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Effect of priming agents on whiteflyresistance

M.Sc. Thesis

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6/27/2013

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M.Sc. Thesis Plant Breeding (PBR-80436)

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Abstract

Plants have capability to prime their immune system after detection of specific environmental signals. In this thesis, we have analyzed the effect of priming (BABA, JA and Fructose) against whitefly in tomato (IL4, Moneymaker, Motelle and FCN93-6-2). For that we have conducted 3 experiments, namely; No-choice, Choice and Gene expression. We have found that whitefly preferred Fructose treated plant more than BABA, JA and H_2O treated plant. The BABA and JA affect negatively on growth of plant. In partially resistant varieties we have found no effect of priming treatment in oviposition rate and free choice essay. From gene expression analyses we have found that upon whitefly infestation induce in JA dependent pathway and suppress in SA dependent pathway. However we have not seen the effect of priming treatments on gene expressions.

Contents

Introduction

In nature, 1556 whitefly species have been identified [1]. Most of the attention has been given to the polyphagous whitefly *Bemisia tabaci* species complex and *Trialeurodes vaporarioum. Bemisia tabaci* is a problem due to its, wide host range, exceedingly eager feeding habit on phloem sap and continuous development of insecticide resistant strain [2]. It is vector for more than 111 virus species [3]. Among the most important, *B tabaci* is vector of the tomato yellow leaf curl (TYLCV) virus. In tropical and subtropical region TYLCV can cause 100% yield loss [4]. Whitefly control is mainly done by insecticide applications. However, negative impact of insecticide on environment and rapid development of insecticide resistance by insect, is a current problem [5]. It pushes to develop alternative protective method [5].

Plants are armed with different defense mechanism to guard themselves against herbivorous insects and pathogens. Those mechanisms can be constitutive or induced upon insect or pathogen attack [6]. The wax layer on leaf surface, trichomes, rigid cell wall, anti-microbial enzyme and secondary metabolite are part of constitutive mechanism [7]. On the other hand, plants can response to the pathogen/herbivory attack. Induced response mechanisms are triggered by the recognition of pathogen-associated molecular pattern (PAMPs), microbe-associated molecular pattern (MAMPs) and herbivore-associated molecular pattern (HAMPs) [8]. The outcome of this turns into PAMP-triggered immunity (PTI) guard the plant against the majority of harmful pathogen. To overcome this defense mechanism pathogens developed the mechanism in which an effector molecule discharge in plant tissue that obstructs the PTI [9]. This possess is known as Effector-trigger Susceptibility (ETS). But this is not all, plants can contra rest and some have the ability to perceive the effector molecules and trigger a defense response [10]. This Immunity based in the recognition of effector molecules is known as Effector-triggered Immunity (ETI) [10]. In ETI, R proteins recognize specific effector activity of virulence of pathogen. ETI protect fully against specific virulence of pathogen. So finally, this arms race or combat between plant and pathogen depends in the ability of pathogens to suppress PTI and the ability of plant to trigger ETI [11].

Plants have capability to prime their immune system after detection of specific environmental signals (called priming) that results in stronger and faster activation of basal resistance after the attack by microorganism [8]. Priming provide broad spectrum of protection against biotic and abiotic stress by boosting the plant basal defense[8]. In that sense, priming can be defined

as the ability of increase the capability of express basal resistance mechanisms [8]. Priming can be done by treatment with biotic inducers (i.e. necrotizing attackers, nonpathogenic root-colonizing pseudomonads) or by the application of natural or synthetic compounds (i.e. salicylic acid, β-aminobutyric acid) [12]. Priming induces resistance after the contact of pathogens, both locally (site of infection) and systemically. In general, induced resistance can be divided into two type which are systemic acquired resistance (SAR) and induced systemic resistance (ISR) [11]. The SAR activates in response to an attack of biotrophs; whereas ISR is activated by necrotrophs, and upon colonization of plant root by beneficial microorganisms. ISR also activates systemically in other plant part also[11].

In plant, jasmonic acid (JA), salicylic acid (SA) and ethylene (ET) are major hormone regulators of plant innate immunity [13]. These hormones intensify the defense related gene expression in plant. For instance in rice, JA intensifies the expression of *PR-1* gene that is activated in response to fungal elicitors [13]. The role of these hormones depends upon host pathogen interactions in basal resistance. The SA defense pathway provide protection against biotrophs but usually fails to protect against necrotrophs [13]. The SA pathway is required for activation of SAR. In which, SA activates large set of genes that encodes pathogenesis-related proteins (PRs) with antimicrobial properties [11]. The JA pathway is activated by JA and its derivatives (collectively called jasmonates), which are ubiquitous plant regulators. In plant JA can act as signals in plant immunity response under biotic and abiotic stresses. It also activates in wounding recovery of plants [13]. The JA defense pathway provides protection against necrotrphs and fails to protect against biotrophs [10]. Also there are exceptions. For instance the JA pathway increase resistance against biotrophic *Erysiphe orontii* and *Oidium neolycopresicino* in *Arabidopsis* plant [13]. The ET pathway play intermediate role in activating ISR which strengthen and induce defense response [14]. The SA and JA pathway are antagonistic. For example, TMV (tomato mosaic virus) infected tobacco plants expressing SA-dependent systematic acquire resistance (SAR) are unable to develop JA-mediated defense responses [13]. There are also examples of synergism between SA and JA pathway. For instance, when *Arabidopsis* plant was treated with low concentration of SA and JA a synergetic effect on the SA and JA responsive genes *PR-1* and *PDF1.2* was found. However, with higher concentration of SA and JA they showed antagonistic effect [11]. It means the effect of SA and JA depends on relative concentration of each hormone [11]. The JA and ET pathways are synergetic and activate ISR in plants[13].

In ISR not direct activation of PR genes. In ISR JA and ET dependent primed genes activates that enhance defense response[11].

The antagonistic mechanism between SA and JA pathway helps to plant to minimize energy cost and it's allowed to fine tune defense action against attacker [6]. However there are several examples of attacker manipulate the plant defense mechanism for their benefits [6]. For instance, silverleaf whitefly (*Bemisia tabaci*) induce SA pathway and suppress JA pathway in *Arabidopsis* [15, 16]. It was also reported that the effect of a priming treatment also depends on genotype and the biotype of the insect [17].
In plant JA plays vital role in initiating defense mechanism after attack of herbivory and in other process such as senescence and flower growth[18]. It has been shown that the external application of methyl Jasmonate (MeJA) produce an increase in the density of trichomes in young leaves, which help in increase resistance level against herbivores in plant[19]. It has been also shown that the secretion of extra floral nectar is increased after exogenous application of JA attracts predator insects [20].

Priming can be induced by the application of semi-chemicals. Among those semi-chemicals it was shown that the application of BABA produce an increase in the resistance level against oomycetes, fungal, bacterial, viral and nematode diseases [21]. The resistance mechanisms induced through BABA application are different from the resistance mechanisms activated through SA or ABA applications. BABA activates different priming pathways in plants depending on the attacker [22]. For example in *Arabidopsis,* BABA induce callose deposition in sites of penetration of *Hyaloperonospora parasitica,* whereas it activate SA-dependent defense mechanism and the accumulation of *NPR1/NIM1/SAI1* proteins *Arabidopsis* against *Botrytis cinerea* [21]. Furthermore in *Arabidopsis* it have being shown that BABA dependent resistance is effective in SA-dependent, SA-independent and ABA-dependent defense mechanisms [21].

Sugars have been also used to induce resistance in plants. Apart from typical roles as carbon and energy sources sugar also involved in plant as signaling molecules and metabolic pathways [23]. In plant interconnected communication in sugar and hormonal pathway may lead to an effective immune response [23]. It is assumed that change in apoplastic sugar level consolidate with PAMPs signal leading to further activation of SAR. When tobacco plant treated with glucose, sucrose and fructose increase in expression level of *PR-Q* and *PR1* [23].

In this study, our objective was to evaluate the effect of priming treatment on partially resistant cultivars against whitefly, host preference and effect of treatment on plant growth in tomato plants. A very few studies has been done so far on effect of priming treatment against whitefly resistance in tomato plant. Additionally we have included fructose as priming agent. To our understanding, no study has been done so far on to see the potential of fructose against whitefly. We have done the factorial experiment in that we have compared the phenotypic data such as ovi-position rate or adult survival rate and fresh plant weight. We also measure the relative gene expression of defense related genes by using real-time PCR. This study generates new knowledge on effect of priming in partially resistant genotypes.

Material and Methods:

Plant material

Four tomato varieties (*Solanum lycopersicum)* were used. The accession FCN93-6-2 selected by its difference in preference with *Trialeurodes vaporarioum* [24], the cv. Motelle selected because it carries the *Mi1-2* gene related with whitefly resistance [25], the accession IL-4 (introgression line derived from the cross between LYC4 and cv. Moneymaker) selected because is resistant to botrytis [26], and the cv. Moneymaker was used as reference.

Whitefly Rearing

A non-viruliferous whitefly rearing (*Bemisia tabaci* Group Mediterranean-Middle East-Asia Minor I) [27] was maintained on the susceptible cv. Moneymaker at the Laboratory of Plant Breeding, Wageningen UR, Wageningen, the Netherlands. The initial inoculum was obtained from a rearing at the Laboratory of Entomology, Wageningen UR, Wageningen, The Netherlands.

Chemicals treatment and concentration

In this study we used three priming compounds, β-aminobutiric acid (BABA, 0.5 mM), Jasmonic acid (JA, 0.05 mM) and fructose (100 ppm). Water was used as control. The concentrations used were the highest concentration that can be applied to cv. Moneymaker with a minimum fitness cost (Luna, E. and Ton J., personal communication). To prime 0.5 mM BABA in 20 ml volume pot, 5 ml of BABA (2 mM) and 15 ml H_2O was injected into the soil. To prime 0.05 mM JA in 20 ml volume pot, 1 ml of JA (1 mM) and 19 ml H_2O was injected into the soil. To prime 100 ppm fructose in 20 ml pot, 2 ml of fructose (1000 ppm) and 18 ml H_2O was injected into the soil. In control 20 ml H_2O was injected into 20 ml pot.

Experimental design and plant treatments

We have conducted three experiments, namely; No-choice, Choice and Gene expression. Each of the experiment was arranged in a randomize complete block design with four genotypes (FCN93-6-2, cv. Motelle, IL-4, cv. Moneymaker), four treatments (BABA, JA, Fructose and H_2O) and eight replications.

For all the experiments, the tomato seeds of each variety were sown in soil compost mixture in trays with 96 pots, where the volume of each pot was 20 ml. The trays were placed in growth chamber with 8 h day (24 ^{0}C) and 16 h (20 ^{0}C) night cycle 60-70% relative humidity

(RH) for 2 weeks. At the moment the plants had reached cotyledons stage, the trays were shifted to greenhouse with 8 h day (24 ^{0}C) and 16 h (20 ^{0}C) night cycle 60-70 % relative humidity (RH). The plants were kept in greenhouse for a week. In this period, the plants were watered (50 ml per pot) every 2 day. One week after priming, the plants where removed from the pot, the roots were washed to remove any remaining of soil and/or priming compounds, and transferred in new pots (500 ml) containing fresh soil and compost but chemical free (repotting). The plants were grown for 10 days from repotting. In mean time, the plants were irrigated every 2 days. Plants were ordered in a randomized complete block design. This was the common procedure followed in all experiments, and a summary of the priming treatment can be found in (Fig 1.0).

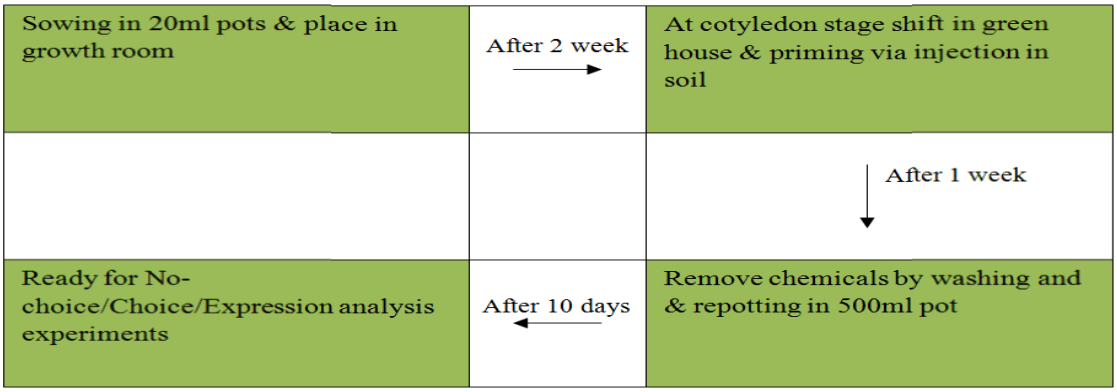

Figure1. Steps in common procedure for no choice experiment, choice experiment and expression analysis.

No-choice Experiment

After priming, five females (four days old) were anesthetized (using CO_2), selected under binocular microscope, and placed into a clip-on cage (2.5 cm in diameter and 1.0 cm high). One cage per plant was put. Five days after inoculation, the number of alive and death whiteflies was recorded and the surviving whiteflies were removed. The number of eggs was counted, and the Oviposition rate (OR) and Adult survival (AS) were calculated according to [28]. The variables AS and OR were Arcsin transformed to fulfill normal distribution and ANOVA was performed. Statistical analyses were done using GENSTAT.

Choice experiment

For this experiment, plants where arranged in a randomized block design and non-sexed

whiteflies where released into the greenhouse at a density of 17.8 whiteflies per plant. For this, the whiteflies were placed in collection tubes (44 tubes with 50 whiteflies each), and the tubes balanced distributed in the greenhouse (Fig 1.1). After 24 h from release of whiteflies, the number of whiteflies present on each plant counted. The data was LOG (x+1) transformed and analyzed by ANOVA. Statistical analysis were done using GENSTAT [28].

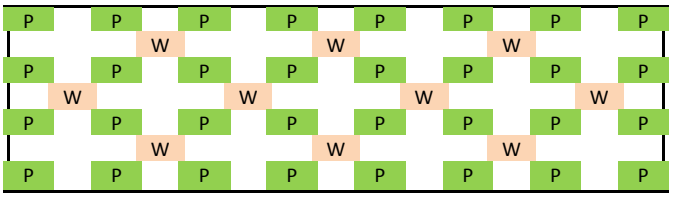

Figure 1.1: Arrangement of plants and whiteflies filled tubes for release in choice experiment. P = Plant, W = whiteflies filled test tube

Expression analysis

To see expression analysis we have harvested leaf sample 2 times, the first was before inoculation of whiteflies and second time 5 days after inoculation (at the moment of removing the adults). Immediately after harvest, the leaf samples were placed in a collection tube and frozen in liquid nitrogen and stored in -80 ^{0}C refrigerator till analysis. The leaf

sample of after inoculation harvested from the plants that were used in no-choice experiment

after the counting of egg deposition the inoculated leaf harvested.

Leaf sample were ground in liquid nitrogen using machine tissuelyser II from QIAGEN. For each treatment, we had eight leaf samples. The eight replicates we pooled in two biological replicates of four different leaf samples per treatment. RNA extraction was done by using the RNeasy Plant Mini Kit, QIAGEN, and following manufacturer protocol. RNA quality was checked on an agarose gel (1.5% TAE), and a DNAse treatment was given to RNA for elimination of DNA. DNAse treated RNA was retro-transcripted into cDNA using the iScript cDNA synthese kit (Biorad) following manufacturer protocol. For real time PCR (Qpcr) cDNA was diluted in RNAse free MQ water to a final concentration of 0.05 ug/ul. All Amplification reactions were done on CFX96 Real-Time PCR Detection System (Biorad). Two technical replicates for each sample and two biological replicates for each treatment

were used, with individual gene specific primers (table 1). PCR performance (E) of primer were estimated from multiple amplification plot using equation $(1+E) = 10^{slope}$ and were confirmed to provide (1+E) values close to two (ranging from 1.9 to 2). Relative gene expression calculated using 2 Δ Ct method where Δ Ct = Ct reference – Ct gene of interest

[29]. Relative gene expression data was LOG (x+1) transformed and alkalized by ANOVA. Statistical analyses were done using the software package Infostat.

Table 1: Genes and primers used for real-time PCR amplification

Gene	Primer name	Sequence	Pathway	Reference
EF1-α	EF-1-F	CTCCGTCTTCCACTTCAGG	Reference gene	[30]
	EF-1-R	TACGTTGTCAAACCAGTAGGG		
PR1	PR1_F1	TGGTGACTTCACGGGGAGGG	SA response	[15]
	PR1_R1	CGGACTGAGTTGCGCCAGAC		
CHI9	CHI9_F1	GTCATCACCGGAAGATGGCAGC	ET response	[31]
	CHI9_R1	CCGATCCTGGACCCTGCTGT		
ICS	ICS_F1	GGCAATAGATGCACTTCAGGCCA	SA biosynthesis	[32]
	ICS_R1	CGCATGGTCCCAAGACGCTTT		
LOX D	LOX-D F	CCGTGGTTGACACATTATCG	JA synthesis	[33]
	LOX-D R	ACAGCAGTCCGCCCTATTTA		
LAP-A	LAP F	ATCTCAGGTTTCCTGGTGGAAGGA	JA response	[34]
	LAP R	AGTTGCTATGGCAGAGGCAGAG		
PIN2	PIN2-F	CTTCTTCCAACTTCCTTTG	JA response	[34]
	PIN2-R	TGTTTTCCTTCGCACATC		
RBOH-D	RBOH-D-F	TCAGGTCAAGCATCAAAGCCGTT	Whitefly induced gene	[35]
	RBOH-D-R	TGGTGAAACCGCAGCACAGT		
MTS2	MTS2-F	ACCAAAGAGGCCTTGGAATC	Monoterpene synthase	[36]
	MTS2-R	ACCGAAGATGTCCCCAAATC		

Results

No-choice test

In no-choice test we measured the three parameters, Adult survival (AS), Oviposition rate (OR) and Fresh weight (FW). For AS, we have not found difference neither at the treatment level nor for the genotype or the interaction between treatment and genotype. For OR, we have detected a genotype effect, being FCN93-6-2 the most susceptible and IL-4 is the most resistant (Table 2). We did not find a treatment or a Genotype by Treatment effect. For FW we have not found interaction between genotype and treatment for FW but the significantly different effect of genotype and treatment individually identified. The FW of FCN93-6-2 was the highest and the weight of IL-4 was the lowest.

Table 2: Mean oviposition rate (no.eggs/day) and fresh weight (g) per genotype (±SE). Different letters indicate statistically significant differences by Fisher's least significant difference (LSD) test; α=0.05

Genotype	Oviposition rate	Fresh Weight
IL-4	4.02 ±0.30 a	13.19 ±0.82 a
cv. Moneymaker	4.57 ±0.30 ab	15.40 ±0.80 b
cv. Motelle	4.81 ±0.26 b	17.40 ±0.71 c
FCN93-6-2	5.78 ±0.26 c	18.37 ±0.67 c

The effect of different chemical on genotype is seen in table 3. Where lower to higher mean FW in BABA, JA, Fructose and H_2O treated plant respectively.

Table 3: Mean fresh weight (g) per treatment (±SE) over all genotypes. Different letters indicates statistically significant differences by Fisher's least significant difference (LSD) test; α=0.05

Treatment	BABA	JA	Fructose	H_2O
FW (g)	11.48 ± 0.72 a	14.57 ±0.51 b	19.28 ±0.58 c	19.23 ±0.50 c

To know how much weight loss due to each treatment we used the water treated plant as standard to correct the weight of plant. Then we calculated the weight reduction in percentage of each treatment in each variety. We have found that the relative weight lost was different

per genotype when treated with BABA (p= 0.015). The weight loss in BABA treated IL4 and cv. Moneymaker was around 50%. The weight loss observed in FCN93-6-2 and cv. Motelle in BABA treated plant around 30 to 35% (Fig 1.2). But we did not see a significantly different effect on genotype due to other treatments.

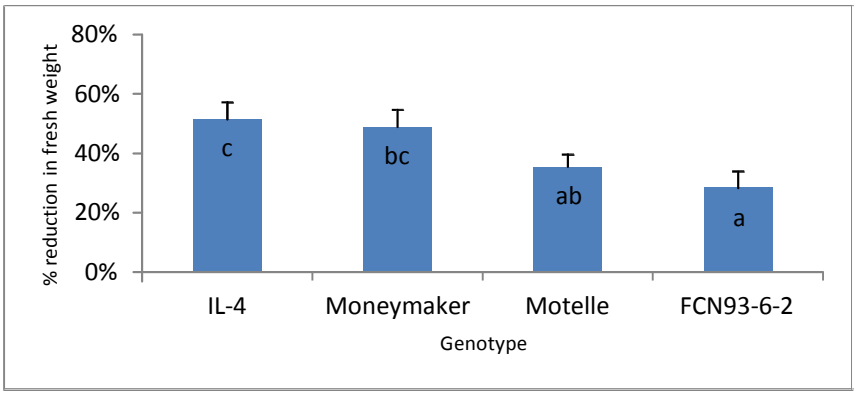

Figure 1.2: Fresh weight reduction in percentage in each genotype (±SE). The different letters indicates statistically significant differences by Fisher's least significant difference (LSD) test; α=0.05

Choice test

In free choice test we have found significant difference in whiteflies preference due genotype and treatment but no significantly different effect due to interaction between genotype and treatment. The genotype IL-4 (12.22 ±9.15) is given first preference, cv. Moneymaker (6.50 ±4.14) and cv. Motelle (5.69 ±2.22) given second and FCN93-6-2 (3.81 ±2.91) given third preference by whiteflies respectively (Fig 1.3).

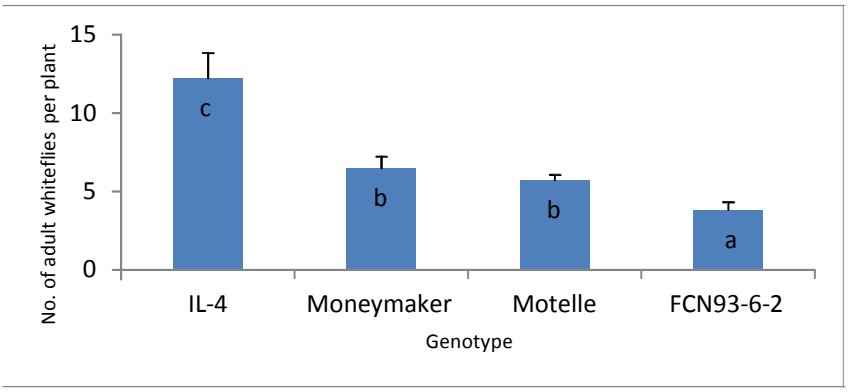

Figure 1.3: Mean number of whiteflies per genotype (±SE). The different letters indicates statistically significant differences by Fisher's least significant difference (LSD) test; α=0.05

With respect to treatment whiteflies could not differentiated between H_2O treated, JA treated or BABA treated plant, but in Fructose treated plants most preferred by whiteflies (Table 4).

Table 4: Mean number of adult whiteflies per treatment (±SD). The different letters indicates statistically significant differences by Fisher's least significant difference (LSD) test; α=0.05

Treatment	JA	H_2O	BABA	Fructose
No. of Whiteflies	5.25 ±3.34 a	6.50 ±6.19 a	6.50 ±5.21 a	9.97 ±8.10 b

Gene Expression

SA, JA and Ethylene inducible gene expressions.

We monitored the activation of the SA, JA and ET pathways by the expression of inducer genes. For the SA pathway we measure the gene expression of pathogenesis-related protein-1 (*PR1*) SA inducible, Isochorismate synthase (*ICS1*) SA biosynthesis. For the JA pathway we measure the gene expression of a protease inhibitor gene (*Pin2*), leucine aminopeptidase gene (*LapA1*) as JA-inducible gene, lipoxygenase D (*LoxD*) JA biosynthesis. For the ET pathway we measure the gene expression of basic chitinase (*Chi9*) ethylene inducible gene. In addition to these three pathways, we have measured the gene expression of the monoterpene synthase 2 gene (*MTS2*) and *RbohD* whitefly induced gene.

The transcript level increased after inoculation except *ICS* and *MTS2*. The transcript level of *ICS* is decreased after inoculation (Fig 1.4). No significant effect of inoculation found in *MTS2.*

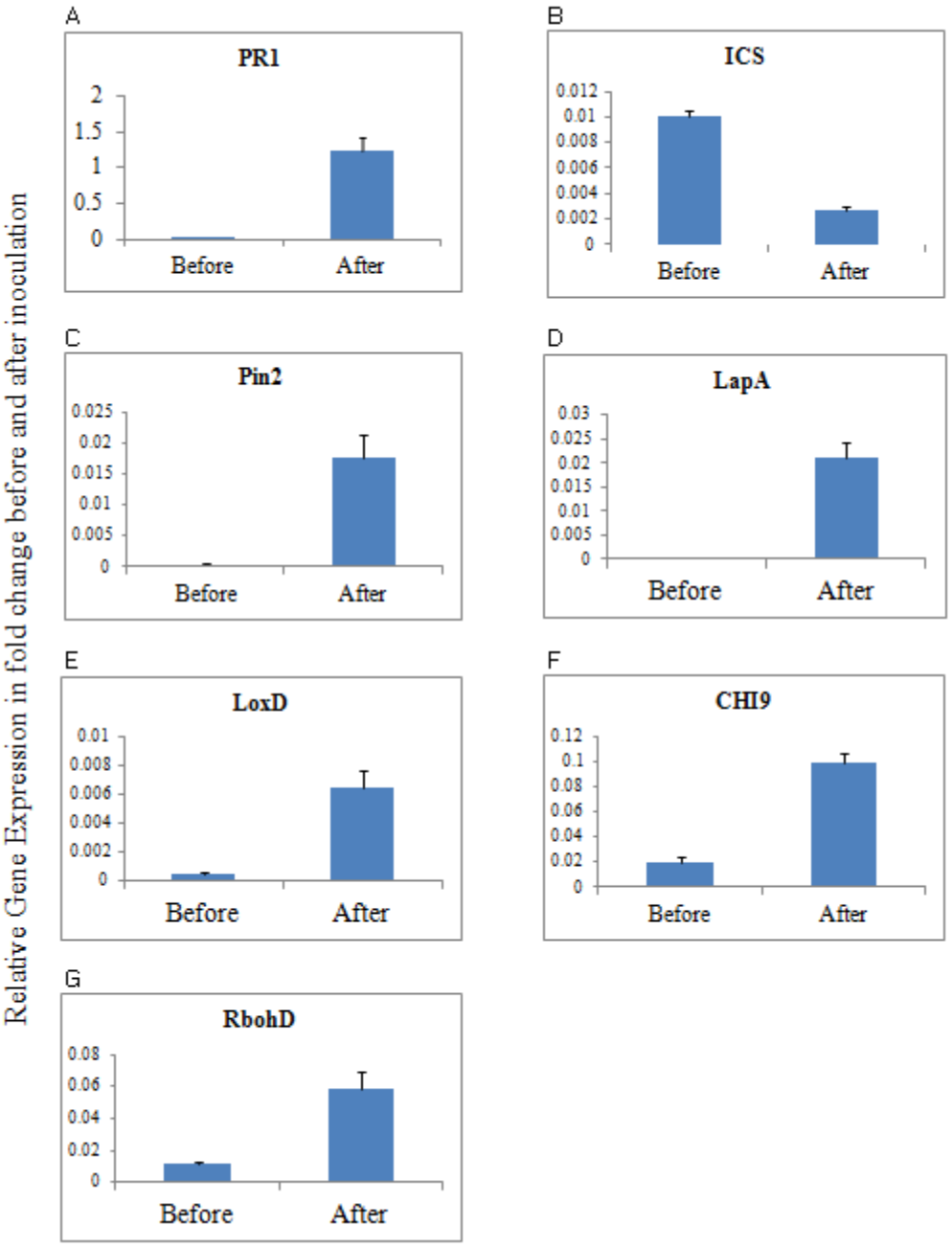

Figure 1.4: Mean relative gene expression of *PR1*, *ICS1*, *Pin2*, *LapA*, *LoxD*, *CHI9* and *RbohD*. The difference is before and after 5 days of feeding of whiteflies. Statistically significant difference by Fisher's least significant difference (LSD) test; α=0.05. The error bars represents the ±SD.

The level after inoculation of the *PR1* gene (SA pathway) was increased due to significantly different effect of genotype being higher in cv. Moneymaker, FCN93-6-2 and cv. Motelle lower in IL4 (Fig 1.5). No treatment or treatment by genotype effect was found.

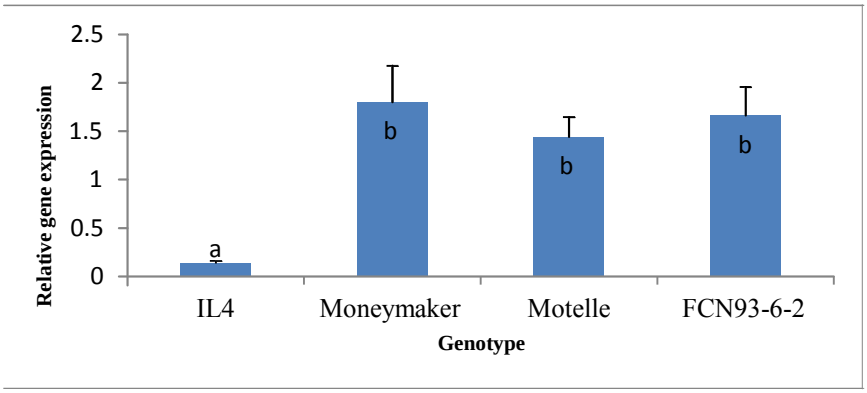

Figure 1.5: Mean relative gene expression of *PR1*. The difference is 5 days after inoculation of whiteflies. Statistically significant difference by Fisher's least significant difference (LSD) test; α=0.05. The error bars represents the ±SE.

In SA biosynthesis, *ICS1* gene the transcript level before inoculation was significantly differed by genotype but not by treatment or treatment by genotype interaction. The transcript level was high in IL4 and FCN93-6-2 and low in cv. Moneymaker and cv. Motelle (Fig 1.6). The overall transcript level of *ICS1* was high before inoculation than after inoculation (Fig 1.4B). After inoculation, the transcript level differs by genotype by treatment effect (Fig 1.7).

In IL4 and FCN93-6-2 the transcript level of H_2O treated plant was high compare to BABA, JA and fructose treated plants. However in cv. Motelle the transcript level of H_2O treated plants was lowest compare to BABA, JA and fructose treated plants. In cv. Moneymaker lowest transcript level estimated in fructose treated plant and the transcript level of BABA treated plant, H_2O treated plant and JA treated plant is not significantly different (Fig 1.7).

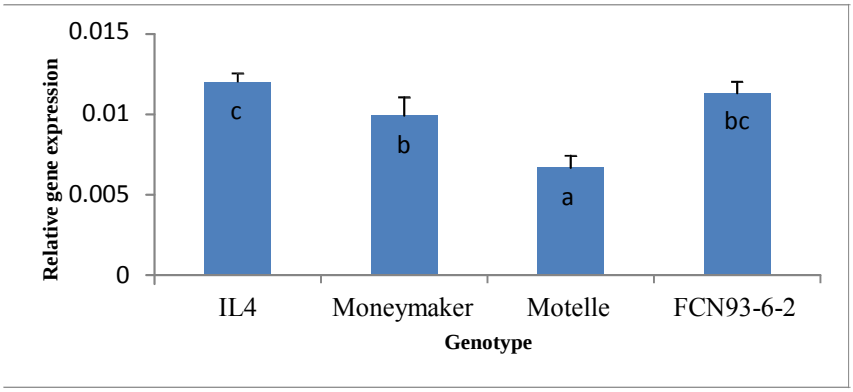

Figure 1.6: Mean relative gene expression of *ICS*. The difference is before inoculation of whiteflies. Statistically significant difference by Fisher's least significant difference (LSD) test; α=0.05. The error bars represents the ±SE.

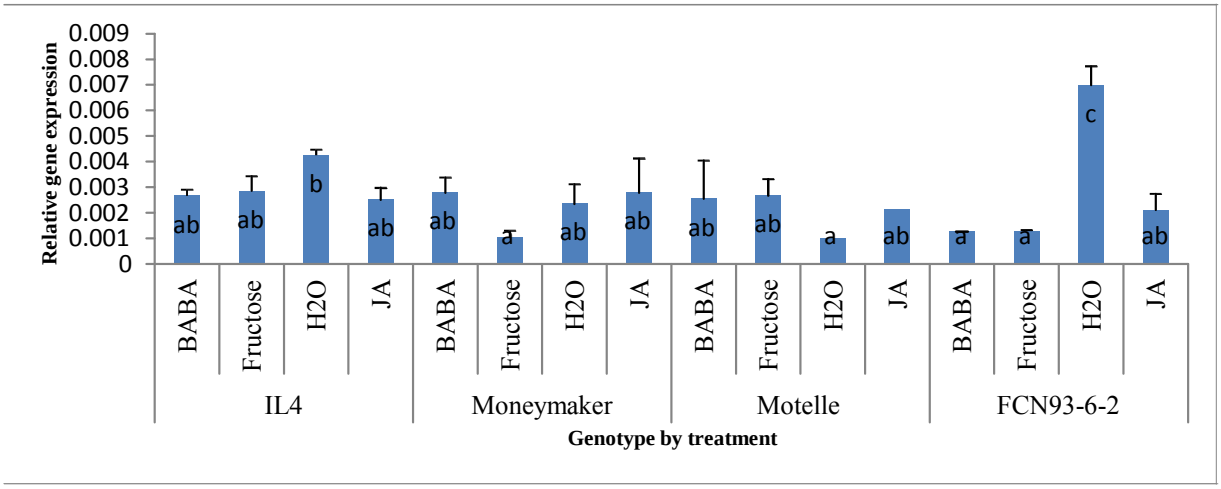

Figure 1.7: Mean relative gene expression of *ICS*. The difference is 5 days after inoculation of whiteflies. Statistically significant difference by Fisher's least significant difference (LSD) test; α=0.05. The error bars represents the ±SE.

In JA inducible *Pin2* gene the transcript level before inoculation was significantly differed by treatment by genotype interaction. The transcript level was higher in H_2O treated cv. Motelle and FCN93-6-2 plant and lower in BABA, JA and fructose treated plant, but there were no significant difference in BABA, JA and fructose treated plants. However, in H_2O treated IL4 and cv. Moneymaker the transcript level was not high compared to BABA, JA and fructose treated plant (Fig 1.8). The overall transcript level of *Pin2* was high after inoculation than before inoculation (Fig 1.4C). After inoculation, the transcript level differed by genotype effect, but not by the treatment or genotype by treatment effect. In cv. Motelle transcript level was high compare to FCN93-6-2 and IL4 and cv. Moneymaker (Fig 1.9). In *LapA* no effect of genotype and treatment each or genotype by treatment found in before and after inoculation. However the overall transcript level was high after inoculation than before inoculation (Fig 1.4D).

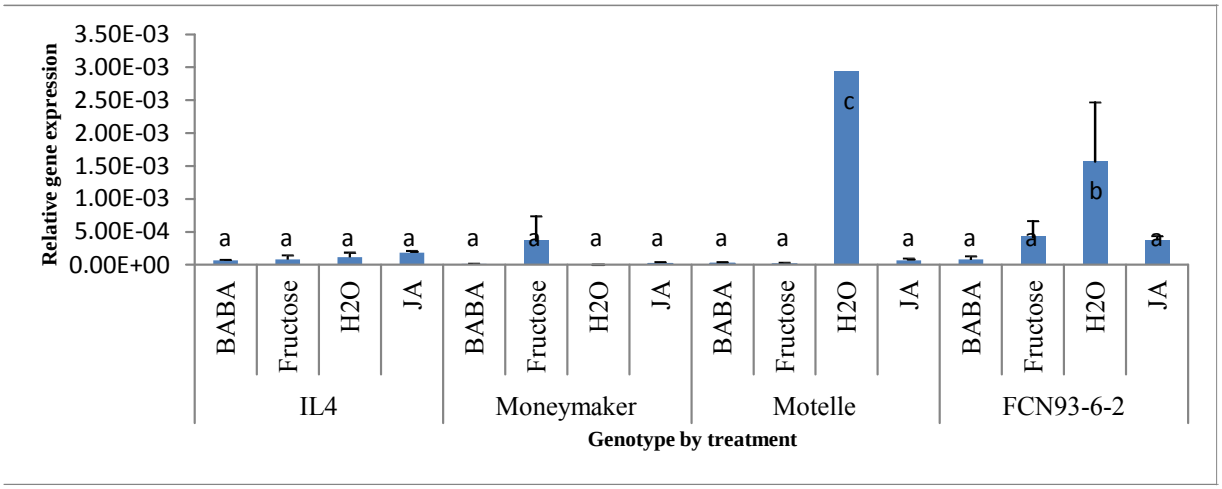

Figure 1.8: Mean relative gene expression of *Pin2*. The difference is before inoculation of whiteflies. Statistically significant difference by Fisher's least significant difference (LSD) test; α=0.05. The error bars represents the ±SE.

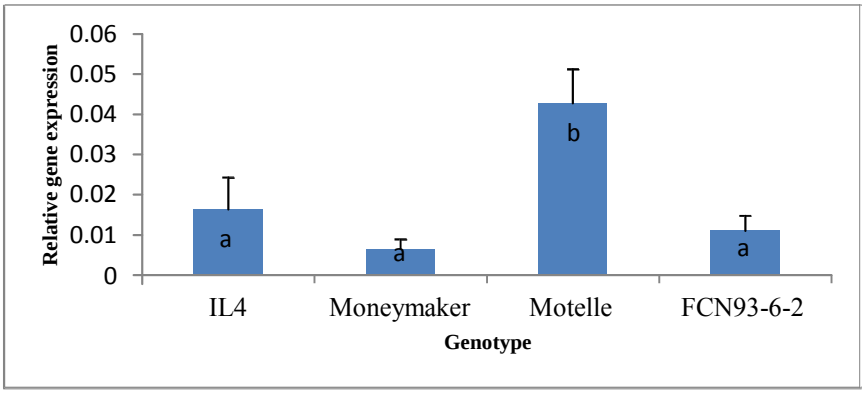

Figure 1.9: Mean relative gene expression of *Pin2*. The difference is 5 days after inoculation of whiteflies. Statistically significant difference by Fisher's least significant difference (LSD) test; α=0.05. The error bars represents the ±SE.

In JA biosynthesis *LoxD* gene the transcript level before inoculation was significantly differed by genotype and treatment individually but not by treatment by genotype interaction. The transcript level high was high in FCN93-6-2 and low in IL4, cv. Moneymaker and cv. Motelle. The transcript level was high in H_2O treated plant and low in BABA, JA and Fructose treated plant. The overall transcript level was high after inoculation than before inoculation (Fig 1.4E), but significant effect of genotype or treatment or genotype by treatment.

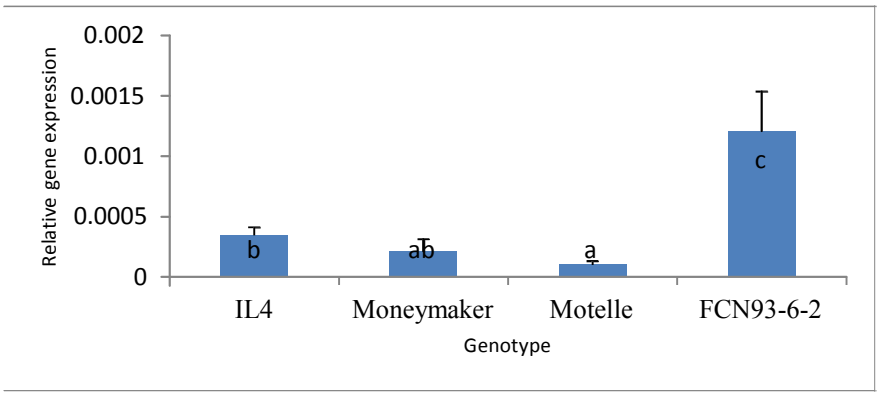

Figure 2.0: Mean relative gene expression of *LoxD*. The difference is before inoculation of whiteflies. Statistically significant difference by Fisher's least significant difference (LSD) test; α=0.05. The error bars represents the ±SE

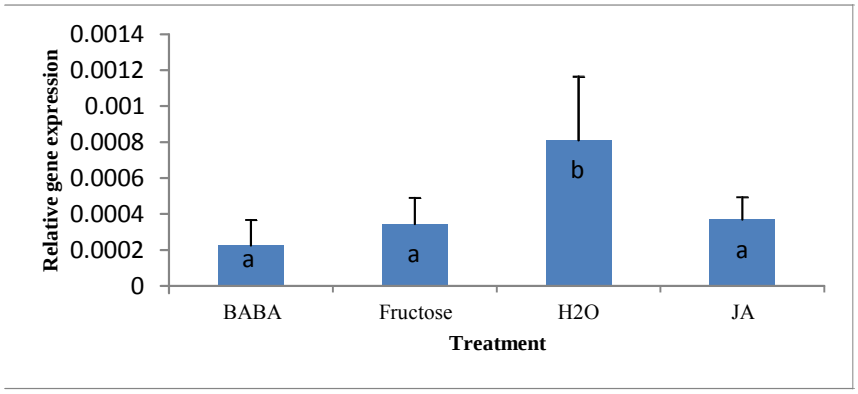

Figure 2.1: Mean relative gene expression of *LoxD*. The difference is before inoculation of whiteflies. Statistically significant difference by Fisher's least significant difference (LSD) test; α=0.05. The error bars represents the ±SE

In ethylene inducible, *CHI9* gene transcript level before inoculation was significantly differed by genotype but not by treatment or treatment by genotype interaction. The transcript level was low in cv. Motelle and high in cv. Moneymaker, FCN93-6-2 and IL4 (Fig 2.2). The overall transcript level of *CHI9* was high after inoculation than before inoculation (Fig 1.4F). After inoculation, the transcript level differs by genotype by treatment effect (Fig 2.3). However no clear effect of any treatment. In IL4 no difference in transcript level due to BABA, JA, H_2O and fructose. In Moneymaker transcript level was high in H_2O treated plant low in BABA, JA and fructose treated plant. In Motelle transcript level was high in JA treated plant low in H_2O, BABA and Fructose treated plant. In FCN93-6-2 transcript level was high in BABA treated plant high low in H_2O, Fructose and JA treated plant (Fig 2.3).

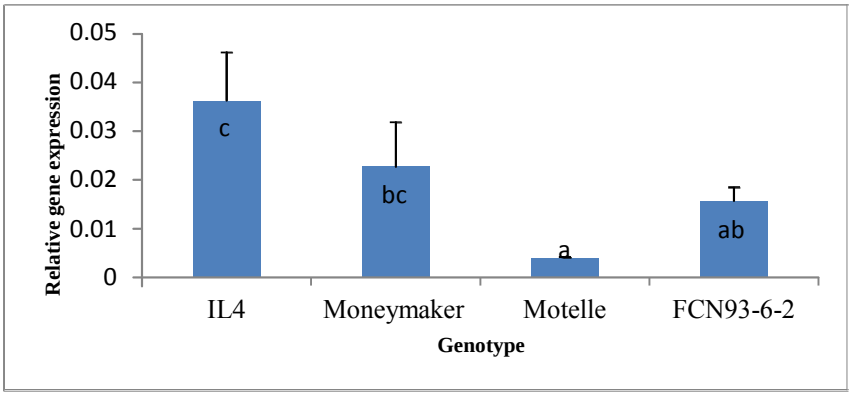

Figure 2.2: Mean relative gene expression of *CHI9*. The difference is before inoculation of whiteflies. Statistically significant difference by Fisher's least significant difference (LSD) test; α=0.05. The error bars represents the ±SE.

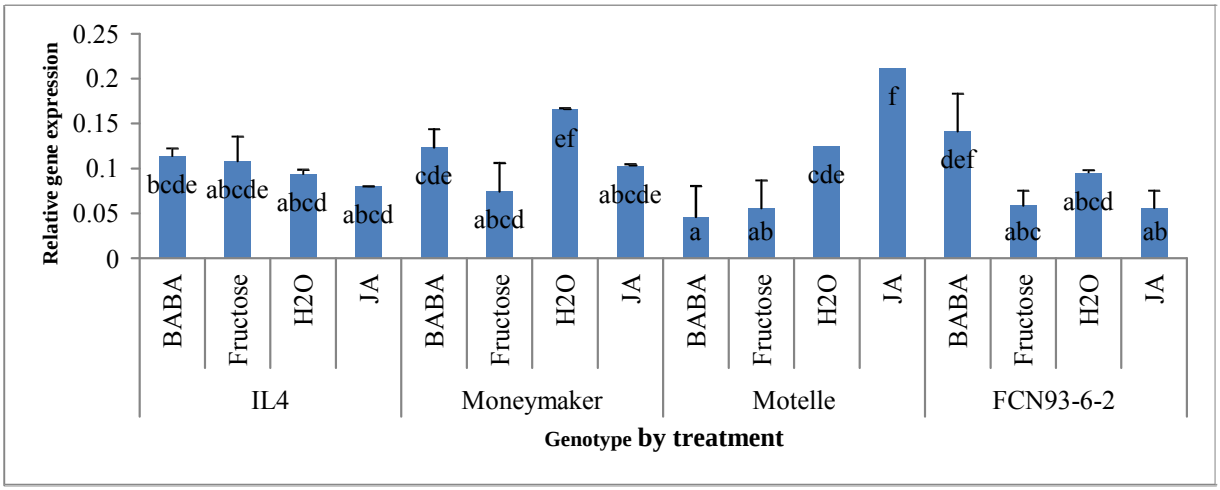

Figure 2.3: Mean relative gene expression of *CHI9*. The difference is 5 days after inoculation of whiteflies. Statistically significant difference by Fisher's least significant difference (LSD) test; α=0.05. The error bars represents the ±SE.

In whitefly induce *RbohD* gene overall transcript level was high after inoculation than before inoculation (Fig 1.4G). However no significant effect of genotype or treatment or genotype by treatment in before and after inoculation found.

In monoterpene pathway *MTS2* no significant effect of inoculation was found but before inoculation significant effect of treatment and after inoculation significant effect of genotype found. The transcript level before inoculation was high in JA treated and low in H_2O,

Fructose and BABA treated plant (Fig 2.4). The transcript after inoculation was high in Motelle and low in FCN93-6-2, IL4 and Moneymaker (Fig 2.5).

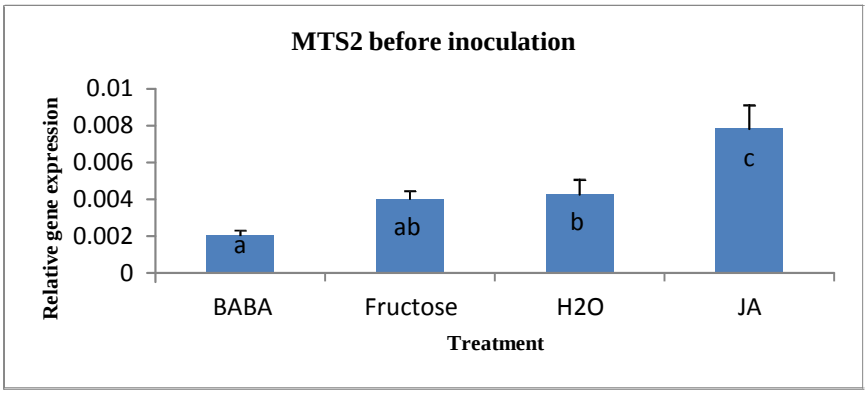

Figure 2.4: Mean relative gene expression of *MTS2* before inoculation. Statistically significant difference by Fisher's least significant difference (LSD) test; α=0.05. The error bars represents the ±SE.

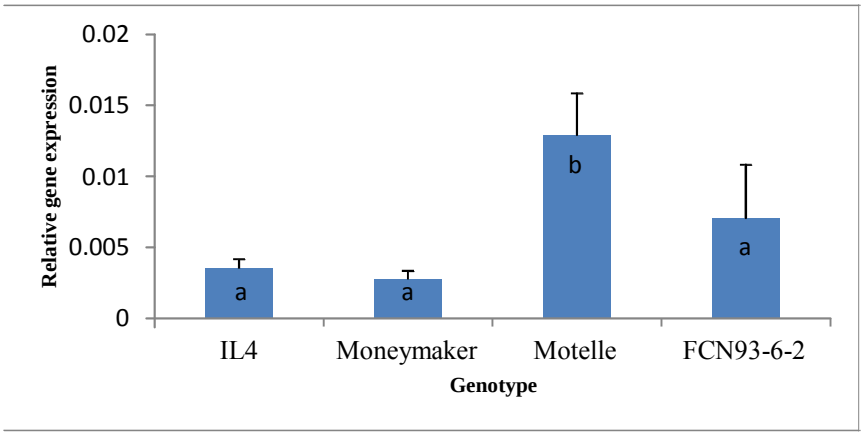

Figure 2.5: Mean relative gene expression of *MTS2* after inoculation. Statistically significant difference by Fisher's least significant difference (LSD) test; α=0.05. The error bars represent the ±SE.

Discussion

The result in this thesis indicate that no effect treatment and genotype on adult survival rate. However the in FCN93-6-2 found intermediate adult survival rate in the experiment conducted by Lucatti *et al*., 2010[24], but author compared FCN93-6-2 with a different whitefly species. We have found the oviposition rate in IL4 was lower compared to the other variety assessed. We have not seen an effect of treatment. The similar finding in the experiment conducted in tomato to see effect of jasmonate-induce defense against potato aphid, *Macrosiphum euphorbiae* [37]. In that study, the authors used cv. Moneymaker and cv. Motelle, the foliar application of 1.5mM JA, and inoculation 2 days after treatment. They found that JA application cv. Motelle did not induce or enhance the resistance and that in cv. Moneymaker it reduces the aphid population growth. In our experiment we used soil drench 0.05mM JA treatment and inoculation done 14 days after treatment. Without consider treatment, we found an oviposition rate in the variety Motelle (*Mi1-2)* and Moneymaker is significantly not different. Nombella *et al*., (2000) observed the similar, where a difference was significantly not different between cv. Moneymaker and cv. Motelle [25]. Our result contradict with experiment conducted by Bingham *et al*., 2013 on effect of cis-jasmone against whitefly on tomato. They found that cis-jasmone produce a reduction of oviposition rate compare to water treatment, but they did not mention on which tomato cultivar they conducted experiment [38]. On infested plant author counted the number of egg laid before spraying, and 5 days after cis-jasmonate spraying they again counted the number of egg laid.

Priming of basal defenses is generally associated with a cost in fitness[39]. We have found the positive effect of fructose and negative effect of BABA and JA on fresh weight of plant. Especially the growth of BABA treated plants in all variety was stunned compared to other treatment. Effect of BABA was also different on different variety (Fig 2.6). In experiment conducted by van Hulten et al., 2006 in *Arabidopsis* plant marginal weight loss observed in BABA treated plant. In the experiment author used 6 week old and 3 week old Arabidopsis plant for priming, the concentration of BABA was 5mg/L, 10mg/L and 25mg/L and they calculated dry weight[40]. However, in our experiment we have found that around 50% weight reduction in IL4 and moneymaker and around 30% to 35% weight reduction in cv. Motelle and FCN93-6-2. It indicates that the effect of BABA differs according to genotype and the treatment concentration.

Figure 2.6: Image 2 week after 0.5mM BABA treatment on different tomato varieties.

In free choice test, we have found that significant difference in whiteflies preference in relation to genotype and chemical treatment. In terms of genotype, we have found completely opposite result from the oviposition rate and fresh weight being the IL4 the most preferable variety and FCN93-6-2 the less preferred one. It was previously reported that the line FCN93-6-2 is non-preferred in a free choice test by T. vaporariorum [24]. In free choice test increased preference of fructose treated plant compared to BABA, JA and H_2O treated plant. However, no difference in the preference of whitefly in BABA, JA and H_2O treated plant. May be whitefly prefer to a plant with better growth. Because if we see a result of FW of plant, In Fructose treated plant, the FW is slightly increased compared to H_2O treated plant. On other hand in wheat plant sprayed with cis-jasmone it becomes less attractive to green aphid S. avenae [41]. In that experiment author conducted field trial for 3 to 4 year, they used the concentration 50gm/ha. In our experiment, we used the soil drenching method for priming treatment. Overall, it indicates that the effect of priming treatment differs by genotype, method of treatment, concentration of priming agent and causal organism.

In SA inducible *PR1* gene increased in transcript level after 5 day of inoculation of whiteflies. The difference in transcript level of *PR1* was found after inoculation of whiteflies. The transcript level was lower in IL4 compare to FCN93-6-2, Moneymaker and Motelle. In transcript level of *PR1* we did not see the effect of treatment. It was reported that the gene expression of *PR1* was increased in response to *T. vaporariorum* and *B. tabaci* feeding and also in response to the exogenous application of methyl jasmonate, ethylene, and salicylic acid [42]. In that experiment author measured the gene expression after 0,1,3,5,7 and 9 day

after infestation. In our experiment we measured the gene expression after 5 days infestation. In SA biosynthesis *ICS1* after inoculation overall transcript level was low. However after 5 days of whiteflies inoculation the less decrease in FCN93-6-2 and IL4 H_2O treated plant apart from this difference overall the decrease in transcript level could not indicate any strong effect of genotype or treatment. The decrease in transcript level of SA biosynthesis *ISC1* after inoculation indicates that SAR induces defense response is suppressed after whitefly feeding. In wound responsive *LapA* and *Pin2* genes overall increased in transcript level after whiteflies feeding it indicate that plant respond to the wounding produced by the whiteflies. The increased level in *LapA* did not show the significant effect of genotype or treatment. In *Pin2* after inoculation the transcript level in Motelle higher compared to IL4, Moneymaker and FCN93-6-2. The increased in transcript level of JA signaling *LoxD* indicate that after the feeding of whiteflies activation of JA signaling pathway. On transcript level after inoculation we did not see the significant effect of genotype or treatment. Also *LoxD* expression increased after feeding of potato aphid and green peach aphid [43]. In ethylene induced *CHI9* the overall transcript level was lower before inoculation than after inoculation. Similar finding in the experiment conducted by Puthoff *et al*,. 2010. The increased in transcript level of *CHI9* indicates that ethylene pathway also activates after whitefly inoculation. In our experiment the transcript level before inoculation different in different genotype but no effect of treatment. After inoculation also we have not seen the clear effect of treatment on transcript level of *CHI9*. However in experiment conducted by Puthoff et al., 2010 [42] they have found that increased in transcript level of methyl jasmonate, ethylene treated plant. In monoterpene *MTS2* we have not seen significant effect of inoculation. It indicates that in tomato plant emission of *MTS2* dependent volatile compound is not affected by whitefly infestation. Similar result observed on infestation of spider mites on tomato plant in experiment conducted by [44]. However in their experiment they *MTS2* did not expressed in leave and JA treated plant. In our experiment the transcript level of *MTS2* in JA treated plant was higher and lowest in BABA treated plant. In *RbohD* increased in transcript level after inoculation indicate that increased in accumulation of hydrogen peroxide. On infestation of cabbage aphid (*Brevicoryne brassicae*) in *Arabidopsis* increased in transcript level of *RbohD*[45]. However on *RbohD* we did not see the effect of genotype or treatment.

In overall we did not see the strong effect of treatment on different variety in relation to resistance. The result of gene expression analysis, adult survival rate and oviposition rate indicate that no strong influence of treatment. We only found the effect of treatment in free

choice test and fresh weight of plant. Free choice test result indicates that fructose increased the preference for whitefly in plant. The result of fresh weight indicate that further research is require to see the mechanism of BABA in plant, because the application of BABA was stressful for all varieties.

Acknowledgements

The author would like to thank Mr. Alejandro Lucatti, Dr. Ben Vosman for their valuable guidance and comments to improve this work.

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Appendix

Mean and SD of relative geneexpression analysis.

Variety		IL4							
Chemical		BABA		Fructose		H_2O		JA	
Ino.	Genes	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Before	CHI9	0.07824	0.02344	0.02103	0.00687	0.01922	0.00477	0.02688	0.01026
	ICS	0.01250	0.00020	0.01098	0.00226	0.01342	0.00066	0.01118	0.00140
	LapA	0.00010	0.00006	0.00003	0.00000	0.00012	0.00006	0.00009	0.00006
	LoxD	0.00015	0.00008	0.00024	0.00006	0.00053	0.00013	0.00048	0.00014
	MTS-2	0.00139	0.00029	0.00554	0.00060	0.00393	0.00257	0.00501	0.00088
	Pin 2	0.00006	0.00002	0.00008	0.00009	0.00011	0.00010	0.00018	0.00004
	PR1	0.00284	0.00241	0.00066	0.00008	0.00070	0.00063	0.00160	0.00098
	RBOHD	0.01037	0.00221	0.00581	0.00039	0.00877	0.00161	0.00952	0.00501
After	CHI9	0.11320	0.01310	0.10809	0.03890	0.09318	0.00803	0.07972	0.00074
	ICS	0.00267	0.00034	0.00286	0.00081	0.00426	0.00031	0.00251	0.00067
	LapA	0.01374	0.00879	0.00564	0.00652	0.01025	0.00814	0.03018	0.00198
	LoxD	0.00176	0.00000	0.00532	0.00600	0.00717	0.00316	0.01673	0.00995
	MTS-2	0.00135	0.00057	0.00299	0.00109	0.00430	0.00158	0.00545	0.00137
	Pin 2	0.03518	0.04427	0.00060	0.00007	0.01002	0.01139	0.01984	0.01065
	PR1	0.09532	0.04178	0.10506	0.05970	0.15941	0.00004	0.18198	0.15366
	RBOHD	0.02906	0.00556	0.04267	0.00201	0.03326	0.01036	0.03374	0.00458

Variety		cv. Moneymaker							
Chemical		BABA		Fructose		H_2O		JA	
Ino.	Genes	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Before	CHI9	0.01442	0.01178	0.02656	0.00199	0.00533	0.00489	0.04485	0.05219
	ICS	0.01338	0.00183	0.00641	0.00193	0.00772	*	0.01107	0.00024
	LapA	0.00007	0.00006	0.00014	0.00011	0.00002	0.00003	0.00031	0.00034
	LoxD	0.00006	0.00000	0.00008	0.00006	0.00067	0.00001	0.00006	0.00004
	MTS-2	0.00174	0.00075	0.00224	0.00030	0.00212	0.00133	0.01022	0.00226
	Pin 2	0.00001	0.00001	0.00037	0.00052	0.00000	0.00000	0.00003	0.00002
	PR1	0.00058	0.00007	0.02186	0.01021	0.00061	0.00072	0.02002	0.02773
	RBOHD	0.00936	0.00368	0.00652	0.00090	0.00404	0.00485	0.03724	0.03433
After	CHI9	0.12370	0.02852	0.07455	0.04495	0.16531	0.00268	0.10245	0.00342
	ICS	0.00278	0.00086	0.00105	0.00036	0.00235	0.00109	0.00278	0.00190
	LapA	0.00736	0.00167	0.02534	0.01156	0.02640	0.02848	0.02353	0.02950
	LoxD	0.00061	0.00039	0.00100	0.00048	0.00682	0.00161	0.00295	0.00298
	MTS-2	0.00146	0.00083	0.00470	0.00012	0.00187	0.00008	0.00300	0.00258
	Pin 2	0.00435	0.00415	0.00459	0.00445	0.00180	0.00132	0.01507	0.01044
	PR1	1.16882	0.51356	1.10580	0.03742	3.25596	0.61357	1.68502	1.06206
	RBOHD	0.05217	0.03554	0.02797	0.01611	0.11354	0.12226	0.03914	0.02431

Variety		cv. Motelle							
Chemical		BABA		Fructose		H_2O		JA	
Ino.	Genes	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Before	CHI9	0.00288	0.00083	0.00440	0.00071	0.00402	0.00027	0.00446	0.00087
	ICS	0.00719	0.00169	0.00677	0.00375	0.00527	0.00074	0.00748	0.00289
	LapA	0.00017	0.00019	0.00007	0.00006	0.00121	0.00163	0.00008	0.00007
	LoxD	0.00003	0.00002	0.00006	0.00003	0.00022	0.00007	0.00010	0.00003
	MTS-2	0.00312	0.00076	0.00417	0.00044	0.00448	0.00232	0.01076	0.00532
	Pin 2	0.00003	0.00001	0.00002	0.00001	0.00293	*	0.00007	0.00004
	PR1	0.00001	0.00000	0.00012	0.00003	0.00013	0.00004	0.00011	0.00001
	RBOHD	0.00531	0.00151	0.00743	0.00137	0.00907	0.00415	0.00687	0.00041
After	CHI9	0.04609	0.04882	0.05573	0.04410	0.12464	*	0.21116	*
	ICS	0.00255	0.00212	0.00267	0.00091	0.00099	*	0.00213	*
	LapA	0.03881	0.03536	0.03967	0.02387	0.00533	*	0.06402	*
	LoxD	0.01030	0.01127	0.01124	0.01348	0.00233	*	0.01210	*
	MTS-2	0.01048	0.00581	0.01718	0.01332	*	*	0.00930	*
	Pin 2	0.03517	0.04208	0.04810	0.02041	0.02912	*	0.06091	*
	PR1	0.97290	0.28571	1.21762	0.23881	1.93483	*	2.33019	*
	RBOHD	0.06446	0.01684	0.02333	0.00240	0.02862	*	0.02599	*

Variety		FCN93-6-2							
Chemical		BABA		Fructose		H_2O		JA	
Ino.	Genes	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Before	CHI9	0.02361	0.00467	0.02114	0.00751	0.00869	0.00254	0.00947	0.00052
	ICS	0.01303	0.00393	0.01158	0.00006	0.01005	0.00119	0.01059	0.00135
	LapA	0.00009	0.00009	0.00049	0.00021	0.00062	0.00047	0.00009	0.00000
	LoxD	0.00112	*	0.00100	0.00002	0.00183	0.00199	0.00084	0.00017
	MTS-2	0.00180	0.00030	0.00403	0.00067	0.00652	0.00184	0.00531	0.00191
	Pin 2	0.00008	0.00007	0.00042	0.00034	0.00157	0.00127	0.00037	0.00009
	PR1	0.03736	0.03571	0.02292	0.02089	0.00116	0.00096	0.00314	0.00180
	RBOHD	0.01268	*	0.02015	0.00336	0.01246	0.00230	0.01698	0.00387
After	CHI9	0.14134	0.05952	0.05864	0.02364	0.09490	0.00492	0.05613	0.02745
	ICS	0.00126	0.00000	0.00130	0.00006	0.00699	0.00105	0.00211	0.00091
	LapA	0.01166	0.00389	0.01524	0.01218	0.01280	0.00906	0.01994	0.01045
	LoxD	0.00618	0.00780	0.00453	0.00432	0.00688	*	0.00815	0.00929
	MTS-2	0.00168	0.00060	0.00262	0.00149	0.02269	0.01141	0.00129	0.00043
	Pin 2	0.00279	0.00026	0.00890	0.00101	0.02475	0.01582	0.00775	0.00229
	PR1	2.36475	1.04525	1.25100	0.11503	2.00571	1.18045	1.03057	0.16837
	RBOHD	0.06245	0.02206	0.05229	0.01882	0.16294	0.15960	0.11251	0.06038