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# Induced Plant Defenses Against Herbivory in Cultivated and Wild Tomato

**Sulav Paudel**[1] · **Po-An Lin**[1] · **Majid R. Foolad**[2] · **Jared G. Ali**[1] · **Edwin G. Rajotte**[1] · **Gary W. Felton**[1]

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## Abstract

Crop domestication and selective breeding have altered plant defense mechanisms, influencing insect-plant interactions. A reduction in plant resistance/tolerance against herbivory is generally expected in domesticated species, however, limited efforts have been made to compare inducibility of plant defenses between wild and domesticated genotypes. In the present study, the inducibility of several plant defense mechanisms (e.g. defensive chemicals, trichomes, plant volatiles) were investigated, and the performance and preference of the herbivore *Helicoverpa zea* were measured in three different tomato genotypes; a) wild tomato, *Solanum pimpinellifolium* L. (accession LA 2093), b) cherry tomato, *S. lycopersicum* L. var. *cerasiforme* (accession Matts Wild Cherry), and c) cultivated tomato, *S. lycopersicum* L. var. Better Boy). Enhanced inducibility of defensive chemicals, trichomes, and plant volatiles in the cultivated tomato, and a higher level of constitutive plant resistance against herbivory in the wild genotype was observed. When comparing the responses of damaged vs. undamaged leaves, the percent reduction in larval growth was higher on damaged leaves from cultivated tomato, suggesting a higher induced resistance compared to other two genotypes. While all tomato genotypes exhibited increased volatile organic compound (VOCs) emissions in response to herbivory, the cultivated variety responded with generally higher levels of VOCs. Differences in VOC patterns may have influenced the ovipositional preferences, as *H. zea* female moths significantly preferred laying eggs on the cultivated versus the wild tomato genotypes. Selection of traits during domestication and selective breeding could alter allocation of resources, where plants selected for higher yield performance would allocate resources to defense only when attacked.

**Keywords** Inducibility · Cultivated · Wild · Constitutive defenses · Trichomes · Volatiles · Plant defensive chemicals

## Introduction

Crop domestication and selective breeding have altered traits of wild ancestors to produce crop plants with superior agricultural characteristics, including higher yield and better quality (Ladizinsky 1998). The focus on yield and quality, however, often has neglected other important traits such as plants' defenses against herbivores. While most breeding programs address plant defense if the pest is particularly threatening, resistance to insects is often not a priority (Chaudhary 2013; Mitchell et al. 2016). However, there is great genetic variation for plant defenses against insects, which could be revealed by comparing wild and domesticated germplasm (Milla et al. 2015; Whitehead et al. 2017).

Through their evolution, plants have developed constitutive and induced defense mechanisms in response to attacks from a wide array of herbivores (Felton and Gatehouse 1996; Gatehouse 2002). While plants may exhibit both types of responses, often there is a negative correlation between constitutive and induced defense responses (Agrawal 1999; Agrawal et al. 2002). Plant defenses may be costly, and thus a genotype with high levels of constitutive defenses would have limited benefits on investing in induced defenses (Karban and Baldwin 1997). Plants use an array of biochemical and morphological responses to attacks by herbivores, which would interfere with development or behavior of herbivores (Bi and Felton 1995; Karban 2011); plants may also produce volatile organic compounds (VOCs) that attract natural enemies of herbivores (Walling 2000; Heil et al. 2001).

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✉ Gary W. Felton
gwf10@psu.edu

[1] Department of Entomology, The Pennsylvania State University, 501 ASI Building, University Park, PA 16802, USA

[2] Department of Plant Science, The Pennsylvania State University, 103 Tyson Building, University Park, PA 16802, USA

Domesticated genotypes (except for some modern cultivars) are often more vulnerable to insect pests, compared to their wild relatives; this is because crop evolution under domestication and selective breeding has presumably narrowed their genetic base (Chen et al. 2015; Whitehead et al. 2017). Plant defenses are considered costly (Herms and Mattson 1992), thus selection and breeding for crop productivity and quality were possibly at the expense of reduced pest resistance (Chaudhary 2013; Chen et al. 2015; Whitehead et al. 2017). Specifically, the capacity to augment direct defenses, such as increasing the concentration of plant defensive chemicals (Gols and Harvey 2009; Meyer et al. 2012) and trichomes (Bellota et al. 2013; Mo et al. 2016), are often reduced. On the contrary, stronger indirect defense responses (volatile emissions) in domesticated species compared to the wild relatives have been reported (Rowen and Kaplan 2016).

Domestication of plants may enhance or reduce rates of inducibility in response to herbivore attacks. For example, the inducibility of chemical defenses against an herbivore was lower in domesticated cabbage, *Brassica oleracea* cv. Stonehead and Cyrus (Harvey et al. 2011) and cv. Acephala and Capitata (Moreira et al. 2018) as well as in domesticated cranberries, *Cacccinium macrocarpon* cv BHS98–23 (Rodriguez-Saona et al. 2011), compared to their corresponding wild relatives, whereas it was higher in natural populations of lima beans, *Phaseolus lunatus* L (Shlichta et al. 2014) compared to their domesticated counterparts. A lower constitutive and a higher induced resistance in domesticated species was also revealed in an extensive experiment with ornamental plant species (Kempel et al. 2011). In general, inducibility of plant volatiles increased with domestication (Rowen and Kaplan 2016). However, in contrast there are a few reports of reductions in herbivore induced plant volatiles (Tamiru et al. 2011; Rodriguez-Sanoa et al. 2011; Chen et al. 2015).

Plant volatiles may not only play a role in indirect defense, but also can directly alter host utilization and preference by herbivores. An enhanced ovipositional preference of Tobacco hornworm (*Manduca sexta*) on domesticated modern tomato cultivars (compared to their wild relatives), which was mediated by plant volatiles, was recently reported by Li et al. (2018). In contrast, ovipositional preference of the silk moth (*Bombyx mori*) was not affected by domestication (Damodaram et al. 2014). These variations in plant defenses based on species and cultivars suggest that the impact of domestication and selective breeding is not as consistent as previously assumed (Meyer et al. 2012).

The cultivated tomato, *Solanum lycopersicum* L. (SL), has been continuously selected for a wide array of desirable traits for centuries (Bas et al. 1992), including fruit size, and fruit sugar and phenolics content (Ilahy et al. 2009; Kanayama 2017). Selection reduced genetic variation within the cultivated species, and thus the ability to withstand biotic and abiotic stresses (Sim et al. 2011). Introduced from the Andean region to Europe in the sixteenth century and then to the United States in nineteenth century, tomato is currently the most popular and economically important vegetable crops in the world (Bergougnoux 2014; www.faostat3.fao.org). There are more than 12 wild species of tomato (Knapp and Peralta 2016), of which the red-fruited species *S. pimpinellifolium* (SP) is thought to be the closest relative, and potentially an ancestor to the cultivated tomato (Peralta et al. 2008; Zuriaga et al. 2009; Blanca et al. 2015). Furthermore, the wild form of the cultivated tomato species, known as *S. lycopercicum* var. *cerasiforme* (SLC), is considered to be an evolutionary intermediate between SL and SP (Ranc et al. 2008; Blanca et al. 2015).

Wild tomato species bear a wealth of genetic variation for numerous characteristics important to crop improvement, in particular resistance and tolerance to biotic and abiotic stresses; much of such genetic variation, however, has remained largely unexploited (Miller and Tanksley 1990; Foolad 2007). Some wild tomato genotypes have been evaluated for resistance/tolerance to insect pests, including whitefly, *Bemisia tabaci* (Rodriguez-Saona et al. 2011; Firdaus et al. 2012, 2013), green peach aphid, *Myzus persicae* (Carter and Snyder 1985; Turcotte et al. 2014), beet armyworm, *Spodoptera exigua* (Turcotte et al. 2014), two-spotted spider mite, *Tetranychus urticae* (Lucini et al. 2015; de Oliveira et al. 2018), South American leaf miner, *Tuta absoluta* (Dias et al. 2013; Lima et al. 2016), cabbage moth, *Mamestra brassicae* (Moreira et al. 2018) and Colorado potato beetle, *Leptinotarsa decemlineata* (Carter et al. 1989). However, evaluations between cultivated and wild tomato genotypes were largely based on comparison of herbivore performance alone; plant defense (constitutive and induced) responses against herbivore have not been adequately examined.

The present study compares anti-herbivore chemical defenses, both constitutive and induced, as well as the inducibility of multiple chemical (defensive proteins, total phenolics, plant volatiles) and physical defenses (leaf trichomes) and their subsequent impact on the growth of herbivore *Helicoverpa zea* and its ovipositional preference in a cultivated tomato, SL (cv. Better boy), a cherry tomato, SLC (cv. Matts Wild Cherry), and a wild tomato, SP (accession LA 2093). Knowledge derived from such comparisons will improve our understanding of the evolutionary ecology of insect-plant interactions in tomatoes and will be helpful in identifying potential resistance traits to be targeted by crop breeding programs.

## Materials and Methods

### Plant Material

Original seeds of *Solanum pimpinellifolium* accesion LA 2093 were received from the C.M. Rick Tomato Genetics Resource Center, UC Davis, Davis CA (https://tgrc.ucdavis.edu/), and seeds of cultivated tomato *S. lycopersicum* cv. Better Boy

(BB) and *S. lycopercicum* var. *cerasiforme* accession Matts Wild Cherry (MWC) were procured commercially. LA 2093 was chosen because it is a well-studied accession of SP with numerous desirable horticultural characteristics, including resistance/tolerance to biotic and abiotic stresses and high fruit quality, and has been used to develop a super-density genetic map of tomato and for studying various desirable horticultural characteristics (Ashrafi and Foolad 2015; Foolad et al. 2015; Gonda et al. 2018). BB is a popular commercial hybrid cultivar and MWC is a widely grown cherry tomato sold commercially. Plants were grown in greenhouse under artificial lights in 3.5 in.-pots filled with Metromix 400 potting mix (Griffin Greenhouse and Nursery Supplies, Tewksbury, MA). In the greenhouse experiments, plants were maintained on a 16-h/8-h light/dark photoperiod, relative humidity (RH) of 55–65% and a temperature of 25 °C ± 2 °C. Plants and seeds were randomly allocated to treatments, and plants were randomly placed on different greenhouse benches. Plants were regularly watered every 2–3 days as needed.

## Herbivore

*Helicoverpa zea* eggs were obtained from Benzon Research (Carlisle, PA, USA) and larvae were reared on wheat germ and casein-based diet (Peiffer and Felton 2009) until they were used for the experiments. *H. zea* (Family: Noctuidae), a generalist herbivore, commonly known as corn earworm or tomato fruit borer is a major agricultural pest on a wide variety of crops, including tomatoes (Fitt 1989). It has been estimated that *H zea* arrived in the New World ~ 1 million years ago, well in advance of any crop domestication. Considering its broad host plant range and broad geographic range including the region covering the wild ancestors of tomato (Hardwick 1965), it is highly likely to have used these plants as hosts. Plant (three genotypes) and herbivore responses were compared simultaneously in a greenhouse at the Pennsylvania State University, University Park, PA, during 2017–2018.

## Analysis of Leaf Defensive Proteins and Phenolics

At the four-leaf stage, fully expanded terminal leaflets of the leaf from the bottom ($n = 7–8$/treatment) were used as the focal leaf for defensive chemical bioassays (Tan et al. 2018). Tomato leaflets were damaged by allowing a single 5th instar *H. zea* larva to feed inside a clip cage (3.15 $\mathrm{cm}^2$) ensuring that the plants receive equal amount of damage across all treatments. An empty cage without herbivore was used on undamaged leaves. Detached leaves (damaged and undamaged) were weighed before being flash-frozen in liquid nitrogen and stored at −80 °C until analysis. Activity of two jasmonic acid (JA)-related defensive proteins, trypsin protease inhibitor (TPI; % inhibition/mg protein) and polyphenol oxidase (PPO; mOD/min/mg protein), were measured and compared after 48 h of caterpillar feeding using a spectrophotometric method (Acevedo et al. 2017). Total phenolics content in leaves (μg/mg tissue) based on colorimetric assay was analyzed using methods from Ainsworth and Gillespie (2007).

## Shoot Biomass

In a separate set of experiments, the entire shoot (all above ground) of 3-week old plants ($n = 6$/treatment) were removed and dried in an oven (60 °C for 48 h) to determine the shoot dry weight (SDW; gm).

## Density and Morphology of Leaf Trichomes

Terminal leaflets of the fourth leaf from the bottom were sampled and damaged by *H. zea* as described above. Fourteen days post caterpillar feeding, plants ($n = 8$) from each treatment were chosen and the youngest terminal leaflets of damaged or undamaged leaf were randomly selected to compare the density of trichomes on the adaxial surface. Two leaf discs of 0.6 cm diameter were punched out from each side of the mid-vein of a leaflet, and the density (number/$\mathrm{cm}^2$) of all glandular and non-glandular trichomes was determined using a light microscope. Morphologies of leaf trichomes among genotypes were also compared using scanning electron microscopy (SEM), using a protocol described by Kang et al. (2010).

## Herbivory Feeding Bioassay

An excised leaf bioassay with damaged and undamaged leaves using 1st instar *H. zea* larvae was conducted to measure the herbivore response. Tomato leaflets (youngest terminal leaflet of the fourth leaf from the bottom) were damaged as described above. After 48 h post-damage, randomly selected 1st instar larvae ($n = 20$/treatment) were individually weighed and placed into plastic cups (30 ml) with a tomato leaflet (fourth leaf from bottom) from each of the three tomato genotypes.

Individual larvae were then weighed after 48 h and relative growth rate (RGR) was calculated as:

$$\mathrm{RGR\ (tissue\ gained/g/day)} = (\mathrm{W2}{-}\mathrm{W1})/((\mathrm{t2}{-}\mathrm{t1})^*\mathrm{W1}),$$

where $W_2$ and $W_1$ are larval weight at times $t_1$ and $t_2$, and $W_1$ is the initial larval weight before the start of the experiment.

## Volatile Organic Compounds (VOCs) Collections and Analysis

Volatiles were collected for a duration of 12 h (from the start of the experiments) from plants either damaged by *H. zea* or left undamaged. Individual 3rd instar larvae were placed inside a 9 L volume glass chamber letting them feed freely on plants for damaged treatment throughout the duration of experiment

(12 h). Control plants did not receive any herbivore treatment. In total, 4–5 plants per genotype and treatment were sampled. Dry weight (DW) of the entire shoot of each plant was measured after volatile collection. Volatiles were collected using a push-pull collection system. Volatiles were first trapped using adsorbent filters containing 45 mg of Super-Q (Alltech Associates, Deerfield, IL, USA) at 0.5 L $\text{min}^{-1}$ and were eluted using 150 μL dichloromethane. Five μL of a standard (nonyl acetate (80 ng $\mu L^{-1}$) was added to the 150 μL of aliquot of the emission solution for analysis (Helms et al. 2019).

The various VOCs were identified with gas chromatography (Agilent 7890A), coupled with a mass spectrometer (Agilent model 5975C). Individual volatile compounds were identified by comparing retention time and mass spectra with data from two published libraries, National Institute of Standards and Technology (NIST), 2014 and Gothenburg Department of Chemical Ecology mass spectral library data bases (Helms et al. 2019). Only those volatiles which are identified by the program with more than 90% confidence were included for the principal component analysis (PCA). The relative abundance of each compound was calculated based on the internal standard (nonyl acetate) within the sample. Individual compound abundance was further standardized by the DW of each plant and expressed as ng/g DW/12 h. Total VOC emission per plant was calculated by adding the concentrations of all the VOCs recorded in the blend.

### Oviposition Preference

In a greenhouse, 2 × 2 × 2 m cages (BugDorm Cages, Megaview Science Co., Ltd. Taichung 40762, Taiwan) were used to examine the oviposition preference of *H. zea* for the three tomato genotypes. Ten pairs of newly emerged female *H. zea* moths were placed in a single cage with three plants, one from each of the three tomato genotypes. Undamaged plants with similar size at the four-leaf-stage were randomly allocated to the cages. The number of eggs on the plants were visually determined daily for three days.

### Statistical Analysis

Using a completely randomized design (CRD), experiments on plant defensive chemicals, density of trichomes, herbivore growth, and ovipositional preference were analyzed using a two-way ANOVA, with the main effects being tomato genotypes and insect feeding treatments plus all interaction effects. Plant biomass among genotypes were tested by one-way ANOVA. Means were separated with Tukey's Honest Significant Differences (HSD) mean comparison tests. Data were checked for normality and analyzed using 'Minitab 18.0' software (Minitab Inc. 2018).

Volatile data were analyzed using the R statistical software program (R Core Team 2017). Random forest analysis (package "*randomForest*") was performed on the full data set of 65 volatiles to identify important volatiles differentiating tomato genotypes and herbivore treatments. Volatiles with the "Mean Decrease Accuracy" larger than zero were selected for further analysis (Supplementary Table 1). Principle component analysis (PCA) was performed using function "*prcomp*" on the selected list of volatiles from both control and damage treatments. Results of the PCA were visualized using package "*ggbiplot*" (Vu 2011). Due to lack of significant differences in volatile profiles of undamaged plants, only volatile data from damaged plants were used to test for statistical significance (Table 1). The non-parametric *Kruskal-Wallis* test was employed to determine differences among quantity of volatile emitted from damaged plants. Percent contribution of individual compounds was calculated by dividing the individual volatile concentration by the total volatile blend concentration.

## Results

### Induction of Leaf Defensive Chemicals

**Trypsin Protease Inhibitors (TPI)** LA 2093 had the highest amount of constitutive TPI followed by MWC and BB (Fig. 1). In contrast, inducibility of TPI was significantly higher in the cultivated tomato BB (18-fold increase) in damaged leaves compared to MWC (2.6-fold increase) and LA 2093 (1.7-fold increase) (insect damage; $F = 206.2$, $df = 1$, $P < 0.001$, genotype; $F = 6.6$, $df = 2$, $P = 0.004$) (Fig. 1a). Additionally, there was a significant interactive effect between genotypes and insect damage (genotype x damage; $F = 20.1$, $df = 2$, $P < 0.001$).

**Polyphenol Oxidase (PPO)** The cultivated tomato BB had significantly higher levels of PPO activity in both damaged and undamaged leaves, compared to MWC and LA 2093 (insect damage; $F = 441.2$, $df = 1$, $P < 0.001$, genotype; $F = 183.8$, $df = 2$, $P < 0.001$) (Fig. 1b). The inducibility for BB was also the highest (4.4-fold increase), followed by MWC (4.2-fold increase) and LA 2093 (2.8-fold increase). There was also a significant interactive effect between genotypes and insect damage (genotype x damage; $F = 82.97$, $df = 2$, $P < 0.001$).

**Total Phenolics Content (TPC)** There was a significant effect of genotype on TPC (genotype; $F = 181.3$, $df = 2$, $P < 0.001$) (Fig. 1c). LA 2093 demonstrated the highest level of constitutive TPC and BB had the lowest. While herbivore damage increased levels of TPC in both BB (1.23-fold increase) and MWC (1.20-fold increase), it did not do so in LA 2093 (insect damage; 1.0-fold increase) (insect damage; $F = 106.5$, $df = 1$, $P < 0.001$). There was also a significant interactive effect between genotypes and insect damage (genotype x damage; $F = 14.6$, $df = 2$, $P < 0.001$).

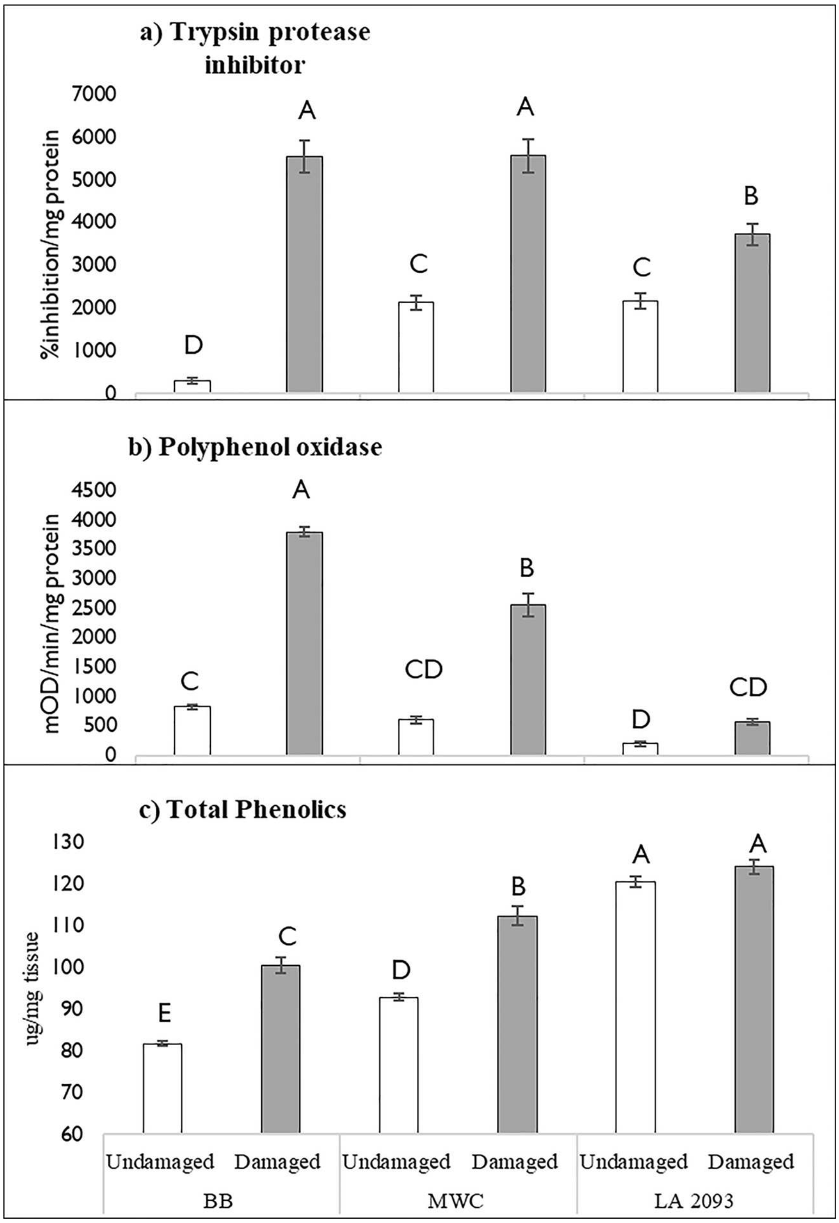
**Table 1** Major Volatiles (ng/g dry weight) emitted by three tomato genotypes, *Solanum lycopercicum* cv. Better Boy (BB), *Solanum lycopersicum var. cerasiforme* (Matts Wild Cherry (MWC)), and *Solanum pimpinellifolium* (LA 2093) upon herbivore damage

| S. N | VOC | BB | | % contribution | MWC | | % contribution | LA 2093 | | % contribution | Kruskal-Wallis Test |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Mean | SE | | Mean | SE | | Mean | SE | | p-value |
| 1 | Limonene (MT) | n.d | n.d | 0.00 | n.d | n.d | 0.00 | 63433 | 3409 | 97.37 | <0.001 |
| 2 | α-Terpinene (MT) | 19244 | 989 | 8.74 | 17864 | 2805 | 19.12 | 374 | 192 | 0.57 | <0.005 |
| 3 | Sabinene (MT) | 33867 | 21467 | 15.39 | 19348 | 19348 | 20.70 | n.d | n.d | 0.00 | 0.465 |
| 4 | β-Caryophyllene (ST) | 3257 | 397 | 1.48 | 1031 | 367 | 1.10 | n.d | n.d | 0.00 | <0.001 |
| 5 | α - Pinene (MT) | 2652 | 542 | 1.20 | 2298 | 440 | 2.46 | n.d | n.d | 0.00 | <0.005 |
| 6 | 2-Carene (MT) | 57604 | 6994 | 26.17 | 16820 | 3834 | 18.00 | 959 | 290 | 1.47 | <0.001 |
| 7 | trans-β- Ocimene (MT) | 1400.4 | 92.4 | 0.64 | 123.2 | 77.5 | 0.13 | n.d | n.d | 0.00 | <0.001 |
| 8 | β - Phellandrene (MT) | 85089 | 18719 | 38.66 | 30276 | 10297 | 32.40 | n.d | n.d | 0.00 | <0.005 |
| 9 | Cymene (MT) | 8795 | 817 | 4.00 | 2931 | 1336 | 3.14 | n.d | n.d | 0.00 | <0.001 |
| 10 | β - Pinene (MT) | 1785 | 345 | 0.81 | 193 | 162 | 0.21 | n.d | n.d | 0.00 | <0.001 |
| 11 | allo-Ocimene | 144 | 40.5 | 0.07 | n.d | n.d | 0.00 | n.d | n.d | 0.00 | <0.001 |
| 12 | γ- Terpinene (MT) | 903 | 348 | 0.41 | 811 | 296 | 0.87 | 324 | 203 | 0.50 | 0.404 |
| 13 | trans-Isolimonene (MT) | 278 | 93 | 0.13 | 287 | 143 | 0.31 | n.d | n.d | 0.00 | 0.087 |
| 14 | α-Humulene (ST) | 571 | 174 | 0.26 | 253 | 128 | 0.27 | n.d | n.d | 0.00 | 0.052 |
| 15 | Cymenene (MT) | 226.2 | 72.3 | 0.10 | n.d | n.d | 0.00 | 13.5 | 13.5 | 0.02 | <0.005 |
| 16 | Terpinolene (MT) | 489 | 364 | 0.22 | n.d | n.d | 0.00 | n.d | n.d | 0.00 | <0.005 |
| 17 | cis-meta-Mentha-2,8-diene (MT) | 298 | 137 | 0.14 | n.d | n.d | 0.00 | n.d | n.d | 0.00 | <0.005 |
| 18 | Copaene (ST) | 5.15 | 5.15 | 0.00 | n.d | n.d | 0.00 | 9.39 | 9.39 | 0.01 | 0.052 |
| 19 | γ- Gurjunene (MT) | 104.1 | 49.2 | 0.05 | 5.27 | 5.27 | 0.01 | n.d | n.d | 0.00 | 0.053 |
| 20 | Trimethyl-1,3(E),7(E),11-tridectetraene (GLV) | 334 | 149 | 0.15 | 152.2 | 93.5 | 0.16 | n.d | n.d | 0.00 | 0.148 |
| 21 | α -Copaene (ST) | 173.7 | 89.7 | 0.08 | n.d | n.d | 0.00 | n.d | n.d | 0.00 | 0.075 |
| 22 | β -Elemene (ST) | 100.7 | 45.4 | 0.05 | n.d | n.d | 0.00 | n.d | n.d | 0.00 | 0.215 |
| 23 | Carvacrol (MT) | 106.9 | 38.2 | 0.05 | 22.5 | 22.5 | 0.02 | n.d | n.d | 0.00 | 0.07 |
| 24 | cis-Calamenene (GLV) | 64.3 | 29.2 | 0.03 | n.d | n.d | 0.00 | n.d | n.d | 0.00 | 0.075 |
| 25 | Cyprene (MT) | 473 | 219 | 0.21 | 25.4 | 25.4 | 0.03 | n.d | n.d | 0.00 | 0.169 |
| 26 | 6-9-Guaiadiene (GLV) | 305 | 146 | 0.14 | 186 | 171 | 0.20 | n.d | n.d | 0.00 | 0.129 |
| 27 | Myrcene (MT) | 491 | 491 | 0.22 | 156.6 | 98.2 | 0.17 | n.d | n.d | 0.00 | 0.409 |
| 28 | γ- Murolene (ST) | 91.3 | 91.3 | 0.04 | 75.5 | 46.3 | 0.08 | n.d | n.d | 0.00 | 0.409 |
| 29 | Indole (A) | n.d | n.d | 0.00 | 76.2 | 67.2 | 0.08 | n.d | n.d | 0.00 | 0.117 |
| 30 | ρ- Cymene (MT) | 46 | 29.2 | 0.02 | n.d | n.d | 0.00 | n.d | n.d | 0.00 | 0.2 |
| 31 | Bicyclo[2.2.1]hept-2-ene, 1,7,7 (GLV) | 91.6 | 48.7 | 0.04 | n.d | n.d | 0.00 | n.d | n.d | 0.00 | 0.075 |
| 32 | α -Gurjunene (MT) | 336 | 153 | 0.15 | 170 | 154 | 0.18 | n.d | n.d | 0.00 | 0.129 |
| 33 | α - Selinene (GLV) | 57.2 | 43 | 0.03 | n.d | n.d | 0.00 | n.d | n.d | 0.00 | 0.2 |
| 34 | Cadalene (GLV) | 17.5 | 11.5 | 0.01 | n.d | n.d | 0.00 | n.d | n.d | 0.00 | 0.2 |
| 35 | Piperitone (MT) | 51.4 | 32.6 | 0.02 | 29.3 | 29.3 | 0.03 | n.d | n.d | 0.00 | 0.412 |
| 36 | 3- Carene (MT) | 560 | 355 | 0.25 | 202 | 202 | 0.22 | 34.8 | 34.8 | 0.05 | 0.751 |
| 37 | Cyclohexene, 1-methyl-4-(1-methlidene) (GLV) | 47.4 | 37.4 | 0.02 | 49.9 | 30.5 | 0.05 | n.d | n.d | 0.00 | 0.396 |
| 38 | δ-Selinene (GLV) | 60.7 | 40.2 | 0.03 | 60.2 | 60.2 | 0.06 | n.d | n.d | 0.00 | 0.505 |

Highlighted VOCs indicate a significant difference among herbivore-damaged genotypes based on Kruskal-Wallis test

*MT* monoterpene; *ST* sesquiterpene; *GLV* C6 aldehydes, alcohols and esters; *A* aromatic; *n.d* Not Detected. Percent (%) contribution of individual compound refers to a value calculated by dividing the individual volatile concentration with the total volatile blend concentration

**Fig. 1** Activity of leaf defensive chemicals in undamaged and damaged (*Helicoverpa zea)* leaves from three different tomato genotypes, *Solanum lycopercicum* cv. Better Boy (BB), *Solanum lycopersicum var. cerasiforme* (cv. Matts Wild Cherry (MWC)), and *Solanum pimpinellifolium* (LA 2093); a) Trypsin protease inhibitor (TPI) (%inhibition/mg protein) b) Polyphenol oxidase (PPO) activity (mOD/min/mg protein), and c) Total phenolics content (ug/mg tissue). Bars are mean ± SEM, and means with different letters are statistically different as determined by a Tukey HSD



## Plant Growth

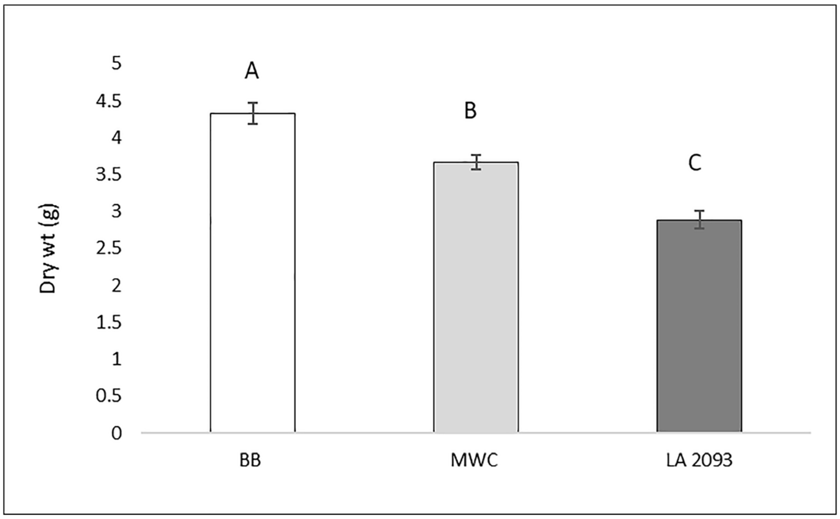
As expected, at 3-weeks of age, BB had the highest shoot biomass (SDW), whereas LA 2093 had the lowest (F = 29.9, df = 2, P < 0.001) (Fig. 2).

## Trichome Density and Morphology

Undamaged leaves of LA 2093 had fewer glandular and non-glandular trichomes than BB and MWC (genotype; F = 181.3, df = 2, P < 0.001) (Fig. 3). In response to caterpillar damage, there was a significantly higher induction in trichome density in BB than MWC and LA 2093 (insect damage; F = 88.48, df = 1, *P* < 0.001). While there was a very low level of induction in LA 2093 (1.07- and 1.04- fold increase) and MWC (1.4- and 1.2-fold increase), density of glandular trichomes and non-glandular trichomes in BB was increased by 1.7- and 1.4-fold increase, respectively.

SEM pictures of trichome types are provided in supplementary materials (Supplementary Fig. 1a-1e). The BB and MWC genotypes had higher number of types VII trichomes and a very low density of Type III trichomes. The MWC and BB exhibited Type III, V, VI VII, and VIII trichomes, but type VI glandular trichomes were the most abundant (Supplementary Fig. 2a & 2b). LA 2093 had very few foliar trichomes and type III, IV and V were mostly observed (Supplementary Fig. 2c).

**Fig. 2** Shoot biomass (dry weight (g)) of three-weeks old plant from three different tomato genotypes, *Solanum lycopercicum* cv. Better Boy (BB), *Solanum lycopersicum var. cerasiforme* (Matts Wild Cherry (MWC)), and *Solanum pimpinellifolium* (LA 2093). Bars are mean ± SEM, and means with different letters are statistically different as determined by a Tukey HSD



## Herbivore Performance

Tomato genotypes varied in their level of resistance to *H. zea* caterpillar feeding. In undamaged leaves, the RGR of *H. zea* was highest when larvae fed on undamaged leaves of BB in comparison with MWC and LA 2093 (F = 124, df = 2, P < 0.001) (Fig. 4). However, when larvae were fed on leaves previously damaged by *H. zea,* the RGR reduction in BB (2.2-fold decrease) was highest followed by MWC (1.3-fold decrease) and LA 2093 (1.09-fold decrease).

## Emission of Volatile Organic Compounds (VOCs)

Overall, a total of 63 VOCs were detected (Table S1), representing a complex blend of alcohols, esters, benzenoids, monoterpenes and sesquiterpenes, with terpenes being the most abundant. Based on the values of variable importance to the projection (VIP) of VOCs (Supplementary Table 1), 38 major VOCs were selected for comparison of quantitative difference in volatile emission among three tomato genotypes (Table 1). Thirteen of the 38 volatiles significantly differed in damaged plants when compared across the three tomato

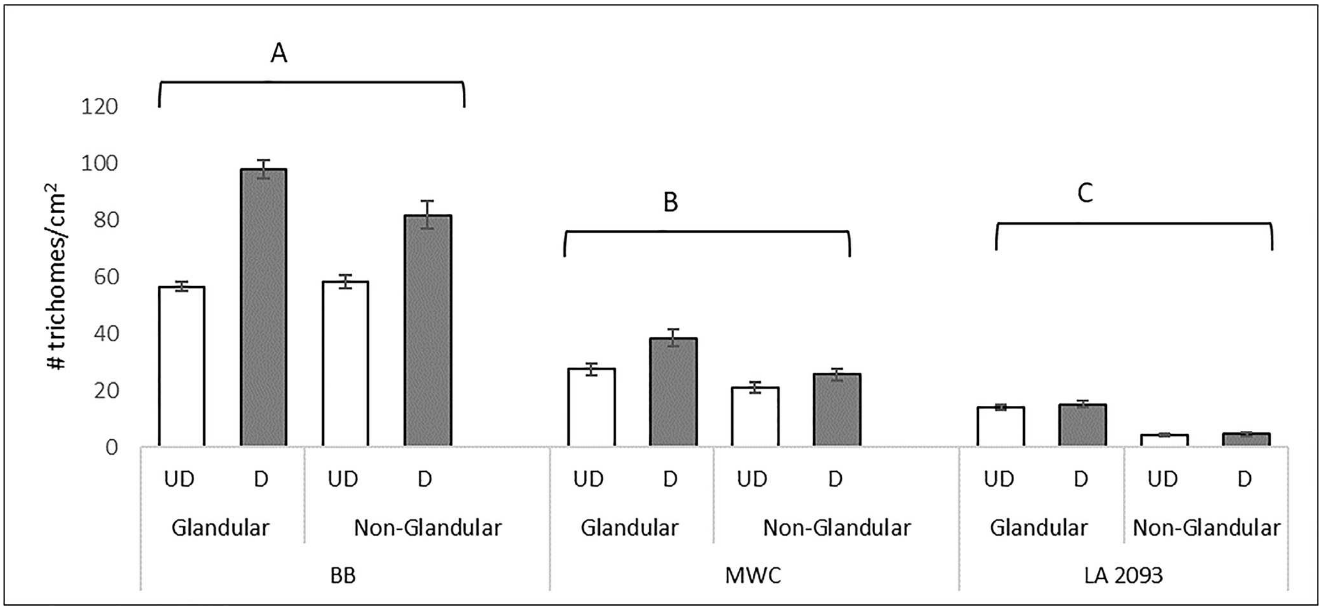


**Fig. 3** Density of glandular and non-glandular trichomes (no of trichomes/cm$^2$) on undamaged (UD) and damaged (D) (*Helicoverpa zea*) leaf surface (adaxial) from three different tomato genotypes, *Solanum lycopercicum* cv. Better Boy (BB), *Solanum lycopersicum var. cerasiforme* (Matts Wild Cherry (MWC)), and *Solanum pimpinellifolium* (LA 2093). Bars are mean ± SEM, and means with different letters are statistically different as determined by a Tukey HSD

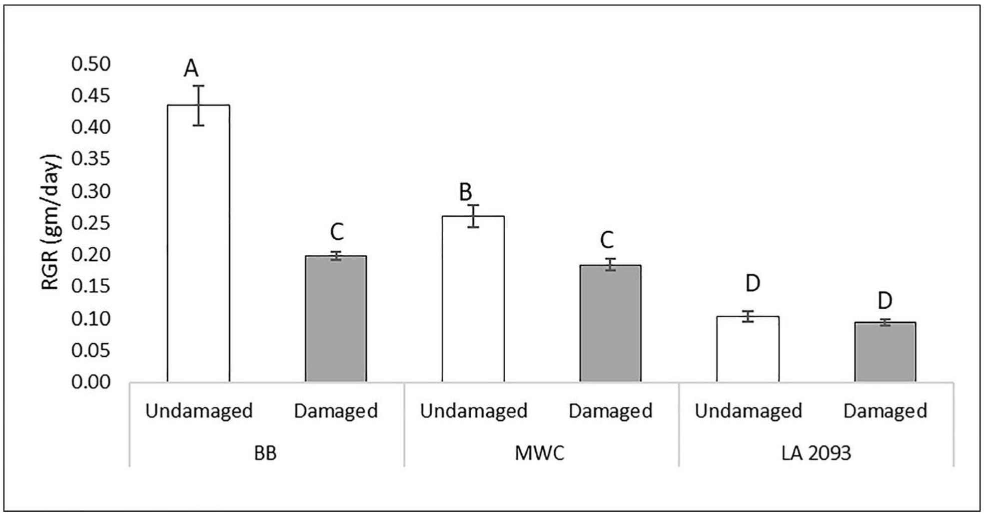


**Fig. 4** Relative growth rate (RGR) (tissue gained/g/day) of *H.zea* larvae fed with detached leaves (damaged and undamaged) from three different tomato genotypes, *Solanum lycopercicum* cv. Better Boy (BB), *Solanum lycopersicum var. cerasiforme* (Matts Wild Cherry (MWC)), and *Solanum pimpinellifolium* (LA 2093). Bars are mean ± SEM, and means with different letters are statistically different as determined by a Tukey HSD

genotypes (Kruskal Wallis test: $P < 0.05$, see Table 1). These compounds were limonene, α-terpinene, β-caryophyllene, α-pinene, 2-carene, trans-β-ocimene, β-phellandrene, cymene, β-pinene, allo-ocimine, cymenene, terpinolene and cis-meta-mentha-2,8-diene.

Total volatile emission in undamaged BB leaves was significantly higher in comparison to MWC and LA 2093 (Fig. 5). Further, a significantly higher inducibility of VOCs was detected when *H. zea* actively fed on BB (23-fold increase) compared with the feeding on MWC (18.3-fold increase) and LA 2093 (15.3-fold increase) ($F = 23.1$, $df = 2$, $P < 0.001$) (Fig. 5). Principal component analysis (PCA) for VOCs from damaged plants presents a clear separation in volatile emission, with the first principal component, separating BB (damaged) and MWC (damaged) from LA 2093 (damaged) and explaining 44.5% of the variance (Fig. 6a and b).

The tomato genotypes BB and MWC, in comparison to LA 2093, produced different patterns of volatiles, both in quality and quantity, during active herbivore feeding (Table 1 and Supplementary Fig, 3a-3c). A total of 36 (out of 38) major volatiles were recorded in the BB leaves. Better Boy leaves emitted 36 of the 38 major volatiles. β-Phellandrene was the most abundant (38.6% of the total blend) followed by 2-carene (26.2%), sabinene (15.4%), α-terpinene (8.7%), and cymene (4.0%). Apart from sabinene, all other compounds were significantly different among genotypes (Table 1). Similarly, 25 major volatiles were recorded in MWC. Like BB, β-phellandrene was the most abundant (32.4%) followed by sabinene (20.7%), α-terpinene (19.2%), 2-carene (18.0%), and cymene (3.14%). Only seven volatiles were recorded in LA 2093 with limonene being the most abundant with at 97% of the total blend followed by 2-carene (1.5%) and α-terpinene (0.6%).

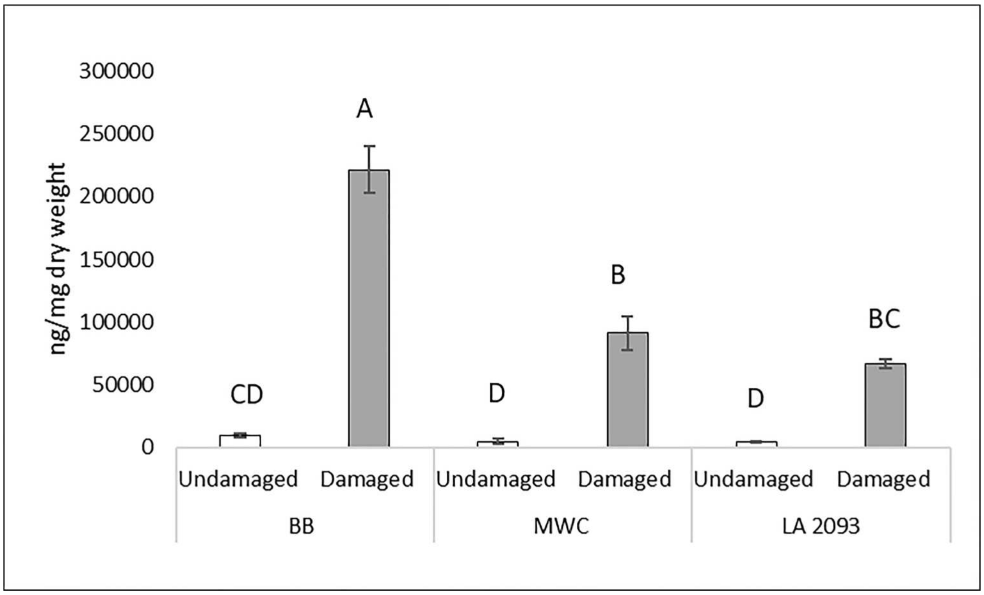


**Fig. 5** Emission of volatile Organic Compounds (VOCs) from undamaged and damaged (*Helicoverpa zea* feeding) plants in three different tomato genotypes *Solanum lycopercicum* cv. Better Boy (BB), *Solanum lycopersicum var. cerasiforme* (Matts Wild Cherry (MWC)), and *Solanum pimpinellifolium* (LA 2093) Bars are mean ± SEM, and means with different letters are statistically different as determined by a Tukey HSD

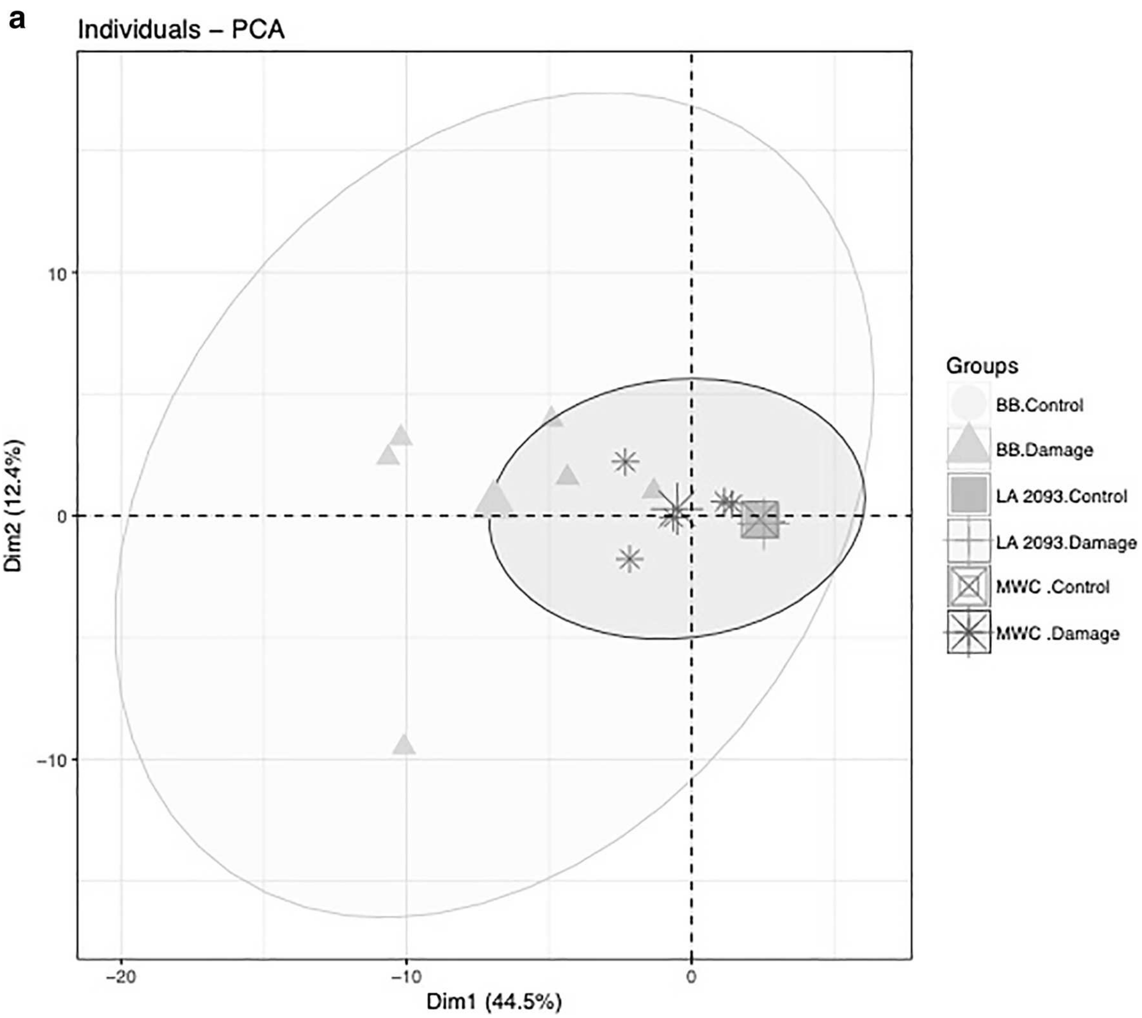


**Fig. 6** Principal component analysis (PCA) a) Individual factor map and b) Variables factor map displaying the variability in headspace volatile collection (measured as ng/g dry weight) of three different tomato genotypes, *Solanum lycopercicum* cv. Better Boy (BB), *Solanum lycopersicum var. cerasiforme* (Matts Wild Cherry (MWC)), and *Solanum pimpinellifolium* (LA 2093) with or without herbivore *(H.zea)* damage. A clear separation at the genotypes level damaged by herbivore applied on sixty-five volatile compositions; BB Damage (yellow circle), MWC damaged (purple circle) and LA 2093 damaged (blue circle). Alignment of arrows representing individual compounds towards a principal component (either PC1 or PC2) indicates a strong association towards that component. The length of an arrow represents how well the parameter explains the distribution of the data. Color indicate the contribution of a variable in explaining the variation in principle component. The darker the color the more significant role a variable plays in explaining variation. Due to a high similarity of volatile profile of undamaged control from different genotypes in fig (a), all symbol for control are overlapped. The location is indicated by the symbol for mean coordinate of LA 2093 Control (filled square)

## Oviposition Preference

Female *H. zea* moths exhibited a significant preference for plants of BB throughout the experiment (F = 263.1, df = 2, P < 0.001) (Fig. 7), suggesting that plants of the other two genotypes were less favorable for the female moths. There was a significant difference in the number of eggs laid on each plant genotype. BB, on average, received a significantly higher number of eggs (21.4 eggs/plant), followed by MWC (11.0 eggs/plant) and LA 2093 (10.2 eggs/plant).

## Discussion

This study provides evidence that the selection and breeding process that produced modern commercial tomato varieties also reorganized their defense strategy as demonstrated by a shift from constitutive to inducible chemical defenses as well as concomitant fitness changes in an herbivore. While this study focuses on a association among primitive to modern tomato genotypes and a single herbivore, it is illustrative of the variation available in a single plant genus. Among the genotypes examined in the present study, the cultivated tomato (BB) had

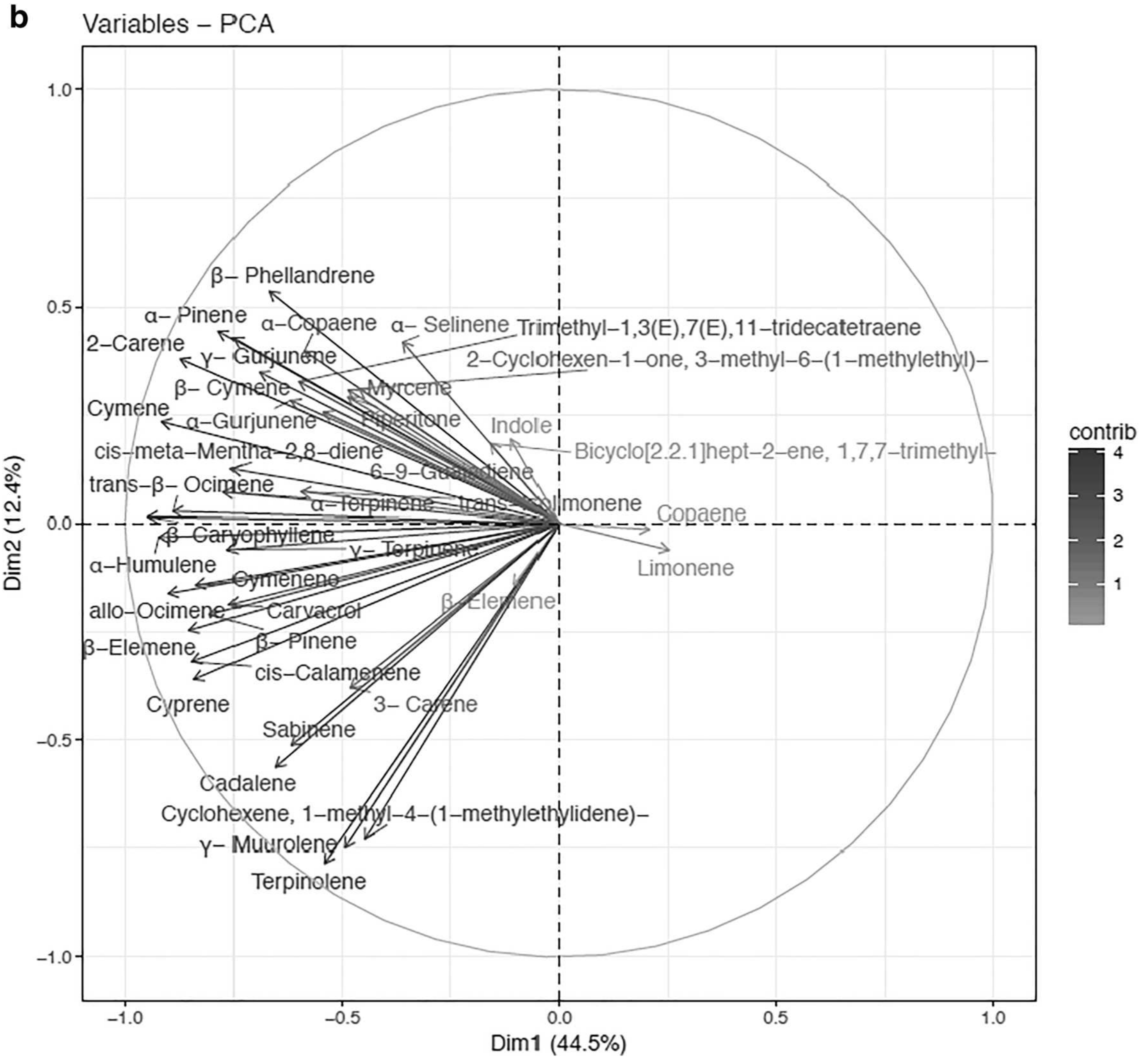


**Fig. 6** (continued)

proportionately higher levels of inducible chemical defenses whereas the cherry tomato (MWC) and the wild tomato accession (LA 2093) had higher levels of constitutive chemical defenses. Undamaged leaves of the cultivated tomato had higher densities of constitutive trichomes, and post herbivory they produced more glandular and non-glandular trichomes, than the two other genotypes. Herbivore performance was poorer on undamaged wild tomato species, but once damaged by herbivory, the cultivated tomato suppressed herbivore performance to a greater degree. While all tomato genotypes demonstrated increased VOC emissions in response to herbivory, the cultivated type responded with higher levels of VOCs. Differences in VOC patterns may have influenced the ovipositional preference of *H. zea* for Better Boy.

The wild tomato genotype (LA 2093) had a proportionately higher level of constitutive defensive chemicals compared with inducible chemicals. Higher levels of total phenolics and TPI, but reduced concentrations of PPO, were recorded in undamaged leaves of LA 2093 and MWC, compared with the cultivated variety BB. Notably, the inducibility of all three defensive chemicals was lowest in LA 2093 upon herbivore feeding. The cultivated variety BB, in contrast, demonstrated a low constitutive amount of both phenolics and TPI, but a higher concentration of PPO. This corresponds with previous reports suggesting that domesticated crops have reduced constitutive defense levels compared to their wild relatives (Turcotte et al. 2014; Moreira et al. 2018; Whitehead et al. 2017). Further, the low inducibility in MWC and LA 2093 was comparable to that observed in cranberry, where a very weak induction of phenolics was found in wild populations (Rodriguez-Saona et al. 2011). Selective breeding that prioritizes yield and desirable traits such as fruit quality during the process of domestication often results in an inadvertent selection against plant defenses, including reduced constitutive chemical defenses in domesticated species (Herms and Mattson 1992; Mirnezhad et al. 2010; Whitehead et al. 2017).

The cultivated tomato BB exhibited significantly higher inducibility for all three defensive chemicals, TPI, PPO and TPC.

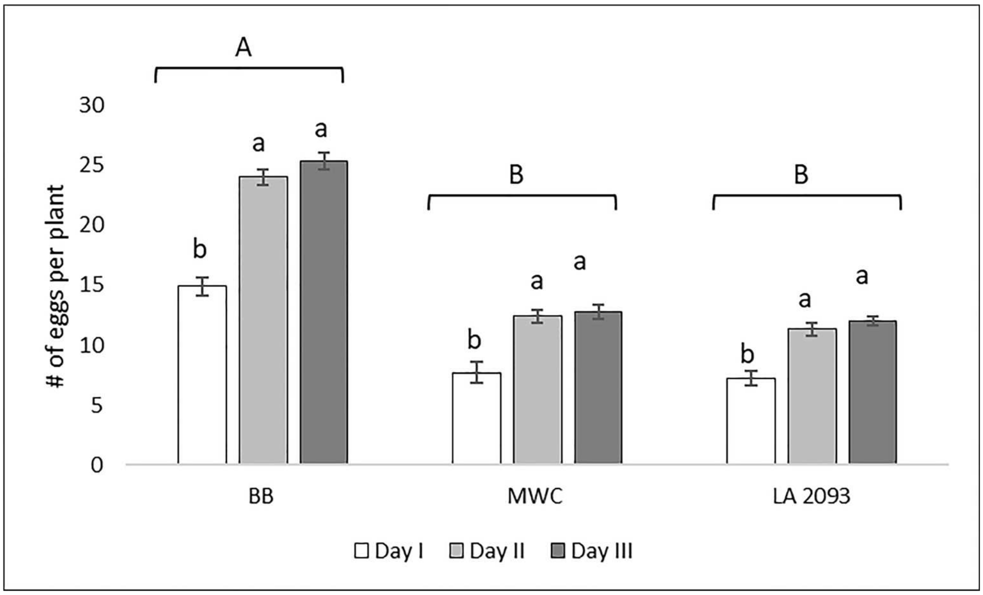


**Fig. 7** Oviposition preference of *H. zea* female moths on three different tomato genotypes, *Solanum lycopercicum* cv. Better Boy (BB), *Solanum lycopersicum var. cerasiforme* (Matts Wild Cherry (MWC)), and *Solanum pimpinellifolium* (LA 2093) calculated by counting number of eggs in plants for three consecutive days (Day I, II and III). Bars are mean ± SEM, and means with different letters are statistically different as determined by a Tukey HSD

While this response is largely consistent with the findings by Kempel et al. (2011) and Brzozowski et al. (2019), it contrasts with results of investigations by as Gols et al. (2008) and Moreira et al. (2018); in the latter studies no significant induction of glucosinolates was found in either the wild or cultivated cabbage against Diamond back moth (*Plutella xylostella)* and Cabbage butterfly (*Pieris brassicae)*. The contradictory finding in cabbage might be due to the narrow scope of the assessment, where only a single defensive compound was considered. Plants tend to utilize multiple defense mechanisms to resist attacks by foreign invaders (Agrawal and Fishbein 2006; Kempel et al. 2011). Furthermore, the cabbage herbivores were specialists, whereas the herbivore used in the present study was a generalist. In general, plant responses to insect herbivores with a common evolutionary history is different from responses to those without a shared evolutionary relationship (Lankau 2007; Desurmont et al. 2011; Danner et al. 2018).

Contrary to the patterns of inducible chemical defenses, the cultivated tomato (BB) had significantly higher levels of constitutive leaf trichomes, both glandular and non-glandular, compared to MWC and LA 2093. While our results were congruent to those of Bellota et al. (2013), they were in contrast with findings of several other studies (e.g Turcotte et al. 2014; Chen et al. 2015; Mo et al. 2016), where trichome density was reduced in the cultivated species. This contradiction may be a cultivar-specific phenomenon, as we only used one cultivated genotype, or may have some broader trend that needs to be studied further.

In the present study, a higher inducibility of both glandular and non-glandular trichomes was observed in the cultivated species following herbivore damage. Wounding and/or herbivore feeding generally would activate jasmonic acid (JA) pathway. Besides the expression of defensive genes (e.g protease inhibitors), JA is also known to induce trichome formation, especially glandular trichomes (Traw and Bergelson 2003; Boughton et al. 2005). While induction of leaf trichomes in response to herbivores is well documented in the cultivated tomato (Peiffer et al. 2009; Tian et al. 2012), studies comparing inducibility of leaf trichomes in the domesticated as well as wild genotypes are limited. Additionally, insects not only influence trichome densities but also the allelochemicals produced by them, which can affect performance of both herbivores and their natural enemies (Escobar-Bravo et al. 2017).

As to the impacts on the herbivore, undamaged leaves from the MWC and LA 2093 were less suitable for the growth of *H. zea* larvae than the leaves from BB. This is in general accordance with several previous similar studies (Meyer et al. 2012; Whitehead et al. 2017). Rosenthal and Dirzo (1997) also reported that cultivated maize is less resistant to insects compared to wild relatives (teosintes), which is a indicative of a tradeoff between productivity and defense. A higher concentration of phenolics and TPI in wild relatives may provide a plausible mechanism for higher resistance against *H. zea* larvae. For instance, various reports have suggested a strong growth inhibition with phenolics and TPI against caterpillars (Felton et al. 1989; Duffey and Stout 1996; Lawrence and Koundal 2002). Surprisingly, higher densities of trichomes in BB failed to affect herbivore growth. While a limited number of studies have demonstrated direct resistance against caterpillars (e.g. Kariyat et al. 2018), leaf trichomes in tomatoes, by and large, have been found to offer resistance against small insects such as whiteflies (Bleeker et al. 2009; Firdaus et al. 2012), leafhoppers (Dellinger et al. 2006; Kaplan et al. 2009) and mites (Maluf et al. 2001).

Interestingly, there was a significant reduction in larval growth when they fed on damaged leaves from BB. Reduced growth rates of *H. zea* on damaged BB leaves were correlated with increased accumulations of PPO and TPI in the leaf tissues. This reveals that the cultivated tomato BB exhibits higher levels of resistance once attacked, which negatively influences herbivore performance. Kempel et al. (2011) and Haak et al. (2014) reported similar correlations, whereas others have reported a reduction in plants' resistance in cultivated crops (Harvey et al. 2011; Moreira et al. 2018). While a strong effect of inducible chemical defenses in BB suggests an important role of inducibility in the cultivated tomato (BB), it makes a relatively weak contribution in LA 2093 and MWC in response to herbivore damage suggesting an increased reliance on constitutive resistance in the cherry tomato and wild accession. Induced defenses are less costly and increases the variability of plants response to herbivores (Karban and Myers 1989; Karban and Baldwin 1997). Therefore, cultivated varieties like BB may benefit with a high level of inducibility as the additional resources could be diverted to growth and reproduction. In contrast, high investments in constitutive defenses seems appropriate in wild genotypes which are exposed to a constant and high pressure from herbivores (Karban 2011; Bixenmann et al. 2016).

The analysis of volatile profiles revealed that herbivore feeding elicited increased levels of VOCs in all three genotypes, although the overall emissions differed quantitatively. The cultivated variety BB demonstrated greater inducibility of volatile emission in comparison to the cherry and wild tomato genotypes (MWC and LA 2093). This result is consistent with a recent meta-analysis that reported a comparatively higher level of herbivore-induced volatile emission in commercial varieties than their wild relatives (Rowen and Kaplan 2016). Terpenes, the most abundant group of compounds in the present study, play a major role in plant defenses that are mostly secreted by glandular trichomes (Kang et al. 2010; Bleeker et al. 2012). A higher density of glandular trichomes in BB, therefore, could possibly explain the enhanced level of volatile compounds in comparison with the two wild genotypes (Spyropoulou et al. 2014; Chen et al. 2018).

The most abundant VOCs in damaged BB and MWC leaves were β-phellandrene, 2-carene, α-terpinene and cymene, whereas limonene and 2-carene dominated in LA 2093. Interestingly, β-phellandrene and cymene were only detected from the leaves of BB and MWC, whereas limonene was found exclusively in LA 2093. In similar studies with several domesticated tomatoes, β-phellandrene, 2-carene, α-terpinene and cymene were found as the major contributors to the volatile blend (Raghava et al. 2009; Bleeker et al. 2009). Limonene has also been previously reported in the wild tomato relatives (Bleeker et al. 2012). Overall, the results suggest that volatile compositions are influenced by domestication and selective breeding (Kariyat et al. 2012).

In the oviposition assay, *H. zea* females preferred the cultivated BB variety over the cherry and wild tomato genotypes. The volatile blend emitted by LA 2093, especially the large contribution of limonene to the blend, may partly explain the low preference of *H. zea* female moths for this tomato species. Limonene can have a strong repellent effect on various insect pests (Li et al. 2014; Afifi et al. 2015; Zarrad et al. 2017). A recent study on tomato with another herbivore, *Manduca sexta,* suggested a similar trend where female moths preferred laying eggs on cultivated rather than the wild or landrace tomatoes (Li et al. 2018). Similar results were observed by McDaniel et al. (2016), where whiteflies preferred laying eggs on the cultivated tomato, when compared with the wild tomato species *S. pimpinellifolium.* However, there was no noticeable effect of a higher volatile level in BB on herbivore performance and preference, as described by previous studies (Paré and Tumlinson 1999; McCallum et al. 2011). In addition to influencing herbivore performance and preference, plant volatiles play a key role in recruitment of predators and parasitoids (Silva et al. 2018; Turlings and Erb 2018). Considering the minimal effect of higher volatile emissions from BB on insect performance and preference, we speculate that those induced volatiles may play an important role in attraction of natural enemies (Walling 2000; Heil et al. 2001).

Higher trichome densities in BB did not affect *H. zea* growth and ovipositional preference, which is contrary to findings of previous studies where trichomes affected performance and preference of herbivores (Horgan et al. 2009; Kessler and Baldwin 2002). However, Tian et al. (2012) observed similar results to ours, when studied tomato resistance to *H. zea* and Colorado potato beetle, *Leptinotarsa decemlineata*. These contrasting results may suggest that the effect of trichomes on oviposition and herbivore performance is variable and depends on both insect and plant genotypes used. It should be noted, however, that trichomes also offer other benefits to the plant, including reflectance, energy balance, ultraviolet protection, drought resistance and gas exchange (Xiao et al. 2017).

Overall, the findings from the present study indicated an enhanced inducibility of defensive chemicals, trichomes and herbivore induced volatiles in cultivated tomato variety BB compared to the other two accessions. This is important as induced plant defenses are economical, flexible and avoid resource allocation to defense production when herbivores are not present (Agrawal 2000). Although the cherry and wild tomato genotypes were more resistant against the herbivore, a higher induced resistance in cultivated tomato is notable and suggests that priming defenses should be further examined in both the wild and cultivated genotypes (Kim and Felton 2013). Inducibility of plant defenses could be exploited as a component of sustainable crop production by either using defense elicitors (Paudel et al. 2014; Strapasson et al. 2014) or through plant breeding approaches (Åhman 2009).

The herbivore *H. zea* exhibited reduced growth and preference on the cherry and wild genotypes, MWC and LA 2093. Identification and characterization of specific genetic traits in these genotypes to deter herbivore could be useful for host resistance breeding purposes (Trapero et al. 2016). Bleeker et al. (2012), for example, successfully developed tomato plants that produced 7-epizingiberene, originally discovered in

*S. habrochaites*, and demonstrated an enhanced level of host resistance against insect pests. Introgression of trichome-based resistance traits from wild tomato *S. pimpinellifolium* to the cultivated tomato resulted in an increased type-IV trichomes following methyl jasmonate (MeJA) treatment, leading to a decreased whitefly incidence (Escobar-Bravo et al. 2016).

This study demonstrated some of the benefits of comparing wild and cultivated genotypes for insect resistance, suggesting potential exploitation of plant-defensive traits against insect herbivore in breeding programs. Because of global climate change, crop losses from insect pests are expected to rise (Deutsch et al. 2018); therefore, identification, characterization and utilization of variation in plant-defensive traits may contribute to the sustainability of crop protection. Further studies, including a broader array of host genotypes and additional defensive traits such as glycoalkaloids, are necessary to bolster the results and conclusions drawn from this study.

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