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Metabolomic profiling of susceptible and resistant genotypes of *Gerbera jamesonii* Bolus to western flower thrip

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Abstract

An infestation of *Frankliniella occidentalis*, commonly known as the Western Flower Thrip (WFT), affects the aesthetic value of *Gerbera jamesonii* Bolus flowers. Hence, developing WFT-resistant strains is an effective means of controlling pests, and it is essential to comprehend the resistance mechanism. Here, we investigated the both biochemical and metabolite-level resistance of latara genotype Gerbera to WFT. Initially, we assessed the rates of WFT mortality on the floral buds of two genotypes of Gerberas, 'latara' and 'faith,' and observed that the former exhibited strong resistance to the WFT soon after infestation. Gas chromatography mass spectrometry (GC-MS) was employed to examine the metabolomic response to WFT infestation. The profiles revealed three key characteristics such as higher antioxidant enzyme activities and concentrations of several secondary metabolites, like flavonoids and alkaloids in latara upon WFT infestation. This work presents the metabolomic profiles of WFT resistant genotype of gerbera will aid in the biochemical and molecular understanding of the mechanisms underlying Gerbera resistance against WFT.

Keywords: Biotic stress, Gerbera, Metabolites, Pest, Thrip

1.Introduction

Complex constitutive and inducible defenses have evolved by plants to protect themselves from arthropod herbivores. These defenses can be chemical components (e.g., secondary metabolites and defense-related enzymes) or physical structures (e.g., trichomes, thorns, and spines) that are either naturally occurring in the plant before herbivory occurs (Rosner and Hannrup, 2004; Franceschi et al., 2005; Mithöfer and Boland, 2012) or are specifically induced upon herbivore attack (Erb et al., 2012). Within and between plant species, there can be differences in the expression of constitutive and inducible defenses (Underwood et al., 2000; Koorneef et al., 2004; Zhang et al., 2020). Therefore, a crucial first step in transferring these defenses into susceptible plant varieties through plant breeding programs is the identification and characterisation of novel sources of pest resistance (Macel et al., 2019; Visschers et al., 2019).*Gerbera* is one of the most popular ornamental plant which can be used as cut-flowers, as well as potted plant.

Gerbera commonly known as Transvaal Daisy, Barberton Daisy or African daisy is cultivated commercially in greenhouses all over the world under wide range of climatic conditions for its attractive colored flowers (Moyer and Peres, 2008; Simpson, 2009). In terms of economic value, *Gerbera* ranks fourth in the global cut flower market, after Rose, *Chrysanthemum*, and Tulip (Teeri et al., 2006).

Exotic invasive insect species threaten biodiversity seems to be major concern nowadays. The invasion of Western Flower Thrip (WFT, *Frankliniella occidentalis* Pergande), a polyphagous pest is threatening several ornamental and crop plants globally (Reitz, 2009). WFT are minute insects and its life cycle consists of the egg, instars, the pre-pupa, pupa, and the adult stages (Lewis, 1973). WFT is one of the serious insect pests limiting crop productivity in ornamental plants, particularly on *Gerbera* causing aesthetic damages and incompatible for global market. In *Gerbera*, WFT cause deformations on growing parts and flowers (silver lesions), resulting in distorted petals, discoloration, and streaks (Bueno, 2005). Being tiny, WFT tends to occupy narrow crevices within or between plant parts (flowers) and gets protected from predators and insecticides as well. Currently, the primary approach employed for controlling WFT is the use of pesticides, which has significant negative effects on both human health and the environment. More ecologically friendly methods of controlling thrips include using alternative agricultural techniques including the use of biological control agents and/or plant genotypes with insect-resistant features (Muñoz-Cárdenas et al., 2017; Bac-Molenaar et al., 2019). Emergence of insecticide resistance globally among WFT made situation even worse in eradication or control (Jensen, 2000).

Plants synthesize an array of defense-responsive metabolites and alter the metabolic profiles of a plant in response to pest attack which can be analyzed with metabolomics approach. Metabolomics is widely used to investigate the tolerance of plants to biotic stresses which provides the opportunity to evaluate pest induced local and systemic alterations in plant metabolite patterns (Wu et al., 2015).

Thus in the given study, it has become quite mandatory to focus on metabolite profiling for untargeted metabolite analysis which provides a more comprehensive view on the differential accumulation of metabolites in WFT resistant/susceptible Gerbera genotypes. In this study, we will deploy the gas chromatography-mass spectrometry (GC-MS) for identification and quantification of the primary metabolites and a wide array of secondary metabolites (Schauer and Fernie, 2006; Shuman et al., 2011). Our proposed study will be crucial in deciphering the precise function of defense secondary metabolites as potential which can be further used as ecofriendly biopesticides against WFT.

2. Materials and methods

Plant material

Tissue culture raised *Gerbera jamesonii* Bolus cv Terraregina genotypes with varied flower color (Latara and Faith) of one month old were procured from Rise N Shine Plant Tissue Culture Laboratory Pvt Ltd, Pune, Maharashtra. These plants are grown under standard polyhouse conditions with regular fertigation.

Infestation of gerbera by WFT

The emerging flower buds of 3 day old were infested with equal number adult WFT (15-20 no's) and left for 3 days in a randomized infestation blocks covered with nylon filter cover (30 µm)

grown under greenhouse conditions

Enzyme extraction and assays

Determination of H_2O_2 content

The control and WFT infested buds from Latara and Faith genotypes of *Gerbera* weighing about 100mg were ground to fine powder in liquid nitrogen and homogenized in 100 mM Na-Phosphate buffer (pH 7.0). The obtained homogenate was centrifuged at 19,000g for 20 min and the supernatant was used for measurement of H_2O_2 content spectrophotometrically after reaction with potassium iodide (KI) as per Alexieva et al. (2001) with slight modifications. The reaction mixture contains 0.5 ml of 0.1% trichloroacetic acid (TCA), 50 µg protein, 100 mM Na-phosphate buffer (pH 7.8) and 2 ml of 1 M KI reagent. The blank consists of 0.1% TCA without protein sample. The reaction mixture was incubated in dark for 1 h and the absorbance was recorded at 390 nm. The quantity of H_2O_2 was calculated using a standard curve prepared with known concentration.

Determination of MDA content

Lipid peroxidation of samples was determined by the amount of malondialdehyde (MDA) content produced involving thiobarbutyric acid (TBA) reaction as per Heath and Packer (1969) with slight modifications. 1 gram of floral buds was homogenized in a mortar and pestle in 1 ml of 0.5% TCA and centrifuged at 19000g for 20min. The supernatant obtained is treated as a crude extract. A mixture of equal volumes (0.4 ml) of crude extract and TBA reagent (15% trichloroacetic acid (w/v) and 0.375% TBA(w/v) in 0.25 M HCl) were heated to 95^{o}C for 15 min, then cooled immediately in an ice bath and centrifuged at 15000 ×g for 15 min. The supernatant obtained is used to read absorbance at 532 nm by subtracting turbidity at 600 nm. The quantity of MDA was calculated from the extinction coefficient of 155 $mM^{-1}cm^{-1}$. Data were expressed as µmol per g FW.

Determination of antioxidant enzyme assays

For enzyme assays, 100 mg of floral buds from control and WFT infested plants were weighed and thoroughly ground to a paste in liquid nitrogen in a mortar and pestle and then transferred to 1 ml of ice cold extraction buffer (100 mM potassium phosphate buffer pH 7.0, 1 mM EDTA). The homogenate was filtered using muslin cloth and centrifuged at 5,000 rpm for 15 min and the collected supernatant was used to analyze activities of SOD, CAT, APX, POD and POX. In all the enzyme assays, the quantity of total soluble protein concentration was determined using bovine serum albumin, BSA (Sigma Aldrich co, USA) as standard at 640nm according to the Lowry's method (1951). Tissue samples (100 mg each) were ground in ice cold potassium phosphate buffer (10 mM, pH 6.8), then centrifuged at 15 000 × g for 20 min. The supernatant was used to determine soluble protein content. Obtained values were expressed as mg per g fresh weight (FW). Catalase activity, CAT (1.11.1.6) was measured as per Aebi et al. (1984), wherein, decrease in H_2O_2 was monitored at 240nm absorbance and quantified by its molar extinction coefficient (36 $M^{-1}cm^{-1}$). The activity of enzyme was expressed as micro moles of H_2O_2 decreased min^{-1} mg^{-1} protein. Ascorbate Peroxidase, APX (1.11.1.1) activity was determined according to Nakano and Asada (1981) at an absorbance of 290 nm and quantified by its molar extinction coefficient (2.8 $mM^{-1}cm^{-1}$). The activity of enzyme was expressed as micro moles of AsA decreased min^{-1} mg^{-1} protein. POD activity was as-

Extraction of metabolites

The WFT treated floral buds (2 g) were crushed in liquid nitrogen and fine powdered samples used for metabolite extraction. The extraction of metabolites for GC-MS analyses was performed following the protocol described as per Kim et al. (2013). Finally lyophilised sample would be subjected to double derivatization for GC-MS analyses as per Lisec et al. (2006).

GC-MS analysis

GC-MS analysis was carried out using an Agilent 7890A gas chromatograph and an Agilent 5975C mass detector (Agilent Technologies, CA, USA). Using an automated sampler (7683B series, Agilent Technologies) with a split ratio of 1:5, a double-derivatized sample (1 µL) was injected into the GC-MS. For the purpose of separating metabolites, an Agilent Technologies DB-5 MS column (5% phenyl methyl polysiloxane: 30 m × 0.25 mm i.d. × 0.25 µm) was utilized. This was the temperature program that ran: The temperature was initially set at 80°C for one minute, then increased to 220°C at a rate of 10°C min^{-1}, then increased to 310°C at a rate of 20°C min^{-1}, and ultimately held at 320°C for ten minutes. The calculated total run time was 39 minutes. A flow rate of 1 mL min^{-1} of helium was employed as the carrier gas. 280°C was specified as the interface and inlet temperatures. The mass range for total ion current was m/z 80–700, the detector voltage was set at 1700 V, and the MS unit was adjusted to its maximum sensitivity. Three duplicates of each sample were used. The scan was initiated at a frequency of 4 S^{-1} (2.0 HZ) following a 7-minute solvent delay.

Metabolite identification

Employing a built-in mass spectral database which includes several secondary metabolites, amino acids, organic acids, and sugar standards, as well as a standard NIST-17 mass spectral library (National Institute of Standards and Technology), metabolites were identified by comparing the mass-to-charge ratios and abundance of each metabolite detected. Especially those instances where the mass spectra comparison matching value was over 70 % and an increase in the relevant peak's size was noted upon spiking the sample with the corresponding pure standard was the metabolite identity given. The detection of co-elution was extensively examined in each mass spectra. There was no evidence of co-elution in any of the recognized peaks.

Metabolite data pre-processing

Using tools available with WsearchPro (www.wsearch.com.au), the Automated Mass Spectral Deconvolution and Identification System (AMDIS) deconvoluted raw GC-MS data files downloaded from Agilent ChemStationTM software. The collected metabolite data were then uploaded to MetaboAnalyst 4.0 (www.metaboanalyst.ca) after being further transformed into the.csv (comma separated values) format. Internal standards were used to standardize the TIC values. Pareto scaling, which divides each variable's mean by its square root of standard deviation, was next carried out. Normalization came next, and finally, statistical analysis. Using SigmaPlot 12 software, one-way analysis of variance (ANOVA) was used to quantify significant differences in metabolite levels, and Tukey's significant-difference test was then applied. The level of statistical significance was established at $p < 0.05$. Principal component analyses (PCA) were carried out using MetaboAnalyst 4.0, an interactive web tool. The PCA data output included loading plots to illustrate the cluster separation and score plots to show the comparison between different time points

of the samples treated with WFT. Using MetaboAnalyst 4.0's interactive heatmap tool, a heatmap was produced. Using data from the Kyoto Encyclopedia of Genes and Genomes (KEGG) database and pathway analysis in MetaboAnalyst 4.0, a streamlined metabolic pathway was created manually.

Quantitative Real-Time PCR

The WFT-infested "Latara" samples were subjected to specific time points (0–48 hpe) of total RNA isolation using the Qiagen (www.qiagen.com) RNeasy Plant Mini Kit. RevertAid H Minus reverse transcriptase (Fermentas; www.thermoscientificbio.com) and Oligo (dT) primers were used to reverse transcribe 1 µg of total RNA at 42°C in order to create cDNA. Using Power UpTM SYBR Green Master Mix (Thermo Fisher Scientific) and the QuantStudio 3 Real-Time PCR System (Thermo Fisher Scientific), quantitative RT-PCR was carried out in accordance with the manufacturer's instructions. 40 cycles of 90°C for 15 seconds, 55°C for 1 minute, and a final extension at 72°C for 1 minute make up the PCR protocol. An analysis of the melt curve was done to assess gene-specific amplification. A total of six serial dilutions of cDNA from all samples were used to calculate the amplification and correlation efficiencies of each PCR. The cycle threshold values were converted into raw data using the PCR efficiency in order to determine relative quantification. Using the gene-specific primers, the expression levels of hydroxycinnamoyl-CoA quinate hydroxycinnamoyl transferase (HQT), phenolalanine ammonia-lyase (PAL), cinnamonate-4-hydroxylase (C4H), 4-coumarate CoA ligase (4CL), and hydroxycinnamoyl-CoA were assessed. Actin 7 gene was used to standardize all samples. The expression level was scaled in accordance with the corresponding accumulation of metabolite levels in the cells used as controls (0 h), where the levels were set to 1. There were three technical repetitions. Efficiency and gene expression levels were estimated using a published mathematical model (Pfaffl, 2001).

Statistical analysis

The data presented in the biochemical studies are the average values (±SE) of results from three experiments and data were subjected to one way ANOVA (Holm-Sidak method) using SigmaPlot Version 14.0.

3. Results

Effect of WFT infestation on H_2O_2 and MDA content

In an attempt to screen the WFT resistant variety, we examined the antioxidant defenses among both genotypes, our findings on estimation of the H_2O_2 (Figure 1a) and MDA (Figure 1b) levels revealed that there was a significantly enhancement in the levels of H_2O_2 and MDA after WFT infestation in faith genotypes signifying the susceptibility of this genotypes towards WFT.

Similarly in this study, the antioxidant enzymes monitored in both genotypes with or without WFT infestation and the observations revealed upon WFT that there was a sudden rise in the activities of Catalase, APX, POX and PPO especially in faith genotype of Gerbera, while the activities of all these enzymes were remained high in latara with or without WFT infestation (Figure 2a to 2c).

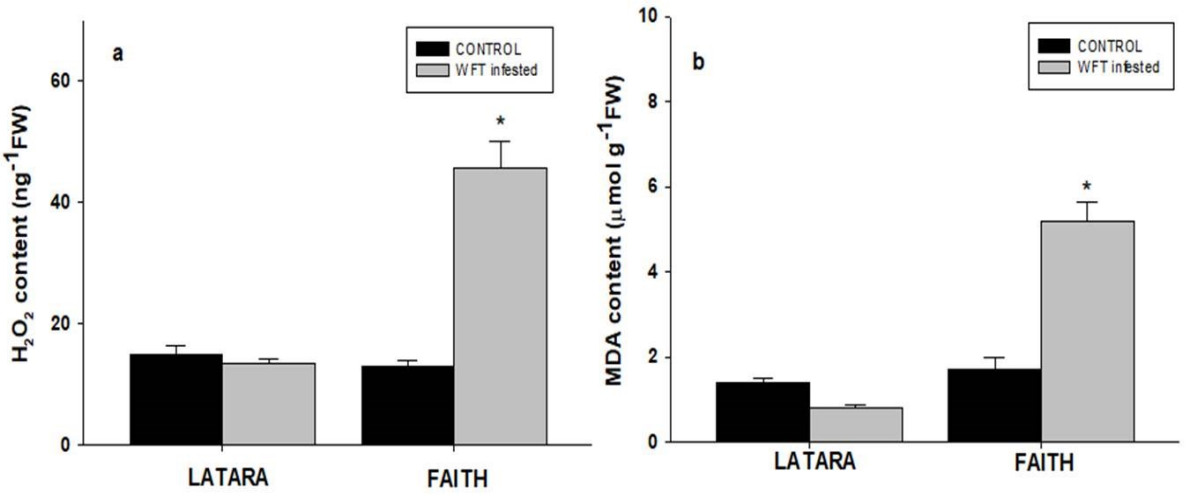

Figure 1. Effect of WFT infestation on H_2O_2 content (a) and MDA content (b) in Latara and Faith genotypes of *Gerbera* floral buds after 3 dpi with WFT: Each bar is represented as mean average ± standard deviation of three replicates per treatment performed randomly at different time periods. Asterisks indicate that the differences ($p<0.05$) between the control (uninfested) and treated samples (WFT infested) are statistically significant as determined by one way ANOVA (Holm-Sidak method).

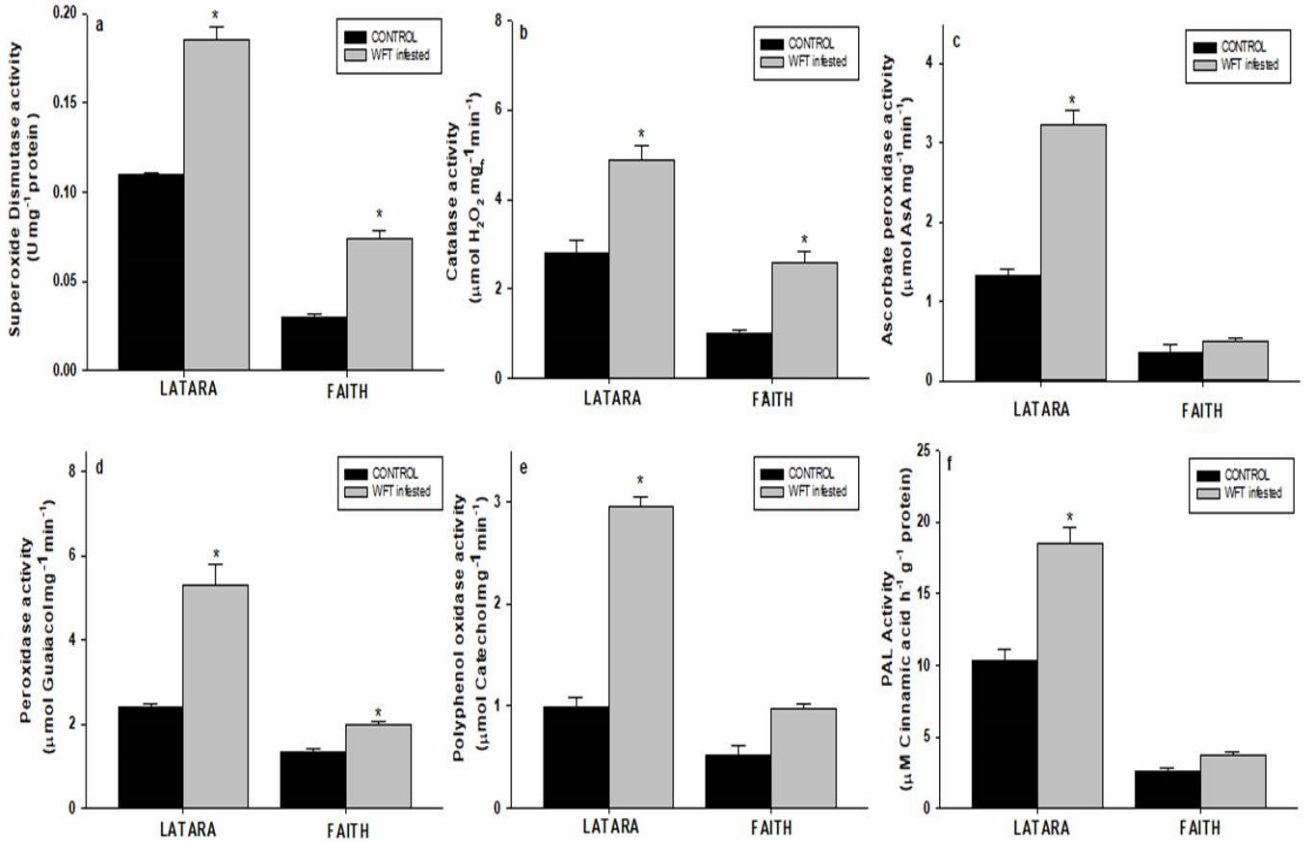

Figure 2. Effect of WFT infestation on activities of crucial enzymes- SOD (a), CAT (b), APX (c) POD (d), PPO (e) and PAL (f) in Latara and Faith genotypes of *Gerbera* floral buds after 3 dpi with WFT: Each bar is represented as mean average ± standard deviation of three replicates per treatment performed randomly at different time periods. Asterisks indicate that the differences ($p<0.05$) between the control (uninfested) and treated samples (WFT infested) are statistically significant as determined by one way ANOVA (Holm -Sidak method).

These findings provided us with insight into how the latara genotype's antioxidant defenses are modulated to defend against WFT. As a result, we examined the function of secondary metabolism in latara resistance to WFT by measuring the activity of POD, PPO, and PAL activities (Figure 2d to 2f). Interestingly, after WFT infestation, we noticed a significant rise in PAL activities, particularly in latara. The significance of secondary metabolites in conferring resistance against WFT in the latara variety was reinforced by this discovery. Further, we performed the metabolite profiling among these two genotypes and the results revealed that 52 % of variance in the principal component score plot measured by GC–MS (Figure 3a). The heatmap derived from the metabolite analysis (Figure 3b) demonstrated 47 differentially accumulated metabolites in latara upon WFT exposure which needed to be identified, quantified and structurally characterized in order to understand precise role of these metabolites. In the given study in order to understand the mechanisms involved in WFT resistance, we performed gas chromatography-mass spectrometry based metabolomics analysis between the WFT resistant gerbera genotype 'Latara' and susceptible 'Faith' upon treatment with WFT.

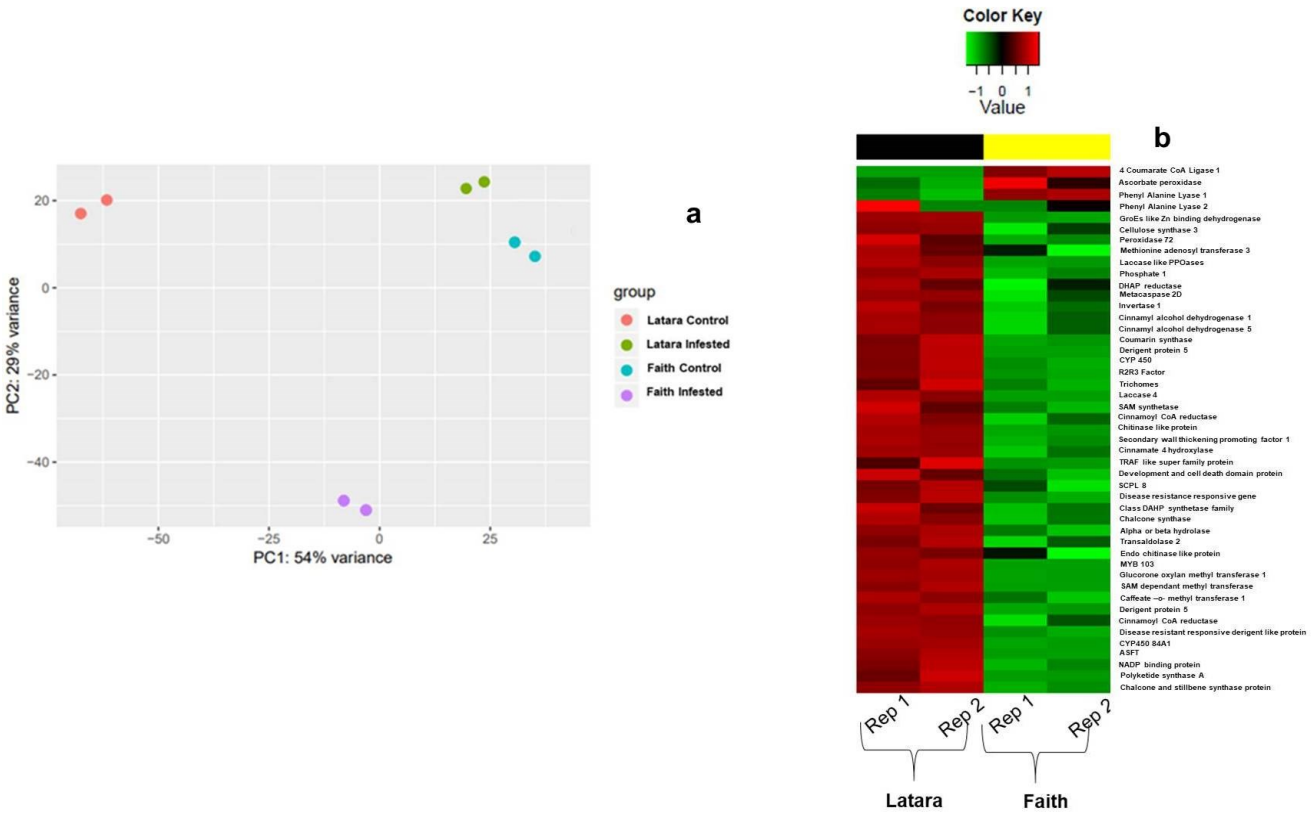

Figure 3a) PCA score plot of floral bud metabolic profiles measured by GC–MS. The floral bud samples from two gerbera genotypes (Latara and Faith) were infested by WFT for 72 h. Red dot represents Latara floral buds without WFT infestation. Green dot represents Latara floral buds infested by WFT, Blue dots represent Faith floral buds without WFT infestation, Violet dot represents Faith floral buds infested by WFT and b) Heatmap analyses of 47 differentially accumulated secondary metabolites from WFT -infested floral bud samples of Latara and Faith genotypes of Gerbera. Similarity assessment for clustering was done on the basis of Euclidean distance coefficient. Rows and columns represent individual metabolites and average samples, respectively.

Table 1 Total number of identified metabolites within each metabolite class.

Class of metabolites	No. of Identified metabolites in Latara	No. of Identified metabolites in Faith
Amino acids	18	14
Flavonoids	4	2
Organic acids	7	2
Phenolics	8	2
Phytoharmones	4	1
Sugars	4	4
Sugar alcohols	2	2
Total	47	27

The "latara" genotype infected with WFT exhibited a distinct buildup of certain organic acids. The concentration alterations of metabolites indicated that the WFT infestation mostly changed six metabolic pathways. Increased glycolysis and TCA cycle turnover numbers, which produce more biosynthetic intermediates, may be linked to the greater level of organic acids (Table 1). A total 47 metabolites were identified to be altered significantly in 'Latara'. Among 47 metabolites, formation of few specialized metabolites such as chlorogenic acid, myceritin, naringenin, Quercetin and Resveratrol were observed only in resistant genotype 'Latara' (Table 2). The alteration in metabolite levels correlated well with the changes in the transcript levels of selected secondary metabolite biosynthetic genes especially the key regulatory genes involved in chlorogenic acid biosynthetic pathway such as PAL, C4H, CL and HQT (Figure 4). The investigation of the 'latara' genotype's qRT PCR-based selected gene expressions before and after the WFT infestation indicated that all four of the genes under study had increased expression levels following the WFT attack. This suggests that the WFT attack led to an increase in the production of flavonoids, phenylpropanoid, and biphenyl biosynthetic products.

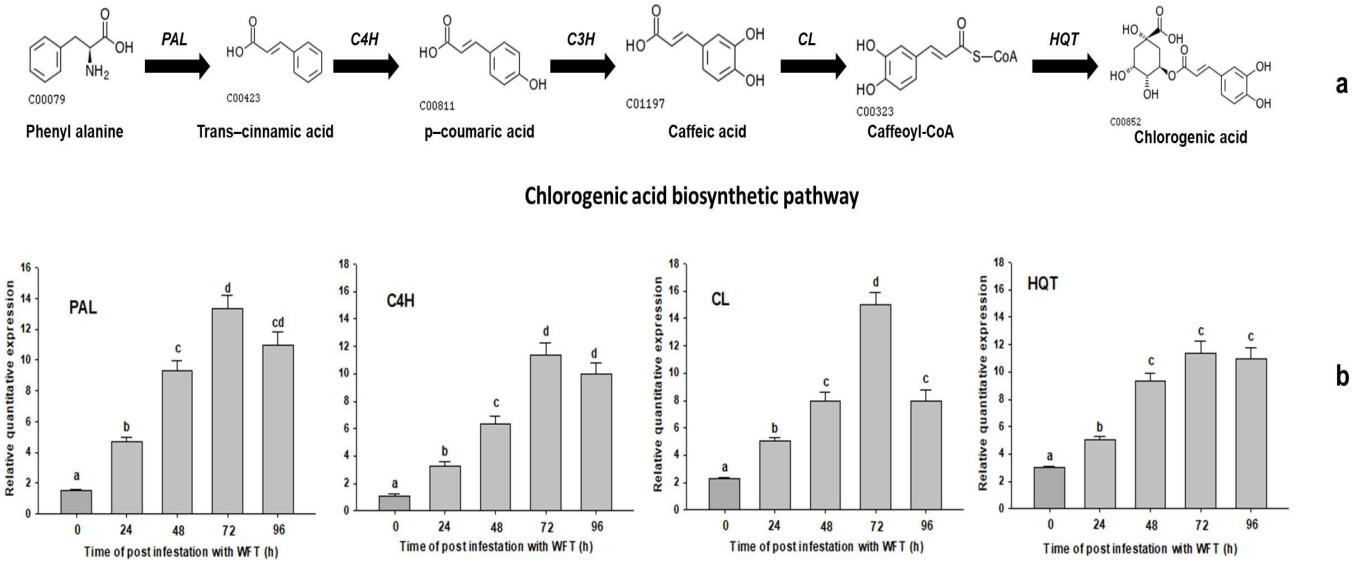

Figure 4. Resistance of latara genotype of gerbera flower buds against WFT (a), the schematic overview of the chlorogenic acid synthesis pathway (b), as well as expression profiles of biosynthetic genes of chlorogenic acid at different times of post infestation by WFT. All data are expressed as mean ± S.D. (n = 3). Significant differences among the different time points after infestation with WFT on latara genotype of gerbera are indicated as different alphabets (Means accompanied by same letters are not significantly different from each other ($p \leq 0.05$, Duncan's test).

S.No.	Differentially accumulated metabolites	TMS Derivate	KEGG ID.	Retention Time	Qualification Ions [m/z]	Signifcance of diferential accumulation	
						Latara	Faith
1	Arginine	3 TMS	C00062	21.51	390, 343	S	S
2	Asparagine	3 TMS	C00152	15.02	349, 316	S	S
3	Aspartic acid	3 TMS	C00049	13.52	349, 218	S	S
4	Ascorbic acid	4 TMS	C00072	18.26	464, 449	S	S
5	Benzoic acid	1 TMS	C00180	13.6	194, 179	S	NS
6	Caffeic acid	3 TMS	C01197	22.86	396, 381	S	NS
7	Catechin	5 TMS	C00199	26.25	649, 576	S	NS
8	Chlorogenic acid	6 TMS	C00852	21.24	786, 712	S	ND
9	Citric acid	4 TMS	C00158	16.53	465, 437	S	S
10	p-Coumaric acid	2 TMS	C00811	22.2	308, 293	S	NS
11	Trans-Cinnamic acid	1 TMS	C00423	17.81	220, 205	S	NS
12	Ferulic acid	2 TMS	C01494	23.6	338, 323	S	NS
13	Fructose	5 TMS	C00095	19.61	569, 307	S	S
14	Jasmonic acid	1 TMS	C08491	17.30	210,269	S	NS
15	GABA	3 TMS	C00334	21.72	319, 311	S	ND
16	Gallic acid	4 TMS	C01424	22.06	458, 281	S	NS
17	Glucose	6 TMS	C00031	19.86	540, 525	S	S
18	Glutamic acid	3 TMS	C00025	17.72	432, 330	S	NS
19	Glycine	3 TMS	C00037	17.77	291, 261	S	S
20	Histidine	3 TMS	C00135	21.91	371, 319	S	S
21	Hydroxybenzoic acid	2 TMS	C00156	18.04	282, 267	S	NS
22	Isoleucine	2 TMS	C00407	11.44	302, 274	S	NS
23	Leucine	2 TMS	C00123	11.32	275, 252	S	S
24	Lysine	3 TMS	C00047	21.24	362, 309	S	S
25	Malic acid	3 TMS	C00149	16.69	350, 335	S	NS
26	Malonic acid	3 TMS	C00383	15.51	305, 231	S	S
27	Mannitol	6 TMS	C00392	24.85	421, 319	S	S
28	Mannose	5 TMS	C00159	18.81	435, 393	S	S
29	Methionine	2 TMS	C00073	17.81	320, 292	S	S
30	Myricetin	6 TMS	C10107	14.85	318, 235	S	ND
31	Naringenin	3 TMS	C00509	30.78	272, 252	S	ND
32	Phenylalanine	2 TMS	C00079	16.09	294, 266	S	NS
33	Proline	2 TMS	C00148	11.85	259, 216	S	S
34	Protocatechuic acid	3 TMS	C00230	20.82	370, 355	S	NS
35	Pyruvic acid	1 TMS	C00022	10.78	145, 116	S	S
36	3-phosphoglyceric acid	3 TMS	C00197	14.58	402, 337	S	S
37	Quercetin	5 TMS	C00389	27.04	302, 235	S	ND
38	Resveratrol	3 TMS	C03582	18.40	228, 243	S	ND
39	Salicylic acid	2 TMS	C00805	11.90	281, 267	S	NS
40	Serine	3 TMS	C00065	12.75	306, 278	S	S
41	D-Sorbitol	6 TMS	C00794	19.48	217, 147	S	S
42	Sucrose	8 TMS	C00089	26.79	437, 361	S	S
43	Succinic acid	2 TMS	C00148	12.03	262, 247	S	S
44	Tyrosine	3 TMS	C00082	20.30	466, 438	S	NS
45	Tryptophan	3 TMS	C00078	20.29	405, 291	S	S
46	Valine	2 TMS	C00183	09.92	117, 146	S	S
47	Vanillic acid	2 TMS	C06672	19.55	312, 297	S	S

S: significant ($p < 0.05$); NS: non-significant ($p > 0.05$); ND: not detected.

4. Discussion

In plant-pest interactions, H_2O_2 is commonly referred to as a signal transduction molecule that responds in the tolerance mechanism. Moreover, MDA is the end result of membrane lipid peroxidation in plants, and it is a direct indicator of the extent of cell damage (Bailly et al. 1996). In comparison to faith, whose MDA and H_2O_2 contents were lower in latara genotype, it was observed that thrips-damage significantly increased the MDA and H_2O_2 contents of both genotypes. This suggests that latara possessed a robust system of enzyme defense that could effectively control ROS levels to ensure less damage to the membrane system by the external environment and, indirectly, reflect better resistance against WFT.

In the relationship between plants and pests, ROS is a signaling molecule that is generated in the initial stages of a pest infestation.

Plant resistance can be physiologically indicated by SOD and CAT, which are the main ROS scavengers in plants. While CAT can primarily scavenge the H_2O_2, SOD regenerates the H_2O_2 and O_2 by a disproportionation reaction, ensuring a low ROS level (Bruce et al. 1989; Penella et al. 2014). The findings of this study indicated that, upon WFT infestation, latara's SOD activities significantly increased in comparison to faith, and its CAT activities were significantly higher than faith's, indicating that latara had a higher resistance due to its ability to quickly eliminate H_2O_2 and generate less ROS.

The given study's findings point to a relationship between SOD activity and latara's defense mechanism against the WFT infestation in contrast to faith. According to earlier studies (Heng et al. 2004; He et al. 2011), there is a strong correlation between plant resistance to pests and the enzymes peroxidase (POD), phenylalanine ammonia-lyase (PAL), polyphenol oxidase (PPO), catalase (CAT), and superoxide dismutase (SOD). POD, which may catalyze the oxidation of phenolic compounds and is crucial for the defense against H_2O_2 and $OH\cdot$, is one of the major enzymes in the formation of lignin (Gulsen et al. 2010).

Plant resistance to insect pests has been tightly linked to both PAL and PPO activities (Zhang et al. 2008). The manufacture of phenols, lignin, and plant protection factors can be catalyzed by PAL, a major and rate-limiting enzyme in the phenylpropane metabolic pathway (Zhang et al. 2005a, b, c). Comparably, PPO can convert phenols into quinones, fortify cell wall architecture, or restore damaged walls to prevent pests from feeding (Mauchmani et al. 1996). Thus, to assess plant-induced resistance, PAL and PPO activity was employed as a physiological indication. Liu (2009) noted that following thrips inoculation, the new strain of alfalfa's POD, PAL, and PPO enzyme activity altered swiftly, allowing the plant to adapt and resume regular development. Upon the aphid infestation, Lu et al. (2017) found that cotton cultivars' POD, PAL, and PPO activities were noticeably increased and that resistant cultivars' enzyme activities were noticeably higher than those of susceptible cultivars.

Previous research has demonstrated that during plant-pathogen interactions, there is an up-regulation of primary metabolites, primarily at the levels of amino acids, sugar, and sugar alcohols (Rojas et al 2014). Elevated levels of organic acid are linked to improved disease resistance since they are known to improve ion absorption (Hudina et al 2000).

In the WFT-infested "latara" genotypes compared to "faith," there was a considerable up-regulation of phenolic and flavonoid contents among secondary metabolites. Nonetheless, the latara genotype has been found to include chlorogenic acid and quercitin, which suggests that phenolic acids are deposited along the cell wall to serve as the initial line of defense against infection (Schwalb and Feucht, 1999).

Enhanced phenylpropanoid production has been linked to increased expressions of the PAL, C4H, and 4CL genes (Cavallini et al 2015; Mukherjee et al 2016). Our results clearly illustrate the accumulation of phenolics and flavonoids (especially chlorogenic acid) which helps in lignification plant cells during pest infestation, while phenolic organic acids exhibit deterrence/ insecticidal properties. Thus our study till now explains the metabolic basis of WFT-resistance in Latara genotype of Gerbera. Further investigation of pesticidal efficacy of these secondary metabolites gives a clear picture on the precise role of these metabolites.

5. Conclusion

Our study is first of its kind to employ metabolomics to evaluate gerbera genotypes infested with WFT using non-targeted GC-MS. Our findings demonstrated the superior approach of the established metabolomics technology in analyzing metabolites from two genotypes of gerberas. Furthermore, our findings suggested that the latara genotype gerbera could serve as a model system for comprehending the interactions between Gerbera and WFT as well as the function of secondary metabolites in the "latara" genotype, which denotes the metabolic reprogramming of particular biosynthetic pathways following a WFT attack. Based on our findings, we can say that the phenyl propanoid production pathway is essential for gerbera ‘latara’ genotype WFT resistance. It would give future metabolomics analyses of gerbera plants an excellent basis for interpreting marker metabolites linked to WFT resistance in a number of other plants.

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CRediT authorship contribution statement

Talla, Sai Krishna: Investigation, Validation, Visualization, Writing – original draft. B, Vidya Vardhini: Conceptualization, Methodology, Funding acquisition, Supervision, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have NO competing financial interests.

Data Availability

Data will be made available on request.

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