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Spodoptera litura-mediated chemical defense is differentially modulated in older and younger systemic leaves of Solanum lycopersicum

Anish Kundu[1] · Shruti Mishra[1] · Jyothilakshmi Vadassery[1]

Abstract

Main conclusion **Metabolite profiling, biochemical assays, and transcript analysis revealed differential modulation of specific induced defense responses in local, older, and younger systemic leaves in *Solanum lycopersicum* upon *Spodoptera litura* herbivory.**

Plants reconfigure their metabolome upon herbivory to induce production of defense metabolites involved in both direct and indirect defenses against insect herbivores. Herbivory mediated leaf-to-leaf systemic induction pattern of primary and non-volatile secondary metabolites is not well studied in tomato. Here, we show that, in cultivated tomato *Solanum lycopersicum* herbivory by generalist insect, *Spodoptera litura* results in differential alteration of primary metabolites, majorly sugars and amino acids and specific secondary metabolites in local, younger, and older systemic leaves. Cluster analysis of 55 metabolites identified by GC–MS showed correlation between local and younger systemic leaves. Re-allocation of primary metabolites like glucose and amino acids from the local to systemic leaf was observed. Secondary metabolites chlorogenic acid, caffeic acid, and catechin were significantly induced during herbivory in systemic leaves. Among specific secondary metabolites, chlorogenic acid and catechin significantly inhibits *S. litura* larval growth in all stages. Local leaf exhibited increased lignin accumulation upon herbivory. Differential alteration of induced defense responses like reactive oxygen species, polyphenol oxidase activity, proteinase inhibitor, cell wall metabolites, and lignin accumulation was observed in systemic leaves. The metabolite alteration also resulted in increased defense in systemic leaves. Thus, comparative analysis of metabolites in local and systemic leaves of tomato revealed a constant re-allocation of primary metabolites to systemic leaves and differential induction of secondary metabolites and induced defenses upon herbivory.

Keywords *Solanum lycopersicum* · *Spodoptera litura* · Direct defense · Metabolite profiling · Primary metabolite · Lignin · Chlorogenic acid

Abbreviations
GC–MS	Gas chromatography–mass spectrometry
HPLC–PDA	High-performance liquid chromatography–photo diode array detector
CGA	Chlorogenic acid
CA	Caffeic acid
ROS	Reactive oxygen species
PPO	Polyphenol oxidase
PI	Proteinase inhibitor
CAD	Cinnamoyl alcohol dehydrogenase
DAB	3, 3′-Diaminobenzidine

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Introduction

Insect herbivory and subsequent damage in economically important plants affects crop productivity. To counter insect herbivores, plants have developed sophisticated direct and indirect defense mechanisms (War et al. 2012). Direct defense includes constitutively expressed morphological features such as thorns, prickles, trichomes, and induced defenses which include secondary metabolites that are repellent, anti-nutritive, and toxic to the herbivores (Howe and Jander 2008; Mithöfer and Boland 2012). Indirect defenses comprise production and emission of volatile organic compounds (VOCs) through which plants attract the natural enemies and predators of insects (Kessler and Baldwin 2001). Both direct and indirect defenses may be constitutive or inducible (War et al. 2012). During plant–biotic interactions, metabolites play a major role and are considered as important components of plant defense (Tenenboim and Brotman 2016). Herbivory-induced defense responses in plants occur by metabolic reprogramming predominantly by secondary metabolites (Marti et al. 2013), while primary metabolites support growth (Schwachtje and Baldwin 2008). However, recent reports implicate primary metabolism as a vital factor in herbivory defense (Zhou et al. 2015) and specific primary metabolites, e.g., sugars, function as signals in defense (Sheen 2014).

Spodoptera litura (cutworm/tobacco caterpillar/asian armyworm), a generalist herbivore, causes a significant crop loss in Asia and other parts of the world by defoliation in several plant families including *Solanaceae* and *Fabaceae* (Cheng et al. 2017). Tomato, *Solanum lycopersicum*, is the second most cultivated and consumed vegetable throughout the world and attack by generalist herbivore, *S. litura* results in huge crop loss (Fand et al. 2015). In tomato, defense responses against wounding and herbivory have been extensively studied and involve constitutive trichome-mediated defense, proteins like polyphenol oxidase (PPO), proteinase inhibitors (PIs) that are anti-herbivore molecules which interfere with insect's metabolism, and production of volatile organic compounds to attract predators (Howe and Jander 2008; Hägg et al. 2013; Constabel et al. 1995; Pearce and Ryan 2003; Constabel and Barbehenn 2008; Bosch et al. 2014). An 18-amino acid peptide called systemin is a key signal for the systemic defense in tomato, which includes PPO activity (Constabel et al. 1995) and proteinase inhibitors (PIs) that disrupt the activity of digestive enzymes in the insect midgut (Green and Ryan 1972). It has been proposed that systemin and jasmonic acid (JA) interact through an amplification loop to propagate a long-distance wound signal (Sun et al. 2011). More recently, fast signals moving in the range of cm min^{-1} have been identified: electro-potential waves at the plasma membrane, Ca^{2+} elevations in the cytosol, and accumulation of reactive oxygen species (ROS) in the apoplast have been proposed to be part of a propagating signaling system (Mousavi et al. 2013; Choi et al. 2014; Kiep et al. 2015).

Defense-related secondary metabolites, especially phenolic compounds, are induced both locally and systemically in rice (Shinya et al. 2016; Alamgir et al. 2016) and *N. attenuata* (Lee et al. 2017). Metabolite alteration in tomato upon *Manduca sexta* and *Helicoverpa zea* herbivory and tritrophic interaction with predators were studied in recent past (Steinbrenner et al. 2011; Errard et al. 2016). It was found that *M. sexta* regurgitate treatment reduced the growth and chlorophyll content in tomato (Korpita et al. 2014) and herbivore attack induces tryptophan accumulation that can be used for the biosynthesis of defense metabolites (Steinbrenner et al. 2011). Specific inducible secondary metabolites play an important role in plant defense (Howe and Jander 2008), and role of nicotine, caffeoyl putrescine, and chlorogenic acid in *Solanaceae* was broadly demonstrated upon herbivore treatment (Steppuhn and Baldwin 2007; Lee et al. 2017). Phenolic (War et al. 2012) and volatile organic compounds (VOCs) (Paré and Tumlinson 1999) also provide defense to plants against herbivore by acting as anti-nutritive and repellant. Induction of VOCs in tomato upon insect herbivory is also involved in indirect defense (Kessler and Baldwin 2001; Zebelo et al. 2014; Bautista-Lozada and Espinosa-García 2013) and their role in cytosolic Ca^{2+} signaling and membrane depolarization-mediated plant-to-plant communication has been investigated in recent past (Zebelo et al. 2014). However, upon actual herbivory (which involves both wounding and deposition of elicitors/effectors in oral secretion), leaf-to-leaf systemic signal-mediated differentiation in metabolome and induced non-volatile defense is poorly understood in tomato. Plant cell wall, an important morphological structure, is involved in plant defense against herbivore attack and reinforcement of the cell wall upon herbivory is reported in chrysanthemum (He et al. 2011). Primary metabolites including sugar molecules are the building blocks for cell wall forming macromolecules, such as lignin, cellulose, hemicellulose, suberin, and callose, and secondary metabolites like phenolics also take part in cell wall formation (Hägg et al. 2013). Hence, alteration of metabolome also has a role in cell wall strengthening. Nevertheless, in tomato, there is no information about alteration of cell wall and cell wall-associated metabolites upon herbivory.

Therefore, to obtain a clear view of differentiation in leaf-to-leaf systemic signal-mediated defense induction and its correlation with metabolite alteration upon herbivory, we used generalist herbivore, common cutworm (*S. litura*) on tomato (*S. lycopersicum*) for untargeted metabolic profiling. We selected herbivore fed local leaf and two adjacent older

and younger systemic leaves to investigate the alteration of major primary metabolites, specific defensive secondary metabolites, and herbivory-induced defense responses, which were significantly altered upon herbivory. Our work shows that *S. litura* herbivory induces differential metabolite alteration along with the specific defensive secondary metabolites and induced defense responses in local and two adjacent systemic leaves of tomato.

Materials and methods

Plant materials and growth conditions

Tomato (*S. lycopersicum*, cv. Pusa Ruby) seeds were presoaked in water overnight and placed on a moist tissue paper for 4 days in dark and then transferred to light to facilitate germination. Three-day-old seedlings of uniform growth were transplanted in plastic pots, filled with agropeat and soilrite (w/w 1:1), and maintained at 24 ± 2 °C (day/night: 16/8 h), relative humidity 60%, and light intensity 330 µmol s^{-1} m^{-2} for ~45 days (until 6–7 true leaves were appeared). *S. litura* eggs were procured from NBAIR, Bangalore and larvae hatched and reared on an agar-based optimal diet (Bergomaz and Boppre 1986). *S. litura* fourth instar larvae were used for plant feeding experiment.

Herbivory treatment and plant sampling

Tomato leaves were numbered from bottom as shown in Fig. 1a, b and third leaf was chosen as local leaf to do *S. litura* feeding assay. The adjacent second and fourth leaves were named as 'older systemic' leaf and 'younger systemic 'leaf, respectively. The local leaf was partially caged using a clip cage, and one pre-starved fourth instar *S. litura* larva was placed inside the cage and allowed to feed for 24 h. Control plants for local, older, and younger systemic leaves were without larval treatment. Three biological replicates were done for both *S. litura* fed and control set of experiments. Each biological replicate is a pool of three individual plants. After 24 h of *S. litura* feeding, the local, older, and younger systemic leaves were excised and flash frozen in liquid nitrogen and stored at −80 °C. A set of local leaves were similarly harvested after 6 h of *S. litura* feeding. The leaves were lyophilized, milled, and equal weights taken for further analysis. All analytical grade standard compounds were purchased from Sigma-Aldrich®.

Metabolite profiling through GC–MS

Lyophilized leaf was weighed and extracted according to a published method (Schauer et al. 2005) with a slight modification. In brief, 10 mg lyophilized leaf was extracted
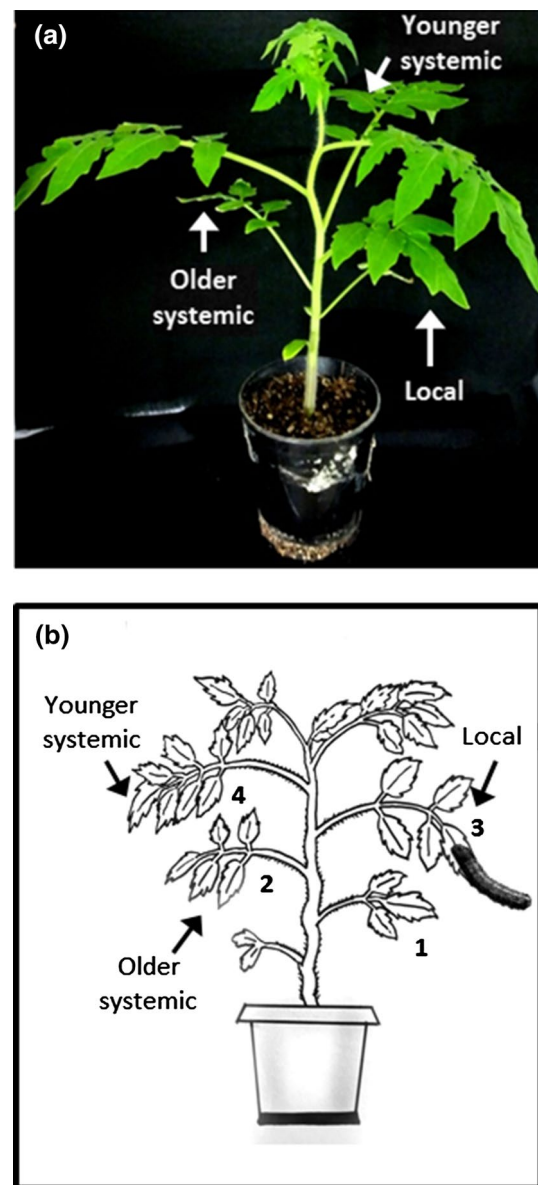

Fig. 1 Representation of *S. litura* feeding on tomato leaves experimental set-up. **a** Tomato plants with 6–7 true leaves were taken for experiments. **b** Numbering was done starting from the lowest true leaf and fourth instar *S. litura* larvae was caged and put on the terminal leaflet of the local leaf (no. 3). Adjacent older (no. 2) and younger (no. 4) leaves were taken as older systemic leaf and younger systemic leaf, respectively

with 480 µL pure methanol and 20 µL of 0.2 mg mL^{-1} ribitol (adonitol) was added to it as an internal standard. The mixture was vigorously shaken for 2 min and then heated 70 °C for 15 min. Thereafter, equal volume of water was added and vigorously shaken, followed by addition of 250 µL of chloroform and thorough mixing. This mixture was centrifuged at 2200*g* for 10 min at room temperature (~25 °C). The upper aqueous phase was taken out and dried in speed vacuum rotator at 35 °C. The dried fraction

was then re-dissolved in 40 μL of 20 mg mL^{-1} methoxamine hydrochloride in pyridine and then incubated for 90 min at 37 °C. After that, 60 μL of MSTFA (*N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide) was added and incubation was done for 30 min at 37 °C. After this derivatization process, 2 μL of sample was used to perform gas chromatography–mass spectrometry (GC–MS) analysis employing a Shimadzu GC–MS-QP2010™ coupled with an auto sampler–auto injector (AOC-20si). Analysis was conducted exploiting Rtx-5® capillary column (Restek Corporation, USA) and helium as carrier gas. A method consisting of 80 °C isothermal heating for 2 min, followed by ramp rate of 5 °C min^{-1} to 250 °C, a withhold of 2 min and a final ramp of 10 °C min^{-1}, a withhold time of 24 min. The chromatogram integration and mass spectra analysis were done through GC–MS solution software (Shimadzu®) and NIST14s and WILEY8 spectral library were used for derivatized metabolite identification; glucose, sucrose, and fructose were confirmed for identification with authentic standards analyzed in GC–MS. Metabolite that was derivatized with different number of trimethylsilyl (TMS) was considered as different metabolite for data analysis (e.g. L-Asparagine-2TMS and Asparagine-3TMS; L-serine-3TMS and serine 2-TMS). For metabolite with multiple peaks, summation of the peak area was taken after confirming the spectral data, according to the published protocol (Lisec et al. 2006). Peak area of each compound was normalized by dividing by peak area of internal standard (IS) ribitol in each GC–MS run. For the metabolites, which were below detection level, a normalized minimal value (0.001) was used for fold-change calculation. Normalized peak area (normalized peak response) of treated sample was divided by normalized peak area of control sample and logarithmic (log$_{10}$) value of this calculated fold change is shown in Online Resource 1: Table S1 and used for creating heat map. For quantification of glucose, sucrose, and fructose, linear calibration curves were prepared using authentic external standard compounds (Online Resource 2: Table S2).

HPLC–PDA analysis of chlorogenic acid

Chlorogenic acid (CGA) was first identified through GC–MS analysis. Furthermore, a HPLC–PDA-based quantification of CGA was done using external linear calibration curve prepared with authentic standard CGA (Additional file 2: Table S2). A gradient method with water and acetonitrile mobile phase (0–20% acetonitrile within 12 min) was used for eluting CGA through Shimadzu CLASS-VP V6.14 HPLC machine equipped with Luna C$_{18}$ RP column of 250 × 4.6 mm with 0.5 μm internal diameter and PDA detector. CGA was detected at 330 nm wavelength.

Detection of H$_2$O$_2$ accumulation

H$_2$O$_2$ accumulation was observed with 3, 3′-Diaminobenzidine (DAB) staining. Control and treated terminal leaflets were subjected to vacuum infiltration with DAB (1 mg mL^{-1}) solution for 5 min and kept in dark at room temperature for 5 h. Leaflets were then destained with a boiling de-staining solution consisting ethanol:acetic acid:glycerol for 15 min.

Polyphenol oxidase (PPO) assay

Polyphenol oxidase assay was done using a modified in vitro published spectrophotometric method (Bosch et al. 2014). After harvesting, 0.5 g leaf sample was ground in liquid nitrogen and protein was extracted in 1 mL of 50 mM sodium phosphate buffer (pH 7.8) containing 2% polyvinyl polypyrrolidone, 0.1 mM EDTA. The extracted sample was centrifuged at 16,000g for 20 min and supernatant collected and kept on ice. Protein concentration was measured using a standard method using Bradford reagent (Bradford 1976). Assay was done by adding 150 μL of protein extract in 300 μL of 100 mM of catechol in sodium phosphate buffer and incubating at 37 °C for 30 min. The reaction was quenched by adding 150 μL of 6 N HCl and absorbance was measured at 525 nm. Specific activity was used for data interpretation.

Lignin quantification and staining

Lignin quantification was performed with a spectrophotometric method adopted from a report described by (Ali et al. 2006). Lyophilized leaf powder (5 mg) was extracted in 500 μL of pure ethanol for 15 min and centrifuged at 12,000g for 10 min at room temperature. The supernatant was discarded and pellet was dried overnight at room temperature. The dried pellet was treated with 500 μL of 2 N HCl and 0.1 mL of thioglycolic acid at 95 °C for 6 h. The pellet was then washed with water and re-suspended in 500 μL of 1 N NaOH. The sample was then kept overnight with gentle shaking at room temperature. Thereafter, it was centrifuged at 12,000g for 15 min at room temperature. To the supernatant, 250 μL of concentrated HCl was added and the mixture was kept overnight at 4 °C (to precipitate lignin thioglycolate). The precipitate was then collected by centrifugation at 12,000g for 5 min. The pellet was dissolved in 500 μL of 1 N NaOH and the amount of lignin was measured spectrophotometrically at 280 nm. The lignin content was represented as the absorption values (A_{280}) measured at 280 nm using 1 N NaOH as the blank. For visualization of lignin thickening, phloroglucinol-based histochemical staining was performed (Xu et al. 2011). Hand-cut petiole cross section of the terminal leaflets of the treated plants and

systemic leaves was incubated in saturated phloroglucinol solution in 20% HCl for 10 min. After incubation, it was mounted in water and visualized under bright-field microscope. Lignin appeared in reddish-brown colour.

RNA extraction and qRT-PCR

Leaves were homogenized using liquid N_2 and total RNA was extracted using TRIzolReagent (Invitrogen). RNA obtained was treated with DNase (TURBO DNase, Ambion) to remove any contaminating DNA, and its quantity was determined using nano drop. Total DNA-free RNA (1 µg) was converted to single-stranded cDNA using iScript cDNA synthesis kit (Bio-Rad). Sequences for the genes were obtained from Sol Genomics Network (https://solgenomics.net/), and gene-specific primers were designed using NCBI primer designing tool (https://www.ncbi.nlm.nih.gov/tools/primer-blast/). For the generation of amplicon, iTAQ universal SYBR green super mix (Bio-Rad) was used and the reaction mixture was prepared according to the manufacturer's protocol. qRT-PCR was performed in 96-well plates on a Bio-Rad CFX connect real-time system. Relative expressions of the genes in the treated samples were calculated as fold change relative to untreated samples. Ubiquitin was used as a reference to normalize the gene expression. Following primer pairs were used:

PI-II   FP, 5′-GGAAAACGAATGTGGCCAGAAC-3′
         RP, 5′-CAGCTGTGCCTGGAGAACC -3′;
AspI   FP, 5′-CTACTTCAGGTAAGCCAGTCC-3′
         RP, 5′-CTCACCTAGGTACACATCACC-3′
CAD   FP, 5′-GGGCCTGATGATGTGCAAGTC-3′
         RP 5′-CTGGCCCTACCTCTACCAC-3′;
UBI3   FP 5′-GAGGCTTCGTAAGGAGTGCC-3′
         RP, 5′-CGCCTCCAGCCTTGTTGTAA-3′.

Insect diet feeding assay

Chlorogenic acid (CGA) was mixed in the artificial diet at 0.25 and 0.016 mg g^{-1} concentration and used to feed 30 ($n=30$) and 18 ($n=18$) *S. litura* larvae, respectively. In another experiment, 0.01 mg g^{-1} catechin and 0.02 mg g^{-1} caffeic acid (CA) were mixed in the artificial diet separately and used to feed *S. litura* ($n=20$). Concentrations of chemicals were taken on the basis of their concentration in the systemic leaf after 24 h of *S. litura* feeding. Control diet contained solvent controls for the chemical used. Each larva was pre-weighed and those having equal weights were selected. The larvae were kept separately in plastic container with air passage at 28 °C temperature and diet changed once in 2 days. After 8 days of feeding, larvae were taken out and

weighted. For pupation and maturation experiment, larvae ($n=30$) were kept for 22 days.

Insect-feeding assay on systemic leaf

After 24 h of *S. litura* herbivory on local (third) leaf, a naïve *S. litura* second instar larvae were allowed to feed on intact older systemic leaf of the plant. Control experiments were set with untreated local leaf. Each larva was pre-weighed for equal weights. Larvae were allowed to feed for 3 days at 28 °C. After that, larvae were taken out and weighted.

Statistical and software analysis

For statistical analyses of GC–MS data, normalized responses were used. MULTIPLE EXPERIMENT VIEWER (MeV, http://mev.tm4.org) was used for heat mapping, cluster analysis, and relevance network analysis. For metabolite pathway mapping, iPath2 (http://pathways.embl.de/iPath2.cgi#) was used using the KEGG annotations of each metabolic pathway. Principal component analysis was done using Multi Variate Statistical Package (https://www.kovcomp.co.uk/mvsp/), a web-based tool for multivariate statistical analysis. Sigma Plot was used for significance analysis and each value is represented as mean ± standard error (SE) of at least three biological replicates of each experiment. Significance analysis was done with *t* test.

Results

Metabolite profiling revealed differential alteration of primary metabolites in local and systemic leaves upon *S. litura* herbivory

To assess the impact of the *S. litura* herbivory on the metabolome of tomato plant, untargeted metabolic profiling was done in local (third leaf), older systemic (second leaf), and younger systemic leaves (fourth leaf) after 24 h of feeding (Fig. 1a, b). Through GC–MS analysis, 52 primary metabolites were identified and quantified in local and systemic leaves (Online Resource 1: Table S1). To obtain the global view of metabolite profile, heat map of 52 primary metabolites and 3 secondary metabolites fold change upon herbivory was generated (Fig. 2a). The primary metabolites identified were carbohydrates and their derivatives, amino acids, TCA cycle intermediates, and octadecanoid pathway-related metabolites and myo-inositol. In local leaf, only four primary metabolites, i.e., tagatose, oxalic acid, 9-octadecadienoic acid, and stearic acid (octadecanoic acid), were up-regulated; 22 primary metabolites, majorly sugars, amino acids, and TCA cycle intermediates were down-regulated, while 26 metabolites did not show any change (Fig. 2a; Online
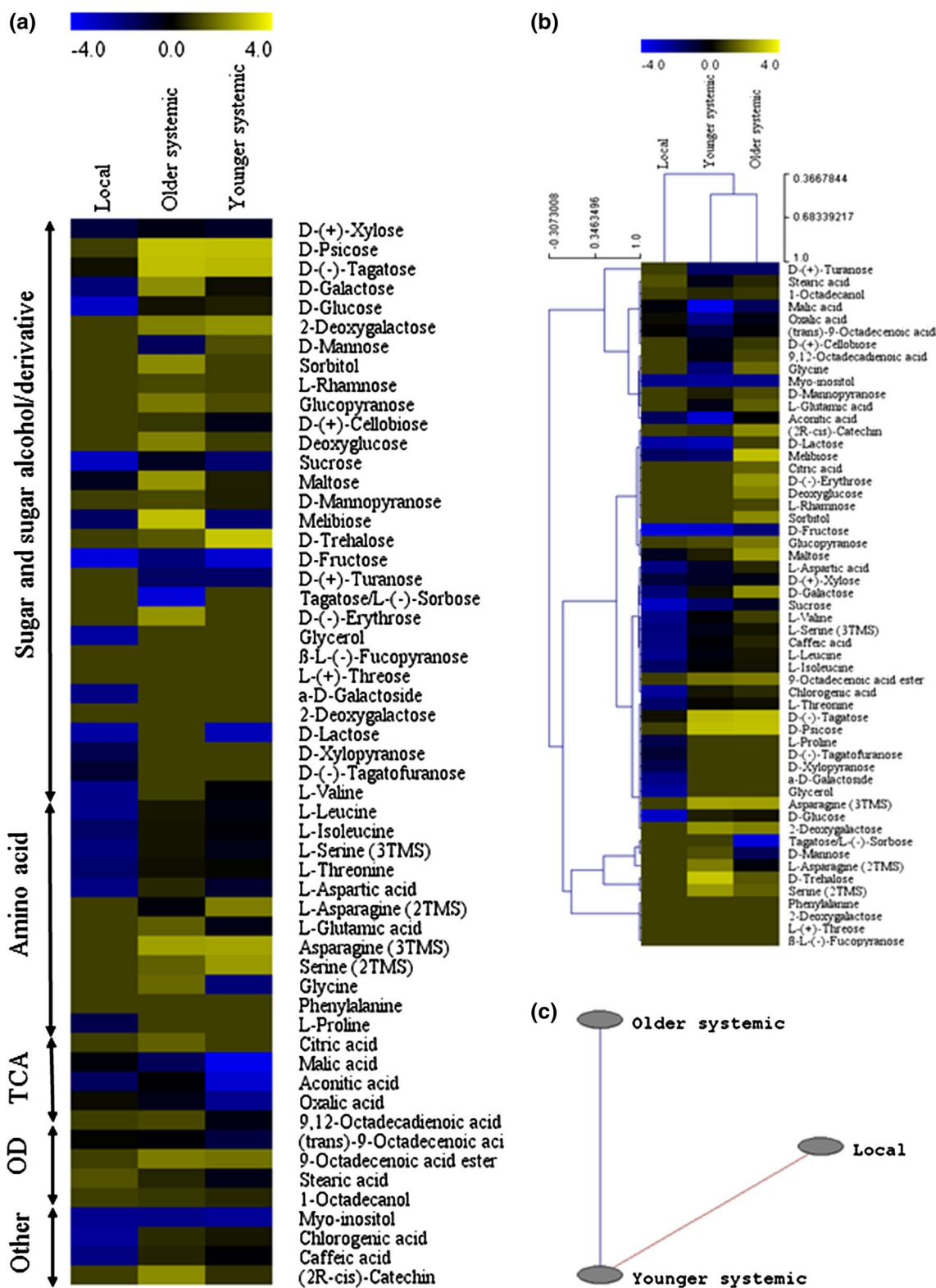
Fig. 2 Major metabolite changes in tomato ($\log_{10}$) local and systemic leaves after 24 h of feeding by *S. litura* larvae. **a** Heat map of fold change of sugar and sugar derivatives, amino acids, intermediates of tri-carboxylic acid (TCA) cycle, metabolites derived from octadeca-noid pathway (OD), myo-inositol, and three secondary metabolites detected through GC–MS. Blue and yellow indicate decreased and increased metabolite levels, respectively. **b** Hierarchical clustering of metabolites based on 'Pearson correlation' shows neighboring corre-lation in between local and younger systemic leaves. **c** Relevance net-work analysis of metabolite changes among local and systemic leaves shows relevance between younger systemic leaf with local leaf

Resource 1: Table S1). Significance analysis showed that 23 metabolites were significantly altered with $P < 0.05$ (Online Resource 1: Table S1). In the older systemic leaf, 29 primary metabolites were up-regulated which are sugars, amino acids, and octadecanoid pathway-related compounds; 12 primary metabolites including 7 sugars, 3 TCA cycle intermediates, and octadecanoic acid were down-regulated; 11 primary metabolites remained unchanged upon 24 h of *S. litura* herbivory (Fig. 2a; Online Resource 1: Table S1). A significance analysis showed that 18 primary metabolites were significantly altered with $P < 0.05$ (Online Resource 1: Table S1). In younger systemic leaf, 16 primary metabolites were up-regulated of which mostly are sugars and amino acids, 21 primary metabolites were down-regulated which included amino acids, TCA cycle intermediates, and octadecanoid pathway-related compounds, and 15 metabolites were not changed (Fig. 2a; Online Resource 1: Table S1). A significance analysis showed that 23 primary metabolites were significantly altered with $P < 0.05$. Hierarchical clustering based on 'Pearson correlation' of the fold change (log_{10}) of each metabolite (including primary and secondary) was done and correlation of younger systemic leaf with the local leaf was identified (Fig. 2b). Furthermore, relevance network analysis by fold change of each metabolite in each leaf, older systemic leaf showed distinct metabolome alteration compared to both the other leaves, and relevance was found in between local and younger systemic leaves (Fig. 2c). Global view through PCA with fold change of metabolites (including both primary and secondary metabolites) showed clear separation in local, older, and younger systemic leaves (Online Resource 2: Figure S1).

To identify primary metabolites that are altered in local and systemic leaves upon herbivory in detail, we plotted most abundant primary metabolites separately from the global view. Glucose, sucrose, and fructose were quantified by external calibration curve prepared with authentic standards (Online Resource 2: Table S2). Local leaves showed a drastic reduction in major sugars, i.e., glucose (Fig. 3a), sucrose (Fig. 3b), and fructose (Fig. 3c). To identify kinetics of sugar alteration, we also performed 6 h *S. litura* feeding. It was observed that glucose level was unchanged, while sucrose and fructose significantly increased at 6 h unlike 24 h treatment (Online Resource 2: Figure S2). This indicates that major sugar levels in local leaf are not drastically reduced upon a shorter herbivory treatment, but, after 24 h, major sugar levels are decreased in local leaf. In case of older systemic leaf, after 24 h of herbivory, glucose level increased (Fig. 3a), while fructose level was decreased (Fig. 3c). Younger systemic leaf also revealed an increased glucose level (Fig. 3a), while both sucrose and fructose levels decreased (Fig. 3b, c). Analysis of amino acid content revealed that, upon 24 h of *S. litura* feeding, amino acid pools were significantly decreased in local leaf (Fig. 3d). Among them, L-valine, L-leucine, L-isoleucine,

L-serine-3TMS, L-threonine, and L-aspartic acid were significantly ($P < 0.001$) reduced. Though, no change of total amino acid pools was found in local leaf after 6 h (Online Resource 2: Figure S2). Total amino acid content was, however, significantly increased in older systemic leaf (Fig. 3d). The amino acids that were significantly ($P < 0.05$) up-regulated in older systemic leaf were L-threonine, L-glutamic acid, asparagine-3TMS, and serine-2TMS. In younger systemic leaf, only L-aspartic acid showed a significant ($P < 0.005$) reduction (Online Resource 1: Table S1).

S. litura herbivory results in alteration of specific secondary metabolites in local and systemic leaves of tomato

In tomato, the induction of volatile organic compounds upon insect herbivory is well studied (Bleeker et al. 2009; Bautista-Lozada and Espinosa-García 2013; Zebelo et al. 2014), but the systemic induction of non-volatile secondary metabolites is unclear. To investigate whether induced direct defenses are differentially altered in local and systemic tomato leaves, we investigated the secondary metabolites upon herbivory. GC–MS analysis revealed three secondary metabolites, i.e., chlorogenic acid (CGA), caffeic acid (CA), and catechin, to be significantly ($P < 0.05$) induced during herbivory in systemic leaves (Online Resource 1: Table S1). CGA accumulation was increased significantly ($P < 0.005$) in both younger and older systemic leaves after 24 h of *S. litura* infestation, with older systemic leaf having a higher CGA accumulation than younger. Interestingly, CGA levels were decreased in local leaves after 24 h of herbivory (Fig. 4a), though they transiently increased in local leaves after 6 h of *S. litura* feeding (Online Resource 2: Figure S2a). CA showed similar trend with increase in local leaves after 6 h (Online Resource 2: Figure S2c), but below detection level after 24 h of *S. litura* herbivory (Fig. 4b). In older systemic leaf, CA increased significantly after *S. litura* treatment (Fig. 4b). Catechin was below detection level in both control- and herbivore-treated local leaves. However, catechin levels increased in older and younger systemic leaves (Fig. 4c). Thus, chlorogenic acid (CGA), caffeic acid (CA), and catechin are systemic leaf-induced metabolites after 24 h of *S. litura* feeding.

Induced defense responses are differentially activated upon local and leaf-to-leaf systemic signaling

In plant–microbe interaction, a rapid production of reactive oxygen species (ROS), phosphorylation events, and hormonal perturbations form a signaling network that coordinately

Fig. 3 Alteration of major primary metabolites upon 24 h of *S. litura* herbivory. Mean ± SE of content of **a** glucose, **b** sucrose, and **c** fructose upon herbivory ($n = 4$ for local leaf and $n = 3$ for systemic leaves; each biological replicate of systemic leaf is a pool of three individual samples). Quantification was done by calibration curve using authentic standard compounds. **d** Mean ± SE of total normalized peak area amino acids ($n = 4$ for local leaf and $n = 3$ for systemic leaves; each biological replicate is a pool of three individual samples). Normalization was done with the peak area of the internal standard (ribitol). Star indicates significant differences calculated by *t* test. *$P < 0.05$, **$P < 0.005$, ***$P < 0.001$, *n.s.* not significant
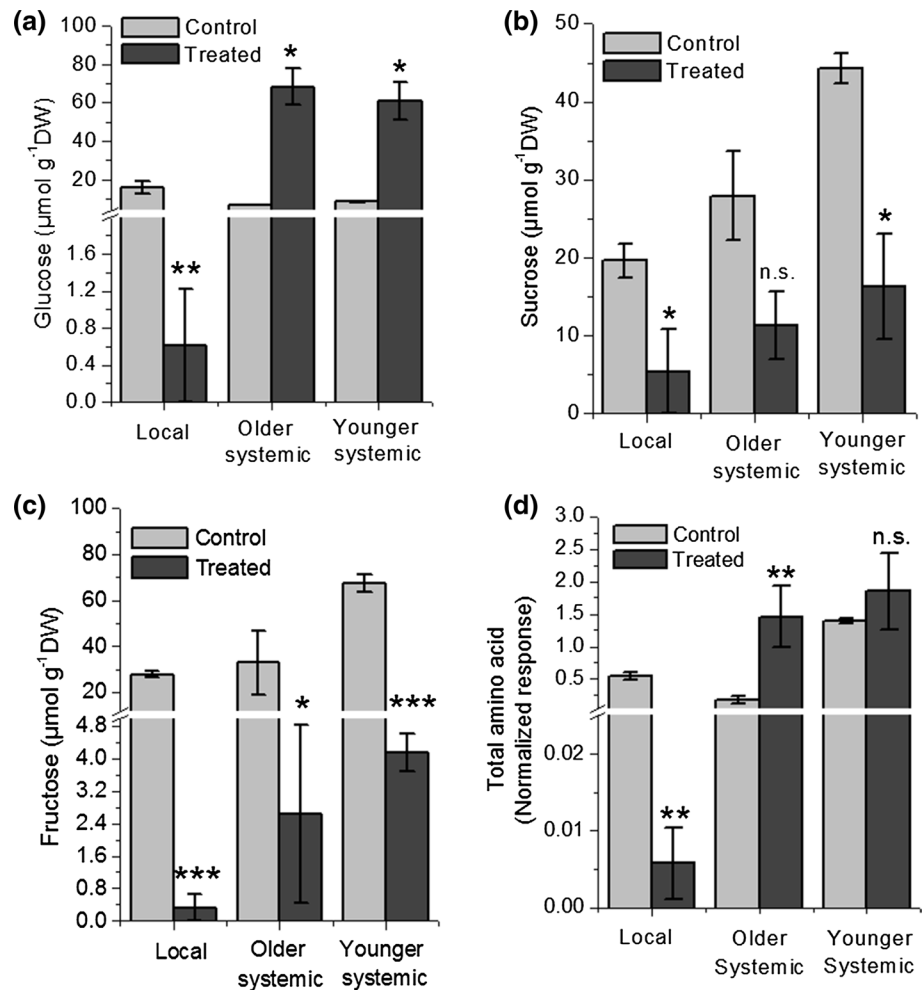
controls local and systemic defense (Howe and Jander 2008). It is known that mechanical wounding in tomato results in systemic H_2O_2 accumulation (Orozco-Cardenas and Ryan 1999). In tomato late-activated defense protein, polyphenol oxidase (PPO) has anti-nutritive effect against herbivores including *Heliothis zea* and *Spodoptera exigua* (Felton et al. 1989). However, the effect of *S. litura* herbivory on systemic induction of PPO and ROS in tomato is not clear. To find differences in leaf-to-leaf systemic responses upon actual *S. litura* herbivory, we investigated H_2O_2 (one of the several reactive oxygen species) accumulation pattern through DAB staining. Local leaves after 24 h *S. litura* feeding accumulated highest amount of the H_2O_2, both adjacent to damaged area and the distal part of the leaflet. In local leaf, H_2O_2 accumulation was distributed throughout the lamina (Fig. 5a). The undamaged leaflet distal from the damaged local leaflet showed H_2O_2 accumulation in tip of the leaf (Online Resource 2: Figure S3). Older and younger systemic leaf accumulated H_2O_2 in the outer lamina, with younger leaf having comparatively higher H_2O_2 accumulation (Fig. 5a). In vitro PPO assay was done by using protein

extract from local, older, and younger systemic leaves with catechin as substrate. Increased PPO activity was observed in both local and systemic leaves upon 24 h of herbivory. Maximum significant increment was observed in younger systemic leaf ($P < 0.005$) compared to local and older systemic leaves (Fig. 5b). This result indicates differential PPO activity in two adjacent systemic leaves when local leaf is damaged by *S. litura*. Proteinase inhibitors (PIs) are one of the principal defense compounds in tomato upon insect herbivory and it was demonstrated that PIs are systemically induced via systemin upon *S. exigua* herbivory (Green and Ryan 1972; Broadway et al. 1986). In tomato, three types of proteinase inhibitors were reported to be induced during wounding and herbivory, i.e., wound-induced proteinase inhibitor 2 (*PI-II*), aspartic proteinase inhibitor (*AspPI*), and cysteine proteinase inhibitor (*CysPI*). Among these three, *PI-II* is majorly involved in defense against wounding (Green and Ryan 1972; Broadway et al. 1986), though, in some recent reports, *AspPI* and *CysPI* showed induction upon herbivory (Chung et al. 2013; Tan et al. 2018). Here, we checked whether the PIs are induced differentially between

Fig. 4 Chlorogenic acid (CGA) and caffeic acid (CA) levels altered upon herbivory. **a** Mean ± SE ($n = 3$, each biological replicate is a pool of three individual samples) of CGA content upon herbivory in local and systemic leaves. Quantification was done through HPLC–PDA, using calibration curve prepared with authentic standard CGA. **b** Mean ± SE ($n = 3$, each biological replicate is a pool of three individual samples) normalized content of CA. Normalization done with the GC peak area of internal standard (ribitol). **c** Mean ± SE ($n = 3$, each biological replicate is a pool of three individual samples) normalized content of catechin. Normalization was done with the GC peak area of the internal standard (ribitol). Star indicates significant differences calculated by *t* test. *$P < 0.05$, **$P < 0.005$. ***$P < 0.001$, *n.s.* not significant, *b.d.l.* below detection level
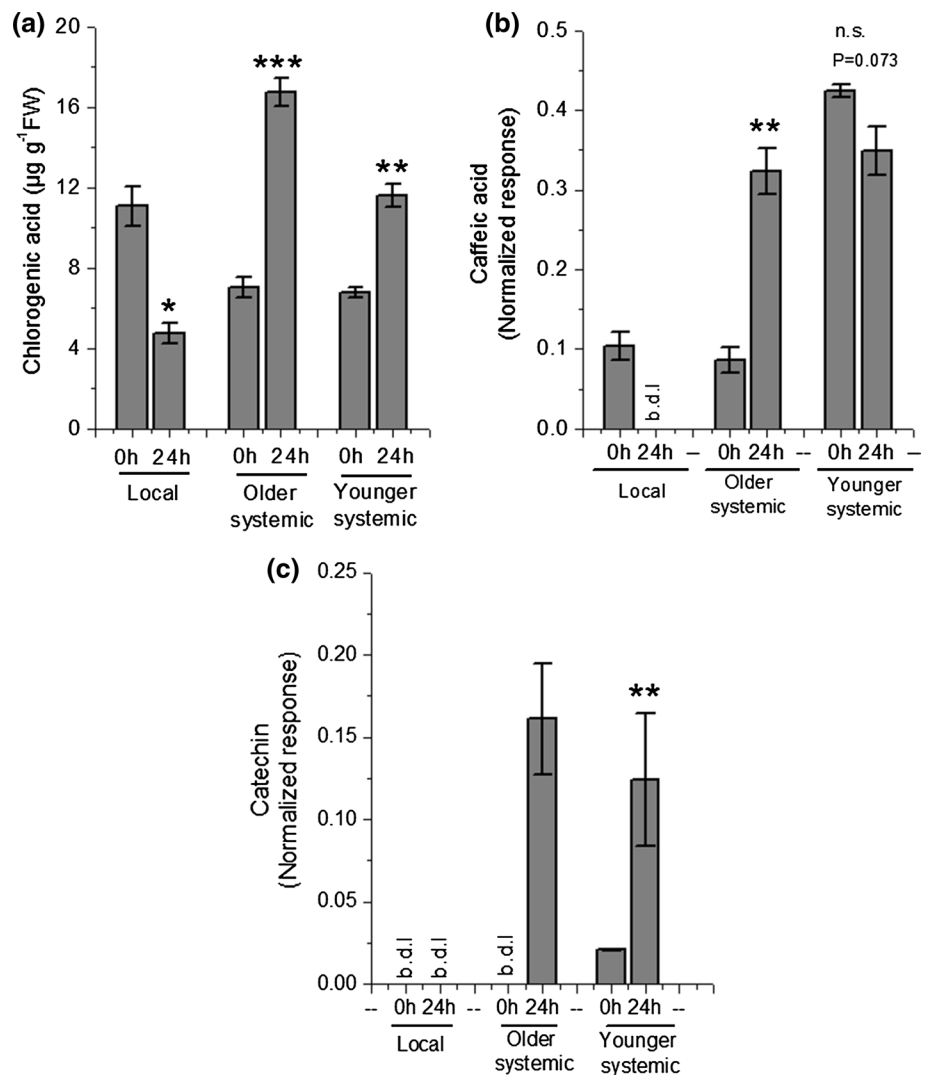
systemic leaves upon *S. litura* herbivory. We analyzed transcript level of *PI-II* in local and systemic leaves upon 24 h of *S. litura* herbivory and its induction was found in both local and systemic leaves. Maximum induction was observed in local leaf. Significance was found in between the induced older and younger systemic leaves ($P < 0.05$) and in between the induced local and older systemic leaves, but no significant difference was found in between the induction of older and younger systemic leaves (Fig. 5c). To obtain a kinetic of *PI-II* induction, we also checked *PI–II* transcript level at an early time point, i.e., after 6 h of *S. litura* herbivory. We found a significant induction in both the local and systemic leaves (Fig. 5c), where maximum induction was observed in local leaf. Here, older and younger systemic leaf showed a significant difference in induction ($P < 0.05$). We also checked *AspPI* transcript level upon 6 and 24 h of herbivory, which showed induction in both local and systemic leaves (Online resource 2: Figure S4). However, older and younger systemic leaves did not show a significant difference in their

induction at any of these two time points. On the other hand, *Cys PI* transcript level did not show any induction upon *S. litura* herbivory in local or systemic leaves.

Cell wall-associated metabolites altered in both local and systemic leaves, while lignification altered only in local leaf

Alteration of cell wall sugars has been demonstrated during plant defense (Zhao and Dixon 2014; Witzel et al. 2015) and xylose is a major precursor for xylan biosynthesis in cell wall (Harper and Bar-Peled 2002; Rennie and Scheller 2014). During carbohydrate profiling through GC–MS analysis, we surprisingly observed a decrease in soluble xylose content in both local and younger systemic leaves (Fig. 6a). We also found that myo-inositol, a precursor of cell wall polysaccharide (Siddique et al. 2014) and structural building block of lipid signaling molecules (Eckardt 2010), was significantly reduced upon 24 h of *S. litura* herbivory in
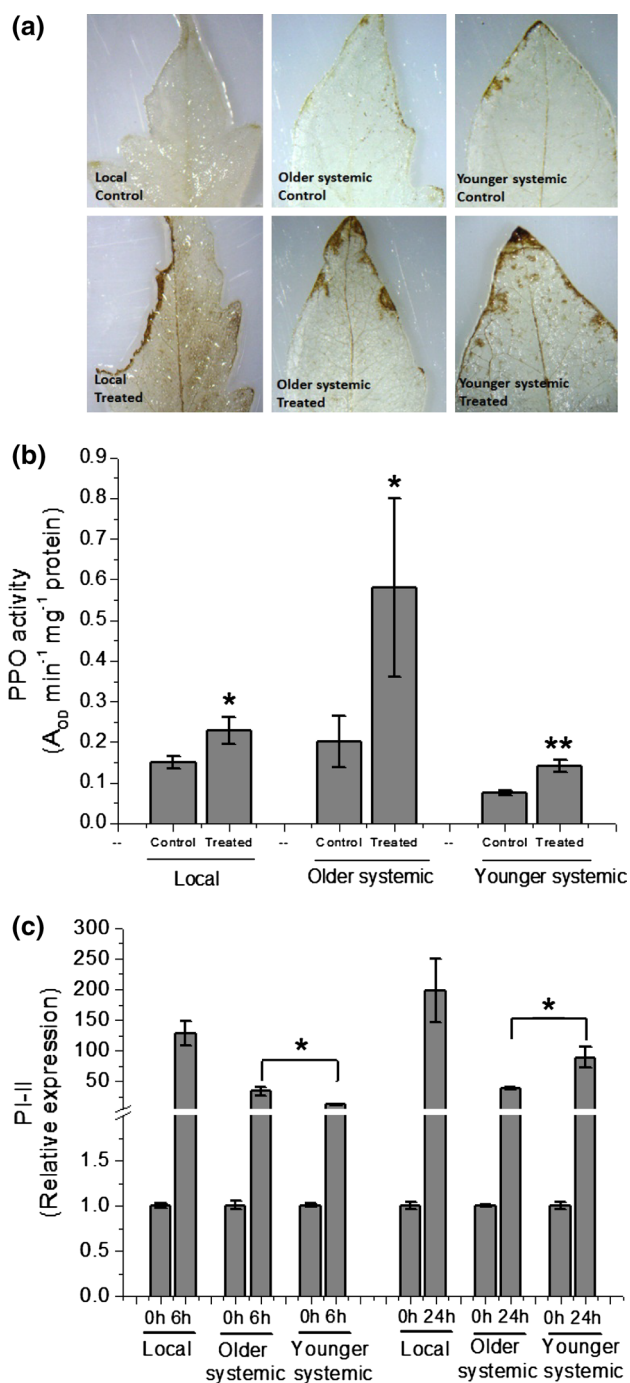
(a)

(b)

(c)

Fig. 5 Systemic ROS, PPO activity, and *PI* transcript accumulation upon *S. litura* herbivory in tomato. **a** DAB staining of tomato leaves after *S. litura* herbivory to detect ROS accumulation. This figure is representative of three independent experiments. **b** PPO activity in local and systemic leaves. Each value indicates mean ± SE ($n = 3$, each biological replicate is a pool of three individual samples). *PI-II* transcript level after **c** 6 and 24 h of *S. litura* herbivory in local and systemic leaves of tomato. Relative expression of transcript in leaves was determined by real-time PCR analysis and normalized to the plant ubiquitin mRNA level. Star indicates significant differences calculated by *t* test. *$P < 0.05$, **$P < 0.005$, ***$P < 0.001$, *n.s.* not significant

both local and systemic leaves (Fig. 6b). Lignin is a major component of secondary cell wall in plants and responsible for the rigidity of the cells (Vanholme et al. 2010). Hence, lignin content was measured spectrophotometrically in local and systemic leaves. Only local leaf showed a significant increase in lignin content after 24 h of *S. litura* feeding (Fig. 7a). For further confirmation, we analyzed the expression of the gene of cinnamoyl alcohol dehydrogenase (*CAD*), involved in the biosynthesis of lignin monomer (Roth et al. 1997), in local and systemic leaves. Up-regulation of *CAD* transcript level was found only in local leaf (Fig. 7b). We also checked *CAD* expression level at an early time point, i.e., after 6 h herbivory, but no alteration was found in any of local and systemic leaves. For further confirmation of 24 h data, we conducted histochemical staining of local leaflet fed by larvae to visualize lignin accumulation using phloroglucinol, a lignin specific stain. We detected lignin thickening in the xylem tissue of the local leaf petiole upon *S. litura* herbivory (Fig. 7c).

Chlorogenic acid and catechin hinders the *S. litura* larval growth and pupation

Metabolite profiling revealed the chlorogenic acid (CGA) as a significantly enhanced secondary metabolite in systemic leaves after 24 h of herbivory. This led us to hypothesize that CGA could have anti-herbivory effect against *S. litura*, as CGA was previously reported as defense metabolite against tomato fruit worm, *H. zea* (Elliger et al. 1981), thrips, *Frankliniella occidentalis* (Leiss et al. 2009), and *S. exigua* (Kumar et al. 2016) Therefore, we examined the effect of chlorogenic acid on *S. litura* larval growth. In older systemic leaf, where maximum CGA increase was observed upon 24 h of *S. litura* feeding, the mean content was 0.25 mg g^{-1} dry weight and 0.016 mg g^{-1} fresh weight. We used both these CGA concentrations for checking its effect on *S. litura* growth. After feeding *S. litura* larvae with artificial diet containing 0.25 mg g^{-1} CGA for 8 days, larval weight was found to be significantly decreased ($P < 0.05$) (Fig. 8a). Interestingly, 0.016 mg g^{-1} CGA containing diet also showed a significant ($P < 0.05$) decrease in growth *S. litura* larvae (Online Resource 2: Figure S5). Furthermore, we continued the feeding to larvae with 0.25 mg g^{-1} CGA containing diet until pupation and about 60% reduction of *S. litura* pupation and conversion of pupa to adult moth was observed (Online Resource 2: Figure S5). These results indicate that CGA, which is increased in tomato leaf upon *S. litura* herbivory, acts as a growth hindering anti-nutritive metabolite in the all stages of *S. litura* growth and development. Apart from CGA, catechin and caffeic acid (CA) were also increased in the systemic leaves upon *S. litura* herbivory. Therefore, we also tested these two metabolites to have anti-herbivore
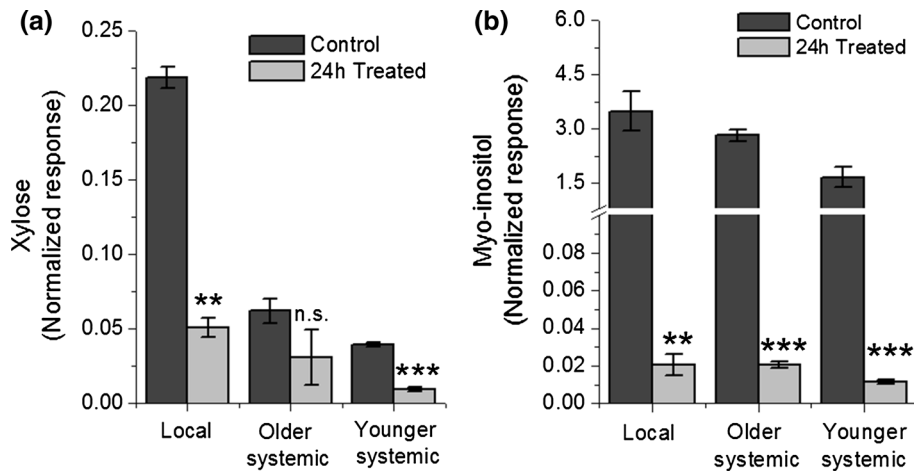
Fig. 6 Alteration of xylose and myo-inositol in tomato local and systemic leaves after 24 h of herbivory. **a** Mean ± SE ($n = 3$, each biological replicate is a pool of three individual samples) normalized xylose content in local and systemic leaves. **b** Mean ± SE ($n = 3$, each biological replicate is a pool of three individual samples) normalized myo-inositol content in local and systemic leaves. Normalization was done with the GC peak area of the internal standard (ribitol). Star indicates significant differences calculated by t test. *$P < 0.05$, **$P < 0.005$, ***$P < 0.001$, *n.s.* not significant

Fig. 7 Lignification upon 24 h of *S. litura* herbivory in local and systemic tomato leaves. **a** Mean ± SE ($n = 3$, each biological replicate is a pool of three individual samples) of lignin content in local and systemic leaves. Star indicates significant differences calculated by t test. *$P < 0.05$. **b** CAD transcript level upon 24 h of *S. litura* herbivory in local and older systemic leaves of tomato. Transcript abundance in leaves was determined by real-time PCR analysis and normalized to the plant ubiquitin mRNA level. **c** Phloroglucinol staining of local (control and treated) leaf petiole. The figure is representative of three individual experiments. Scale bar: 100 μm
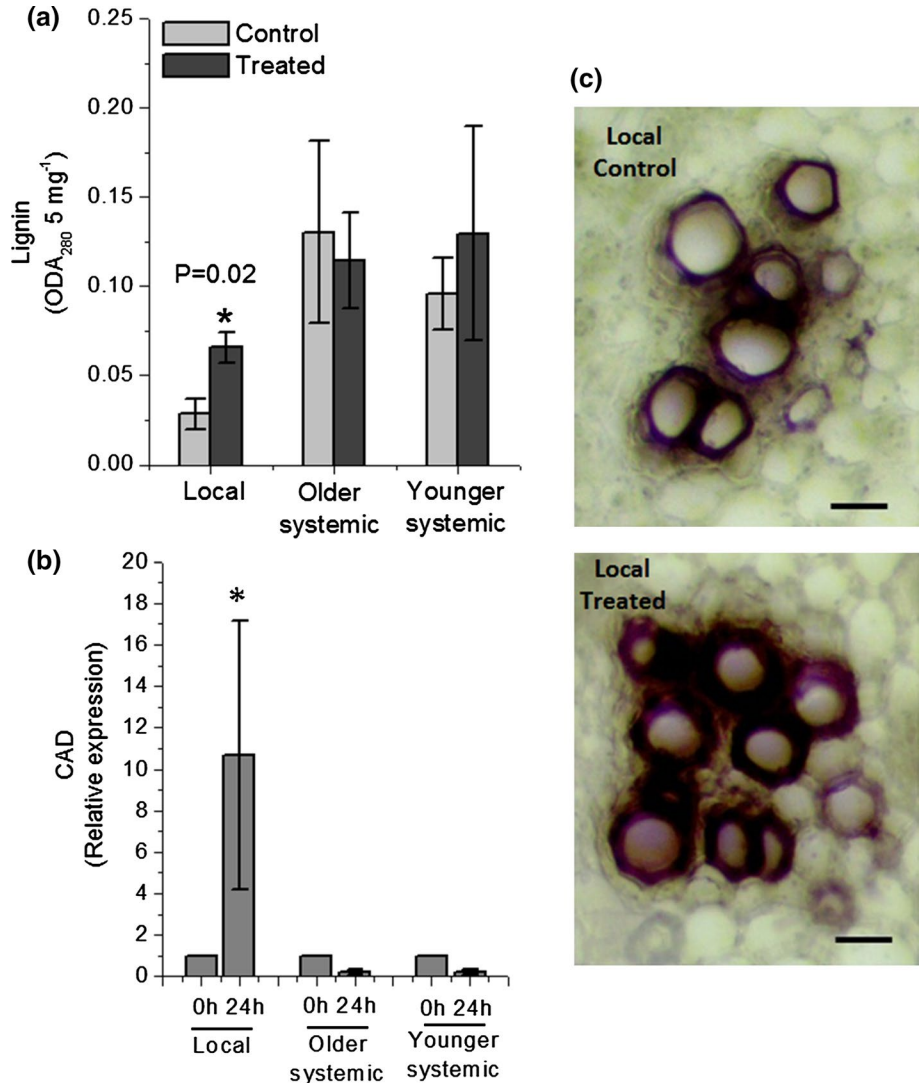
Fig. 8 Effect of CGA, catechin, and caffeic acid on larval growth and pupation. **a** Mean ± SE ($n=30$) *S. litura* larval weight after feeding on control and CGA (0.25 mg g^{-1}) added diet for 8 days. **b** Mean ± SE ($n=17$) *S. litura* larval weight after feeding on control and catechin (0.01 mg g^{-1}) added diet for 8 days. **c** Mean ± SE ($n=17$) *S. litura* larval weight after feeding on control and caffeic acid (0.02 mg g^{-1}) added diet for 8 days. **d** Larval feeding assay on systemic leaves of previously infested ($n=21$) and non-infested control plants ($n=28$) (each value represents mean ± SE of replicate experiments). *$P<0.05$, **$P<0.005$, ***$P<0.001$
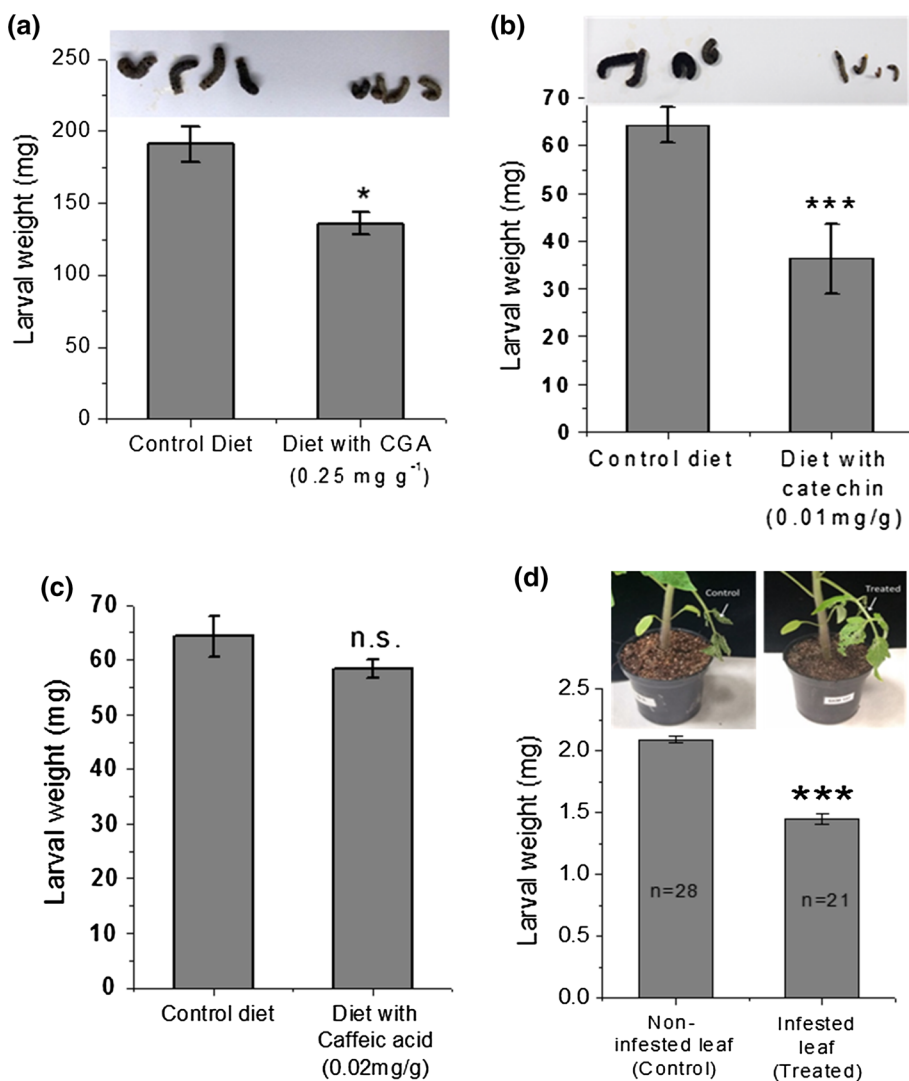
effect against *S. litura*. Catechin exhibited a strong growth inhibition on insect growth, while caffeic acid did not show any significant impact on insect growth (Fig. 8b, c). These results suggest that both CGA and catechin induced in the systemic leaves of tomato are a defense metabolite-hindering larval growth during *S. litura* herbivory.

Larvae exhibited low weight gain upon feeding on systemic leaf of pre-infested tomato plants

Since systemic leaf undergoes changes in distribution and content of metabolites, we wanted to test if this provides resistance to them for future herbivory. To test this hypothesis, *S. litura* larvae were allowed to feed on the systemic leaves of infested and non-infested (control) tomato plants. We found that *S. litura* larvae that fed on older systemic leaf of the infested tomato plant showed less weight gain, compared to one feeding non-infested tomato plant (with

no local wounding) and leaf phenotype also clearly exhibited lower larval performance on pre-infested older systemic leaf (Fig. 8d). This result confirms that feeding on local leaf induced systemic defense responses in the tomato plant that provides resistance to future herbivory.

Discussion

Sugar, amino acid, and secondary metabolite re-allocation in tomato upon *S. litura* herbivory

Induced defenses and growth require energy which is captured through photosynthesis, transported to sink tissues in the form of sucrose, cleaved to glucose and fructose and, used for glycolysis, and stored as starch (Machado et al. 2017; Braun et al. 2014). Reduction in photosynthetic activity is an active plant response to insect herbivory and to

meet energy demands many plants respond by promoting local catabolism of energy storage compounds (e.g., sucrose or starch) (Zhou et al. 2015). Metabolite profiling upon *S. litura* herbivory in tomato local and two systemic leaves (older and younger) revealed that both primary and secondary metabolic pathways were altered during herbivory as shown in the metabolic pathway map (Online Resource 2: Figure S6). After 24 h of *S. litura* herbivory, the local fed leaf showed a drastic reduction of sugars like glucose, sucrose, and fructose. Meanwhile, in young and old systemic leaves, the level of glucose increased, indicating a possible re-allocation from the local to systemic leaf. The levels of fructose are reduced in both local and two systemic leaves, whereas sucrose levels are reduced only in local and younger systemic leaves. The substantial decrease in leaf sugar content after 24 h of *S. litura* feeding is indicative of reduction in carbon assimilation as well as re-allocation from wounded to unwounded leaf. The decrease in fructose level is also reported in tomato leaves upon *Verticillium dahliae* infection (Buhtz et al. 2015), which might also indicate reduction in nutrition value of local leaves to starve the larvae. It has been demonstrated in multiple studies that leaf herbivory in diverse plants reduces the sugar accumulation in distal roots (Sampedro et al. 2011; Machado et al. 2013; Tytgat et al. 2013; Ferrieri et al. 2015; Machado et al. 2015, 2017). *M. sexta* and *H. zea* herbivory on tomato, however, resulted in no consistent changes in sugar content in systemic roots (Steinbrenner et al. 2011). In tomato (*S. lycopersicum*; *Solanum peruvianum*), both soluble and cell wall bound invertases play crucial role in alteration of sugar pool upon herbivory by breaking down sucrose into glucose and fructose (Zhang et al. 1996; Ohyama and Hirai 1999). These sugars act as signaling molecule along with the jasmonates during induction of plant defense (Ahn et al. 1999; Ahn and Lee 2003; Tauzin and Giardina 2014). Glucose, which, in our data, is reduced in local leaf and re-allocated to systemic leaves, is a signaling molecule that controls gene expression, central and secondary metabolism, as well as growth and developmental programs (Sheen 2014) and, hence, could have crucial role in leaf-to-leaf systemic defense activated upon *S. litura* herbivory.

Apart from sugars, a significant alteration in amino acid content was also found upon *S. litura* herbivory. Reduction in total amino acid content in local leaf and increased amino acid content in older systemic leaf upon 24 h of herbivory was observed. Amino acids serve as growth-limiting nutrient and precursor for the production of plant defense compounds during plant–herbivore interactions (Zhou et al. 2015). In tomato, *H. zea* herbivory induced significantly higher levels of aspartate content in leaves, while transport amino acid levels (glutamine, glutamate, asparagine, and aspartate) decreased in the sink tissue (apex and root), while tryptophan levels increased (Steinbrenner et al. 2011).

Reduction of total amino acid as well as protein content in local leaf was previously found in cotton (*Gossypium hirsutum*) upon *H. zea* herbivory (Bi et al. 1997). In a report by Gómez et al. (2010), tomato leaves exported amino acid pool out of the leaves upon treatment with a methyl jasmonate which indicated re-allocation of nitrogen pool towards the undamaged area like root. Transport of nitrogen pool away from the insect-feeding site was also observed in common milkweed (*Asclepias syriaca*) upon feeding by *Tetraopes tetrophthalmus* (Tao and Hunter 2013). In our experiment, the re-allocation of amino acids from local to older systemic leaf could be to limit access of nutrients to herbivores, while, in systemic leaf, their increased content could be used for the synthesis of defensive metabolites. Correlation and relevance network analysis of metabolites showed that metabolite alteration between local and younger systemic leaves follows similar pattern, whereas older systemic leaf showed distinct pattern, which suggests differential metabolic reconfiguration upon *S. litura* herbivory. Two prominent hypotheses to explain spatial and temporal variation in plant defense expression are the optimal defense theory (ODT) (Rhoades 1991) and the growth-differentiation balance hypothesis (GDBH) (Herms and Mattson 1992), and our data point to ODT via sugar and amino acid alterations.

Variation in the early and late-activated induced defense responses in local and systemic leaves

One of the earliest signaling events after herbivory perception is rapid changes in reactive oxygen species (ROS), which can act as a local signal for hypersensitive cell death and also as a diffusible signal for the induction of defensive genes in adjacent cells (Alvarez et al. 1998). Wounding results in H_2O_2 accumulation in both local and systemic leaves of tomato (Orozco-Cardenas and Ryan 1999), and ROS accumulation was demonstrated to be locally induced in lima bean (*Phaseolus lunatus*) upon *S. littoralis* herbivory (Maffei et al. 2006). H_2O_2 accumulation in tomato upon herbivory is yet to be investigated. Herbivore-induced signaling differs from simple wounding signal as elicitor and effector molecules in larval oral secretion also play important role in modulating defense during herbivory (Maffei et al. 2006; Mithöfer and Boland 2008). In situ detection of H_2O_2 by DAB staining revealed that local leaf showed maximum H_2O_2 accumulation in the *S. litura* fed area, as it was damaged maximally. Both the older and younger systemic leaves showed H_2O_2 accumulation but with differential intensity and distribution; H_2O_2 itself acts as second messenger for defense induction, and our results indicate that H_2O_2 induced differentially in local and systemic leaves could activate different signaling pathways. Polyphenol oxidase (PPO) is known to be part of

the jasmonate-dependent inducible defense system against lepidopteran insects in tomato and is induced locally upon both wounding and *S. exigua* herbivory (Constabel et al. 1995; Bosch et al. 2014). In the insect gut, PPOs reduce the nutritive quality and digestibility of dietary proteins and the availability of essential amino acids (Felton et al. 1989). In our study, we show PPO activity in both local and systemic leaves upon *S. litura* herbivory, and found that activity is higher in both local and systemic leaves. The younger systemic leaf had increased significance of PPO induction which suggests that it is highly defended. Proteinase inhibitor, one of the major inducible defense responses in tomato (Green and Ryan 1972; Broadway et al. 1986), showed induction upon both 6 and 24 h of herbivory. We checked *PI-II*, *AspPI,* and *CysPI* transcript accumulation upon *S. litura* herbivory as these three are reported to be induced upon herbivory (Green and Ryan 1972; Chung et al. 2013; Tan et al. 2018). *PI-II* and *AspPI* significantly induced upon herbivory, but no induction was found in *Cys PI* transcript level. Interestingly, *PI-II* was differentially induced in older and younger systemic leaves, but no significant differentiation was found in *AspPI* transcript induction in between older and younger systemic leaves, which indicates that differential induction of proteinase inhibitors in older and systemic leaves is mostly mediated by *PI-II*.

Alteration in cell wall metabolites in local and systemic leaves signifies differential defense signaling, while lignification alters locally upon *S. litura* herbivory

Although alteration in cell wall sugars due to cell wall invertase activity upon wounding is reported in tomato (Ohyama and Hirai 1999), the effect of herbivory on plant cell wall is not explored yet. During metabolite analysis, xylose and myo-inositol were observed to be decreased upon 24 h of *S. litura* herbivory in local and systemic leaves of tomato, which indicated a probable over-utilization of these building blocks for strengthening the cell wall. In a previous report, xylose content was decreased in tomato after being infected with soil-borne fungus *V. dahliae* (Buhtz et al. 2015). Role of lignification as induced plant defense against herbivores is emerging from studies in *N. attenuata* and tropical trees, suggesting that herbivores select leaves based upon their digestibility rather than upon their nutritive value (Gaquerel et al. 2014; Poorter and Arets 2003). An increase in total lignin content was observed only in local leaf after 24 h of *S. litura* herbivory. Even transcript expression analysis of *CAD* and histochemical staining exhibited increased lignification in local leaf. Recently, it has been shown in cotton that over-expression of lignin polymerization enzyme, laccase (GhLac1), leads to increased lignification, and increased tolerance to the fungal

pathogen *V. dahliae* and to cotton bollworm (*Helicoverpa armigera*) and cotton aphid (Hu et al. 2017).

Chlorogenic acid and catechin are strong growth inhibitor of *S. litura*

Differential re-allocation of specific secondary metabolites like chlorogenic acid (CGA), caffeic acid (CA), and catechin from local leaf to systemic leaf was observed after 24 h of *S. litura* feeding. In local leaf, both CGA and CA were reduced over their basal levels, whereas catechin was undetected. However, CGA, CA, and catechin are induced in systemic leaf after *S. litura* herbivory with CGA and catechin being induced in both systemic leaves, while CA being specific to older systemic leaf. Decreased accumulation of CGA in local leaf was accompanied by an increase of its levels in older and younger systemic leaves. The higher accumulation in CGA in systemic leaves indicates plant's choice to produce/re-allocate defensive metabolites in systemic leaves to prevent future herbivore attack, rather than in already demolished local leaf. CA is a major phenylpropanoid involved in the biosynthesis of lignin (Boerjan et al. 2003), and a precursor of CGA (Olthof et al. 2001) and catechin is an induced polyphenol during herbivore attack (Moctezuma et al. 2014). This result also supports the hypothesis that plant prefers to induce defensive metabolite biosynthesis in unaffected systemic leaves to prevent future herbivore attack.

Among the induced phenolic compounds, chlorogenic acid (CGA) was previously proven to have anti-nutritive properties against tomato fruit worm, *H. zea* (Elliger et al. 1981), *F. occidentalis* (Leiss et al. 2009), and *S. exigua* (Kumar et al. 2016). CGA from ground nut (*Arachis hypogaea*) was also reported to resist *S. litura* larval growth and facilitate mortality rate in neo-natal stage (Mallikarjuna et al. 2004). In a recent study, a drastic increase in CGA accumulation has been reported in the pith of *Nicotiana attenuata* upon herbivory with *Trichobaris mucorea*, a stem borer, which showed decrease in herbivore performance (Lee et al. 2017). Feeding assay with CGA displayed a strong hindrance to all stages of *S. litura* growth with significantly delayed the growth of larvae, pupation, and conversion of pupa to mature. We also checked catechin and caffeic acid (CA) to have anti-nutritive effect against *S. litura*. Caffeic acid did not show any significant effect on larval growth, but catechin showed a strong inhibition against the larval growth. Hence, both plant metabolites, CGA and catechin, act as anti-nutritive agent against *S. litura* larvae.

S. litura feeding on local leaf induces defense in systemic leaf against future herbivory

We found from the current work that, to resist *S. litura* herbivory, tomato re-allocates primary metabolites, increases

lignification, and differentially produces defensive secondary metabolites in systemic leaves. Furthermore, feeding assay of *S. litura* larvae on systemic leaf of the infested plant showed a clear inhibition of larval growth, which indicated that induced defense responses hinder the *S. litura* larvae from feeding on the systemic tissue.

Conclusion

In conclusion, metabolite profiling revealed that *S. litura* herbivory in tomato activates a suite of primary and secondary metabolites and induced defenses differentially in both local and systemic leaves. To resist *S. litura* herbivory, tomato reinforces its lignification and differentially produces defensive metabolites in systemic leaves, so that they could resist the future herbivore attack. The role of lignin accumulation in defense against *S. litura* herbivory and associated genes involved in the process is an exciting area of research, apart from the metabolite re-allocation of primary metabolites.

Author contribution statement JV and AK designed the experiments and analyzed the data. AK and SM performed the experiments. JV and AK wrote the manuscript. All authors have read and approved the final version of the manuscript.

Compliance with ethical standards

Conflict of interest Authors have no conflict of interest to declare.

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