cellular components, as well as some unknown functions. The 250 ESTs were subsequently analyzed using the COG database, and 19 ESTs (17.27%) were found to be involved in transportation of carbohydrates and metabolism, and 18 ESTs (16.36%) were found to be involved in translation, ribose structure, and biogenesis (Fig. 1). Within the cellular component categories "cell" and "cell part" were the most abundant terms. In molecular function categories "binding" and "catalytic activity" were the most abundant terms. In biological process categories "metabolic process" was the most abundant term (Fig. 2). According to gene function analysis, the functions of *R. solani* AG1-IA genes included transcriptional regulation, signal transduction, transport, metabolism, defense, energy, cell division and chromosomal structure, protein and nucleic acid synthesis,

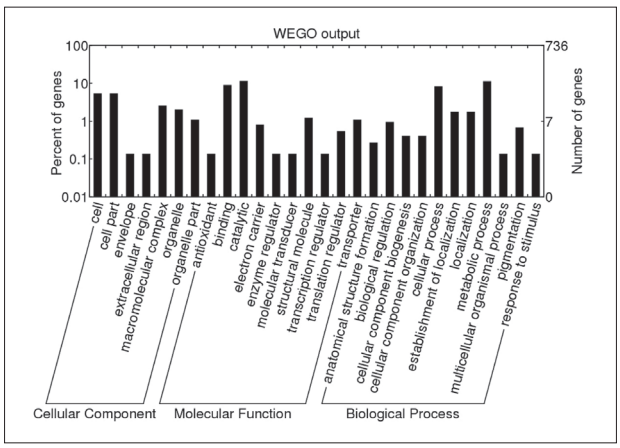

Fig. 2. Different gene ontology (GO) terms that indicate gene functions with respect to biological process, cellular component and molecular function. The ordinate presents the GO enrichment term and the abscissa presents the number of genes annotated to specific term. Different areas are used to distinguish between biological processes, cell components, and molecular functions.

cell components, and other aspects of fungal growth, and development that reflect the gene expression of *R. solani* AG1-IA.

Validation of genes involved in sclerotia formation of *R. solani* AG1-IA by qRT-PCR

Twelve genes (Acyl-CoA oxidase 1, catalase, alpha-1,3 glucan synthase, mitogen-activated protein kinase HOG1, isocitrate dehydrogenase 1, triose-phosphate isomerase, cytochrome P450, G-protein α subunit, exo-beta-1,3-glucanase, mitogen-activated protein kinase, chitin synthase D, NADPH oxidase) were selected for qRT-PCR analysis. The results showed that during the three stages of sclerotial formation (mycelium, initiation of sclerotium, and maturation of sclerotium), five genes (catalase, acyl-CoA oxidase 1, G-protein α subunit, isocitrate dehydrogenase 1, alpha-1,3 glucan synthase) showed increased expression; five genes (NADPH oxidase, mitogen-activated protein kinase HOG1, exo-beta-1,3-glucanase, chitin synthase D, triose-phosphate isomerase) initially showed increased expression, followed by decreased expression, and two genes (mitogen-activated protein kinase, cytochrome P450) showed decreased expression (Fig. 3).

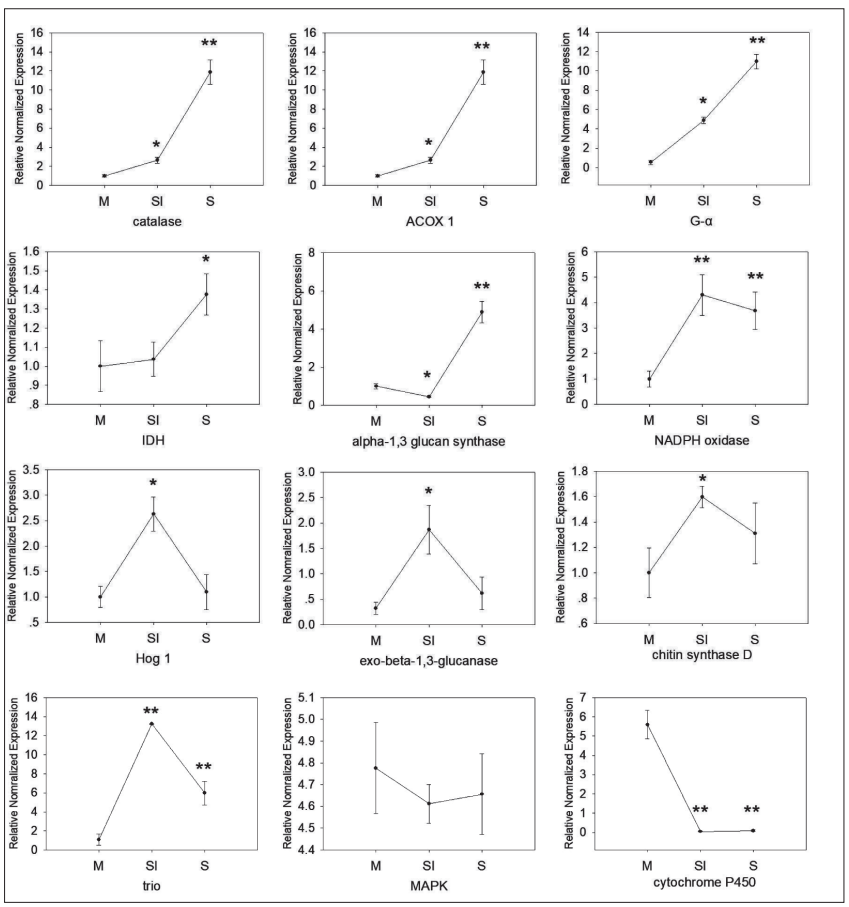

Fig. 3. Quantitative RT-PCR analysis of expression of 12 genes in the three sclerotial developmental stages (M, SI, S) of *Rhizoctonia solani* AG1-IA. M – mycelium; SI – initiation of sclerotium; S – maturation of sclerotium. The asterisk indicates that the mean value is significantly different from that of the mycelium stage (*P<0.05; **P<0.01).

DISCUSSION

Georgiou hypothesized that sclerotium development in filamentous fungi is caused by oxidative stress. According to the hypothesis, when oxygen enters the cells of a sclerotium-forming fungi, the cells are incapable of handling the high concentrations of ROS and form an unstable hyperoxidant state [26]. Oxidative stress activates a signal transduction pathway, causing cells to differentiate to isolate oxygen molecules. Simultaneously, the cells undergo a series of synergistic mechanisms, including increase in

consumption of oxygen molecules, limiting the entry of oxygen molecules, or generating antioxidants to balance peroxide concentration. During ROS production, cytochrome P450, NADPH oxidase and catalytic extracellular O2 generate superoxide radicals and use these as a base to generate a series of byproduct ROS. Superoxide radicals are converted into hydrogen peroxide and oxygen by superoxide dismutase (SOD) to balance the highly oxidized substances in the cells [26]. Acyl-coenzyme oxidase 1 (ACOX1) catalyzes the first rate-limiting step in peroxisomal beta-oxidation of medium and very long straight-chain fatty acids [27]. In this study, cytochrome P450 and NADPH oxidase 1 are highly expressed in the mycelium and initiation stages, suggesting that the early developmental stage of sclerotium generates ROS. In contrast, CAT and ACOX1 showed increased expression in the maturation stage, suggesting that the maturation stage increases the consumption of oxygen molecules and limits oxygen molecules from entering the cells to balance the peroxide content.

The signal transduction pathway induced by oxidative stress is mainly regulated by MAPK, HOG 1 and the G-protein α subunit. A conserved family of fungal MAPKs (in *Saccharomyces cerevisiae* Hog1, *S. pombe* Spc1, and possibly in filamentous fungi) is activated by many stress signals, including oxidative stress [28]. *R. solani* with a knockout G-protein α subunit showed a change in morphology, and the ability to form sclerotium was completely lost [11]. In the present study, the changes in expression levels of MAPK, HOG 1, and G-protein α subunit were evident, which is in accordance with their suggested role in ROS-induced signal transduction pathway.

Cell walls are composed of β-glucan and chitin, and the enzymes involved in their metabolism are chitin synthase D, exo-beta-1,3-glucanase, and protein, which have been hypothesized to preserve water content and energy [29]. In this study, chitin synthase D and exo-beta-1,3-glucanase were increased in expression in the initiation stage, suggesting that these two genes play a role in the thickening of cell walls.

In conclusion, we constructed a cDNA library of *R. Solani* AG1-IA and analyzed 12 genes that play different roles in the different stages of sclerotial growth. Our study shows that the genes with significant changes in expression may be associated with the formation of sclerotia. The findings of this study can be used as information that may be useful for developing methods for the prevention and control of diseases by *R. solani* AG1-IA.

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Author contributions: Bo Liu, Zhoujie Ma and Zenggui Gao conceived and designed the experiments; Xiaotong Gai and Shidao He contributed reagents and materials; Bo Liu collected and interpreted the data; Bo Liu and Yanqiu Sun analyzed the data statistically; Bo Liu wrote the paper; Yanfeng Wang and Zenggui Gao provided a guideline for the work.

Conflict of interest disclosure: The authors declare no conflict of interest.

REFERENCES

1. Zheng A, Lin R, Zhang D, Qin P, Xu L, Ai P, Ding L, Wang Y, Chen Y, Liu Y. The evolution and pathogenic mechanisms of the rice sheath blight pathogen. Nat Commun. 2013;4:1424.
2. Ogoshi A. Ecology and pathogenicity of anastomosis and intraspecific groups of *Rhizoctonia solani Kuhn*. Annu Rev Phytopathol. 1987;25(1):125-43.
3. Sattari A, Fakheri B, Noroozi M. Breeding for resistance to sheath blight in rice. Intl J Farm Alli Sci. 2014;3(9):970-79.
4. Gautam H, Bhardwaj M, Kumar R. Climate change and its impact on plant diseases. Curr Sci. 2013;105(12):1685-91.
5. Renfro B, Ullstrup A. A comparison of maize diseases in temperate and in tropical environments. PANS. 1976;22(4):491-8.
6. Prasanna B, Pixley K, Warburton ML, Xie CX. Molecular marker-assisted breeding options for maize improvement in Asia. Mol Breed. 2010;26(2):339-56.
7. Bruce A, McDonald, Celeste L. Pathogen population genetics, evolutionary potential, and durable resistance. Annu Rev Phytopathol. 2002;40(1):349-79.
8. Moni ZR, Ali MA, Alam MS, Rahman MA, Bhuiyan MR, Mian MS, Iftekharuddaula KM, Latif MA, Khan MA. Morphological and genetical variability among *Rhizoctonia solani* isolates causing sheath blight disease of rice. Rice Sci. 2016;23(1):42-50.
9. Townsend BB, Willetts HJ. The development of sclerotia in certain fungi. Trans Br Mycol Soc. 1954;37(3):213-21.
10. Kwon YS, Kim SG, Chung WS, Bae H, Jeong SW, Shin SC, Jeong MJ, Park SC, Kwak YS, Bae DW. Proteomic analysis of *Rhizoctonia solani* AG-1 sclerotia maturation. Fungal Biol. 2014;118(5-6):433-43.
11. Charoensopharat K, Aukkanit N, Thanonkeo S, Saksirirat W, Thanonkeo P, Akiyama K. Targeted disruption of a G protein α subunit gene results in reduced growth and pathoge-

nicity in *Rhizoctonia solani*. World J Microbiol Biotechnol. 2008;24(3):345-51.
12. Peraza L, Hansberg W. Neurospora crassa catalases, singlet oxygen and cell differentiation. Biol Chem. 2002;383(3-4):569-75.
13. Kim HJ, Chen C, Kabbage M, Dickman MB. Identification and characterization of *Sclerotinia sclerotiorum* NADPH oxidases. Appl Environ Microbiol. 2011;77(21):7721-9.
14. Soares MB, Bonaldo MF, Jelene P, Su L, Lawton L, Efstratiadis A. Construction and characterization of a normalized cDNA library. Proc Nati Acad Sci. 1994;91(20):9228-32.
15. Gasic K, Hernandez A, Korban SS. RNA extraction from different apple tissues rich in polyphenols and polysaccharides for cDNA library construction. Plant Mol Biol Rep. 2004;22(4):437-8.
16. Wiemann S, Mehrle A, Bechtel S, Wellenreuther R, Pepperkok R, Poustka A, Consortium GC. cDNAs for functional genomics and proteomics: the German Consortium. C R Biol. 2003;326(10):1003-9.
17. Zhu Y, Machleder E, Chenchik A, Li R, Siebert P. Reverse transcriptase template switching: A SMART™ approach for full-length cDNA library construction. Biotechniques. 2001;30(4):892-7.
18. Gunning P, Ponte P, Okayama H, Engel J, Blau H, Kedes L. Isolation and characterization of full-length cDNA clones for human alpha-, beta-, and gamma-actin mRNAs: skeletal but not cytoplasmic actins have an amino-terminal cysteine that is subsequently removed. Mol Cell Biol. 1983;3(5):787-95.
19. Seki M, Narusaka M, Ishida J, Nanjo T, Fujita M, Oono Y, Kamiya A, Nakajima M, Enju A, Sakurai T. Monitoring the expression profiles of 7000 Arabidopsis genes under drought, cold and high-salinity stresses using a full-length cDNA microarray. Plant J. 2002;31(3):279-92.
20. Rioux R, Manmathan H, Singh P, De los Reyes B, Jia Y, Tavantzis S. Comparative analysis of putative pathogenesis-related gene expression in two *Rhizoctonia solani* pathosystems. Curr Genet. 2011;57(6):391-408.
21. Zhulidov P, Bogdanova E, Shcheglov A, Shagina I, Wagner L, Khazpekov G, Kozhemyako V, Lukyanov S, Shagin D. A method for the preparation of normalized cDNA libraries enriched with full-length sequences. Russ J Bioorganic Chem. 2005;31(2):170-7.
22. Huang X,Madan A. CAP3: A DNA Sequence Assembly Program. Genome Res. 1999;9(9):868-77.
23. Martin DM, Bohlmann J. Identification of Vitis vinifera (−)-α-terpineol synthase by in silico screening of full-length cDNA ESTs and functional characterization of recombinant terpene synthase. Phytochem. 2004;65(9):1223-9.
24. Tatusov RL, Galperin MY, Natale DA, Koonin EV. The COG database: a tool for genome-scale analysis of protein functions and evolution. Nucleic Acids Res. 2000;28(1):33-6.
25. Tatusov RL, Fedorova ND, Jackson JD, Jacobs AR, Kiryutin B, Koonin EV, Krylov DM, Mazumder R, Mekhedov SL, Nikolskaya AN. The COG database: an updated version includes eukaryotes. BMC Bioinformatics. 2003;4(1):41.
26. Georgiou CD, Patsoukis N, Papapostolou I, Zervoudakis G. Sclerotial metamorphosis in filamentous fungi is induced by oxidative stress. Integr Comp Biol. 2006;46(6):691-712.
27. Morais S, Knoll-Gellida A, André M, Barthe C, Babin PJ. Conserved expression of alternative splicing variants of peroxisomal acyl-CoA oxidase 1 in vertebrates and developmental and nutritional regulation in fish. Physiol Genomics. 2007;28(3):239.
28. Aguirre J, Rios-Momberg M, Hewitt D, Hansberg W. Reactive oxygen species and development in microbial eukaryotes. Trends Microbiol. 2005;13(3):111-8.
29. Willetts HJ, Bullock S. Developmental biology of sclerotia. Mycol Res. 1992;96(10):801-16.

Supplementary Data

Supplementary Tables S1. and S2.
Available at: http://serbiosoc.org.rs/sup/SupplementaryData_TablesS1S2.pdf