# Differential molecular response of maize and Johnson grass against maize dwarf mosaic virus and bermuda grass southern mosaic virus

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**Summary. –** Maize dwarf mosaic virus (MDMV) and bermuda grass southern mosaic virus (BgSMV) is the most important cereal potyvirus in Iran. Expression of some key genes in maize plants susceptible and tolerant to MDMV or BgSMV and gene expression profile of MDMV and BgSMV compatible or incompatible to Johnson grass plants were studied. Time points of 1, 9, 24 and 72 h after inoculation with both viruses were investigated as well. By analyzing the expression of the genes, it was identified that in maize infected by MDMV and BgSMV, the transcript levels of the *peroxiredoxin*, *GLP*, *SAM*, *NPR1* and *chlorophyll a-b binding* genes were significantly higher in the tolerant than in susceptible plants during the entire experiment. In the BgSMV inoculated Johnson grass plants, some of the genes related to plant defense responses including *NPR1*, *peroxiredoxin* and *SAM* had higher expression level than the Johnson grass plants inoculated by MDMV. Important genes in maize tolerance like *NPR1* and *MT-LP*, were analyzed by trilinear decomposition analysis and genes clustering. The upregulated expression of genes at one-hour post inoculation showed that the plant response to viruses was activated at the early stage of infection.

**Keywords:** MDMV; BgSMV; gene expression; quantitative real-time PCR; trilinear decomposition analysis

## Introduction

Sweet corn (*Zea mays* L.) is a cereal grain first domesticated in Mexico. Actually**,** it is one of the most widely cultivated crops in Iran, used for animal feed, corn ethanol production and corn starch feeding. maize dwarf mosaic virus (MDMV) and bermuda grass southern mosaic virus (BgSMV) are the most yield-limiting factors in cultivated sweet corn varieties in Iran.

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**Abbreviations:** BgSMV = bermuda grass southern mosaic virus; dpi = days after inoculation; FKBP = FK506-binding protein; GF14-6 = G-box factor 14-6; GLP = germin-like protein; hpi = hours post inoculation; MDMV = maize dwarf mosaic virus; MT-LP = metallothionein like protein; NPR1 = nonexpressor of pathogenesis related genes 1; qRT-PCR = quantitative real-time PCR; SAM = S-adenosyl methionine synthase

Both viruses are ssRNA positive strand viruses of the family *Potyvirus* that cause serious disease on cereals and can be spread via aphids in non-persistent transmission manner (Uzarowska *et al.*, 2009; Masumi *et al.*, 2011a; Cueto-Ginzo *et al.*, 2016).

Bermuda grass mosaic virus disease is caused by two viral groups including: bermuda grass southern mosaic virus (BgSMV) and bermuda grass mosaic virus (BgMV). BgSMV has a widespread distribution across temperate and tropical regions of southern Iran. BgSMV is similar to MDMV but serological, molecular, host range and vector transmission studies had demonstrated that BgSMV is a new species of the genus *Potyvirus* (Zare *et al.*, 2005). Both viruses are able to infect maize and other cereals. Johnson grass plants can be infected by MDMV and it is known as overwintering host of MDMV, however BgSMV virus is not able to infect Johnson grass plants (Masumi *et al.*, 2011b; Mostafavi *et al.*, 2015).

In sensitive and tolerant plants during viral infection, the expression level of defense-related genes are altered based on genetic abilities to recognize pathogen attack (Hao *et al.*, 2012).

The elevated expression of defense-related genes such as *SAMs* and *G-box factor 14-6* (*GF14-6*) leads to production of resistance proteins which are considered as molecular responses of maize to variety of biotic stresses (Chen *et al.*, 2006; Uzarowska *et al.*, 2009; Campo *et al.*, 2012).

Recently, functional genomic analysis has revealed the molecular mechanisms of virulence-related genes involved in the plant resistance pathways (Wu *et al.*, 2013).

Microarray data analysis for gene expression profiling has shown that the expression level of some putative defense-related genes such as *metallothionein-like* protein, *S-adenosyl methionine synthetase*, *germin-like* protein and *26S ribosomal RNA* gene were increased in corn plants infected by sugarcane mosaic virus (SCMV) and MDMV (Uzarowska *et al.*, 2009).

The post transcriptional analysis by proteomic approach has demonstrated a high expression level of *peroxiredoxin* and ascorbate peroxidase genes in maize infected with sugarcane mosaic virus (Wu *et al.*, 2013).

*Metallothionein-like* protein, *S-adenosyl methionine synthetase*, *germin-like* protein, *chlorophyll a-b binding* protein, *peroxiredoxin*, *FK506-binding protein* (*FKBP*), *GF 14-6* and *NPR1* genes are involved in different points of plant defense signaling pathways and response to stress including metabolism and photosynthesis processes.

*Metallothionein* proteins (*MTs*) with a low molecular weight and rich in cysteines are known to play an important role in the regulation of metabolic pathways and also to detoxify heavy metals in maize (Hamer, 1986; Singh *et al.*, 2011). Increase of *germin-like proteins* (GLPs) encoded by multigene families has been reported in several plant species stressed by biotic factor as pathogenic agents and insect feeding and also by application of different biologically active compounds such as salicylic acid, $H_2O_2$ or ethylene (Marathe *et al.*, 2004; Lane, 2002; Zimmermann *et al.*, 2006). Ethylene is responsible for plant disease resistance signaling pathways and *S-adenosyl methionine synthase* (*SAMs*) which is a key enzyme in ethylene biosynthesis (Gomez-Gomez and Carrasco, 1998).

*Peroxiredoxins* (*Prx*) are thiol-dependent antioxidant enzymes that play primary roles in the regulation of peroxide levels in cellular redox signaling (Haslekas *et al.*, 2003).

*G-box factor 14-6* (*GF14-6*) as a member of 14-3-3 family contributes to enhanced defense mechanisms and also is able to influence biological processes involved in stress-related mechanisms (Gökirmak *et al.*, 2010; Oh *et al.*, 2010; Campo *et al.*, 2012). Ability of *GF14-6* gene to recognize parts of viral coat protein has been demonstrated and important regulatory role of this gene during plant defense has been suggested (Konagaya *et al.*, 2004).

The role of *FKBPs* genes have been recently demonstrated in plant stress tolerance and immunology (Wang *et al.*, 2012). In many plants, *FKBPs* genes are a critical regulators of biotic and abiotic stress response and normal growth and development (Hartl and Hayer-Hartl, 2002).

Gene expression analysis by quantitative real-time PCR (qRT-PCR) using defense-related genes could be a reliable way of improving plant tolerance to certain abiotic stress factors as viral infection. Some genes are consistently expressed in plants and are altered at stress conditions. In this study, the expression level of some key genes in both susceptible and tolerant maize genotypes against MDMV and BgSMV were studied. Also, we evaluated the expression levels of these genes in Johnson grass plants compatible or incompatible to MDMV and BgSMV to illustrate the role of these genes in virus host adaptation.

## Materials and Methods

*Plant material and virus infection*. The seeds of maize SC705 cultivar susceptible to MDMV and BgSMV and 8-hybrid seeds (KLM75010/4-4-1-2-1-1-1×MO17) from cultivar tolerant to the both viruses were used. Maize and Johnson grass seeds were obtained from Agricultural and Natural Resources Research Center of Golestan province, Iran. Seeds were surface sterilized in 3% chloramines T (Sigma, France) for 5 min and washed three times in sterile distilled water. Surface sterilized seeds were planted in plastic pots (20 cm deep × 15 cm top diameter) containing autoclaved sand-loam-clay soil (1:2:1). The plants were cultured in greenhouse under controlled conditions at 25–28°C for 15 days before virus inoculation and were protected against insect attack. Maize and Johnson grass plants were mechanically inoculated with MDMV (GenBank Acc. No. JQ280313) and BgSMV (GenBank Acc. No. KU372146) particles at the three-leaf stages and were maintained in a separate greenhouse to avoid cross-contamination. Inoculums were prepared from the sap of the MDMV and BgSMV infected maize leaves in two volumes of 0.05 mol/l phosphate buffer pH 7 and were applied by gentle rubbing with a finger by using carborundum powder. Mock-inoculation was carried out using the inoculation buffer alone. The four parallel replications, each consisting of three pots, were performed for each treatment. Leaves were collected from MDMV and BgSMV-inoculated and mock-inoculated plants at five-time intervals (0, 1, 9, 24 and 72 h post inoculation (hpi)). Harvested leaves from infected and control plants were immediately frozen in liquid nitrogen and then were stored at -80°C until RNA extraction.

*Gene expression changes*. The expression levels of tested genes were measured 1, 9, 24 and 72 hpi using qRT-PCR measurement. Fifty grams of infected maize cultivar leaves at the different time intervals after inoculation were harvested for total RNA extraction by P-Biozol (Bio Flux) solution according to the manufacturer's instructions. The RNA was verified by 1.0 % agarose gel

**Table 1. Primers used in qPCR**

| Primer name | Sequence (5'–3') | Annealing temperature | Product length (bp) | Acc. No. |
|---|---|---|---|---|
| **Peroxiredoxin-F** | CGGCAAGAAGGTCATCC | 59°C | 120 | NP001149765 |
| **Peroxiredoxin-R** | AGCAGGATCTCGTCTACA | | | |
| **GLP-F** | CGAGATCATCTTCGTCCT | 59°C | 130 | AF032974 |
| **GLP-R** | GTTCTGCTGGAAGTGGA | | | |
| GF14-6-F | GGAGCTGAGAGAAAGGA | 59°C | 109 | P49106 |
| GF14-6-R | CCAGTCCAAGCCTAATAG | | | |
| SAM-F | GCTGACCACTGCAAGG | 59°C | 160 | BT054969 |
| SAM-R | GGCTGAGGGGCATCA | | | |
| MTL-F | CGGAGGCAACTGTGG | 59°C | 90 | Q5U7K6 |
| MTL-R | CCTGCTCAGCCATGTC | | | |
| Chlorophyll a-b–F | CCATGTTCGGATTCTTCG | 59°C | 115 | NM001154761 |
| Chlorophyll a-b–R | AAGTTGGTGGCGTAGG | | | |
| NPR1-F | TACCCATCCACCGCT | 59°C | 160 | NM001154115 |
| NPR1-R | CCTCCATCTTGTACCGC | | | |
| FKBP-F | AGTGCCCGCTGGAG | 59°C | 120 | EU958134 |
| FKBP-R | GTCGAACACCGTGCC | | | |
| Actin-F | AAGAGAGGCATCCTGACA | 59°C | 120 | J01238 |
| Actin-R | CAGGGTGATCTTCAGGC | | | |

electrophoresis and quality checked by NanoDrop™ 100 UV-V spectrophotometer (Thermo Scientific, USA). Total RNA was subjected to cDNA synthesis using Sinaclon cDNA synthesis kit (SinaClon, Iran) according to the manufacturer's protocol. Maize viral infection was verified by using a specific primer pair (MDF3:5'-GATGAGTTRAAYGTYTATGCACGAC-3' and MDR1: 5'-RTGCATRATTTGTCTGAAAGTTGG-3').

The real time reaction contained 5 μl of SYBR Green PCR master mix, 2 μl of cDNA template and 1 μl of 10 μmol/l of each forward and reverse primer.

The qRT-PCR was performed in duplicates using an iCycler instrument (iQ5, BioRad, USA).

The thermal cycling condition for qRT-PCR were as follows: 5 min at 94°C followed by 40 cycles of denaturation at 94°C for 10 s, annealing at 59°C for 10 s and extension at 72°C for 2 min. For the standard curves, the threshold cycle (Cq) values were calculated using the iCycler software provided by the qPCR equipment manufacturer. Melting curve analysis and electrophoresis pattern was used to determine the specificity of the PCR products. Table 1 shows the characterization of primers used in this study. The *Actin* was used as the house-keeping gene. For calculation of real-time efficiencies (E), the slope of the standard curve was fitted into the following equation: $E = 10^{-1/slope}$. The relative changes were calculated according to $\Delta\Delta C_T$ method of Pfaffl (Pfaffl, 2001; Ludmerszki *et al.*, 2015). The $2^{-\Delta\Delta CT}$ value was given to estimate the fold change of gene expression. Each value was the mean of three independent biological experiments. The data were analyzed using the GenEx6 software and the chart was designed by Microsoft excel software.

*Trilinear (3-way) decomposition analysis.* Three datasets were provided based on trilinear decomposition for two susceptible and tolerant maize plants and Johnson grass plants by GenEx6 software. The data were analyzed by trilinear decomposition which assumes that there is a limited number (*n*) of underlying factorial expression patterns.

Three-way decomposition calculated three sets of responses: one for data columns which show the expression changes over the genes, one for rows which reflect the expression changes over the samples (combination of two viruses with four points of hpi) and one for the variable that distinguishes the data file that shows gene expression variation over the genotypes.

## Results

*Detection of MDMV and BgSMV*

RNAs quality determination on the agarose gel indicated a high quality of extracted RNA for qRT-PCR analysis. Single qRT-PCR product of the expected size was yielded using specific primers to MDMV and BgSMV (MDF3, MDR1) (Fig. 1a; Table 1).

Symptoms of infection and differences between the susceptible and tolerant infected maize plants were monitored for 20 days after the first inoculation. To verify the success of infection, ELISA technique was used.

Maize SC705 cultivar and Johnson grass plants showed chlorotic streaks on green tissues approximately 15 and 18
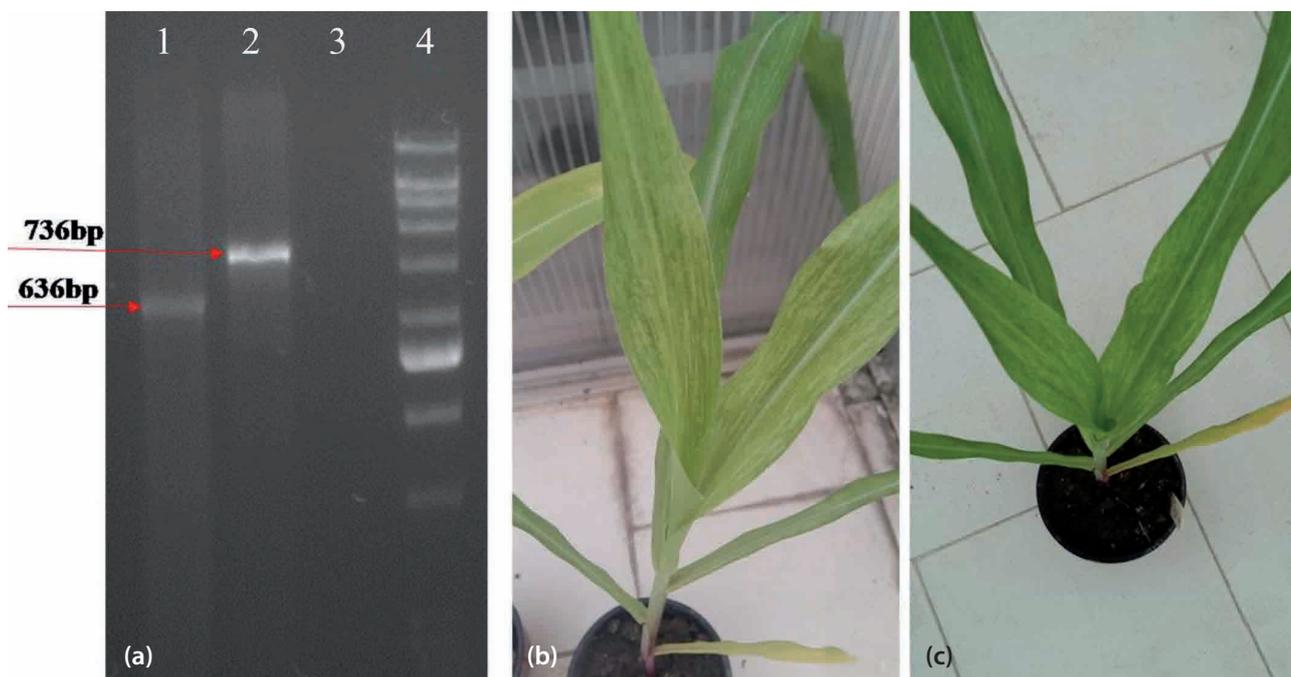
**Fig. 1**

**MDMV and BgSMV symptoms in maize plants**
(a) PCR products from infected maize with two viruses (1: MDMV, 2: BgSMV, 3: negative control, 4: DNA Ladder 100bp plus GelPilot, Qiagen). Severe mosaic symptoms in susceptible maize genotype inoculated by MDMV (b) and BgSMV (c).
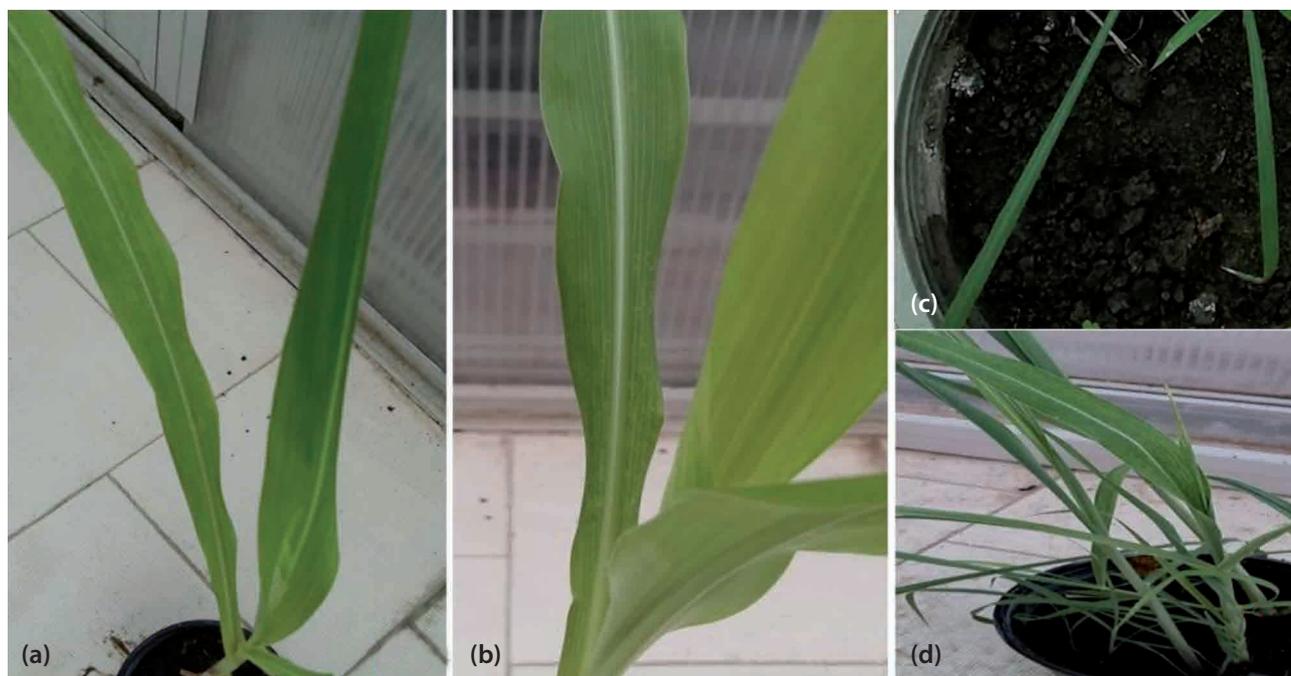


**Fig. 2**

**Symptoms in maize and Johnson grass infected with MDMV and BgSMV**
Mild mosaic in tolerant maize hybrid inoculated by MDMV (a) and BgSMV (b), mosaic symptoms in Johnson grass inoculated by BgSMV (c) and without symptoms in Johnson grass inoculated by MDMV (d).
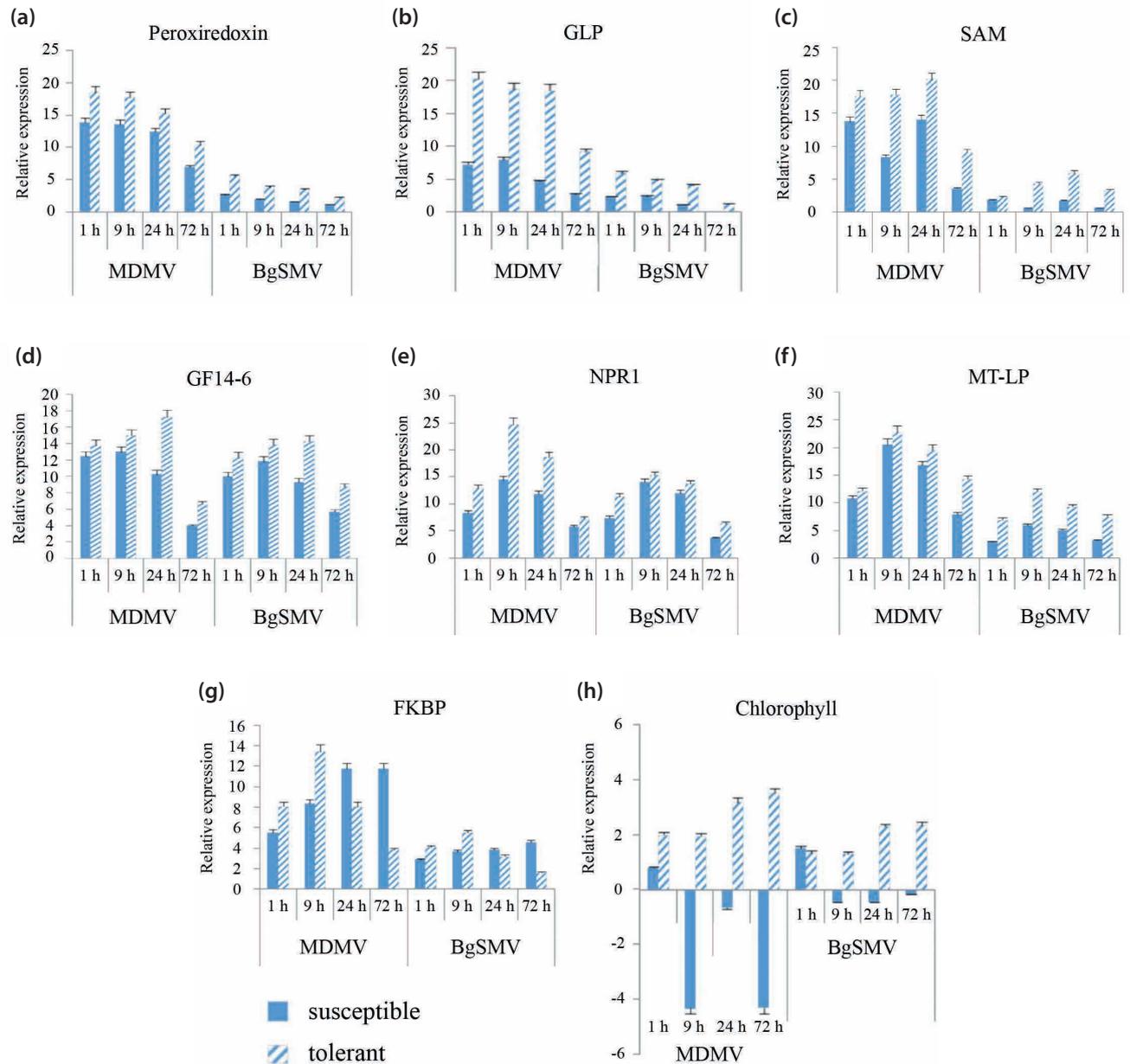
**Fig. 3**

**Gene expression pattern in susceptible and tolerant maize seedlings inoculated by MDMV and BgSMV at different time points**
In the tolerant maize plants, gene expression is significantly higher than in the susceptible plants during entire experiment. *Peroxiredoxin* (**a**), *GLP* (**b**), *SAM* (**c**), *GF14-6* (**d**), *NPR1* (**e**), *MT-LP* (**f**), *FKBP* (**g**), *chlorophyll a-b binding* (**h**).

days after inoculation (dpi) with MDMV inoculums provided from infected field plants. Maize SC705 cultivar infected with BgSMV showed visible diseases symptoms at 18 dpi. 8-hybrid cultivars showed low systemic chlorotic symptoms at 19 dpi with MDMV. In maize leaf samples inoculated with BgSMV, these symptoms were very weak when compared to MDMV infection (Fig. 1b,c and Fig. 2).

qRT-PCR was used to determine the transcript levels of some genes including *peroxiredoxin, metallothionein-like protein (MTL), S-adenosyl methionine synthetase (SAM), germin-like protein (GL-P), FKBP, GF 14-6, NPR1* and *chlorophyll a-b binding* protein in the maize infected by MDMV and BgSMV at 0, 1, 9, 24 and 72 hpi (Fig. 3).

QRT-PCR analysis in the infected maize samples with MDMV and BgSMV, showed a similar pattern of the rela-
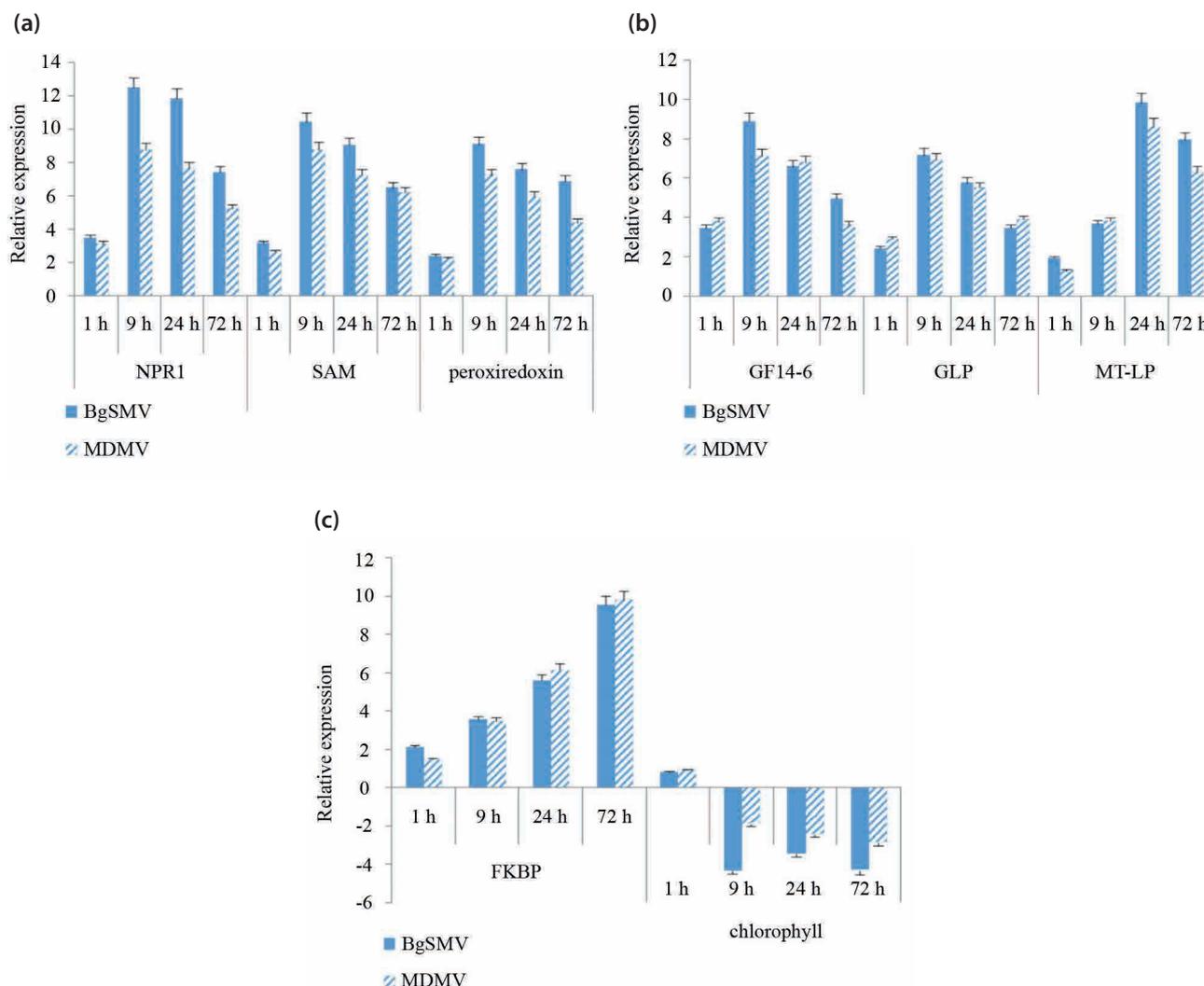
**Fig. 4**

**Gene expression pattern in the Johnson grass plants inoculated by MDMV and BgSMV at different time points**
*NPR1* (**a**), *SAM* (**b**), *peroxiredoxin* (**c**), *GF14-6* (**d**), *GLP* (**e**), *MT-LP* (**f**), *FKBP* (**g**), *chlorophyll a-b binding* (**h**). In the Johnson grass plants inoculated by BgSMV, *NPR1*(**a**), *peroxiredoxin* (**c**) and *SAM* (**b**) show higher expression than the plants inoculated by MDMV.

tive expression levels of each investigated gene. One hour after maize inoculation an increase of expression level for all tested genes was seen. During the progresses of MDMV and BgSMV infection, significant changes in all genes has occurred in tolerant maize infected plants compared to sensitive maize infected plants.

At 1 hpi gene expression level of *peroxiredoxin* and *GLP* was up-regulated and then followed by a reduction at the 9, 24 and 72 hpi (Fig. 3a,b).

The expression levels of *SAM* and *GF14-6* genes were elevated in a moderate rate with a continuous increase in the infected maize from first time point after inoculation until last time point of sampling. A maximum 23 and 18-

fold rise in expression of *SAM* and *GF14-6* was noted at 2 dpi (Fig. 3c,d).

The expression level of *GF14-6* gene didn't show a statistically significant difference in infected tolerant and susceptible maize plants at 1 and 9 hpi while in the 3rd time point after inoculation a significant increase was observed in tolerant cultivar compared to susceptive cultivars (Fig. 3d).

Maximum expression level of three *NPR1* (26-fold), *MT-LP* (19-fold) and *FKB* (14-fold) genes was measured at 9 hpi. A considerable reduction of gene expression of three genes was observed in the 2–3 dpi.

In susceptible maize cultivars infected with MDMV, the expression level of *chlorophyll* gene increased at all sampling

**(a)**



**(b)**



**(c)**
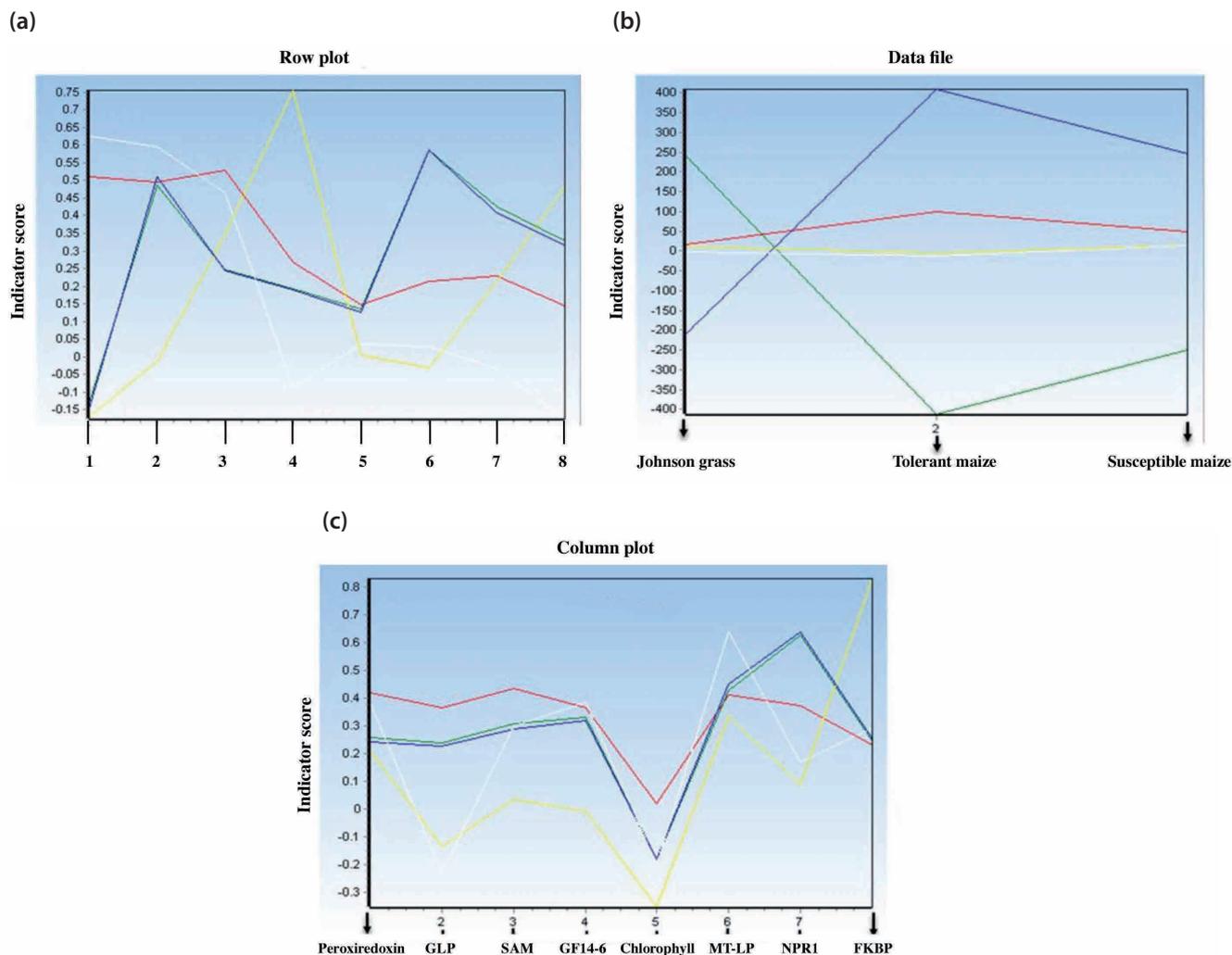


**Fig. 5**

**Trilinear decomposition analysis**

**(a)** Row plots indicates the expression changes over the samples (combination of two viruses with four time points of hpi). Number 1–4: MDMV; 1, 9, 24 and 72 hpi. Number 5–8: BgSMV; 1, 9, 24 and 72 hpi. **(b)** Data file plot indicates gene expression variation over the genotypes and **(c)** column plot indicates the expression changes over the genes.

time points. However, unlike in tolerant maize infected plants, *chlorophyll* gene was suppressed at 9, 24 and 72 hpi while in the susceptible cultivars the expression levels were unregulated at 1 hpi (Fig. 3h).

As showed in Figure 3, a similar pattern of gene expression but with a moderate rate has occurred in the tolerant and susceptible maize infected with BgSMV.

In total, the highest expression level in the infected tolerant maize with MDMV (24.68-fold) and BgSMV (15.2-fold) was related to *NPR1* gene at 9 hpi when compared to the control.

The lowest transcription level was seen for *chlorophyll* gene in both maize genotypes infected with MDMV and BgSMV.

*Changes of gene expression in Johnson grass plants inoculated by MDMV and BgSMV*

In nature, Johnson grass is not infected with BgSMV. For better understanding of molecular action on this compatibility, we investigated the expression levels of previously tested genes in Johnson grass infected with MDMV and BgSMV. QRT-PCR analysis in infected leaf samples from Johnson grass plants inoculated with MDMV and BgSMV showed that all investigated genes except *chlorophyll* gene were increased.

The maximum expression level for majority of tested genes has occurred at 9 hpi (Fig. 4). Maximum rate of *MT-LP*
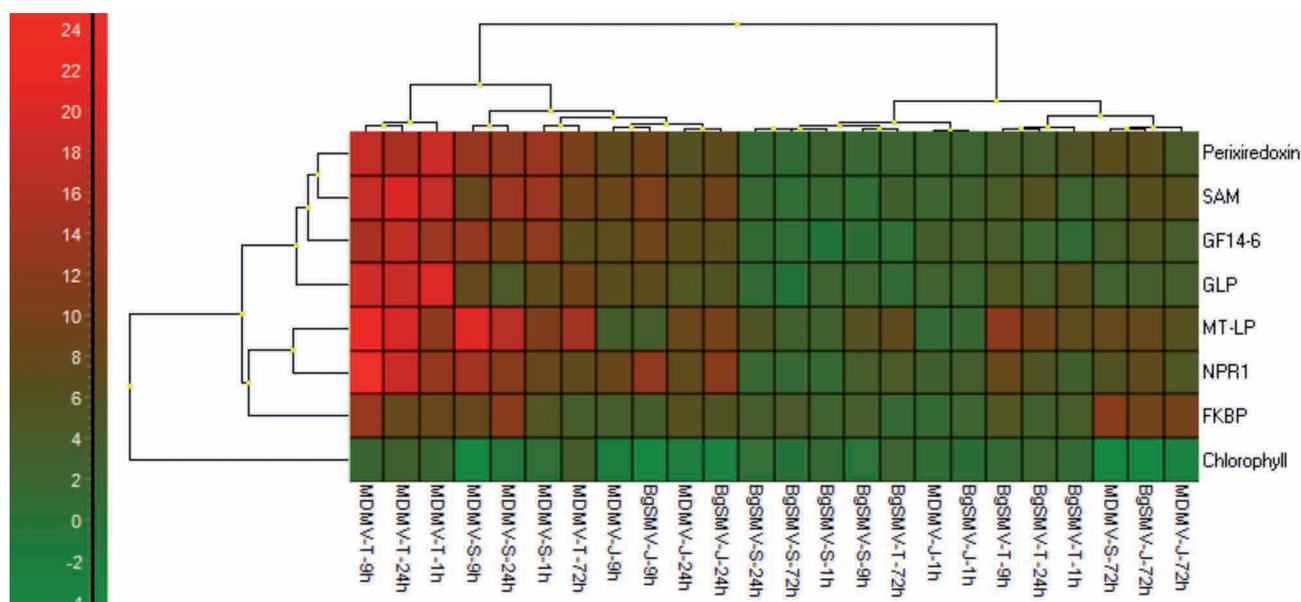
**Fig. 6**

**Gene clustering chart**

The genes are located in 4 groups. 1. *NPR1* and *MT-LP* genes; 2. *peroxiredoxin*, *SAM*, *GF14-6* and *GLP*; 3. *chlorophyll a-b binding* and 4. *FKBP* are in separator groups. ***MDMV-S-1h (9, 24 and 72 h) = MDMV and susceptible maize at 1h (9, 24 and 72) hpi, MDMV-T-1h (9, 24 and 72 h) = MDMV and tolerant maize at 1h (9, 24 and 72) hpi, BgSMV-S-1h = BgSMV and susceptible maize at 1h (9, 24 and 72) hpi, BgSMV-T-1h = BgSMV and tolerant maize at 1h (9, 24 and 72) hpi, MDMV-J-1h = MDMV and Johnson grass at 1h (9, 24 and 72) hpi, BgSMV-J-1h = BgSMV and Johnson grass at 1h (9, 24 and 72) hpi.

and *FKBP* genes expression was recorded at the 24 and 72 hpi, respectively. The *chlorophyll a-b binding* transcripts in Johnson grass infected with MDMV and BgSMV did not show significant difference at 1 hpi compared to non-infected plants. Gene expression level was significantly decreased at the 9, 24 and 72 hpi and at these time points after inoculation with both viruses, the *chlorophyll* gene was suppressed. The rate of this suppression was approximately similar in third time of sampling after inoculation. All changes of expression in compatible or non-compatible Johnson grass plants infected by MDMV and BgSMV are shown in Fig. 4.

*Trilinear decomposition analysis and clustering of genes*

Inspecting the underlying components, green and blue principle component (PC) curves were quite similar in the row plot (Fig. 5a). Looking at the data file plot, PCs show extreme values for tolerant maize genotype, therefore, the maximum variation in gene expression belongs to this genotype (Fig. 5b). It was concluded from the row plot that a maximum gene expression response occurred against BgSMV at 9 hpi. Based on the highest indicator score of green and blue PCs, it was understood that *NPR1* and *MT-LP* genes are important in tolerant maize genotype by looking at the columns plot (Fig. 5c).

Gene clustering chart designed based on host genotype, time of infection, and viral strains showed that all investigated genes are located in 4 groups based on their expression. *NPR1* and *MT-LP* genes are placed in one group, *peroxiredoxin*, *SAM*, *GF14-6* and *GLP* are in other separated group and *chlorophyll a-b binding* and *FKBP* are in two single groups (Fig. 6).

Results of gene clustering showed that *NPR1* and *MT-LP* genes are located in one group, indicating that related expression of these genes could be co-regulated in the different states (Fig. 6).

**Discussion**

Maize dwarf mosaic virus (MDMV) is one of the most important biotic stressors of cultivated maize varieties. The infection usually causes a considerable loss of crop. In this study, molecular changes at the level of gene expression were studied to illustrate host specificity of maize and Johnson grass infected by MDMV and BgSMV viruses. The development of MDMV was monitored over 18 days after virus inoculation. The mosaic symptoms were observed in new appearing leaves. The results were similar to the reported data by other researchers (Uzarowska *et al.*, 2009). In maize com-

patible to virus infection, biotic and abiotic stress responses are detectable in the infected leaves (Ludmerszki *et al.*, 2014). The BgSMV is very similar to MDMV with some differences at the level of disease symptoms and biochemical traits. It has been reported that BgSMV has one 90 nucleotide region (position 108 to 198) related to 5′-region of coat protein gene more than MDMV. The differentiation between the two viruses by ELISA is not possible. Also, BgSMV is compatible, while MDMV is incompatible to Johnson grass host. In this paper, the seedling leaves sample infected by MDMV and BgSMV were harvested at 1–72 hpi and then the leave-less plants were monitored up to 18 dpi. In the all susceptible and tolerant plants, the expression level of all investigated genes was increased at the first-hour post infection. This is a rapid response indicator of proliferation and replication of MDMV and BgSMV viruses. This virus progress at the first time point after inoculation quickly makes the defense reaction a lot stronger, because the production of pathogenesis related proteins increase resistance mechanism pathways. In infected plants with various viruses belonging to the *Potyvirus* family; activation of some related-defense genes has been immediately increased after the first time point of infection (Maule *et al.*, 2002; Marathe *et al.*, 2004; Love *et al.*, 2005; Uzarowska *et al.*, 2009) and these results are in concordance with our data in this work. Specific symptoms in same plants infected with related viruses displaying common symptoms suggest linked changes to resistance responses and could help us to identify common resistance candidate genes (Dardick, 2007). Gene expression analysis by microarray approach in infected maize (*Zea mays* L.) with MDMV and SCMV viruses indicated that the *SAM* and *GF14-6* genes coding for *metallothionein-like* protein, *S-adenosylmethionine synthetase*, and *germin-like protein 4*, respectively were differentially expressed at significant levels (Użarowska *et al.*, 2009). In this research, the expression level of *SAM* and *GF14-6* genes in tolerant plants differentially changed at different time points after both viral infections. Increased expression level of putative genes in infected plants during virus development; propose that expression changes of defense-related genes might be associated with symptom development. Our findings show that the majority of investigated genes were up-regulated in Johnson grass plants inoculated by BgSMV compared to MDMV infection. Based on these results, we can suggest a possible identification of plant resistance using these genes as biomarkers.

Normally, in resistant and tolerant plants, a virus resistance gene has to be expressed before pathogen attacks. So, this state can lead to rapid resistance response after infection. Based on this theory, a high expression level of defense-related genes at the first time point after inoculation is anticipated. In this study the highest regulation was observed for *NPR1* gene. *NPR1* as a key regulator of systemic acquired resistance (SAR) which can enhance the expression

level of a large numbers of pathogenesis related proteins (PR protein) to general stress responses. Proteomic analysis of infected *Capsicum annum* with TMV revealed 7 up-regulated PR-proteins through *NPR1* pathway (Elvira *et al.*, 2008). Trilinear decomposition analysis showed the maximum variation of *NPR1* and *MT-LP* genes among three infected genotypes with both MDMV and BgMV viruses. In previous work we showed the important and key role of *NPR1* gene in resistance induction in infected tomato plants with *Ralstonia solanacearum* by using virus induced gene silencing (VIGS) method (Bolok Yazdi *et al.*, 2018). To confirm the critical role of *NPR1* and *MT-LP* genes microarray and VIGS analysis with infected tolerant maize plants with different virus from same family is proposed. Gene clustering pattern indicated that *NPR1* and *MT-LP* are placed in a common group, so we could conclude that both genes have the same importance in enhancement of plant disease resistance against viral infection.

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