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Polyphenolic genes expression pattern and their role in viral resistance in tomato plant infected with *Tobacco mosaic virus*

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Flavonoids, a class of secondary metabolites, are derived from phenylalanine and acetate metabolism to protect higher plants from biotic and abiotic stress. By using a sensitive and reliable quantitative Real-time PCR technique, the transcriptional changes of thirteen tomato genes encoded for the polyphenol biosynthetic pathway enzymes were evaluated at 12 and 15-day post *Tobacco mosaic virus* (TMV) inoculation (dpi). Compared to mock- plants, only four genes were up-regulated at 12 dpi while at 15dpi the expression level changes of almost genes was up-regulated. Intriguingly, flavonoid biosynthetic enzyme chalcone isomerase (CHI) showed the highest relative expression levels at 12 and 15dpi with 242 and 369-fold respectively. Our results provide valuable information on key polyphenol biosynthetic genes and tomato immune response to TMV. This data could be a benefit to developing tomato defence system against viral infection.

Keywords: TMV; Tomato; Defence system; Flavonoid; Gene expression.

INTRODUCTION

Plant viruses cause various diseases of an international impact and are responsible for huge losses of crop production and quality worldwide (Abdelkhalek et al., 2018). Annual global losses due to virus diseases in plants/crops have been estimated by some authors and reached to be close to US $60 billion (Srivastava and Prasad, 2014) so that, viral diseases need to be controlled. In plants, Phenolics, Terpenes, N and S containing compounds are the major three groups of secondary metabolites that involved in defence against biotic and abiotic stress (Rosenthal et al., 1991). Flavonoids are a large group of polyphenolic compounds that produced through the phenylpropanoid pathway and its specific branching reactions (Singh et al., 2010). Flavonoids are one of the earliest defence mechanisms in the plant that through transporting to the infection site, inducing the hypersensitivity reaction and activating cell death programmed (Blount et al., 1992; Dai et al., 1996; Beckman, 2000; Mierziak et al., 2014). Moreover, flavonoids can inhibit viral polymerases through intercalating of the B ring with the viral nucleic acid bases or its capsid proteins (Selway, 1986; Wu et al., 2013; Mierziak et al., 2014). Tomato (*Solanum lycopersicum* L.) is one of the most important vegetable crops that used as a model plant for the research and study of biological and metabolic processes (Giovannoni, 2007). *Tobacco mosaic virus* (TMV, genus *Tobamovirus*), positive-sense

ssRNA, is one of the most important plant virus in the poll of the plant virology community (Scholth et al., 2011). TMV infects tomato plants systemically causing mosaic and chlorosis symptoms that often lead to plant deformations or alteration in plant structure and morphology. In this study, we used real-time PCR technique to understand how tomato cells (either infected or non-infected) organize polyphenol metabolic pathways through measuring expressions of the known tomato flavonoid genes upon TMV infection.

MATERIALS AND METHODS

Mechanical transmission and plant samples collection

Two true leaves of 35-day-old tomato seedlings Heinz 1706 cultivar were dusted with carborundum and the surface of the leaf was gently abraded with 1 ml of TMV according to Hafez et al., (2013). Inoculated plants were kept under greenhouse conditions and observed for viral symptoms development. Three biological replicates of each treatment were collected at 3 and 12 days post-inoculation (dpi) and kept at -80 ^{o}C until use. Mock-treated plants inoculated with buffer only were used as a control.

Total RNA extraction and cDNA synthesis

Total RNA from mocked and infected tomato tissue was extracted using the RNeasy Mini Kit according to the manufacturer's instructions (QIAGEN, Germany). Each biological sample is a mix of three samples derived from three different plants. Two micrograms of total RNA for each sample were reverse transcribed to cDNA using oligo (dT) primer with Reverse Transcriptase Enzyme of Super-Script II (Invitrogen, USA) in a Thermal Cycler (Eppendorf, Germany). The amplified cDNA was then stored at -20 °C until further used.

Expression of polyphenol-related genes and Real-time PCR data analysis

Different primer sets specific for phenolic-related genes were synthesised according to previous studies by Andre et al. (2009) (Table 1). For normalization of the transcript expression levels, β-actin gene (Table 1) was used as an internal control. Each sample on all reactions run in triplicate on a Rotor-Gene 6000 (QIAGEN, ABI System, USA) using the SYBR Green PCR Master Mix (Fermentas, USA). The amplification program of thermal cycling included an initial denaturation step at 95 °C for 10 min, followed by 40 cycles consisting of; denaturation at 95 °C for 15s, annealing at 60 °C for 30s and extension at 72 °C for 30s. The relative expression ratio was accurately quantified and calculated according to Livak and Schmittgen (2001). Values higher than 1 demonstrated an increase (up-regulated in expression) while values lower than 1 express a decreased (down-regulation in expression) for a parameter.

Table (1) List of primer sequences of the polyphenol biosynthetic genes and the housekeeping gene (β-actin) used in real-time PCR.

Gene name	Direction	Primer sequences 5′……..3′
PAL	Forward	ACGGGTTGCCATCTAATCTGACA
	Reverse	CGAGCAATAAGAAGCCATCGCAAT
C4H	Forward	CCCAGTTTTTGGAAATTGGCTTCA
	Reverse	GCCCCATTCTAAGCAAGAGAACATC
HQT	Forward	CCAATGGCTGGAAGATTAGCTA
	Reverse	CATGAATCACTTTCAGCCTCAACAA
HCT	Forward	TCTCCAACCCCTTTTAACGAACC
	Reverse	CAACTTGTCCTTCTACCACAGGGAA
C3H	Forward	TTGGTGGCTACGACATTCCTAAGG
	Reverse	GGTCTGAACTCCAATGGGTTATTCC
CHS	Forward	CACCGTGGAGGAGTATCGTAAGGC
	Reverse	TGATCAACACAGTTGGAAGGCG
CHI	Forward	GGCAGGCCATTGAAAAGTTCC
	Reverse	CTAATCGTCAATGATCCAAGCGG
F3H	Forward	CCAAGGCATGTGTGGATATGGACC
	Reverse	CCTGGATCAGTATGTCGTTCAGCC
FLS	Forward	CCTCCTTCCTACAGGGAAGCAAA
	Reverse	CAAGCCCAAGTGACAAGCTCCTAA
DFR	Forward	TCACAGGAGCAGCTGGATTTATCG
	Reverse	TCAGGATCACGAACAGTAGCATGG
F3′H	Forward	TGCGTATACCCAAACTCATTCCG
	Reverse	AAAAGCCCAAAGTTGATGTGAAAGG
AN1	Forward	CCTCAACCTCAGAAATTCAGAAGC
	Reverse	TCGTTGTTGTCGTTCGATGC
AN2	Forward	ACAAGATGCCACTTTCCTTCACC
	Reverse	TGTGCATCGTTGGGAGTTAGG
β-actin	Forward	CTCGCCTTTGCCGATCC
	Reverse	GATCTTCATGAGGTAGTCAGTC

RESULTS AND DISCUSSON

In this study, the tomato inoculated plants showed symptoms identical to those observed on naturally TMV-infected plants including systemically mosaic and chlorosis symptoms on leaves (data not shown). The relative expression level of TMV-CP results indicated that the viral propagation increased by the time that reflects the needs for more plant defence activity. Accordingly, the transcript levels of thirteen genes encoded for functional and regulatory enzymes of the polyphenol biosynthetic pathway were evaluated at both 12 and 15dpi compared to mocked plants. Among the major route of polyphenol compounds, there are three pathways (i) phenylpropanoid pathway, (ii) chlorogenic acid pathway and (iii) flavonoid pathway.

The core phenylpropanoid pathway

Depending on of the correlation between rates of phenylpropanoid accumulation and expression of resistance in vivo, phenylpropanoid compounds play important roles in plant defence against many microbial pathogens (Dixon and Paiva, 1995; La Camera et al., 2004; Kim and Hwang, 2014). All phenylpropanoids are derived from cinnamic acid that constitutes from phenylalanine (Vogt, 2010). Phenylalanine ammonia-lyase (PAL) and cinnamate 4-hydroxylase (C_4H) are the key enzymes in the two first steps of the phenylpropanoid pathway. Consequently, PAL and C_4H are the main linkers between primary and secondary metabolism through catalysing the non-oxidative deamination of phenylalanine to *trans*-cinnamate (Hahlbrock and Scheel, 1989; Huang et al., 2010). Compared to mocked inoculated plants, PAL was induced (up-regulated) with a relative expression level of 5.52-fold and 17.21-fold at 12dpi and 15dpi respectively (Fig.1). In contrast, the expression level of C_4H was down-regulated or repressed by 0.68-fold at 12dpi. At 15dpi C_4H relative expression was rapidly increased by 11.08-fold compared with mocked ones (Fig.1).The induction of both PAL and C_4H at 15 dpi is probably related to defence responses, as two-first enzymes in phenylpropanoid pathway leads to precursors for all flavonoids compounds. Beside its role in plant defence, PAL involved in the salicylic acid (SA) biosynthesis that is an essential signal in plant systemic resistance (Mauch-Mani and Slusarenko, 1996; Nugroho et al., 2002; Chaman et al., 2003). We assume that, the PAL is the inducer for C_4H and its response is faster than the C_4H upon viral infection. As shown in the dendogram (Fig. 2), C_4H came after PAL in the pathway, which means that C_4H was not expressed until the day 12-post infection. Our results agree with Gutha et al., (2010) who showed higher transcript levels of these genes in leafroll-associated virus 3-infected grapevine.

The chlorogenic acid pathway

In solanaceous plant species, hydroxycinnamoyl CoA quinate transferase (HQT) acting on the principal route of chlorogenic acid synthesis (Niggeweg et al., 2004). TMV infection causes a rapid induction for HQT with a significant relative expression level of 12.82-fold than mocked at 12dpi (Fig. 1). After 15 days, the expression was decreased to 8.97-fold but still up-regulated (Fig. 1). Niggeweg et al., (2004) reported that increasing the chlorogenic acid content upon over-expression of HQT in tomato plants. The slightly decreasing of HQT expression by 30% at 15dpi does not mean that HOT gene became silencing because the gene is still expressing but in a low manner of 12dpi. Our suggestion that the decrease in expression may due to the cell became completely infected and symptoms had appeared, so energy should be saved for another strategy to compete for either the viral propagation or the viral movement. Otherwise, the virus became exhausted which resulted in no need for high amounts of chlorogenic acid inside the cell. This suggestion corroborates the finding of Leiss et al., (2009) that the chlorogenic acid plays an important role as an inhibitor for plant pathogens. Moreover, Tsao et al. (2005) suggested that these compounds could help in host-plant resistance. The relative expression pattern of HCT was similar for the C_3H while the two genes were repressed at 12dpi. At 15dpi C_3H continues in decreasing but HCT showed lower up-regulation (increased). The transcript levels of the C_3H gene were 0.68-fold at 12dpi and 0.38-fold at 15dpi. The 12-day expression level of the HCT gene was 0.45-fold lower than the expression level at 15dpi that gave 1.12-fold in comparison with the mocked. Based on the previous results we can assume that the TMV was not able to make complete suppression for chlorogenic acid biosynthesis and had a fluctuated effect on the two genes that control chlorogenic acid synthesis. This finding agrees with Malli et al., (2002) who observed that more reduction in total phenols in susceptible genotypes than in resistant genotypes infected with yellow mosaic virus.

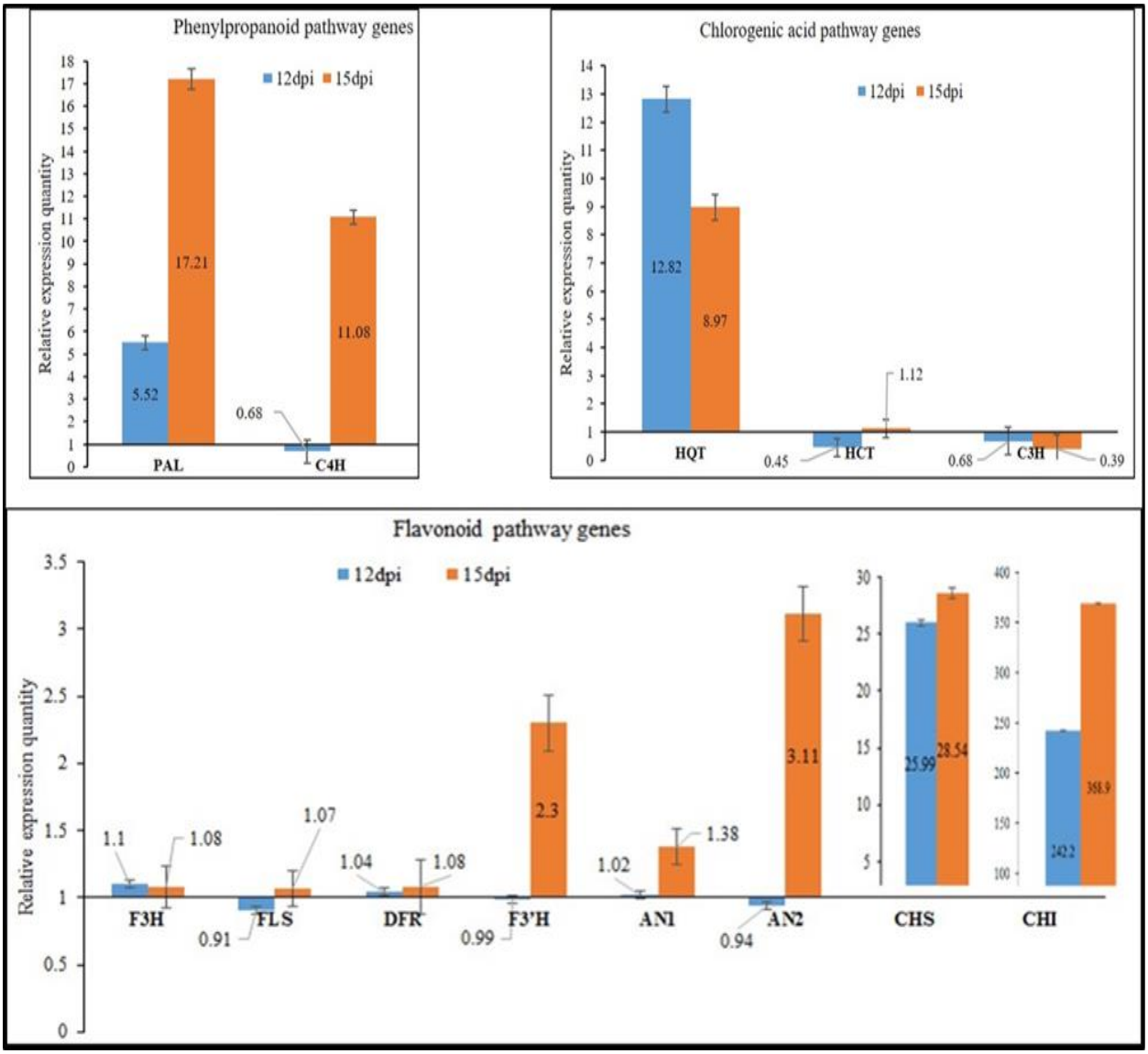

Figure1. The expression levels of the genes included in phenylpropanoid, chlorogenic, and flavonoid pathways using Real-time PCR.

The flavonoid pathway

Chalcones and dihydrochalcones are considered the primary precursors and they constitute the main intermediates for flavonoid synthesis (Marais et al., 2006). Chalcone synthase (CHS), which synthesizes naringenin chalcones, and chalcone isomerase (CHI), that catalyses the transformation of naringenin chalcone to naringenin,are encoding genes for the first two steps of the flavonoid pathway and are strictly required for flavonoid production in multiple tissues of tomato (Andre et al., 2009; Kang et al., 2014). In virus-infected leaves, CHS and CHI showed the highly expressed genes compared to other pathway genes (Fig.1, 2). The relative expression level of CHS gene was 25.99-fold and 28.54-fold at 12dpi and 15dpi respectively in comparing to mocked plants. At 12dpi, CHI showed very high relative expression level with 242.19-fold more than mocked. Likewise, at 15 dpi the relative expression level continues to increase until reached to 368.90-fold (Fig. 1). Our result agrees with Gutha et al., (2010) and Hanssen et al., (2011) that CHS was highly induced gene after plant viral infection. It was reported that the suppression of CHS with over-

expression of virus-encoded silencing suppressor protein increase mottling symptoms of virus-infected soybean plants (Teycheney and Tepfer, 2001; Senda et al., 2004; Koseki et al., 2005). In line with the given assumptions, both of CHS and CHI play important roles in viral resistance and the high transcriptional levels of the two genes could be indicators of how strong the plant immune system. The phenylpropanoids contents and their rate of metabolism were enhanced in plants under stress conditions (Kulbat, 2016). Moreover, it may help to strengthen the mechanical properties of the cell walls (Michalak, 2006). As shown in Figure (1) flavonoid pathway related genes were slightly induced at 15dpi with an exception for F3'H and AN2 that showed moderate expression levels with 2.30-fold and 3.11-fold respectively. In contrast, at 12dpi the three genes (F3'H, FLS and AN2) were slightly repressed with 0.99-fold, 0.91-fold and 0.94-fold expression levels comparing to mocked. We accept that genes control flavonoid synthesis are induced and are expressed with many folds when compared with the non-infected leaves.

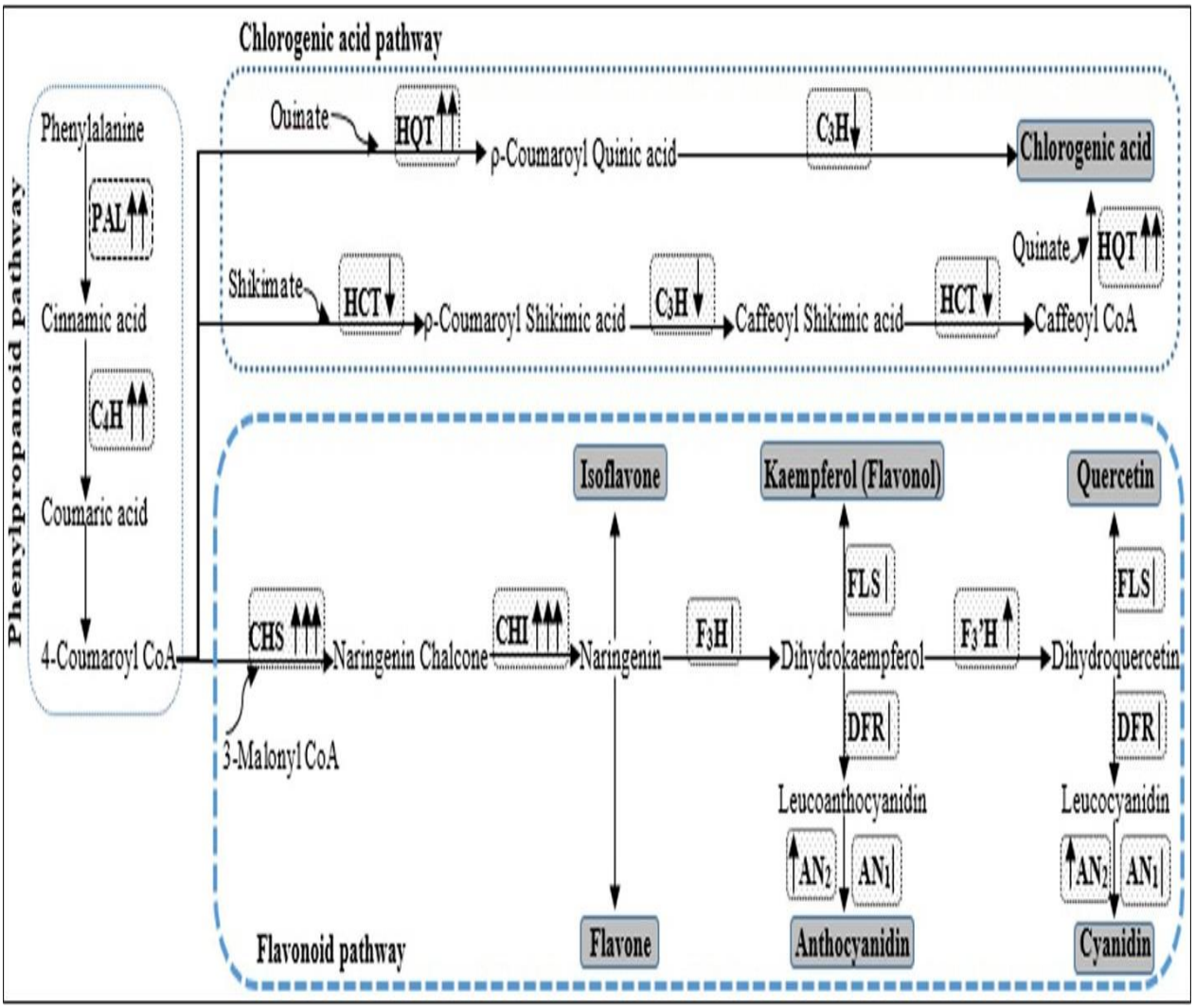

Figure 2. Diagram summarized the expression pattern of the genes regulate polyphenol pathway synthesis in the infected tomato plant with TMV based on the data obtained in this study. Arrows up: high gene expression. Arrows down: low gene expression.

Results obtained in this study agree with those obtained by Gutha et al., (2010) who postulated that the expression levels of CHS, F3'5'H, F3H, LDOX, LAR1 and MybA1 genes ranged from two- to fifty-fold increase in virus-infected leaves. The flavanone 3-hydroxylase (F_3H), DFR and AN1 genes showed relative expression levels at 12dpi with 1.10-fold, 1.04-fold and 1.02-fold respectively (Fig.1). At 15dpi, the three genes continue to express with a slight increase in the expression (1.08-fold for both F3H and DFR and 1.38-fold for AN1). Dardick et al., (2007) showed that overlapping expression patterns of the lignin pathway genes CCR and C3H with CHS and DFR that differentially expressed in the viral infected tissues. Gutha et al. (2010) reported that F_3H, which converts hydroxylate naringenin to dihydroflavonol or dihydrokaempferol, and $F_3'H$, which transforms hydroxylate dihydrokaempferol into dihydroquercetin, was expressed by more than 5-fold higher in virus-infected leaves compared to mock. We suggest that the expression of these genes that regulate enzymes in the pathway of polyphenols may differ according to the type and the site of the tissues in the same plant and the rate of induction and the copy number of the invading virus. Dihydroflavonol4-reductase (DFR) is located in an important regulatory branching point in the pathway and catalyses the reactions upstream of cyanidin and anthocyanidin production (Himi and Noda, 2004). This enzyme is the key enzyme responsible for the NADPH-dependent reduction of the dihydroflavonols to colourless leucoanthocyanidins (flavan-3, 4-cis-diols). The two transcription factors anthocyanin 1 (AN1) and anthocyanin 2 (AN2) are involved and play an important role in the regulation of anthocyanin gene transcription (Allan et al., 2007). It is clear that Flavonoids are the defensive materials inside the plant and its role in stopping and controlling the virus propagation and movement are still not completely understood.

CONCLUSION

Despite TMV being able to induce many polyphenols in high expression manner in infected tomato tissues, the produced polyphenols were not enough to stop the viral propagation and symptoms limitation during the 15dpi. At the same time, the completely silenced gene was not clear among the examined genes in this study. Therefore, the future study plan nominates the use of biosynthesized nanomaterial coated with polyphenols in plant protection.

CONFLICT OF INTEREST

The present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

All authors contributed equally in all parts of this study.

REFERENCES

Abdelkhalek A, ElMorsi A, AlShehaby O, Sanan-Mishra N, Hafez E (2018). Identification of genes differentially expressed in Iris yellow spot virus infected onion. Phytopathologia Mediterranea 57(2):334-340.

Allan A, Hellens R, Laing W (2007). MYB transcription factors that colour our fruit. Trends Plant Sci 13: 99-102.

Andre CM, Schafleitner R, Guignard C, Oufir M, Alvarado C, Nomberto G, Hoffmann L, Hausman JF, Evers D, Larondelle Y (2009). Modification of the health-promoting value of potato tubers field grown under drought stress: emphasis on dietary antioxidant and glycoalkaloid contents in 5 native Andean cultivars (*Solanum tuberosum* L.). J Agric Food Chem 57: 599-609.

Beckman CH (2000). Phenolic-storing cells: Keys to programmed cell death and periderm formation in wilt disease resistance and in general defence responses in plants? Physiol Mol Plant Pathol 57: 101-110.

Blount JW, Dixon RA, Paiva NL (1992). Stress responses in alfalfa (*Medicago sativa* L.) XVI. Antifungal activity of medicarpin and its biosynthetic precursors; implications for the genetic manipulation of stress metabolites. Physiol Mol Plant Pathol 41:333-349.

Chaman ME, Copaja SV, Argandona VH (2003).

Relationships between salicylic acid content, phenylalanine ammonia-lyase (PAL) activity, and resistance of barley to aphid infestation. Journal of Agricultural and Food Chemistry51:2227-2231.

Dai GH, Nicole M, Andary C, Martinez C, Bresson E, Boher B, Daniel JF, Geiger JP (1996). Flavonoids accumulate in cell walls, middle lamellae and callose-rich papillae during an incompatible interaction between Xanthomonas campestris pv. malvacearum and cotton. Physiol Mol Plant Pathol 49: 285-306.

Dardick C (2007). Comparative expression profiling of Nicotiana benthamiana leaves systemically infected with three fruit tree viruses. Mol Plant-Microbe Interact 20:1004-1017.

Dixon RA, Paiva N (1995). Stress-induced phenylpropanoid metabolism. Plant Cell 7:1085–1097.

Giovannoni JJ (2007). Fruit ripening mutants yield insights into ripening control. Curr Opin Plant Biol 10:283-289.

Gutha LR, Casassa LF, Harbertson JF, Rayapati AN (2010). Modulation of flavonoid biosynthetic pathway genes and anthocyanins due to virus infection in grapevine (*Vitis vinifera* L.) leaves. BMC Plant Biol 10:187.10.1186/1471-2229-10-187.

Hafez EE, Abdelkhalek AA, Abeer SA, Galal, FH (2013). Altered gene expression: induction/suppression in leek elicited by iris yellow spot virus infection (IYSV) Egyptian isolate. Biotechnol 27(5):4061-4068.

Hahlbrock K, Scheel D (1989). Physiology and molecular biology of phenylpropanoid metabolism. Annual Review of Plant Physiology and Plant Molecular Biology 40: 347–369.

Hanssen IM, Peter van Esse H, Ballester AR, Hogewoning SW, Parra NO, Paeleman A, Lievens B, Bovy AG, Thomma BPHJ (2011). Differential tomato transcriptomic responses induced by pepino mosaic virus isolates with differential aggressiveness. Plant Physiol 156:301-318.

Himi E, Noda K **(**2004). Isolation and location of three homoeologous dihydroflavonol-4-reductase (*DFR*) genes of wheat and their tissue-dependent expression. Journal of Experimental Botany 55:365-375.

Huang J, Gu M, Lai Z, Fan B, Shi K, Zhou YH, Yu JQ, Chen Z (2010). Functional analysis of the Arabidopsis PAL gene family in plant growth, development, and response to environmental tress. Plant Physiology 153:1526-1538.

Kang JH, McRoberts J, Shi F, Moreno JE, Jones AD, Howe GA (2014). The flavonoid biosynthetic enzyme chalcone isomerase modulates terpenoid production in glandular trichomes of tomato. Plant Physiol 164:1161-1174.

Kim DS, Hwang BK (2014). An important role of the pepper phenylalanine ammonia-lyase gene (*PAL1*) in salicylic acid-dependent signalling of the defence response to microbial pathogens.Journal of Experimental Botany 65:2295-2306.

Koseki M, Goto K, Masuta C, Kanazawa A (2005). The star-type color pattern in Petunia hybrida 'red Star' flowers is induced by sequence-specific degradation of chalcone synthase RNA. Plant Cell Physiol 46(11):1879-1883.

Kulbat K (2016). The role of phenolic compounds in plant resistance. Biotechnol Food Sci 80(2): 97-108.

La Camera S, Gouzerh G, Dhondt S, Hoffmann L, Fritig B, Legrand M, Heitz T (2004). Metabolic reprogramming in plant innate immunity: the contributions of phenylpropanoid and oxylipin pathways. Immunological Reviews 198:267-284.

Leiss KA, Maltese F, Choi YH, Verpoorte R, Klinkhamer PGL (2009). Identification of Chlorogenic Acid as a Resistance Factor for Thrips in Chrysanthemum. Plant Physiology 150(3):1567-1575.

Livak KJ, Schmittgen TD (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2–ΔΔCt method. Methods25:402-408.

Malli PC, Uday B, Satish L (2002). Effect of planting dates and development of yellow mosaic virus on biochemical constituents of moth bean genotypes. Indian Phytopathol 53:379-383.

Marais JPJ, Deavours B, Dixon RA, Ferreira D (2006). The Stereochemistry of Flavonoids. In The Science of Flavonoids, ed. Grotewold E. Columbus, Ohio, USA: Springer.

Mauch-Mani B, Slusarenko AJ (1996). Production of salicylic acid precursors is a major function of phenylalanine ammonia-lyase in the resistance of Arabidopsis to Peronospora parasitica. Plant Cell 8:203-212.

Michalak A (2006). Phenolic Compounds and Their Antioxidant Activity in Plants Growing

under Heavy Metal Stress. Pol J Environ Stud 15:523-530.

Mierziak J, Kostyn K, Kulma A (2014). Flavonoids as Important Molecules of Plant Interactions with the Environment. Molecules 19:16240-16265.

Niggeweg R, Michael AJ, Martin C (2004). Engineering plants with increased levels of the antioxidant chlorogenic acid. Nat Biotechnol 22:746–754.

Nugroho LH, Verberne MC, Verpoorte R (2002). Activities of enzymes involved in the phenylpropanoid pathway in constitutively salicylic acid-producing tobacco plants. Plant Physiology and Biochemistry 40:775-760.

Rosenthal GA (1991). The biochemical basis for the deleterious effects of L-canavanine. Phytochemistry 30:1055-1058.

Scholthof K, Adkins S, Czosnek H, Palukaitis P, Jacquot E, Hohn T, Hohn B, Saunders K, Candresse T, Ahlquist P, Hemenway C, Foster GD (2011). Top 10 plant viruses in molecular plant pathology. Mol Plant Pathol 12:938-954.

Selway JW (1986). Antiviral activity of flavones and flavans. Prog Clin Biol Res 213:521-536.

Senda M, Masuta C, Ohnishi S, Goto K, Kasai A, Sano T, Hong JS, MacFarlane S (2004). Patterning of virus-infected Glycine max seed coat is associated with suppression of endogenous silencing of chalcone synthase genes. Plant Cell 16(4):807-818.

Singh R, Rastogi S, Dwivedi UN (2010). Phenylpropanoid metabolism in ripening fruits. Comprehensive Reviews in Food Science and Food Safety 9:398-416.

Srivastava S, Prasad V (2014). Induction of Defence Responses for Biological Control of Plant Diseases, in Biological Controls for Preventing Food Deterioration: Strategies for Pre- and Postharvest Management (ed N. Sharma), John Wiley & Sons, Ltd, Chichester, UK.

Teycheney PY, Tepfer M (2001). Virus-specific spatial differences in the interference with silencing of the chs-A gene in non-transgenic petunia. J Gen Virol 82(5):1239-1243.

Tsao R, Marvin CH, Broadbent AB, Friesen M, Allen WR, McGarvey BD (2005). Evidence for an isobutylamide associated with host-plant resistance to western flower thrips, Frankliniella occidentalis, in chrysanthemum. J Chem Ecol 31:103-110.

Vogt T (2010). Phenylpropanoid biosynthesis. Mol Plant 3:2-20.

Wu T, Zang X, He M, Pan S, Xu X (2013). Structure-activity relationship of flavonoids on their anti-Escherichia coli activity and inhibition of DNA gyrase. J Agric Food Chem 61:8185-8190.