Original Article

TIPS Trends in Pharmaceutical Sciences 2017: 3(2): 71-82.

Analysis of the expression level of aquaporins under acetylene treatment and pathogen attack

Shiva Hemmati[1,2,*]

[1]Department of Pharmaceutical Biotechnology, School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran.

[2]Pharmaceutical Sciences Research Center, Shiraz University of Medical Sciences, Shiraz, Iran.

Abstract

Fusarium wilt disease and Sigatoka leaf spots threaten global market of *Musa sp*. Major Intrinsic Proteins (MIP) consisting aquaporins (AQPs) facilitate the transport of water and molecules like H_2O_2, CO_2, silicon, boron, urea, and ammonia. Biotic and abiotic stresses affect the expression level of MIPs and influence the transportation of water and nutrients, which results in the susceptibility of plants to diseases. Expression level of *MIP* genes in *Musa acuminata* (*MaMIPs*) fruits during development and under acetylene treatment; expression of *MaMIPs* in the corms of banana infected with *Fusarium oxysporum* cubense (Foc), and the expression of *MaMIP* genes in the leaves treated with *Mycosphaerella fijiensis* were retrieved from the banana genome hub database. Expression data of roots, treated with virulent Focs at 3, 27, and 51 hours post-inoculation (hpi) were downloaded from Gene Expression Omnibus. The expression data were analyzed using MeV 4.9 program. Expression level of *MaMIPs* was mainly suppressed by acetylene and biotic treatments. Twenty seven and 51 hpi of roots with Foc, 88% and 63% of *MaMIPs* were down-regulated. However, *MaNIP2-1* expression showed a significant up-regulation in all conditions. Infection of banana corms resulted in the suppression of *MaMIPs*. A low decrease in the expression of *MaMIPs* was observed, when the leaves were under *Mycosphaerella fijiensis* attack. Suppression of *MaMIPs* might be in line with repression of plant defense by banana pathogens as an approach for infection progression. Identification of the *MIPs* influenced by stresses provides the opportunity for the production of transgenic resistant cultivars.

Keywords: Aquaporin, Banana, Biotic stress, Expression profile, *Fusarium*, *Mycosphaerella*.

1. Introduction

Banana is one of the major stocks of food for millions of people throughout the world. Besides nutritional values, this species has numerous medicinal usages including ameliorating gastric disorders (1). Banana plants consist of an underground plant system named corm, serving as a storage organ and tightly packed sheaths (driven from petioles), which form a false stem (pseudo-stem) (2). The consequence of inter- or intra-specific crosses between wild species is the high ploidy level in banana plants (diploid, triploid, and tetraploid). Wild diploid species of banana, *Musa acuminata* (AA genome) and *Musa balbisiana* (BB genome) are the parent of the cultivated bananas, which are mostly triploid varieties of AAA (sweet bananas), AAB (cooking), and ABB (plantain bananas) (3). Compared to other cultivars, Cavendish (AAA) is the most favored cultivar of sweet banana because of enhanced growth and vigor (4).

With a production over 100 million tons annually, one of the most destructive fungal

Corresponding Author: Shiva Hemmati, Department of Pharmaceutical Biotechnology, School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran.
Email: hemmatish@sums.ac.ir

Recieved: 01/05/2017; Accepted: 20/05/2017

diseases in the agricultural history that threatens banana is *Fusarium oxysporum* cubense (Foc); a soil born ascomycete fungus, which is the cause of *Fusarium* wilt disease (5). The pathogenic isolates of Foc are classified as races, indicating the pathogenicity on specific banana cultivars. There are four main races of Foc. Race 1 is virulent to "Rasthali" (AAB) and "Gros Michel" (AAA) cultivars. "Bluggoe" (ABB) cultivar is susceptible to race 2. Race 3 does not affect banana and race 4 is virulent to "Cavendish" (AAA) cultivars. Subtropical race 4 and tropical race 4 (TR4) are subgroups of race 4 (6). Foc TR4 is the greatest threat to global banana production and the most important hazard to Cavendish production (2). Chlamydospore form of the pathogene survives in the soil after plant decay and is able to attack other plants. (7). Once the soil is infected, susceptible cultivars cannot be planted for at least 30 years (6). Soil spores germinate, penetrate to the root hairs and larger roots, then reaches the xylem vessels via cortex (8). While Foc attacks banana roots, the airborn fungal leaf spot disease, called black sigatoka disease (caused by the ascomycete fungus *Mycosphaerella fijiensis*) is the most destructive foliar disease in banana. *M. fijiensis* penetrates via stomata (9). Damage to the leaves affects photosynthetic area and results in 33-69% of yield loss (10). Three to four weeks before appearance of the lesion, pathogen settles among mesophyll cells.

Major intrinsic proteins (MIPs), consisting aquaporins (AQPs) and aquaglyceroporins, are involved in the transportation of water and small molecules from metalloids, such as boron and silicon (Si) to gaseous molecules, namely CO_2, as well as ammonia (NH_3), hydrogen peroxide (H_2O_2), and urea (11). Plasma membrane intrinsic proteins (PIPs), tonoplast intrinsic proteins (TIPs), nodolin-26 like intrinsic proteins (NIPs), and small basic intrinsic proteins (SIPs) are the most studied MIPs in plants (12). It has been shown that after *Fusarium* attack in different plant species, such as wheat, cotton, rice, cowpea, watermelon, and cucumber, *MIP* channels, involved in the resistance or susceptibility of the corresponding species, were either over-expressed or repressed (13-15). Identification of the molecular basis of AQP involvement in banana-Foc and banana-*Mycosphaerella* interaction by gene expression profiling analysis brings insight into the genetic basis of banana defense against these pathogens. For example, it has been demonstrated that H_2O_2 content in banana resistant cultivars elevated drastically in comparison to susceptible lines, after inoculation of Foc (16). Application of Si to the banana growth medium resulted in a 27% decrease in the signs of *Fusarium* wilt on the roots (17). Identification of MIPs involved in H_2O_2 transportation in banana provides the opportunity to increase banana resistance against biotic stress. Identification of Si transporters in banana provides a new approach to produce genetically modified crops with improved Si uptake capacity.

We have previously predicted the functionality of *MIP* genes in *Musa acuminata* (*MaMIPs*) (18). Herein, the transcriptional levels of *MaMIPs* in different organs and in the presence of *Fusarium* and *Mycosphaerella* (biotic) stresses and acetylene (abiotic) treatment based on available digital expression data have been analyzed.

2. Materials and methods

The expression data of *M. acuminata* was available at banana genome hub database, under "Search" (http://banana-genome-hub.southgreen.fr/transcriptomics) (19). Using accession numbers, indicated in Table 1, (GSMU_AchrxTxxxxx_001) (T represents Transcript) the transcript levels of *MaMIPs* in "Cavendish" (AAA) banana fruits during development and under acetylene treatment were retrieved from the banana genome hub. Different conditions were annotated as A-D. A (fruits 40 days after flowering), B (fruits 60 days after flowering), and C (fruits 90 days after flowering) were control groups without treatment. D (treated fruits 40 days after flowering), E (treated fruits 60 days after flowering), and F (treated fruits 90 days after flowering) were conditions after treatment with acetylene. Expression levels of the *MaMIPs* in the corms of "Pahang" (AA) banana infected with a virulent form of Foc TR4 (called R condition by banana genome database) compared to corms treated with the culture medium without pathogen as the control group, which is called mock (S) ,

Table 1. Nomenclature of the identified *MIP* genes in banana. Accession numbers, locus name, the corresponding gene ID in the GenBank, and the presence on either positive or negative strand is defined.

Gene name	Accession number	Genome location	Gene ID (GenBank)	Strand
MaPIP1-1	GSMUA_Achr1G00370_001	chr1:309949..311086	103968911	+
MaPIP1-2	GSMUA_Achr5G12400_001	chr5:8900863..8902050	103984478	+
MaPIP1-3	GSMUA_AchrUn_randomG06140_001	chrUn_random:25903674..25905413	103973264	-
MaPIP1-4	GSMUA_Achr11G07560_001	chr11:5891847..5893288	103970640	+
MaPIP1-5	GSMUA_Achr2G03980_001	chr2:9514063..9515412	103971568	+
MaPIP1-6	GSMUA_Achr10G06770_001	chr10:17047443..17048918	104000197	-
MaPIP1-7	GSMUA_AchrUn_randomG07520_001	chrUn_random:32391574..32392970	103973378	+
MaPIP1-8	GSMUA_AchrUn_randomG09120_001	chrUn_random:42488936..42490444	103973536	-
MaPIP1-9	GSMUA_Achr8G11540_001	chr8:8339009..8340316	103993246	+
MaPIP2-1	GSMUA_Achr8G18910_001	chr8:23819427..23820979	103994968	-
MaPIP2-2	GSMUA_Achr11G00590_001	chr11:351856..353504	103970064	-
MaPIP2-3	GSMUA_Achr4G20780_001	chr4:21312580..21314126	103983149	+
MaPIP2-4	GSMUA_AchrUn_randomG16230_001	chrUn_random:76546853..76548320	103974205	+
MaPIP2-5	GSMUA_Achr2G11190_001	chr2:14157672..14159101	103975821	-
MaPIP2-6	GSMUA_Achr5G11480_001	chr5:8226359..8227864	103984551	+
MaPIP2-7	GSMUA_Achr1G02360_001	chr1:1907059..1908631	103994286	-
MaPIP2-8	GSMUA_Achr8G33990_001	chr8:34900930..34902313	103995557	+
MaPIP2-9	GSMUA_Achr8G33980_001	chr8:34897878..34899390	103995558	+
MaPIP2-10	GSMUA_Achr1G26380_001	chr1:21887614..21890184	103998800	-
MaTIP1-1	GSMUA_Achr1G01270_001	chr1:964964..966054	103981942	-
MaTIP1-2	GSMUA_Achr10G13510_001	chr10:22108780..22109758	104000923	+
MaTIP1-3	GSMUA_Achr6G15380_001	chr6:10252515..10253899	103987892	+
MaTIP1-4	GSMUA_Achr5G19470_001	chr5:21119271..21120205	103985757	+
MaTIP1-5	GSMUA_Achr11G02240_001	chr11:1541891..1543020	103970208	-
MaTIP1-6	GSMUA_Achr8G12920_001	chr8:9684386..9685522	103993130	+
MaTIP2-1	GSMUA_Achr11G04570_001	chr11:3457818..3458973	103970395	+
MaTIP2-2	GSMUA_Achr11G21010_001	chr11:21805537..21806767	103972007	-
MaTIP2-3	GSMUA_Achr10G22240_001	chr10:27491957..27493172	103968936	+
MaTIP2-4	GSMUA_Achr9G28940_001	chr9:32753332..32754457	103999323	+
MaTIP2-5	GSMUA_Achr6G05830_001	chr6:3923145..3924353	103987068	+
MaTIP3-1	GSMUA_Achr4G26010_001	chr4:25303207..25304217	103982686	+
MaTIP3-2	GSMUA_Achr2G10640_001	chr2:13693713..13694806	103975851	-
MaTIP4-1	GSMUA_Achr9G06260_001	chr9:4002288..4003638	103997079	-
MaTIP4-2	GSMUA_Achr1G13800_001	chr1:10530095..10531480	103986437	-
MaTIP4-3	GSMUA_Achr10G26280_001	chr10:29916414..29917868	103969275	-
MaTIP5-1	GSMUA_Achr4G24600_001	chr4:24336496..24337748	103983448	+
MaNIP1-1	GSMUA_Achr6G14730_001	chr6:9771332..9773347	103987833	+
MaNIP1-2	GSMUA_Achr9G00710_001	chr9:545396..546827	103996599	+
MaNIP2-1	GSMUA_Achr10G29740_001	chr10:32140004..32143007	103969574	+
MaNIP2-2	GSMUA_Achr5G23340_001	chr5:25095141..25103807	103985432	+
MaNIP2-3	GSMUA_Achr6G02660_001	chr6:1725352..1727663	103986794	+
MaNIP2-4	GSMUA_Achr9G27460_001	chr9:31648075..31651067	103999196	-

Continued.

MaNIP3-1	GSMUA_Achr1G04130_001	chr1:3446141..3449511	103975807	+
MaNIP3-2	GSMUA_Achr11G16670_001	chr11:18372342..18373688	103972534	-
MaSIP1-1	GSMUA_Achr11G14150_001	chr11:15211602..15215046	103971426	+
MaSIP2-1	GSMUA_Achr5G27600_001	chr5:28050849..28055213	103986235	-
MaSIP2-2	GSMUA_Achr5G21450_001	chr5:23588489..23597854	103985586	-

were extracted from the banana genome hub. In addition, the expression level of "Pahang" *MaMIP* genes in leaves treated with mock as the control group (H) and *M. fijiensis* (L) were extracted from the banana genome database.

According to the banana genome hub database, the expression level values equal to 1 are assumed as extremely low expression, values from 2 to 5 is considered as very low expression, and 6-10 is called low expression. Values in the range of 11-25 and 26-100 are related to moderate and moderately high expression transcripts. Finally, an expression value around 101-500 is a high expression level.

Expression data of "Cavendish" banana roots, treated with Foc1 and FocTR4, at 3, 27, and 51 hrs post-inoculation (hpi) were downloaded from NCBI Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/) under accession no. GSE48563 or from EBI Array Express (https://www.ebi.ac.uk/arrayexpress/) under accession no. E-GEOD-48563 (20). Plants whose roots were immersed in the culture medium without pathogen (mock inoculation) were used as the control group. We have compared *MaMIP* gene expression pattern after infection in comparison with the control group.

The expression level of each *MaMIP* gene was analyzed using MeV 4.9 program (21). Gene sets were clustered using hierarchical clustering (HCL) using Pearson correlation, and the heatmap was displayed based on the transcript abundance pattern.

3. Results

3.1. Expression of MaMIPs in Cavendish banana fruit, according to the developmental stage (comparison of A, B, and C conditions)

The gene expression data of those *MaMIPs*, which were available for banana fruit, were retrieved from the banana genome database. Transcript level of *MaMIPs* in banana fruits, harvested 40 (A), 60 (B), and 90 (C) days after flowering (before any treatment) showed that 13% of *MaMIPs* (*MaPIP1-6*, *MaPIP2-6*, *MaPIP1-2*, and *MaPIP1-1*) had high abundant transcript levels during Cavendish fruit development and ripening (Figure 1a). Nineteen percent of *MaMIPs* (*MaPIP2-5*, *MaPIP2-2*, *MaTIP2-5*, *MaTIP1-5*, *MaTIP4-1*, and *MaTIP4-2*) had moderately high abundant transcript levels during Cavendish fruit development and ripening (Figure 1a). Comparison of A and B conditions showed that during the earlier stages of fruit development, *MaTIP4-1* and *MaTIP1-6* has a low up-regulation in transcript abundance. Comparison of B and C conditions showed that during the later stages of fruit development *MaPIP2-6*, *MaPIP1-6*, *MaTIP1-6*, and *MaTIP4-1* have a moderately high up-regulation in transcript abundance. *MaPIP1-2* and *MaPIP1-1* had high and moderately high down-regulation during fruit development (Figure 1a).

3.2. Effect of acetylene treatment on the expression level of MaMIPs in Cavendish banana fruits (comparison of A-D, B-E, and C-F conditions)

Effect of acetylene on banana fruits, 40 days after flowering (D), in comparison to the control group (A) showed that 71% of *MaMIPs* were down-regulated. Sixty and 90 days after flowering, a high suppression of *MaPIP1-2*, *MaPIP1-6*, and *MaPIP1-1* was observed under acetylene treatment (Figure 1a). Moderately high down-regulation was observed for *MaTIP2-5* and *MaTIP1-5*. For *MaPIP2-6* and *MaPIP2-3* high and moderately high transcript abundance was observed, respectively (Figure 1a). The effect of acetylene on banana fruits, 60 days after flowering (E) in comparison to the control group (B), showed that 53% of *MaMIPs* were down-regulated, and 22% of the studied *MaMIPs* did not have any changes in the expression level. A high suppression of *MaPIP1-2*,

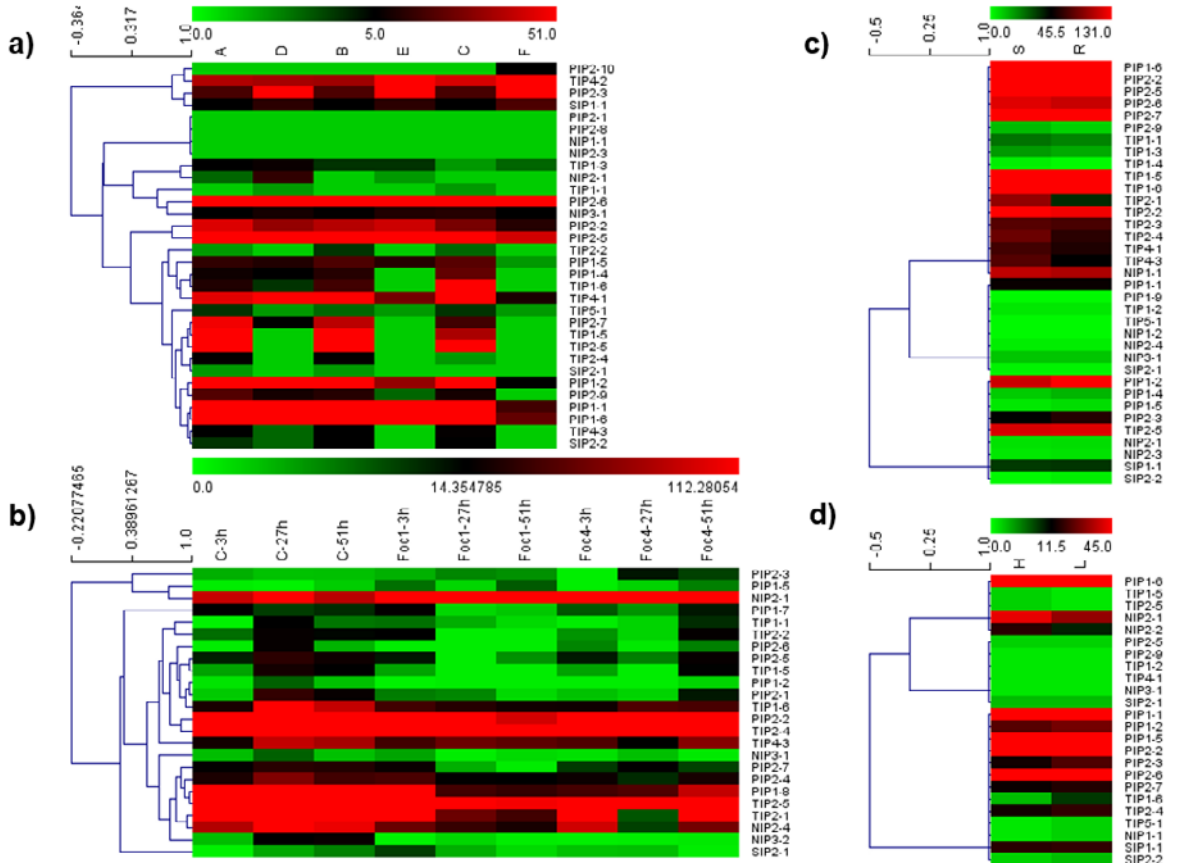

Figure 1. Expression profile of *MaMIP* genes. a) Transcript levels of *MaMIPs* in Cavendish banana fruits, during development and under acetylene treatment. A, B, and C (fruits 40, 60, and 90 days after flowering) are control groups without treatment. D, E, and F (fruits 40, 60, and 90 days after flowering) are conditions after treatment with acetylene. b) *MaMIP* gene expression pattern after infection of Cavendish roots with Foc1 and Foc TR4, at 3, 27, and 51 hpi. c) Expression levels of the *MaMIPs* in Pahang banana corms, infected with Foc TR4 (R), compared to the corms treated with mock (S). d) Expression level of Pahang *MaMIP* genes in treated leaves with mock as the control group (H) and *M. fijiensis* (L). Note: mock is the culture medium without pathogen, which serves as the control group.

MaPIP1-6, and *MaPIP1-1* was observed 90 days after flowering under acetylene treatment (Figure 1a). Moderately high down-regulation was observed for *MaTIP2-5*, *MaTIP1-5*, and *MaTIP4-1*. *MaPIP2-3* and had high transcript abundance (Figure 1a). The effect of acetylene on banana fruits, 90 days after flowering (F), in comparison to the control group (C) showed that 75% of *MaMIPs* were down-regulated. *MaPIP1-6*, *MaPIP1-1*, and *MaPIP1-2* were highly suppressed. A moderately high down-regulation was observed for *MaTIP4-1*, *MaTIP2-5*, *MaTIP1-6*, *MaPIP2-5*, and *MaTIP1-5*. High up-regulation of *MaPIP2-3* and moderately high increase for MaTIP4-2 was detected.

3.3. MaMIP response in Cavendish roots to Foc1 and Foc TR4

Expression level of Cavendish root genes was retrieved from GEO. Comparison of 24 *MaMIP* genes, expressed 3, 27, and 51 hpi of Cavendish roots with mock (the culture medium without pathogen), showed that the expression level of 92% of *MaMIP* genes were increased at 27 hpi. This level of *MaMIP* expression at 27 hpi remained consistent or partially decreased until 51 hpi (Figure 1b).

Three hpi of root with Foc1, 25% (6 out of 24) of genes were suppressed, while 63% (15 out of 24) were up-regulated. *MaNIP2-1* had a moderately high up-regulation in comparison to the control group. Three hpi of banana roots with TR4, 63% (15 out of 24) and 33% (8 out of 24) of genes had down- and up-regulation, respectively. Moderately high down-regulation of *MaPIP1-8* was observed. Moderately high increase in *MaNIP2-1* expression was detected (Figure 1b).

Twenty seven hpi of roots with Foc1 and TR4, 88% of *MaMIPs* (21 out of 24) were down-regulated. In the presence of Foc1 and Foc TR4, *MaTIP2-1*, *MaPIP1-8*, were highly suppressed. Moderate high down-regulation was observed for *MaTIP1-6*, *MaTIP4-3*, and *MaNIP2-4*. Fifty one hpi, *MaNIP2-1* expression showed a high increase after treatment with Foc1 and a moderately high expression in the presence of Foc TR4 (Figure 1b).

A pattern similar to 27 hpi has been observed for 51 hpi in the presence of Foc1. Fifty one hpi of roots with Foc TR4, 63% of *MaMIPs* (15 out of 24) were down-regulated and 37% (9 out of 24) were up-regulated. In the presence of Foc1, *MaTIP2-1* was highly suppressed 51 hpi. Moderate high down-regulation was observed for *MaPIP1-8*, *MaTIP1-6*, and *MaNIP2-4*. *MaNIP2-1* expression showed a high increase after treatment with Foc TR4.

3.4. Effect of Foc TR4 on the expression level of MaMIP in the corms of banana "Pahanag"

The extracted transcriptomic data of *MaMIPs* from *M. accuminata* DH Pahang Genome Project showed that 52% (19 out of 36) of *MaMIPs* have a high or moderately high abundance in the corm. In the presence of Foc TR4 (R) compared to the control group (S), out of 35 expressed genes, 22% showed unchanged expression. Three percent, 4%, and 2% showed extremely low, very low, and moderate increases in transcript abundance. Fifty one percent of MaMIPs were down-regulated (Figure 1c).

3.5. Response of MaMIP expression in banana leaves under M. fijiensis attack

Extracted transcriptome of *MaMIPs* from the banana genome project showed that 8% (2 out of 25) of *MaMIPs* consisting *MaPIP1-6* and *MaPIP1-1* have high abundance in the leaves. Sixteen percent (2 out of 25) including *MaPIP2-2*, *MaPIP1-5*, *MaPIP2-6*, and *MaNIP2-1* have moderately high expression in the leaves. In comparison to the control group (H) and in the presence of *M. fijiensis* (L), 20% of genes were unchanged. Twenty one percent showed extremely low, 21% had very low and 8% had low up-regulation after pathogen treatment. However, a moderate and a low decrease in the expression of *MaNIP2-1* were observed, respectively (Figure 1d).

4. Discussion

4.1. Why banana plants need MIPs?

Banana is the 4th important crop in developing countries (http://faostat.fao.org) following rice, wheat, and corn. Optimum availability of water and solutes is important for the health and growth yield of *Musa*. Higher salt tolerance has been achieved when *MaPIP2-6* was over-expressed in banana (22). Increased tolerance to salt and drought stress has been observed when *MaPIP1-1* and *MaPIP1-2* were over-expressed in *Arabidopsis* and banana, respectively (23, 24). Banana has a high nitrogen demand in the form of NH_3 and urea, transported by AQPs (25). This plant is susceptible to boron deficiency and toxicity (26). Sigatoka leaf spots and *Fusarium* wilt disease threaten regional and global market of banana (7,27). Susceptibility to pathogens such as *Fusarium* or *Mycosphaerella* results in H_2O_2 production. H_2O_2 is important to initiate signaling against pathogen, but at the same time can be toxic to the plant host cells (16). Decrease in CO_2 transport and photosynthetic efficiency, which in turn suppresses banana growth and fruit production, is the consequence of pathogen invasion to banana. It has been shown that Si can alleviate disease symptoms in banana (28). All the mentioned situations persist on the presence of a sensitive transport system such as MIP channels to regulate water and small solutes membrane permeability. Presence of MaMIPs is the prerequisite of normal development and provides tolerance to various threats that banana encounters.

4.2. Expression analysis of banana MIP genes

Effect of abiotic and biotic treatments on the *MaMIP* expression level was analyzed based on the available transcriptomic sources from banana genome database or GEO at NCBI. These include analysis of transcript profile of *MaMIPs* in the fruits of "Cavendish" (AAA) cultivars under acetylene treatment. The expression levels of *MaMIPs* in the roots of "Cavendish" (AAA) cultivar treated with Foc1 and FocTR4 were also compared. The effect of inoculation of the corm of

"Pahang" (AA) with Foc TR4 and leaves of "Pahang" (AA) variety with *M. fijiensis* on the *MaMIP* expression is discussed.

4.3. Expression of MaMIP genes under acetylene treatment

Transcription of plant *MIP* genes might be constitutive, inducible, or repressed upon environmental stimuli. Acetylene treatment is a strategy to initiate banana ripening. *MaPIP1-1*, *MaPIP1-2*, *MaPIP1-6*, *MaTIP2-5*, and *MaTIP1-5* were suppressed by acetylene in all stages of fruit development. *MaPIP2-3* was highly up-regulated by acetylene in all stages of fruit development. Although acetylene acts as an inhibitor of banana AQP channels, contra-indicatory results are reported on the effect of ethylene/acetylene treatment on the *AQP* expression level (29-31). One might conclude that transcription would be down-regulated, under some type of stress conditions to adjust intracellular osmotic pressure and protect cell death.

4.4. MaMIP response to Foc invasion

Resistant, tolerant, and susceptible plants, hinder, minimize, and flop the effect of pathogens, respectively (8). Foc, a soil born fungal pathogen invades the xylem vessels of banana, resulting in wilting and death of the plant (8). Understanding the nature of interaction between pathogen and host helps cultivar improvement. Most banana cultivars are resistance to Foc1, but Foc TR4 is still highly virulent on a vast number of *Musa sp*. Although it is claimed that *M. acuminata* (AA) and Cavendish (AAA) are resistant / tolerant to Foc1 and FocTR4, this behavior is highly dependent on the environmental conditions (20). Early infection process between Foc1 and Foc TR4, is not well clarified, because the first sign of disease comes out several weeks after infection. Li *et al*. inoculated a Cavendish banana cultivar that was relatively resistant to Foc1 and susceptible to Foc TR4 with the pathogen. The extracted RNA 3, 27, and 51 hpi was submitted to deep sequencing (20). We have mined and analyzed the transcription data of *MaMIPs* stored in GEO from the above experiment. The expression levels of 33% of more *MaMIP* genes were decreased 3 hpi under Foc TR4 attack in comparison to Foc1. Most of these genes (with a significant role for *MaPIP1-8* and *MaPIP2-2*) are probably involved in resistance toward Foc1 and susceptibility toward Foc TR4. The increase observed in the expression level of *MaNIP2-1* in the presence of Foc1 was twice the amount observed after treatment with Foc TR4, 3hpi. This observation persists on the role of MaNIP2-1 as a Si transporter, which probably provides a higher resistance against attack (18). *MaPIP2-2*, which was down-regulated to 51 hpi in the presence of Foc1, was up-regulated in the presence of Foc TR4, at the same incubation time. Successful invasion of pathogens can occur by suppressing defense related genes (32). Sometimes, induction of defense response is too late -in susceptible species in comparison to resistant ones- to afford an effective resistance mechanism. This phenomenon is observed 51 hpi for root-Foc TR4 interaction. In root-Foc interaction, down-regulation was stronger at 27 hpi than 3 hpi. This shift in gene expression might be due to the changes in the pathogen state, where an endophytic early stage of pathogen is converted to higher aggressive phase. Using transgenic Focs encoding a green fluorescent protein (GFP), the attachment of spores and hyphae to the root was at 27 hpi. Spreading of the hyphae into vascular tissue was at 51 hpi (20). On the other hand, one might conclude that a high similar pattern in root-Foc interaction, at early stages, does not provide enough evidence to discriminate the resistant lines. Differences in later infection stages are the cause of resistance or susceptibility of Cavendish cultivars to Foc1 or Foc TR4. For example, it has been shown in a separate study that Cavendish root inoculation with Foc TR4 enhanced the expression level of genes involved in the phenylpropanoid pathway, which results in the formation of precursors for lignin biosynthesis. Then, lignin acts as a barrier against pathogen penetration (33).

Comparison of the *MaMIP* gene expression in the inoculated Cavendish roots showed that the number of *MaMIP* genes that were up-regulated upon interaction with Foc1 was higher than the induced *MaMIPs* with Foc TR4. Since Cavendish is partially resistant to Foc1, this shows the important role of MaMIP channels under Foc attack. Induced genes might be involved in defense mech-

anisms. Comparison of resistant and susceptible genotypes of wheat to *Fusarium* has revealed that a gene from the *PIP1* subfamily has been over-expressed in the resistant lines (34). PIP1 over-expression in rice resulted in cuticle thickness (35). Involvement of a TIP, as a defense protein in the seed exudates of cowpea against *Fusarium f.* sp. *faseoli* has been demonstrated (36). An *AQP* from the *PIP2* subfamily has been expressed when cucumber was inoculated with *Fusarium f.* sp. *cucumerinum* (37). Transient production of ROS is the first response after pathogen attack. Li *et al*. compared the transcriptome profile of banana roots from susceptible wild type (barazilian) and resistant mutant (Nongke) treated with Foc TR4 (38). During the early stages of Foc TR4 attack, a rapid accumulation of H_2O_2 was observed (16). ROS induces downstream signaling molecule and mediates triggering of systemic defense. Lesion spread is limited in the tolerant banana cultivars by mediating the ROS signal (39, 40). ROS scavenging system had a higher expression in susceptible banana wild type, which suggests inhibitory role of ROS in pathogen colonization (38). Resistant plant lines to Foc have shown reinforcement of plant cell wall and phytoalexin production. Cell wall strengthening is the general mechanism after pathogen attack. Increased lignin (as a cell wall thickening polymer) deposition after interaction of tolerant banana cultivars to Foc TR4 has been observed (41). We have observed repression of *MIP* genes involved in H_2O_2 transportation in susceptible roots, which might result in suppression of lignin biosynthesis. On the other hand, the produced H_2O_2 after pathogen invasion can repress *MIP* gene expression. Treatment of *Arabidopsis* with H_2O_2 down-regulated the *AtPIP2* subfamily isoforms (42). There are evidences that upon ROS production after biotic and abiotic attack, PIP internalization occurs, which consequently down-regulate root water uptake capacity (43). Suppression of the predominant number of *MIPs*, which are involved in the transport of water and solutes in the banana root under Foc attack, is in line with wilt symptom progression. Suppression of *MaPIP2* subfamily, which are mainly water transporters across plasma membrane and *MaTIPs*, which are important to water transport across tonoplast, results in water deficiency and facilitates disease development in a similar way as *ZmPIP2-1* and *ZmPIP2-2* suppression in maize (12). Plasma and tonoplast membranes are important for water homeostasis and salt/drought tolerance. Water deficiency decrease hydraulic conductance and stomatal closure, which in turn affect stomatal and mesophyll conductance and decrease transpiration and photosynthesis (44).

Repression of *MIPs* in response to Foc attack is not a much known response in a pathogen-host interaction. This might be specific to vascular wilt disease and the development of wilt symptoms. However, similar observations have been reported previously for cotton-*Fusarium*, watermelon-*Fusarium* and wheat-*Fusarium* interactions (13-15).

4.5. Expression of MaMIPs in banana leaves after M. fijiensis attack

MaPIPs like *MaPIP2-2*, *MaPIP2-6*, *MaPIP1-1*, *MaPIP1-5*, and *MaPIP1-6*, which show constitutive expression levels in leaves, have solid role in total leaf water transport and growth. *MaPIP1-5* and *MaPIP2-2* showed a moderately high and moderate up-regulation, respectively. A suppression subtractive hybridization (SSH) followed by EST analysis has been performed at the late stages of leaf disease by portal et al., in which susceptible cultivar "Grande naine" was incubated with *M. fijiensis* (27). Genes involved in the phenylpropanoid and flavonoid biosynthetic pathways as well as the pathogenesis related proteins and jasmonate/ ethylene signaling transduction pathway were activated. Like other pathogen invasions, ROS production is one of the first responses after *Mycosphaerella* attack to the leaves (45). Reduction of disease percentage and delay in disease development was observed when Si has been added to the nutrient solution of banana inoculated with *M. fijiensis* (10). According to our analysis, *MaNIP2-1* has a moderately high expression level in the leaves, which is suppressed after *M. fijiensis* attack. Since this protein is predicted to be a Si transporter, down-regulation of *MaNIP2-1* gene after pathogen attack results in the disease progression. However, in contrast to a strong disruption of *MaMIP* gene expression after Foc invasion

to banana roots and corm, it seems that *MaMIPs* are not affected as much as *M. fijiensis* attack to the leaves.

4.6. The origin and expansion of MaPIP and MaTIP subfamilies

It is clear that *MaPIP* and *MaTIP* subfamilies have been expanded in comparison to that of rice and maize (18). Banana with an enormous food security role and trade potential is sensitive to any kind of water deficit (24). This tropical plant experiences drastic seasonal drought and flooding. Since plasma and tonoplast membranes are permeable to water, part of the ability to tolerate such climate variations is due to the role of PIP and TIP channels. For example, in gradual drought stress, most *PIPs* are down-regulated. Higher salt tolerance has been achieved when *MaPIP2-6* was over-expressed in banana. An increased tolerance to salt and drought stress has been observed when *MaPIP1-1* and *MaPIP1-2* were over-expressed in *Arabidopsis* and banana, respectively (22-24). When banana roots were under Foc attack, all of the studied *MaPIPs* and *MaTIPs* were either induced or suppressed by various degrees, at least in one condition. This amount was 85%, when banana corm was under Foc attack. Acetylene treatment resulted in down or up-regulation of all *MaPIPs* and *MaTIPs* in fruits, at least in one condition. All these reveal that MaPIP and MaTIP channels play substantial roles under harsh conditions. Under biotic attack, in parallel with other mechanisms, ROS species such as H_2O_2 are produced (46). H_2O_2 is both a toxic metabolite and a signaling intermediate. Along with our predictions, other studies have shown that PIPs and TIPs are able to transport H_2O_2 efficiently. PIPs and TIPs might have a role in signal propagation, as described (12). Expansion of these two subfamilies in banana might be correlated with the pathogenicity of Focs. It has been demonstrated that some *AQP* isoforms are not essential under optimal growth condition, since other members of the family compensate the role (47). Under optimal conditions, some *MaPIPs* and *MaTIPs* might be redundant, while under stress, specific isoforms make a significant contribution to the whole plant hydraulics. Sometimes high numbers of duplicated genes are under expression sub-functionalization. *MaTIP2-1* and *MaTIP2-2* are duplicated genes. The former gene is expressed in the roots and the latter in the corm. *MaTIP1-5* and *MaTIP1-6* are the other duplicated genes with expression in the roots and in the corm, respectively. Limited duplication of the *MaNIP* subgroup might be due to the fact that some minerals are toxic to plants. Since NIP proteins transport not only Si but also arsenic and germanium, high number of permeable MaNIPs might be lethal to the plants (48).

5. Conclusion

Musa sp. encounters various biotic and abiotic threats, which makes banana as an intriguing crop to investigate aspects of AQPs that cannot be studied in other monocots such as rice or maize. Herein, we have shown dominant numbers of the *MaPIP* and *MaTIP* genes were suppressed under acetylene treatment and pathogen attack, which demonstrate that these channels play substantial roles under harsh conditions. Although, down-regulation of *MIP* genes might help the progression of disease under pathogen attack in susceptible cultivars, suppression of *MIPs* under some type of stress conditions adjust intracellular osmotic pressure and protect cell death in resistant lines. Manipulation of the corresponding *AQPs* in susceptible lines is suggested for the formation of resistant cultivars.

Acknowledgements

Authors would like to thank Shiraz University of Medical Sciences, Shiraz, Iran.

Conflict of Interest

None declared.

6. References

1. Kumar KS, Bhowmik D. Traditional and medicinal uses of banana. *J Pharmacogn Phytochem*. 2012;1:51-63.

2. Jones KW. Silicon in banana plants: uptake, distribution and interaction with the disease fusarium wilt. Thesis. The University of Queensland. 2014.

3. Davey MW, Gudimella R, Harikrishna JA, Sin LW, Khalid N, Keulemans J. A draft *Musa balbisiana* genome sequence for molecular genetics in polyploid, inter- and intra-specific Musa hybrids. *BMC Genomics.* 2013;14:683.
4. Ravi I, Uma S, Vaganan MM, Mustaffa MM. Phenotyping bananas for drought resistance. *Front Physiol.* 2013;4:9.
5. Ploetz RC. Fusarium wilt of banana. *Phytopathology.* 2015;105:1512-21.
6. Ploetz RC. Fusarium wilt of banana is caused by several pathogens referred to as *Fusarium oxysporum f. sp. cubense*. *Phytopathology.* 2006;96:653-6.
7. Guo L, Han L, Yang L, Zeng H, Fan D, Zhu Y, et al. Genome and transcriptome analysis of the fungal pathogen *Fusarium oxysporum f. sp*. cubense causing banana vascular wilt disease. *PLoS One.* 2014;9:e95543.
8. Swarupa V, Ravishankar KV, Rekha A. Plant defense response against *Fusarium oxysporum* and strategies to develop tolerant genotypes in banana. *Planta*. 2014;239:735-51.
9. Balint-Kurti PJ, May GD, Churchill AC. Development of a transformation system for *Mycosphaerella pathogens* of banana: a tool for the study of host/pathogen interactions. *FEMS Microbiol Lett.* 2001;195:9-15.
10. Kablan L, Lagauche A, Delvaux B, Legrève A. Silicon reduces black Sigatoka development in banana. *Plant Disease.* 2012;96:273-8.
11. Kaldenhoff R, Bertl A, Otto B, Moshelion M, Uehlein N. Characterization of plant aquaporins. *Methods Enzymol.* 2007;428:505-31.
12. Maurel C, Santoni V, Luu DT, Wudick MM, Verdoucq L. The cellular dynamics of plant aquaporin expression and functions. *Current Opin Plant Biol.* 2009;12:690-8.
13. Golkari S, Gilbert J, Prashar S, Procunier JD. Microarray analysis of *Fusarium graminearum* induced wheat genes: identification of organ specific and differentially expressed genes. *Plant Biotech J.* 2007;5:38-49.
14. Lü G, Guo S, Zhang H, Geng L, Song F, Fei Z, et al. Transcriptional profiling of watermelon during its incompatible interaction with *Fusarium oxysporum f. sp. niveum*. *Eur J Plant Pathol.* 2011;131:585-601.
15. Dowd C, Wilson IW, McFadden H. Gene expression profile changes in cotton root and hypocotyl tissues in response to infection with *Fusarium oxysporum f. sp. vasinfectum. MPMI.* 2004;17:654-67.
16. Li W-M, Qian C-M, Mo Y-W, Hu Y-L, Xie J-H. Tolerance of banana for Fusarium wilt is associated with early H_2O_2 accumulation in the roots. *Afr J Biotechnol.* 2013;10:11378-87.
17. Fortunato AA, da Silva WL, Rodrigues FA. Phenylpropanoid pathway is potentiated by silicon in the roots of banana plants during the infection process of *Fusarium oxysporum f. sp. cubense. Phytopathology.* 2014;104:597-603.
18. Hemmati S. Predicting the functionality of major intrinsic proteins: An *in silico* analysis in *Musa. Trends in Pharmaceutical Sciences.* 2016;2:139-150.
19. Droc G, Lariviere D, Guignon V, Yahiaoui N, This D, Garsmeur O, *et al.* The Banana Genome Hub. *Database.* 2013;2013:1-14.
20. Li C, Shao J, Wang Y, Li W, Guo D, Yan B, et al. Analysis of banana transcriptome and global gene expression profiles in banana roots in response to infection by race 1 and tropical race 4 of *Fusarium oxysporum f. sp. cubense*. *BMC Genomics*. 2013;14:851.
21. Saeed AI, Sharov V, White J, Li J, Liang W, Bhagabati N, *et al*. TM4: a free, open-source system for microarray data management and analysis. *BioTechniques.* 2003;34:374-8.
22. Sreedharan S, Shekhawat UK, Ganapathi TR. Constitutive and stress-inducible overexpression of a native aquaporin gene (MusaPIP2;6) in transgenic banana plants signals its pivotal role in salt tolerance. *Plant Mol Biol.* 2015;88:41-52.
23. Xu Y, Hu W, Liu J, Zhang J, Jia C, Miao H, et al. A banana aquaporin gene, MaPIP1;1, is involved in tolerance to drought and salt stresses. *BMC Plant Biol.* 2014;14:59.
24. Sreedharan S, Shekhawat UK, Ganapathi TR. Transgenic banana plants overexpressing a native plasma membrane aquaporin MusaPIP1;2 display high tolerance levels to different abiotic stresses. *Plant Biotech J.* 2013;11:942-52.
25. Kojima S, Bohner A, von Wiren N. Molecular mechanisms of urea transport in plants. *J Membr Biol.* 2006;212:83-91.
26. Shapira OR, Israeli Y, Shani U, Schwartz A. Salt stress aggravates boron toxicity symptoms in banana leaves by impairing guttation. *Plant Cell Environ.* 2013;36:275-87.

27. Portal O, Izquierdo Y, De Vleesschauwer D, Sanchez-Rodriguez A, Mendoza-Rodriguez M, Acosta-Suarez M, *et al.* Analysis of expressed sequence tags derived from a compatible *Mycosphaerella fijiensis*-banana interaction. *Plant Cell Rep*. 2011;30:913-28.
28. Fortunato AA, Rodrigues FÁ, Baroni JCP, Soares GCB, Rodriguez MAD, Pereira OL. Silicon suppresses Fusarium wilt development in banana plants. *J Phytopathol.* 2012;160:674-9.
29. Ma N, Xue J, Li Y, Liu X, Dai F, Jia W, *et al*. Rh-PIP2;1, a rose aquaporin gene, is involved in ethylene-regulated petal expansion. *Plant Physiol*. 2008;148:894-907.
30. Aroca R, Porcel R, Ruiz-Lozano JM. Regulation of root water uptake under abiotic stress conditions. *J Exp Bot.* 2012;63:43-57.
31. Chervin C, Tira-Umphon A, Terrier N, Zouine M, Severac D, Roustan JP. Stimulation of the grape berry expansion by ethylene and effects on related gene transcripts, over the ripening phase. *Physiol Plant.* 2008;134:534-46.
32. Ponce de Leon I, Montesano M. Activation of Defense Mechanisms against Pathogens in Mosses and Flowering Plants. *Int J Mol Sci.* 2013;14:3178-200.
33. Wang Z, Zhang J, Jia C, Liu J, Li Y, Yin X, *et al.* De novo characterization of the banana root transcriptome and analysis of gene expression under *Fusarium oxysporum f. sp. Cubense* tropical race 4 infection. *BMC Genomics.* 2012;13:650.
34. Foroud N, Ouellet T, Laroche A, Oosterveen B, Jordan M, Ellis B, *et al.* Differential transcriptome analyses of three wheat genotypes reveal different host response pathways associated with Fusarium head blight and trichothecene resistance. *Plant Pathol.* 2012;61:296-314.
35. Hanba YT, Shibasaka M, Hayashi Y, Hayakawa T, Kasamo K, Terashima I, *et al.* Overexpression of the barley aquaporin HvPIP2;1 increases internal CO_2 conductance and CO_2 assimilation in the leaves of transgenic rice plants. *Plant Cell Physiol.* 2004;45:521-9.
36. Rose TL, da Silva Conceicao A, Xavier-Filho J, Okorokov LA, Fernandes KV, Marty F, *et al.* Defense proteins from *Vigna unguiculata* seed exudates: characterization and inhibitory activity against *Fusarium oxysporum*. Plant Soil. 2006;286:181-91.
37. Zhou X, Wu F. Differentially expressed transcripts from cucumber (*Cucumis sativus* L.) root upon inoculation with *Fusarium oxysporum f. sp. cucumerinum* Owen. *Physiol Mol Plant Path.* 2009;74:142-50.
38. Li CY, Deng GM, Yang J, Viljoen A, Jin Y, Kuang RB, *et al.* Transcriptome profiling of resistant and susceptible Cavendish banana roots following inoculation with *Fusarium oxysporum f. sp. cubense* tropical race 4. *BMC Genomics.* 2012;13:374.
39. Lu Y, Liao D, Pu J, Qi Y, Xie Y. Proteome analysis of resistant and susceptible Cavendish banana roots following inoculation with *Fusarium oxysporum f. sp. cubense. Physiol Mol Plant Path.* 2013;84:163-71.
40. Li X, Bai T, Li Y, Ruan X, Li H. Proteomic analysis of *Fusarium oxysporum f. sp. cubense* tropical race 4-inoculated response to Fusarium wilts in the banana root cells. *Proteome Sci.* 2013;11:41.
41. de Ascensao AR, Dubery IA. Soluble and wall-bound phenolics and phenolic polymers in Musa acuminata roots exposed to elicitors from *Fusarium oxysporum f. sp. cubense. Phytochemistry*. 2003;63:679-86.
42. Hooijmaijers C, Rhee JY, Kwak KJ, Chung GC, Horie T, Katsuhara M, *et al*. Hydrogen peroxide permeability of plasma membrane aquaporins of *Arabidopsis thaliana. J Plant Res.* 2012;125:147-53.
43. Boursiac Y, Boudet J, Postaire O, Luu DT, Tournaire-Roux C, Maurel C. Stimulus-induced downregulation of root water transport involves reactive oxygen species-activated cell signalling and plasma membrane intrinsic protein internalization. *Plant J.* 2008;56:207-18.
44. Sade N, Vinocur BJ, Diber A, Shatil A, Ronen G, Nissan H, *et al.* Improving plant stress tolerance and yield production: is the tonoplast aquaporin SlTIP2;2 a key to isohydric to anisohydric conversion? *New Phytol.* 2009;181:651-61.
45. Passos MA, de Cruz VO, Emediato FL, de Teixeira CC, Azevedo VC, Brasileiro AC, *et al.* Analysis of the leaf transcriptome of *Musa acuminata* during interaction with *Mycosphaerella musicola*: gene assembly, annotation and marker development. *BMC Genomics.* 2013;14:78.
46. Vanhove AC, Vermaelen W, Panis B, Swennen R, Carpentier SC. Screening the banana biodiversity for drought tolerance: can an *in vitro*

growth model and proteomics be used as a tool to discover tolerant varieties and understand homeostasis. *Front Plant Sci*. 2012;3:176.
47. Martinez-Ballesta MdC, Carvajal M. New challenges in plant aquaporin biotechnology. *Plant Sci*. 2014;217:71-7.
48. Mitani N, Yamaji N, Ma JF. Identification of maize silicon influx transporters. *Plant Cell Physiol*. 2009;50:5-12.