Current Science International Volume : 06 | Issue : 01 | Jan.- Mar. |2017
ISSN: 2077-4435 Pages**:** 86-92

Effects of *Fusarium oxysporum* Infection on Chloroplast and Mitochondrial Functions in Resistant Soybean Plants

Heba M. M. Ibrahim

Genetics Department, Faculty of Agriculture, Cairo University, Giza, Egypt.

Received: 25 December 2016 / Accepted: 05 February 2017 / Publication date: 15 February 2017

ABSTRACT

Fusarium wilt (*Fusarium oxysporum*) is one of the most important plant pathogens of soybean. However, molecular processes associated with the soybean-fusarium interaction are still needed to be studied. In this research, effects of Fusarium wilt infection on the mitochondrial and chloroplast functions in resistant soybean plants were sought in order to understand the mechanism the plant adopt for quick response to the *F. oxysporum* invasion. Fusarium wilt affects the function of mitochondria and chloroplasts, causing gene expression disruption until one day after infection. The rate of mitochondrial function decreased from 100% in control leaves to almost 50% at 1 day after infection (dai). Significant decreases in the activities of cytochrome c oxidase and malate dehydrogenase (MDH) were observed in the fusarium wilt-infected leaves, suggesting serious impair in the tricarboxylic acid cycle (TCA) and electron transfer capacity. Chlorophyll and catalase enzyme dramatically decreased in the infected leaves from first and second days after infection. However, the mitochondrial and chloroplast functions were fully restored at the 2 dai except for the catalase enzyme, which exhibited a fully recovered expression 3 dai. These results provide new insights into understanding the mechanism of soybean-fusarium resistance interaction.

Key words: Soybean, Mitochondria, Photosynthesis, *Fusarium oxysporum*.

Introduction

Fusarium wilt *is* known to be an important plant disease that cause severe damages to many host plants including soybean. The causative of fusarium wilt is *F. oxysporum,* a pathogen that is persistently borne and live in the soil for many years without a host. Healthy plants can easily get infected by *F. oxysporum* if they are growing in soil infested with the pathogen (Ignjatov *et al*., 2012). *F. oxysporum infection* is also associated with some other symptoms such as the root rot damping-off, wilt, cortical decay, and vascular discoloration (Farias and Griffin, 1989; French and Kennedy, 1963; Leslie and Summerell, 2006; Nelson, 1999). Pathogenicity effects of *Fusarium oxysporum* on the mitochondrial and chloroplast functions have not been fully demonstrated in neither the resistant nor the susceptible plants.

Soybean (*Glycine max* L.) is one of the most important sources of oil and protein worldwide. Its protein content is considered the highest among leguminous crops (Abady *et al.,* 2008). Of all legume crops, soybean in particular, is infected with many different plant parasites and pathogens (Ibrahim *et al*., 2010 and 2011; Matthews, 2013). Multiple *Fusarium* species have been found to be associated with multiple soybean diseases (Arias *et al*., 2013). The annual losses of soybean yield caused by Fusarium wilt disease in particular, is still difficult to be quantified worldwide because the often combination of this infection with other pathogens or plant health problems (Arias et al., 2013).

Plants recognize the pathogen and respond to the infection via induction of several defense mechanisms (He *et al.*, 2013). One of the quick responses is the rapid production of active oxygen species (AOS) at the very early stages of infection. It has been considered and characterized as a common feature in the programmed cell death in many plant-pathogen interaction mechanisms (Greenberg *et al.*, 1994). In addition, some important aerobic metabolic processes such as respiration and photosynthesis mainly participate in the production of reactive oxygen species in mitochondria and chloroplasts (Apel & Hirt, 2004). Though *Fusarium oxisporum* fungi attack the plant roots, they also affect the leaves causing wilting of the stem and upper leaves, accordingly, affecting the

Corresponding Author: Heba M. M. Ibrahim, Genetics Department, Faculty of Agriculture, Cairo University, Giza, Egypt.
Email: heba.ibrahim@staff.cu.edu.eg

photosynthesis and respiration efficiency in the leaf tissue leading to premature leaves drop. Photosynthesis, respiratory pathways and defense responses to fusarium wilt pathogen have not been well covered in soybean or other legume plants. However, other leaf infecting fungi such as powdery mildew have been well documented in different plants (Wang *et al*., 2014). Hence, we reasoned that plant susceptibility to fusarium wilt could be related to malfunctions of mitochondria and/or chloroplasts.

Gene expression studies may provide insights into fusarium wilt disease pathogenicity and identify potential biomarkers for the relationship between fusarium and their host plants. In this study, a series of temporal molecular indices related to mitochondrial and chloroplast activities were investigated extensively in fusarium-inoculated resistant soybean plants using real-time PCR (RT-PCR), to examine the expression profiles of mitochondrial cytochrome C oxidase and malate dehydrogenase (MDH), as well as the chloroplast catalase, and chlorophyll a and b coding genes, at different time points of infection, to better understand the physiological effects of the infection and the rapid response of the resistant species against fusarium infection.

Materials and Methods

Plant material, fungal isolates and inoculation

Soybean seedlings, var. Giza 111 were used to study chloroplast and mitochondrial functions after inoculation with *Fusarium oxysporum*. *Fusarium* isolate used in the study was obtained from soybean root samples collected during 2014 in the department of plant protection, Faculty of Agriculture, Cairo University, Egypt. Inoculation was performed on 2-weeks soybean seedlings. Seedlings of the same age without inoculation were used as control, and kept in separate illuminated incubators to avoid cross infection by the fungus. The inoculated and control seedlings were grown at 25°C, 16 h day light and 80% relative humidity in green house. Three seedlings per treatment, and the control were used in each experiment . Three leaves from each plant were used for RNA extraction. The inoculated and control leaves were collected at 6 different time points before and after treatment (0, 1, 2, 3, 5, 7 dpi) for further study. qRT-PCR was performed in three biological replicates and each reaction was replicated three times.

RNA Extraction and cDNA Synthesis

Total mRNA was extracted using the High Pure RNA Isolation Kit (Thermo Fisher Scientific, MA, USA) according to the manufacturer's protocol, and the products were transferred to −80°C for storage. Total cDNA was produced using first strand cDNA synthesis Kit (Thermo Fisher Scientific, MA, USA), according to the manufacturer's protocol. The products were transferred to −20°C for storage. The cDNA was used for subsequent real time-PCR amplification of the mitochondrial cytochrome C oxidase and malate dehydrogenase (MDH), and chloroplast catalase, and chlorophyll a and b coding genes. Glyceraldehyde 3-phosphate dehydrogenase and beta–actin were used as the reference genes for gene expression analysis.

Real time-PCR Analysis

Relative quantification of the transcript levels of the genes encoding the cytochrome C oxidase, malate dehydrogenase (MDH), catalase, chlorophyll a and b, was investigated using real time-PCR. 100–300 ng of cDNA from the 6 time points and controls were subjected to real time-PCR amplifications in 20 µl reactions containing 1X absolute SYBER Green ROX Mix, and 100 nM of each specific primer (Table 1). All primer pairs were initially tested via standard RT-PCR using the same conditions as described for real-time RT-PCR. Amplification of single products of expected size was verified by electrophoresis on 3% agarose gel and ethidium bromide staining (data not shown). The real time-PCR amplifications were carried out in a Strategen MX 3000p apparatus (Stratagen, La Jolla, CA., USA) according to the following protocol: 15 seconds at 95°C, followed by 40 cycles of denaturation for 30 seconds at 95°C, annealing for 1 minute at 60°C and extension for 30 seconds at 72°C. Each experiment was performed in 3 replicates, and was repeated three times. Standard curves

were generated in triplicates for each primer using ten-fold serial dilutions of a cDNA sample prepared from total RNA. The efficiencies of the real time-PCR Startegen MX3000p software was used to determine the amplification cycle in which product accumulation was above the threshold (Ct). Real time-PCR Ct values were analyzed using the $2^{-\Delta\Delta Ct}$ method according to Rutledge and Stewart (2008) and Ibrahim *et al.* (2010). In each experiment, the mean Ct of the triplicates for a given gene was normalized to the mean Ct value of the reference genes.

Table 1: Primers used in RT-PCR reaction to quantify the transcript level of some molecular indices related to mitochondrial and chloroplast activities.

Transcript	Primers	
	Forward	Reverse
Cytochrome Oxidase	AGTCTGACCCTGCCACCTAT	CCGGCTTCCTTGAGTTGAGT
MDH	GGTGTGGCGGTTTTGTTGTT	AAGGGTACAACACCCTGCAA
Catalase	CTGCGGAGTCAGAAAGCCAT	ACGCTCGTGGATAACAGTGG
Chlorophyll a-b	TTTTCTCTCAACCGGCCCAA	TCTTCGCAAGGTTCTGGTCC

Results

Infection with *Fusarium oxysporum* affects chloroplast and mitochondrial functions in soybean plants

The *Fusarium oxysporum fungi could* successfully infect the light green leaves of 2-weeks old soybean seedlings and resulted in slight root rot and further leaves necrosis and wilting at later time points (Fig. 1). At 1 dai, all transcripts of the tested genes were noticeably down regulated.

Fig. 1: Giza 111 resistant soybean plants infected with *F. oxysporum* exhibiting mild symptoms after infection with slight necrosis spots on the leaves (Arrows) at 4 dai.

Cytochrome c oxidase transcripts decreased sharply by more than 50% in the infected leaves comparing with the un-inoculated control and quickly increased at 2 and 3 dai (Fig. 2). Similar results were detected for chlorophyll a and b transcripts. MDH transcripts was 2-fold higher in the control sample than that in the infected leaves at 1 dai and also had a recovered level of expression at 2 and 3

dai. Moreover, catalase enzyme transcript decreased at early time point of infection and sharply dropped at 2 dai (Fig. 2). The catalase transcript level increased up to reach back the same level of expression as the control at the 3 dai.

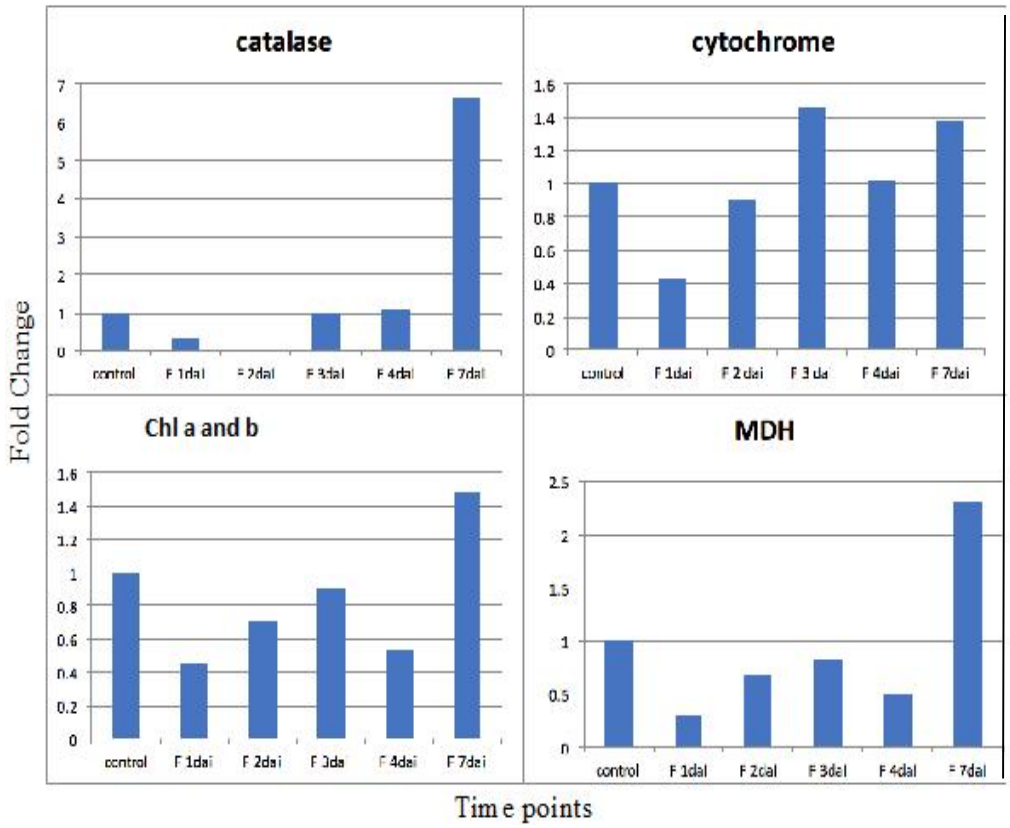

Fig. 2: The effect of *Fusarium oxysporum* on mitochondrial and chloroplast functions at different time points after infection compared to control. Bars with different letters showed significant differences at $P<0.01$ of fold changes.

Discussion

Fusarium wilt disease caused by *F. oxysporum* is one of the most important diseases in Egypt that affect a wide spectrum of plant species including soybean and cause root rot of soybean, leaf necrosis and premature dropping for leaves (Aries, 2012). Though this pathogen is soil-borne fungi and affect mainly the plant roots, the infection is followed by a rapid wilting of plant leaves leading to decline in photosynthesis, energy production, and regulation of other cellular metabolism processes. In the present study, the effect of fusarium invasion on the mitochondrial and chloroplast activities at different time points of infection was investigated by quantifying the level of gene expression of genes that code for mitochondrial and chloroplast enzymes to better understand the physiological effects of the infection. Four genes were chosen in this study, (cytochrome C oxidase, Malate Dehydrogenase (MDH), catalase, chlorophyll II a and b) and their products were identified and quantitatively measured in qRT-PCR using specific designed primers (Table 1) at different time points after infection with *fusarium oxysporum.*

Both cytochrome c oxidase and malate dehydrogenase are mitochondrial enzymes that have primary roles in the tricarboxylic acid (TCA) cycle and energy production. Cytochrome c oxidase is the last respiratory complex of the electron transfer chain in mitochondria and is responsible of transferring electrons to the final acceptor, oxygen, in the respiratory pathway. Mitochondrial malate dehydrogenase catalyzes the interconversion of malate and oxaloacetate to form citrate, reduces OAA to malate during conversion of the amino acid Gly to Ser, and catalyzes a reversible NAD^+-dependent-dehydrogenase reaction. Deficiency in MDH results in reducing leaf respiration and negatively affects the photorespiration process and plant growth (Tomaz *et al*., 2010; Wang *et al.,*

2015). Our results suggest interruption of the TCA cycle in mitochondria during infection with fusarium.

The chlorophylls are the main light absorbing pigments of the photosynthetic apparatus. In the tricarboxylic acid (TCA), an active malate dehydrogenase is required to facilitate carbon flow from phosphoenolpyruvate to oxaloacetate by reversibly catalyzing the oxidation of malate to oxaloacetate utilizing the reduction of NAD+ to NADH.

Catalase is the enzyme which releases gaseous oxygen from hydrogen peroxide and known to occur in chloroplasts (Mhamdi *et al.*, 2010). It catalyzes the decomposition of hydrogen peroxide to water and oxygen. The catalase activity can be detected in chloroplasts due to the presence of peroxisomes (Mhamdi *et al.*, 2010). Knocking out CAT2 enzyme-encoding gene using gene-specific T-DNA confirmed that, this major isoform of the leaf catalase gene is closely linked to photorespiration in Arabidopsis (Queval *et al.*, 2007). Its activity can also be determined by continuous monitoring of the decomposition of H_2O_2 at 240 nm (Wang *et al*., 2014). Our findings showed that the catalase transcript decreased at 1 dai, and almost disappeared at 2 dai. This is in accordance with the slight rotting of the roots and leaf necrosis (Fig. 1) observed at 1 dai. The catalase enzyme severely decreased on the second day of infection unlike the rest of the other tested transcripts, which exhibited accumulation of H_2O_2 leading to the release of ROS, and subsequent death of leaves. In plants, hydrogen peroxide (H_2O_2) plays a major signaling role in triggering both a defense response and cell death. In barley, Catalase-deficiency resulted in leaf bleaching (Kendall *et al.*, 1983), while in tobacco, *CAT1* antisense-transformed plants exhibited necrotic lesions due to the activation of certain defense responses against *Pseudomonas syringae* (Chamnongpol *et al.*, 1996; Takahashi *et al.*, 1997).

Moreover, the major decline in chlorophyll content indicated that *F. oxysporum* infection had a severe impact on photosynthetic efficiency of soybean leaves at the first exposure to the fungi. But, the noticeable and rapid straight increase of the chlorophyll content suggests a speedy response and recovery of the resistant Giza 111 variety from that infection, which implies a resistance characteristics against *F. oxysporum,* consistently with the study reported by Abdel-Monaim *et al*. (2011) which stated that Giza 111 is the least susceptible cultivar to *Fusarium oxysporum* among all the tested soybean cultivars (). However, another study showed that Giza 111 exhibited a susceptible reaction to fusarium infection (Abd El-Hai *et al*., 2016).

To date, detailed mechanisms of *F. oxysporum* infection and resistance in soybean are mostly unknown. The results reported here indicate that it could be concluded that the infection negatively affected both mitochondrial and chloroplasts function severely on the first day after infection, but the Giza 111 plants were able to retrieve the complete function of their mitochondria and plastids at the third day of infection. These results may lay foundations for further investigations of the mechanism of fusarium wilt (*F. oxysporum*) X Plant interactions at the transcriptome, proteome, and metabolome levels.

Acknowledgements

The authors thank Prof. Dr. Said Safina for providing the soybean seeds, and Dr. Maryan Makram for providing the *Fusarium oxysporum* isolate.

References

Abd EL-Hai, K.M., M. S. Elhersh, and M. K. Mahmoud, 2016. Incidence of Soybean Root and Stalk Rot Diseases as a Result of Antioxidant and Biotic Agents." Biotechnology 15.3/4: 52.

AbdeL-Monaim, M. F., M. E. Ismail, and K. M. Morsy, 2011. Induction of systemic resistance in soybean plants against Fusarium wilt disease by seed treatment with benzothiadiazole and humic acid. African Journal of Agricultural Research Vol. 6(26), pp. 5747-5756,

Apel, K., H.Hirt, 2004. Reactive oxygen species: Metabolism, oxidative stress, and signal transduction. Annual Review of Plant Biology 55:373-399.

Arias, M.M.D., L.F. Leandro and G.P. Munkvold. 2013. Aggressiveness of Fusarium Species and Impact of Root Infection on Growth and Yield of Soybeans. Phytopathology 103 (8), 822-832.

Chamnongpol, S., H. Willekens, C. Langebartels, M. Van Montagu, D. Inzé and W. Van Camp. Transgenic tobacco with a reduced catalase activity develops necrotic lesions and induces pathogenesis-related expression under high light. The Plant Journal, 1996, vol. 10 (pg. 491-503)

El-Abady, M.I., S.E.Seadh, A.N.Attia and E.A. El-Saidy Aml, 2008. Impact of foliar fertilization and its time of application on yield and seed quality of soybean; The 2nd Field Crops Conference, FCRI, AV.

Farias, G. M. and G. J. Griffin, 1989. Roles of *Fusarium oxysporum* and *Fusarium solani* in Essex disease of soybean in Virginia. Plant Dis. 73:38- 42.

French, E. R., and W. B. Kennedy, 1963. The role of *Fusarium* in the root rot complex of soybean in Minnesota. Plant Dis. Rep. 47:672-676.

Greenberg, J.T., A. Guo, D.F.Klessig, F.M. Ausubel, 1994. Programmed cell death in plants: A pathogen-triggered response activated coordinately with multiple defense functions. Cell 77:551-563.

He, P., H.Qin, M. Wu, B.Wu, J.Wei and D. Wang, 2013. Identification of genes differentially expressed in the roots of rubber tree (*Hevea brasiliensis* Muell. Arg.) in response to phosphorus deficiency. Molecular Biology Reports 40:1397-1405.

Ibrahim, H.M.M., N.W.Alkharouf, S.L.F.Meyer, M.A.M.Aly, A.Y.Gamal El-Din, E.H.A.Hussein and B.F. Matthews, 2010. Post-transcriptional gene silencing of root-knot nematode in transformed soybean roots. Experimental Parasitology. 127(1):90–99. [PubMed]

Ibrahim, H.M.M., P. Hosseini, N.W.Alkharouf, E.H.A.Hussein, A.Y.Gamal El-Din, M.A.M.Aly and B.F. Matthews, 2011. Analysis of gene expression in soybean (Glycine max) roots in response to the root knot nematode Meloidogyne incognita using microarrays and KEGG pathways. BMC Genomics. 12: 220.

Ignjatov, M., D. Milosevic, Z. Nikolic, J. Gvozdanovic-Varga, D. Jovicic and G.Zdjelar, 2012. Fusarium oxysporum as causal agent of tomato wilt and fruit rot. Pestic. Phytomed. (Belgrade), 27(1), 25–3. DOI: 10.2298/PIF1201025I.

Kendall AC, Keys AJ, Turner JC, Lea PJ, Miflin BJ. The isolation and characterization of a catalase-deficient mutant of barley (*Hordeum vulgare* L.), *Planta* , 1983, vol. 159 (pg. 505-511)

Leslie, J. F., and B. A. Summerell, 2006. The Fusarium Laboratory Manual. Blackwell Publishing, Oxford. Pages 35-36

Li-Feng, W., M. Wang and Y. Zhang. 2014. Effects of powdery mildew infection on chloroplast and mitochondrial functions in rubber tree. Tropical Plant Pathology 39 (3).

Matthews, B.F., H.M.M.Ibrahim, P.Hosseini, N.Alkharouf, and S.S. Natarajan, 2013. Response of Soybean Roots to Soybean Cyst Nematode at the Molecular Level. Roots: The Hidden Half, Fourth Edition.

Mhamdi, A., G.Queval, S. Chaouch, S.Vanderauwera, F. V.Breusegem and G.Noctor, 2010. Catalase function in plants: a focus on *Arabidopsis* mutants as stress-mimic models. J Exp Bot., 61 (15): 4197-4220.

Nelson, B. D., 1999. Fusarium blight or wilt, root rot, and pod and collar rot. in: Compendium of Soybean Diseases, 3rd ed. American Phytopathological Society, St. Paul, MN.

Queval, G., Issakidis-Bourguet, E., F.A.Hoeberichts, M.Vandorpe, B.Gakière, H.Vanacker, M.Miginiac-Maslow, F.Van Breusegem and G. Noctor, 2007. Conditional oxidative stress responses in the Arabidopsis photorespiratory mutant *cat2* demonstrate that redox state is a key modulator of day length-dependent gene expression, and define photoperiod as a crucial factor in the regulation of H2O2-induced cell death, The Plant Journal , vol. 52 (pg. 640-657)

Rutledge, R.G. and D.Stewart, 2008. A kinetic-based sigmoidal model for the polymerase chain reaction and its application to high-capacity absolute quantitative real-time PCR. BMC Biotechnology 8 (47).

Takahashi, H., Z.Chen, H. Du, Y. Liu, D.F. Klessig, 1997. Development of necrosis and activation of disease resistance in transgenic tobacco plants with severely reduced catalase levels, The Plant Journal, vol. 11 993-1005.

Tomaz, T., M. Bagard, I. Pracharoenwattana, P. Lindén, C. P. Lee, A. J. Carroll, E. Ströher, S. M. SteSmith, P.Gardeström and A. H. Millar, 2010. Mitochondrial Malate Dehydrogenase Lowers Leaf Respiration and Alters Photorespiration and Plant Growth in Arabidopsis. Plant Physiology November, 154 (3): 1143-1157.

Zhi-An Wang, Qing Li, Xiao-Yang Ge, Chun-Lin Yang, Xiao-Li Luo, An-Hong Zhang, Juan-Li Xiao, Ying-Chuan Tian, Gui-Xian Xia, Xiao-Ying Chen, Fu-Guang Li and Jia-He Wu, 2015. The mitochondrial malate dehydrogenase 1 gene GhmMDH1 is involved in plant and root growth under phosphorus deficiency conditions in cotton. Scientific Reports 5 (10343).