Host plant resistance of tomato plants to western flower thrips

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Chapter 5

Thrips resistance and NMR-based metabolic profiling of a Solanum pennellii x lycopersicum introgression population

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Abstract

Interspecific chromosomal-substitution lines, also referred to as introgression lines (ILs), represent an excellent platform to explore the genetics of multiple biological and chemical traits of agronomical importance. Using a NMR metabolomics approach we have chemotyped a *Solanum pennelii* x *lycopersicum* introgression population to investigate the genetic and chemical basis of thrips resistance in tomato. ILs were screened for thrips resistance in a choice bioassay and subjected to ^{1}H NMR profiling. A total of 24 primary and secondary foliar metabolites were identified. Both the concentration of the metabolites and thrips damage varied significantly throughout the 76 ILs. Nine quantitative trait loci (QTL) were identified for thrips resistance and 268 for the metabolic traits. Neither multivariate data analysis nor correlation tests evidenced an association between thrips resistance and any of the metabolites. Trait mapping did not show either consistent co-localization of thrips resistance and the profiled metabolites. Metabolic correlations were detected mostly within classes of compounds, *e.g.* lipids, amino acids and phenolics. Interesting QTL were identified for valuable phytochemicals such as chlorogenic acid and rutin. Particularly important were IL10-1 and 10-1-1, which respectively showed a 2.6 and 4.4 fold increase in the chlorogenic acid content compared to the recurrent parent *S. lycopersicum* M82.

Introduction

A long period of selection in tomato for yield and taste-related traits has greatly reduced phenotypic and genetic diversity in domesticated tomatoes. Compared with the rich reservoir in

wild species, domesticated tomatoes contain only a very small part of the genetic variation that is accessible in related wild species (Tanksley & McCouch, 1997). Thus, tomato breeding may have led to loss of resistance traits (Kennedy & Barbour, 1992). Indeed, there is a great source of resistance traits available in the wild tomato species (Bai & Lindhout, 2007). It has, therefore, become important to screen wild genetic resources for valuable traits, including resistance, that could be introduced into commercial varieties (Zamir, 2001). Having identified the valuable agricultural traits of wild species these can be transferred by introgression breeding (Zamir, 2001). To introgress the favorable wild allele into domesticated tomato, marker-assisted selection plays an important role, and the map positions and markers linked to the QTL provide a basis for breeders to design optimal breeding strategies (Bai & Lindhout, 2007).

In tomato domestication traits have been studied for growth and fruit characteristics, and the underlying qualitative genes and quantitative trait loci (QTL) have been identified. A QTL is a polymorphic location of a chromosome containing alleles that differentially control the expression of a phenotypic trait. Many important tomato traits are genetically controlled by a combined action of QTL with favorable alleles often present in the wild species (Bai & Lindhout, 2007). This includes resistance to pathogens and insects. Regarding pathogens, QTL for resistance to the tomato powdery mildew, *Oidium lycopersici* in *Lycopersicon parviflorum* (Bai et al., 2003), to the gray mold, *Botrytis cinerea* in *S. lycopersicoides* (Davis et al., 2009), to the bacterial wilt, *Pseudomonas solanacearum*, in a cross of *L. esculentum* and *L. pimpinellifolium* (Thoquet et al., 1996) and to the bacterial spot, *Xanthomonas* spp. in *S. lycopersicun* var. *cerasiforme* (Hutton et al., 2010) have been identified. In regard to insects QTL for resistance to the sweetpotato whitefly, *Bemisia tabaci*, in a cross of *S. lycopersicum* and *S. habrochaites* (Momotaz et al., 2010) were reported. The molecular genetics of thrips resistance is not well understood. Only a few studies have been reported in various crops. QTL for resistance to the flower bud thrips, *Megalurothrips sjostedti* (Omo-Ikerodah et al., 2008) and for resistance to *Thrips tabaci* and *Frankliniella schultzei* in cowpea (Muchero et al., 2010), for resistance to *Thrips palmi* in common bean (Frei et al., 2005) and for resistance to *T. palmi* and *Megalurothrips usitatus* in potato (Galvez et al., 2005) have been identified.

Interspecific chromosomal-substitution lines or introgression lines (ILs), are more powerful compared with interspecific crosses in QTL identification. These lines carry a single introgressed genomic region, and are otherwise identical for the rest of their genome. As a result, the phenotypic variation in these lines can be associated with individual introgression

segments (Bai & Lindhout, 2007). In tomato, several sets of ILs have been developed for wild relatives of tomato. These include introgressions with *S. lycopersicodes* and *S. sitiens* (Canady et al., 2006), *S. lycopersicum* and *S. hirsutum* (Monforte & Tanksley, 2000) and *S. lycopersicum* and *S. pennellii* (Eshed & Zamir, 1995). The set of ILs with *S. pennellii* (accession LA716) comprises 76 lines. In these ILs a marker-defined genomic region of the domesticated variety *S. lycopersicum* M82 was replaced with its homologous interval in the wild species. Over a series of field studies on these lines a number of phenotypic traits were quantified and QTL identified (Eshed & Zamir, 1995; Gur et al., 2004). Metabolic profiles of *S. esculentum* and *S. pennellii* as well as of six ILs were compared by Overy et al. (2005). Schauer et al. (2006) established a metabolic profile of the *S. pennellii* introgression population to identify loci associated with fruit metabolism and yield. Kamenetzky et al. (2010) determined the genetic basis of metabolic regulation in tomato fruit by constructing a detailed physical map of genomic regions spanning previously described metabolic QTL of the *S. pennellii* introgression population.

In this study we are interested in the genetics of secondary metabolite markers related to thrips resistance. We thus used the *S. pennellii* x *lycopersicum* introgression population to detect QTL for WFT resistance and secondary metabolites, and their co-localization.

Material and methods

Plants and thrips bioassay

Seeds of the 76 ILs and the recurrent parent *S. lycopersicum* M82 were provided by the C. M. Rick Tomato Genetic Resource Center at the University of California Davis, USA. Seeds were directly sown in 13 cm diameter pots with potting soil. Seedlings were thinned to one plant per pot after one week. Six replicates for each line were grown in a randomized fashion in a glass house during the months of June and July in 2008. Temperature was recorded and was 20.5°C on average with a maximum of 37.5°C and a minimum of 13.1°C. All windows on the glass house were covered with nylon gauze of 120 µm mesh size to make it thrips-proof. The thrips herbivory bioassay was started when the plants had in average six fully expanded leaves. Three replicates of each IL were kept in the glass house for the thrips bioassay while the other three replicates were taken away for the NMR metabolomics. Per plant, 15 adult thrips, reared on flowering chrysanthemum, were released into the glass house. Three weeks later thrips herbivory, expressed as mm^2 of silver damage, was scored by eye for each plant.

Sample collection and extraction procedure

For the ^{1}H NMR metabolomic analysis the third oldest leaf was taken from each plant at the beginning of the thrips bioassay. Leaves were harvested throughout the morning. Immediately after collection leaves were flash frozen with liquid nitrogen and kept at -80 °C until freeze-dried. Samples were ground to a fine powder with a Retsch ball-mill (Retsch GmbH, Haan, Germany). Fifty mg of each sample, weighed in 2 mL Eppendorf tubes, was extracted under ultrasonication (15 min) with 1.5 mL of 70% methanol-d_4 in potassium phosphate buffer (90 mM, pH 6.0) containing 0.02% (w/v) 3-trimethylsilyl propionic acid sodium salt-d_4 (TMSP). After centrifugation (13 krpm, 15 min) an aliquot of 800 µL was taken for NMR analysis.

NMR measurements and data analysis

^{1}H NMR spectra were recorded at 25 °C on a 600 MHz Bruker DMX-600 spectrometer (Bruker, Karlsruhe, Germany) operating at a proton NMR frequency of 600.13 MHz. Deuterated methanol was used as the internal lock. Each ^{1}H NMR spectrum consisted of 128 scans requiring 10 min and 26 s acquisition time with the following parameters: digital resolution (DR)=0.16 Hz per point, pulse width (PW30°)=11.3 µs, and relaxation delay (RD)=1.5 s. A pre-saturation sequence was used to suppress the residual water signal with low power selective irradiation at the water frequency during the recycle delay. Free induction decay (FIDs) were Fourier transformed with a line broadening (LB)=0.3 Hz. The resulting spectra were manually phased, baseline corrected and calibrated to the internal standard TMSP at 0.0 ppm using XWIN NMR (version 3.5, Bruker).

For the multivariate data analysis the optimized ^{1}H NMR spectra were automatically binned by AMIX software (v. 3.7, Bruker Biospin). Spectral intensities were scaled to TMSP and reduced to integrated regions of equal width (0.04 ppm) from δ 0.3–10.0. The regions of δ 4.7–5.0 and δ 3.24–3.33 were excluded from the analysis due to the residual signals of water and methanol, respectively.

Metabolites were quantified by integrating an optimum signal of each compound, or class of compounds, selected according to minimal crowdedness and signal overlapping. These signals are shown in bold characters in Table 1. Peak integration was performed with MestReNova software (v. 6.1.1, Mestrelab Research SL, Santiago de Compostela, Spain). Relative concentrations of the metabolites were determined in each sample dividing the peak areas by that of the internal reference, TMSP, in each replicate.

Statistical analysis

Principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) were performed with SIMCA-P software (v. 12.0, Umetrics, Umeå, Sweden) with scaling based on the Pareto method. To determine the significance of differences in thrips damage and metabolite concentration between each IL and the recurrent parent, *S. lycopersicum* M82, *T*-tests were performed at a confidence level of 0.05. Correlations were verified through Spearman's ρ coefficient. All statistical tests were performed using SPSS v. 17.0 (SPSS Inc., Chicago, IL, USA). Concentration averages were determined for each metabolite in all ILs and transformed into folds of the corresponding value in M82. These ratios were centered to the unity and plotted in a color-scaled heat map using the public-domain open-source software Multi Experiment Viewer, MeV v 4.5 (Saeed et al., 2003). Euclidean distance and complete linkage were used for the hierarchical cluster analysis.

Results and discussion

Quantitative trait loci (QTL) for thrips resistance

In the whole plant choice bioassay thrips herbivory varied significantly throughout the introgression lines, ranging from zero to a maximum of 200 mm^2 of silver damage per plant (Fig. 1). However, the variability in silver damage between replicates was high in most cases, which contributes to obscuring performance differences in the IL population against WFT. Lines were designated resistant or susceptible as their silver damage levels were respectively lower or higher than that of *S. lycopersicum* M82 at $P \leq 0.05$. Insect damage on M82, with 33.6 mm^2, was on average 5.8 times higher than on the resistant lines, with 5.8 mm^2, (t=6.674 df =35, P<0.0001) and 4.3 times lower than on the susceptible ones, with 143.3 mm^2, (t=7.573 df =14, P<0.0001). Resistant lines must contain specific polymorphic genomic segments or QTL from the wild parent that are associated with an increased level of thrips resistance compared to *S. lycopersicum*. Susceptible lines, on the other hand, may have either lost genomic fragments related to thrips resistance in M82 or gained QTL from the donor parent associated with an increased level of susceptibility to WFT.

Only two QTL were detected for increased susceptibility to WFT, IL7-4-1 and IL11-2 (Fig. 2). Interestingly, the number of resistant lines was much higher and most of them derived from a

few chromosomes. Particularly relevant were ILs 1-3, 4-1-1, 4-3, 4-4, 5-5, 8-1-D, 8-1-3, 8-2, 10-2 and 10-2-2. Fine mapping for thrips resistance allowed the identification of several clearly defined shorter trait loci (Fig. 2). No previous reports are known to date on direct QTL analysis for pest resistance in the *pennellii* x *lycopersicum* introgression population. Indirect mapping, however, has been performed through QTL analysis for the accumulation of acylsugars. First on an interspecific cross F2 population (Mutschler et al., 1996) and second on the *pennellii* x *lycopersicum* introgression population (Schilmiller et al., 2010). Acylsugars are proven trichomal defense compounds of *Solanum* spp. (Simmons & Gurr, 2005; Mirnezhad et al., 2010). In the interspecific cross five genomic regions associated with different aspects of acylsugar production were identified. Two on chromosome 2 and one each on chromosomes 3, 4 and 11. While in the introgression population QTL for acylsugars were detected on four different chromosomes, 1, 5, 8 and 11. QTL overlapping occurred then only on chromosome 11, specifically at IL11-3, which was consistently reported in both studies to be associated with higher levels of acylsugars. In another interspecific cross F2 population between *S. habrochaites* (LA1777) and *S. lycopersicum* four QTL were identified for tomato resistance to *B. tabaci* (Momotaz et al., 2010). Based on this IL11-3 was expected to be thrips resistant. Surprisingly, this was not the case, suggesting either that IL11-3 did not yield amounts of acylsugars high enough to deter thrips or that other factors, morphological or chemical, play a more important role in tomato resistance to thrips.

Multivariate analysis on the NMR data of the ILs

To identify the putative chemical bases for resistance to WFT in the IL system ^{1}H NMR spectra of all lines were obtained and subsequently analyzed with pattern recognition multivariate data analysis. Principal component analysis was applied to the ^{1}H NMR spectra of resistant and susceptible lines, along with M82 as control, to identify differences in the NMR fingerprint of these sample groups. The scatter plot for the first two components, accounting for 62% of the variance, unfortunately shows no apparent clustering of the classes (Fig. 3). Such overlapping of principal component scores indicates that the ^{1}H NMR spectra of *S. lycopersicum* and the resistant and susceptible lines are rather homogeneous. As a second approach to unveil the chemistry of thrips resistance partial least square discriminant analysis followed. The obtained model, however, failed the cross validation tests confirming the lack of consistent spectral differences between susceptible, resistant and control samples. According to these results the

variation in resistance to WFT among the introgression lines may have its origin either in compounds present at concentrations below the NMR detection limit or in morphological traits such as hairiness and toughness. Unfortunately, none of these physical features were surveyed in our harvest. Glandular trichomes in particular have been extensively reported as effective defenses against many different pests (Simmons & Gurr, 2005), and their exudates usually go undetected in NMR analysis of whole leaf samples (see chapter 2).

Metabolic profiling of the ILs

In a further step the ^{1}H NMR spectra of the ILs were thoroughly scrutinized to identify and quantify the maximum possible number of metabolites extracted from the tomato leaves. Table 1 summarizes the spectral data of all compounds or classes of compounds that were unequivocally identified. The list comprises 24 major plant metabolites, primary and secondary, of very diverse structures and polarities. Most of these compounds can be classified into one of five main classes: lipids, amino acids, free sugars, organic acids or phenolics. Among the latter only catechol-like metabolites could be detected: chlorogenic acid (CGA), the four regioisomers of caffeoylglucaric acid (CQA) and the flavonoid glycoside rutin. The exact position of the caffeoyl moiety on the CQA regioisomers was not determined.

Correlation analysis was performed between all pair combinations of quantified metabolites. Thrips damage was included as well in order to verify the results from the multivariate data analysis. The minimum value of the Spearman correlation coefficient (ρ) to consider two variables as correlated was set at 0.5, for which probability values (P) were in all cases lower than 0.0001. Under this criterion no single correlation was detected between silver damage and any of the listed metabolites. Strong correlations were mostly observed within classes of metabolites. Unsaturated fatty acids, for instance, were correlated with sterols (ρ=0.852) and saturated fatty acids, mainly as palmitic acid, (ρ=0.591). These correlations between lipids may result from their co-accumulation as membrane building blocks rather than from a metabolic network connection. Amino acids in general were also correlated. Coefficient values between phenylalanine and threonine, alanine and aspartic acid were 0.513, 0.653 and 0.457, respectively. A significant correlation was also detected between threonine and alanine (ρ= 0.552). Although these amino acids have diverging biosynthetic pathways and are the precursors of very different secondary metabolites their metabolism in plants is highly regulated (Galili & Hofgen, 2002), resulting in false correlations. Regarding the small organic acids, formic

acid and acetic acid did not correlate with any other metabolite whereas malic acid was positively correlated with fumaric acid (ρ=0.664). Both fumaric and malic acid were negatively correlated with the signal of unsaturated fatty acids (ρ=-0.545 and -0.528, respectively) and sterols (ρ =-0.550 and -0.429, respectively). Fumaric acid was also negatively correlated with rutin and glucose (ρ=-0.698 and -0.510, respectively) but positively with alanine and phenylalanine (ρ=0.623 and 0.646, respectively). The highest coefficients were obtained for correlations between the regioisomers of CQA, ranging from 0.851 to 0.690. Such close structures are undoubtedly genetically linked, *i.e.* sharing the same set of QTL, and for that their expression levels are expected to be highly correlated. CQA isomers were in turn correlated with the other phenolics, rutin and CGA (ρ=0.511 and 0.530, respectively). All these phenolic compounds share the same catechol-like hydroxycinnamic moiety, which relates them all upstream in their metabolic biosynthetic pathway, explaining in this way their correlation. A matrix plot showing some of the most relevant correlations is presented in Figure 4. The results from the multivariate analysis were confirmed as no strong correlations were observed between thrips damage and any of the identified metabolites. The highest correlation coefficients obtained were -0.327 (*P*=0.004) and 0.380 (*P*=0.0007) for sterols and the nucleotide uridine-diphosphate glucose, respectively.

Correlation analysis, however, does not provide any information about the genetic regulation of metabolite concentrations. To close in on the genetic bases for increased or decreased content of relevant metabolites their relative concentrations must be compared throughout the entire IL population. A very simple visual method is the use of color-scaled expression maps or heat maps (Fig. 5), which allow a quick identification of ILs with lower or higher content of any metabolite relative to the control, M82. An optimized hierarchical clustering analysis has been additionally applied. In such analysis both metabolites and ILs have been organized according to maximum similarity in concentration levels. The grouping of all isomers of CQA somehow validates this type of clustering as a similarity analysis. Some false positive linkage can also be recognized with clustering analysis. In this case threonine and fatty acids have been placed next to each other due to a matrix interference in the ^{1}H NMR spectra. The proton integration signals of these metabolites overlap with that of a very broad band corresponding to all non-reactive methylenes, at around 1.3 ppm, which obscures the concentration differences of these metabolites throughout the IL population.

Genetic mapping of QTL for phenolics content

To verify whether the differences detected in metabolic concentration levels were significant *T*-tests between each line and M82 were conducted. A total of 268 QTL for all the quantified metabolites in the introgression population were obtained (Table 2). Confirmed QTL involved in the foliar accumulation of phenolics were mapped as presented in Figure 6. The overall concentration ratios varied between 0.2 and 2 times relative to M82, with a few exceptional cases such as CGA, which reached an increase of 2.6 and up to 4.4 times in ILs 10-1 and 10-1-1, respectively. These two lines define therefore a quantitative locus for increased content of CGA of about 12 centimorgans located between markers TG230 and TG303 on chromosome 10 (Fig. 6). CGA is a ubiquitous secondary metabolite in the plant kingdom with a broad spectrum of alleged and proven biological activities. Its bioavailability (Scalbert et al., 2002; Williamson et al., 2000) and antioxidant properties (Rice-Evans et al., 1997) have drawn a lot of attention to CGA not only for its potential in nutraceutics (Wildman, 2007) but also as a plant defense compound (Leiss et al., 2009; Summers & Felton, 1994). The identified QTL for increased levels of CGA represents a very important finding as it may provide one of the missing links to solve the controversial puzzle of CGA biosynthesis and accumulation in plants (Comino et al., 2009). Expression of this genetic segment may help identify the key enzyme(s) necessary for a plant to accumulate CGA. Three main routes for the synthesis of CGA in plants have been proposed, involving caffeoyl-CoA quinate caffeoyl transferase, HQT (Ulbrich & Zenk, 1979), caffeoyl D-glucose: quinate caffeoyl transferase, HCGQT (Villegas & Kojima, 1986) and *p*-coumaroyl-CoA quinate *p*-coumaroyl transferase, HCT (Hoffmann et al., 2003) as the respective rate-limiting enzymes. Although Niggeweg et al. (2004) demonstrated that HQT catalyzes the main route of CGA production in Solanaceous spp. genes encoding for these hydroxycinnamoyl-CoA shikimate/quinate tranferases have been detected only on chromosome 7 of tomato (Sol Genomics Network, http://solgenomics.net), which did not bear any QTL for high levels of CGA (Fig 6). No other phenolic compounds, rutin or CQA, colocalized on IL10-1-1, suggesting that this locus may control aspects of quinic acid metabolism, either synthesis, accumulation or, most likely, condensation to the hydroxycinnamic group.

QTL for phenolics in tomato fruits have also been identified before in this same *pennellii* x *lycopersicum* introgression population (Rousseaux et al., 2005). Authors identified a total of 20 QTL associated with antioxidants, five for antioxidant capacity, six for ascorbic acid and nine for total phenolics, of which only IL10-1 for antioxidant capacity overlapped with our QTL map for

catechol-like phenolics, specifically for higher content of CGA. In some cases QTL are in contradiction. For instance, IL7-4 for increased total phenolics coincides with one of our QTL for lower rutin levels. Such discrepancies may indicate that different unlinked sets of genes control these same traits on leaves and fruits, or may partly result from the fact that Rouseaux et al. (2005) did not profile any specific phenolics. Antioxidant activity and total phenolic content were determined through general non-chromatographic methods, which on the one hand include more compounds than those quantified in our study and on the other hand are more susceptible to interference from other unrelated metabolites. High variation between year trials in the work of Rouseaux et al. (2005) may account as well for the discrepancies.

QTL analysis confirmed that thrips resistance was not correlated with any of the phenolic compounds. Silver damage did not map consistently with any metabolic trait. In some cases opposing associations were observed.

In order to validate our findings their stability against external variables such as radiation, humidity, air-borne elicitors and pollutants, among others, must be verified. Ideally several harvests from different years should be considered in this kind of studies. Using the same introgression population it has been shown in similar metabolic genomics studies conducted on tomato fruit that environmental conditions have a greater impact on the metabolome compared to genotype (Phuc et al., 2010).

Introgression populations represent an excellent tool to study the genetics of not only pest resistance but also many other relevant traits, including the accumulation of phytochemicals of high added value. The application of NMR metabolomics to a stable marker-assisted introgression population enabled us to discover QTL for differential expression of important secondary metabolites, such as chlorogenic acid and rutin that could easily be incorporated into new domesticated tomatoes for crop improvement purposes. Therefore, this kind of approaches represents an effective alternative to genetic manipulation in plant metabolic engineering.

Table 1. ^{1}H NMR data of the metabolites identified in the *Solanum pennellii* x *lycopersicum* introgression population. Signals in bold characters were used for relative quantification. Dried leaf samples were directly extracted with MeOD-D_2O 7:3 for ^{1}H NMR (600 MHz) analysis.

	Leaf metabolite	Chemical shift of main signals
1	Sterols	0.81 (s), **0.84** (s)
2	SFA	0.88 (brs), **1.26** (brs)
3	UFA	0.95 (t, *J* = 7.5 Hz), **1.30** (brs), 5.35 (brs)
4	Threonine	1.33 (d, *J* = 6.6 Hz)
5	Alanine	1.48 (d, *J* = 7.2 Hz)
6	Acetic acid	1.99 (s)
7	Aspartic acid	**2.65** (dd, *J* = 17.4, 9.3 Hz), 2.83 (dd, *J* = 17.4, 3.5 Hz)
8	GABA	1.91 (m), 2.35 (t, *J* = 7.2 Hz), **3.00** (t, *J* = 7.2 Hz)
9	Choline	3.21 (s)
10	Malic acid	2.54 (dd, *J* = 15.8, 8.3 Hz), 2.78 (dd, *J* = 15.8, 3.9 Hz), **4.29** (dd, *J* = 8.3, 3.9 Hz)
11	Glucose	5.16 (d, *J* = 3.7 Hz)
12	Sucrose	4.14 (d, *J* = 8.7 Hz), **5.40** (d, *J* = 3.8 Hz)
13	Rutin	**6.30** (d, *J* = 2.5 Hz), 6.51 (d, *J* = 2.5 Hz), 6.95 (d, *J* = 8.4 Hz), 7.67 (d, *J* = 2.5 Hz)
14	CGA	**6.34** (d, *J* = 15.9 Hz), 7.11 (d, *J* = 2.5 Hz), 7.59 (d, *J* = 15.9 Hz)
15	CQAa	6.36 (d, *J* = 15.9 Hz)
16	CQAb	**6.37** (d, *J* = 15.9 Hz), 7.61 (d, *J* = 15.9 Hz)
17	Fumaric acid	6.64 (s)
18	PAL	**7.32** (d, *J* = 8.0 Hz), 7.36 (d, *J* = 8.0 Hz)
19	CQAc	6.45 (d, *J* = 15.9 Hz), **7.63** (d, *J* = 15.9 Hz)
20	CQAd	6.45 (d, *J* = 15.9 Hz), **7.65** (d, *J* = 15.9 Hz)
21	UDPG	7.99 (d, *J* = 8.0 Hz)
22	Formic acid	8.45 (s)
23	AMP	8.23 (s), **8.55** (s)
24	Trigonelline	8.10 (dd, *J* = 8.2, 6.3 Hz), 8.85 (d, *J* = 6.2 Hz), 8.89 (d, *J* = 8.1 Hz), **9.16** (s)

SFA: saturated fatty acids, UFA: unsaturated fatty acids, GABA: γ-aminobutyric acid, CGA: chlorogenic acid, CQAa-d: regioisomers of caffeoylglucaric acid, PAL: phenyalanine, UDPG: uridine diphosphoglucose, AMP: adenosine monophosphate.

Table 2. Foliar metabolic QTL in tomato identified through the *Solanum pennellii* x *lycopersicum* introgression population. X-fold values represent the concentration ratio between each line and the recurrent parent *S. lycopersicum* M82, determined at a significance level of 0.05.

Metabolite	Locus	x-Fold	Metabolite	Locus	x-Fold	Metabolite	Locus	x-Fold
Acetic acid	IL 1-4	0.69 ± 0.04	CQAd	IL 1-1	0.79 ± 0.02	PAL	IL 1-1	0.85 ± 0.04
	IL 6-2-2	0.74 ± 0.05		IL 1-1-3	1.59 ± 0.17		IL 1-1-3	0.77 ± 0.00
	IL 9-2	1.31 ± 0.03		IL 1-3	0.73 ± 0.09		IL 1-3	0.74 ± 0.02
Alanine	IL 1-1	0.67 ± 0.04		IL 2-3	0.77 ± 0.04		IL 1-4-18	0.87 ± 0.00
	IL 1-1-2	0.69 ± 0.01		IL 2-6-5	1.27 ± 0.03		IL 2-1-1	0.78 ± 0.09
	IL 1-2	0.67 ± 0.09		IL 3-2	1.46 ± 0.03		IL 2-5	0.77 ± 0.01
	IL 2-1	1.70 ± 0.12		IL 3-3	0.72 ± 0.03		IL-3-1	0.68 ± 0.05
	IL 2-1-1	0.62 ± 0.09		IL 4-1	0.79 ± 0.06		IL 3-2	0.83 ± 0.04
	IL 2-4	0.70 ± 0.02		IL 4-1-1	1.38 ± 0.05		IL 3-5	0.84 ± 0.05
	IL 2-5	0.73 ± 0.05		IL 4-2	1.45 ± 0.00		IL 4-1-1	0.79 ± 0.00
	IL 2-6-5	0.67 ± 0.11		IL 5-5	0.78 ± 0.06		IL 4-4	0.86 ± 0.03
	IL-3-1	0.62 ± 0.07		IL 6-2	1.22 ± 0.07		IL 5-2	0.76 ± 0.03
	IL 3-3	0.67 ± 0.08		IL 6-2-2	0.67 ± 0.03		IL 5-5	1.17 ± 0.04
	IL 4-1-1	0.61 ± 0.01		IL 7-4	1.25 ± 0.10		IL 6-1	1.15 ± 0.03
	IL 5-2	0.41 ± 0.02		IL 7-5-5	0.78 ± 0.06		IL 6-2-2	1.55 ± 0.10
	IL 5-4	0.71 ± 0.05		IL 8-2	0.67 ± 0.01		IL 7-5	1.19 ± 0.02
	IL 5-5	1.27 ± 0.06		IL 9-1	0.81 ± 0.00		IL 8-3	1.39 ± 0.12
	IL 6-2	0.70 ± 0.06		IL 9-3-1	1.29 ± 0.05		IL 9-1	0.77 ± 0.07
	IL 7-4-1	1.63 ± 0.21		IL 10 1-1	0.62 ± 0.05		IL-9-1-2	0.79 ± 0.07
	IL 8-3	0.70 ± 0.03		IL 11-1	0.70 ± 0.02		IL 9-1-3	1.76 ± 0.20
	IL 9-1-3	2.03 ± 0.45		IL 11-2	0.78 ± 0.00		IL 9-2	0.83 ± 0.03
	IL 9-3-2	1.82 ± 0.13		IL-11-4-1	0.76 ± 0.08		IL 9-2-6	1.17 ± 0.05
	IL 10-1	1.80 ± 0.06		IL 12-1	1.37 ± 0.09		IL 9-3-1	1.56 ± 0.06
	IL 10 1-1	1.37 ± 0.14		IL 12-3	0.81 ± 0.04		IL 9-3-2	1.34 ± 0.16
	IL-11-4-1	0.54 ± 0.02		IL 12-3-1	0.77 ± 0.02		IL 10-1	1.25 ± 0.03
	IL 12-1	1.54 ± 0.27	Choline	IL 1-4-18	1.00 ± 0.12		IL 10-2	0.83 ± 0.00
	IL 12-1-1	0.66 ± 0.05		IL 3-3	0.76 ± 0.03		IL 11-2	0.81 ± 0.07
AMP	IL 1-4	1.18 ± 0.01		IL 4-1	0.78 ± 0.02		IL-11-4-1	0.81 ± 0.01
	IL 1-2	0.82 ± 0.02		IL 5-1	1.29 ± 0.04		IL 12-1	1.31 ± 0.15
	IL 1-3	0.67 ± 0.00		IL 8-2	0.64 ± 0.01		IL 12-1-1	0.71 ± 0.05
	IL 2-2	1.17 ± 0.04		IL 9-2	0.76 ± 0.00		IL 12-4-1	1.18 ± 0.07
	IL 2-5	0.76 ± 0.10	CG3A	IL 1-1-3	1.70 ± 0.35	Rutin	IL 1-2	1.85 ± 0.02
	IL-3-1	0.78 ± 0.06		IL 1-3	0.66 ± 0.07		IL 3-2	2.18 ± 0.04

	IL 3-4	0.76 ± 0.05		IL 3-2	1.70 ± 0.01		IL 4-1-1	1.41 ± 0.02
	IL 5-2	0.72 ± 0.11		IL 4-2	1.83 ± 0.37		IL 4-2	1.83 ± 0.20
	IL 5-4	0.78 ± 0.08		IL 4-3-2	1.41 ± 0.07		IL 4-3	1.52 ± 0.13
	IL 5-5	0.80 ± 0.04		IL 5-4	0.69 ± 0.03		IL 5-1	1.63 ± 0.08
	IL 6-1	1.24 ± 0.05		IL 5-5	0.64 ± 0.05		IL 5-5	0.68 ± 0.00
	IL 6-2-2	0.70 ± 0.03		IL 7-5-5	0.48 ± 0.05		IL 7-4-1	0.68 ± 0.02
	IL 7-5	1.33 ± 0.09		IL 8-2	0.58 ± 0.02		IL 7-5-5	0.40 ± 0.08
	IL 8-1-1	0.67 ± 0.07		IL 10-1	2.56 ± 0.21		IL 8-1-1	0.53 ± 0.01
	IL 8-2-1	0.84 ± 0.01		IL 10 1-1	4.48 ± 1.00		IL 9-1-3	0.62 ± 0.01
	IL 8-3	1.35 ± 0.11		IL 11-1	0.57 ± 0.03		IL 9-3	0.62 ± 0.06
	IL-9-1-2	0.73 ± 0.06		IL-11-4-1	0.68 ± 0.04	Sterols	IL 1-1	1.44 ± 0.01
	IL 9-1-3	1.26 ± 0.06		IL 12-3	0.52 ± 0.00		IL 1-2	1.64 ± 0.01
	IL 9-2-6	1.42 ± 0.22		IL 12-3-1	0.58 ± 0.03		IL 2-4	0.60 ± 0.03
			Formic					
	IL 11-2	1.22 ± 0.06	acid	IL 8-3-1	0.53 ± 0.09		IL 4-4	0.69 ± 0.09
	IL-11-4-1	0.81 ± 0.02		IL 9-2-5	1.53 ± 0.18		IL 7-3	1.11 ± 0.10
Metabolite	**Locus**	**x-Fold**		**Metabolite**	**Locus**	**x-Fold**	**Metabolite**	**Locus**	**x-Fold**
	IL 12-2	1.17 ± 0.06			IL 11-3	1.46 ± 0.00		IL 7-4-1	0.74 ± 0.01
				Fumaric					
	IL 12-3	1.18 ± 0.06		acid	IL 1-1	0.32 ± 0.02		IL-9-1-2	1.08 ± 0.26
CQAa	IL 1-1-3	1.39 ± 0.06			IL 5-2	0.37 ± 0.00		IL 9-1-3	1.15 ± 0.15
	IL 2-6-5	1.22 ± 0.01			IL 7-4-1	1.92 ± 0.23		IL 9-3	1.11 ± 0.10
	IL 3-2	1.30 ± 0.03			IL 7-5	2.32 ± 0.20		IL 10-1	0.88 ± 0.07
	IL 4-1-1	1.33 ± 0.05			IL 8-1 D	1.84 ± 0.29		IL 12-2	1.42 ± 0.03
	IL 4-2	1.39 ± 0.07			IL 8-3	1.83 ± 0.04	Sucrose	IL 2-1	0.72 ± 0.08
	IL 7-1	1.30 ± 0.12			IL-9-1-2	0.37 ± 0.09		IL 2-6	0.83 ± 0.01
	IL 7-5-5	0.68 ± 0.10			IL 9-1-3	2.54 ± 0.05		IL 3-3	0.85 ± 0.03
	IL 8-1-1	0.75 ± 0.01			IL 9-3	2.10 ± 0.20		IL 4-3	1.22 ± 0.00
	IL 8-2	0.72 ± 0.03			IL 9-3-2	1.76 ± 0.15		IL 6-2	1.21 ± 0.05
	IL 8-3	0.73 ± 0.04			IL 10-1	1.66 ± 0.05		IL 8-1 D	0.78 ± 0.04
	IL 9-1-3	0.68 ± 0.04		GABA	IL 2-4	0.56 ± 0.07		IL 8-3	1.20 ± 0.01
	IL 10 1-1	1.35 ± 0.12			IL 3-4	1.42 ± 0.06		IL 9-1	0.79 ± 0.08
	IL 11-1	0.78 ± 0.03			IL 6-1	0.56 ± 0.07		IL 9-3	0.77 ± 0.06
	IL 11-2	0.82 ± 0.02			IL 8-3	0.51 ± 0.07		IL 11-1	0.78 ± 0.04
CQAb	IL 1-1	0.61 ± 0.11		Glucose	IL 1-1	1.32 ± 0.10		IL 11-4	1.23 ± 0.07
	IL 1-1-3	1.48 ± 0.08			IL 1-2	1.46 ± 0.02		IL 12-1-1	1.37 ± 0.01
	IL 1-4-18	1.28 ± 0.09			IL 2-1	0.61 ± 0.02	Threonine	IL 2-1	1.18 ± 0.01
	IL 2-2	1.28 ± 0.09			IL 2-4	0.52 ± 0.17		IL 3-3	0.85 ± 0.05
	IL 2-3	0.62 ± 0.19			IL 2-5	1.41 ± 0.07		IL 4-4	0.86 ± 0.05

	IL 2-6-5	1.24 ± 0.04		IL-3-1	1.25 ± 0.04		IL 5-2	0.89 ± 0.00
	IL 3-2	1.39 ± 0.06		IL 3-3	0.48 ± 0.03		IL 7-1	1.10 ± 0.01
	IL 4-1-1	1.34 ± 0.01		IL 4-3	1.64 ± 0.28		IL 9-3-1	1.11 ± 0.02
	IL 4-2	1.44 ± 0.12		IL 5-4	1.45 ± 0.19		IL 9-3-2	1.10 ± 0.01
	IL 7-4	1.25 ± 0.10		IL 5-5	0.69 ± 0.04		IL 10 1-1	1.10 ± 0.01
	IL 7-5-5	0.73 ± 0.12		IL 6-1	0.61 ± 0.06		IL-11-4-1	0.83 ± 0.03
						Trigonelli		
	IL 8-1-1	0.78 ± 0.02		IL 6-2-2	0.66 ± 0.07	ne	IL 2-1	1.17 ± 0.00
	IL 8-2	0.73 ± 0.01		IL 8-1 D	0.55 ± 0.02		IL 3-2	1.39 ± 0.00
	IL 8-3	0.76 ± 0.08		IL 8-1-3	0.62 ± 0.06		IL 3-3	0.20 ± 0.00
	IL 9-1-3	0.72 ± 0.03		IL 8-2	0.65 ± 0.06		IL 3-4	0.30 ± 0.11
	IL 11-1	0.77 ± 0.00		IL 8-3	0.73 ± 0.07		IL 4-1	1.20 ± 0.06
	IL-11-4-1	0.66 ± 0.04		IL 9-2-5	0.59 ± 0.01		IL 6-2-2	1.68 ± 0.09
				IL 9-3	0.36 ± 0.04		IL 6-3	1.18 ± 0.03
				IL 10 1-1	1.33 ± 0.05		IL 8-1-1	0.67 ± 0.01
				IL 11-1	0.63 ± 0.01		IL 9-2-5	1.25 ± 0.06
				IL 11-3	0.64 ± 0.11		IL 9-3-1	1.69 ± 0.02
				IL 12-1-1	1.76 ± 0.14		IL 10-1	0.75 ± 0.04
				IL 12-2	0.67 ± 0.03		IL 12-2	0.79 ± 0.01
							IL 12-3	0.83 ± 0.01
						UDPG	IL 1-1	0.93 ± 0.32
							IL 2-3	0.58 ± 0.10
							IL 3-2	1.54 ± 0.06
							IL 6-2-2	2.87 ± 0.05
							IL 8-1-3	0.62 ± 0.09
							IL 8-3-1	0.61 ± 0.08
							IL 11-3	1.36 ± 0.00
							IL-11-4-1	0.63 ± 0.07
						UFA	IL 1-2	1.15 ± 0.06
							IL 7-4-1	0.81 ± 0.07
							IL-9-1-2	1.15 ± 0.06
							IL 9-1-3	0.84 ± 0.02

UFA: unsaturated fatty acids, GABA: γ-aminobutyric acid, CQA: caffeoylquinic acid (chlorogenic acid), CGA: caffeoylglucaric acid, PAL: phenyalanine, UDPG: uridine diphosphoglucose, AMP: adenosine monophosphate.

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Figure 1. Thrips herbivory as silver damage on the *Solanum pennellii* x *lycopersicum* introgression population. ANOVA for silver damage was performed against the parent *S. lycopersicum* M82.

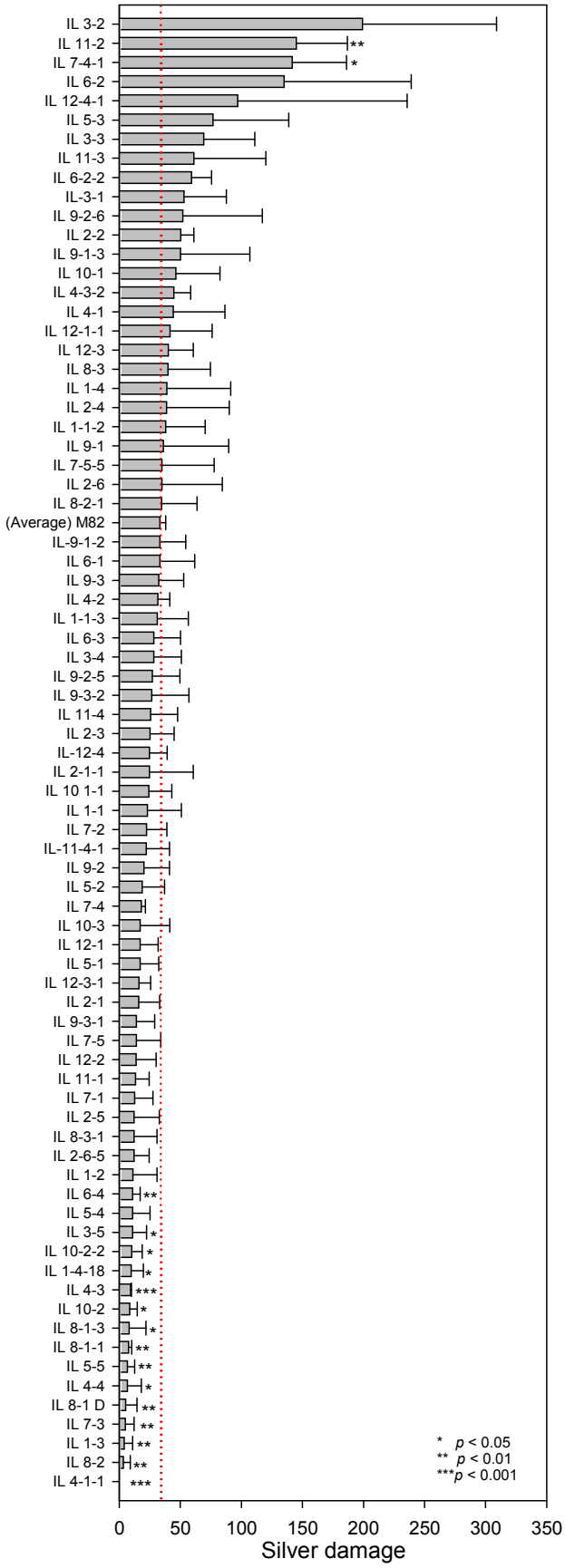

Figure 2. Genetic map of QTL for thrips performance on the *Solanum pennellii* x *lycopersicum* introgression population. Red bars matching QTL for increased thrips damage are located on the left side of each chromosome while those on the right side correspond to QTL for decreased thrips damage relative to the parent *S. lycopersicum* M82.

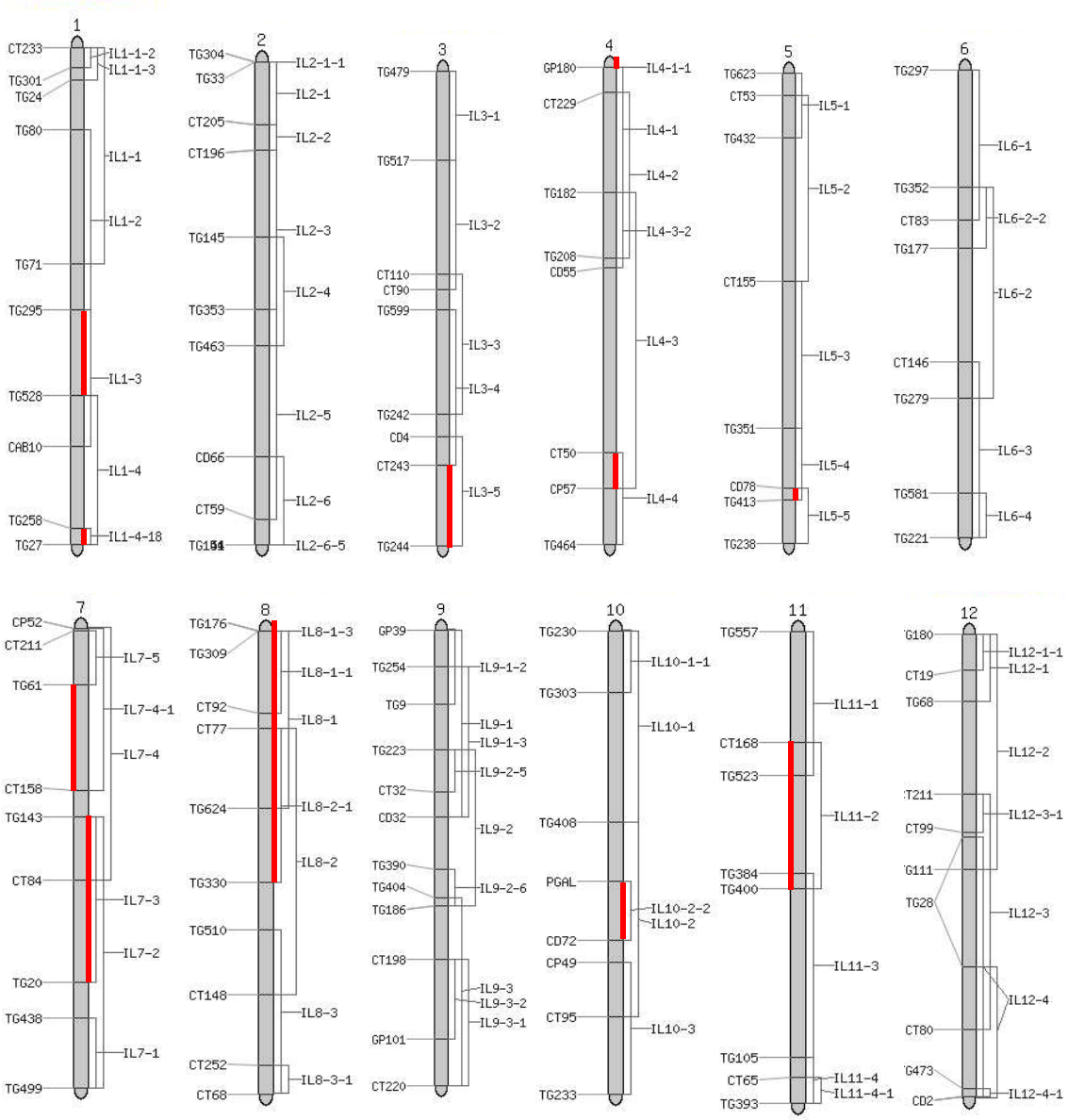

Figure 3. Principal component analysis performed on the ^{1}H NMR data of susceptible and resistant introgression lines. Points represent the scores of the first two principal components for lines with levels of thrips damage significantly higher (susceptible, S, red) and lower (resistant, R, blue) than the parent *Solanum lycopersicum* M82 (control, C, green). Each point represents the average of three (S and R) or five (C) biological replicates.

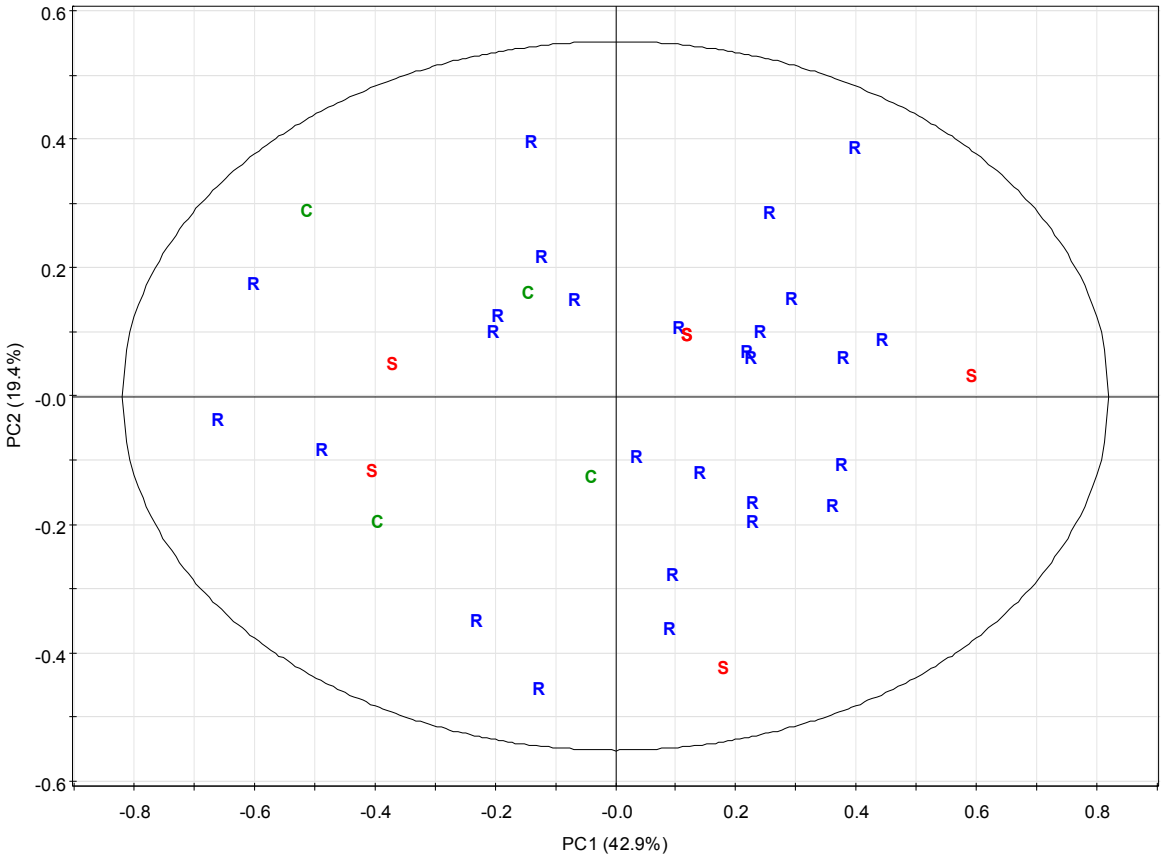

Figure 4. Metabolic correlation matrix. Each box contains a correlation plot between the relative concentrations, as determined by ^{1}H NMR, of relevant metabolites in the *Solanum pennellii* x *lycopersicum* introgression population. Silver damage (SD) was also included as the single ecological variable. Dots in every scatter plot represent the mean of three replicates for each of the 76 lines. Ellipses represent the 90% confidence contour. Narrow ellipses indicate strong correlations. Histograms on the diagonal show the data distribution for each trait. SFA: saturated fatty acids, UFA: unsaturated fatty acids, CGA: chlorogenic acid, CQA: caffeoylglucaric acid.

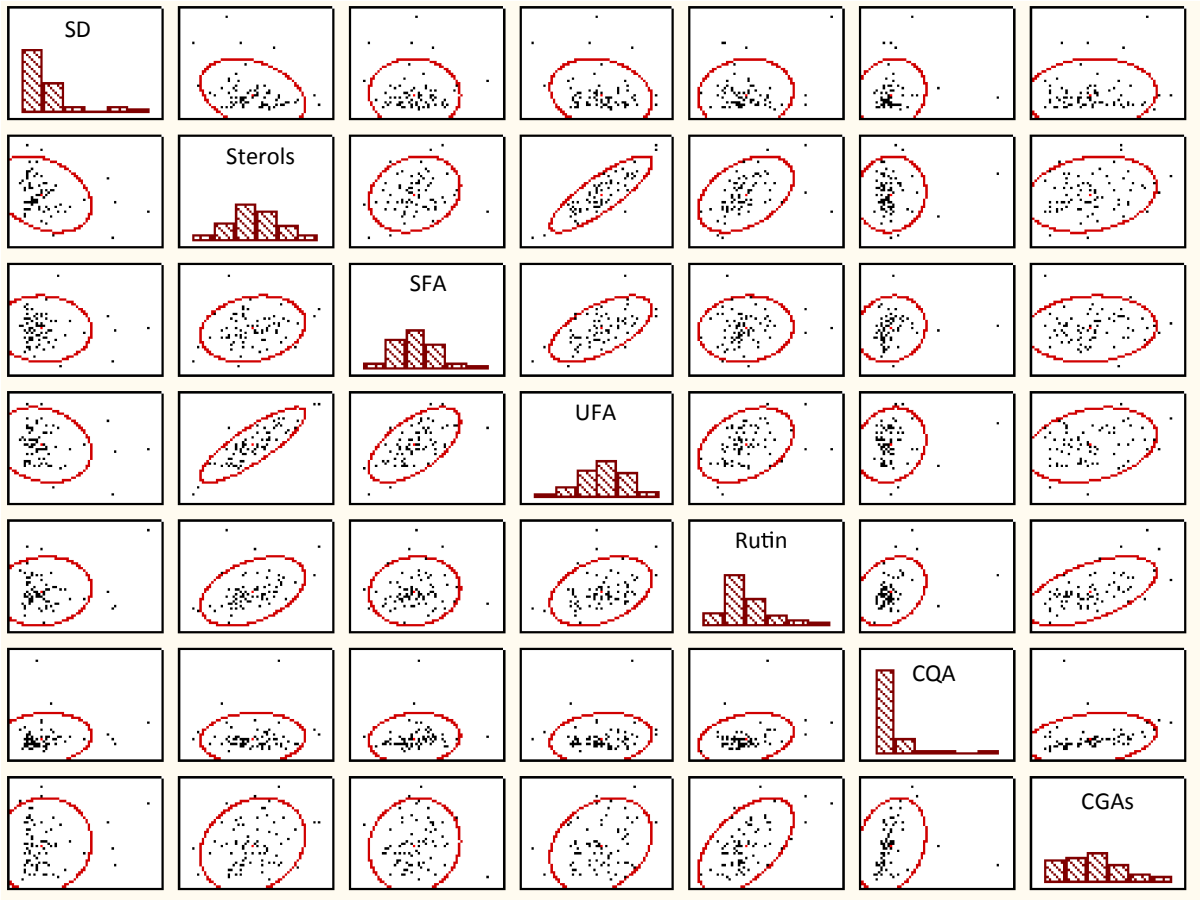

Figure 5. Metabolic heat map of the *Solanum pennellii* x *lycopersicum* introgression population. Relative concentrations of each metabolite, as determined by ^{1}H NMR, were expressed as folds of the corresponding mean in the parent *S. lycopersicum* M82 and plotted in a true color scale. Red and blue respectively denote increase and decrease in the content of a metabolite after a genomic region of *S. lycopersicum* M82 has been replaced by its homologous from *S. pennellii.* Complete linkage was used for the hierarchical cluster analysis. SFA: saturated fatty acids, UFA: unsaturated fatty acids, GABA: γ-aminobutyric acid, CGA: chlorogenic acid, CQA: caffeoylglucaric acid, PAL: phenyalanine, UDPG: urid

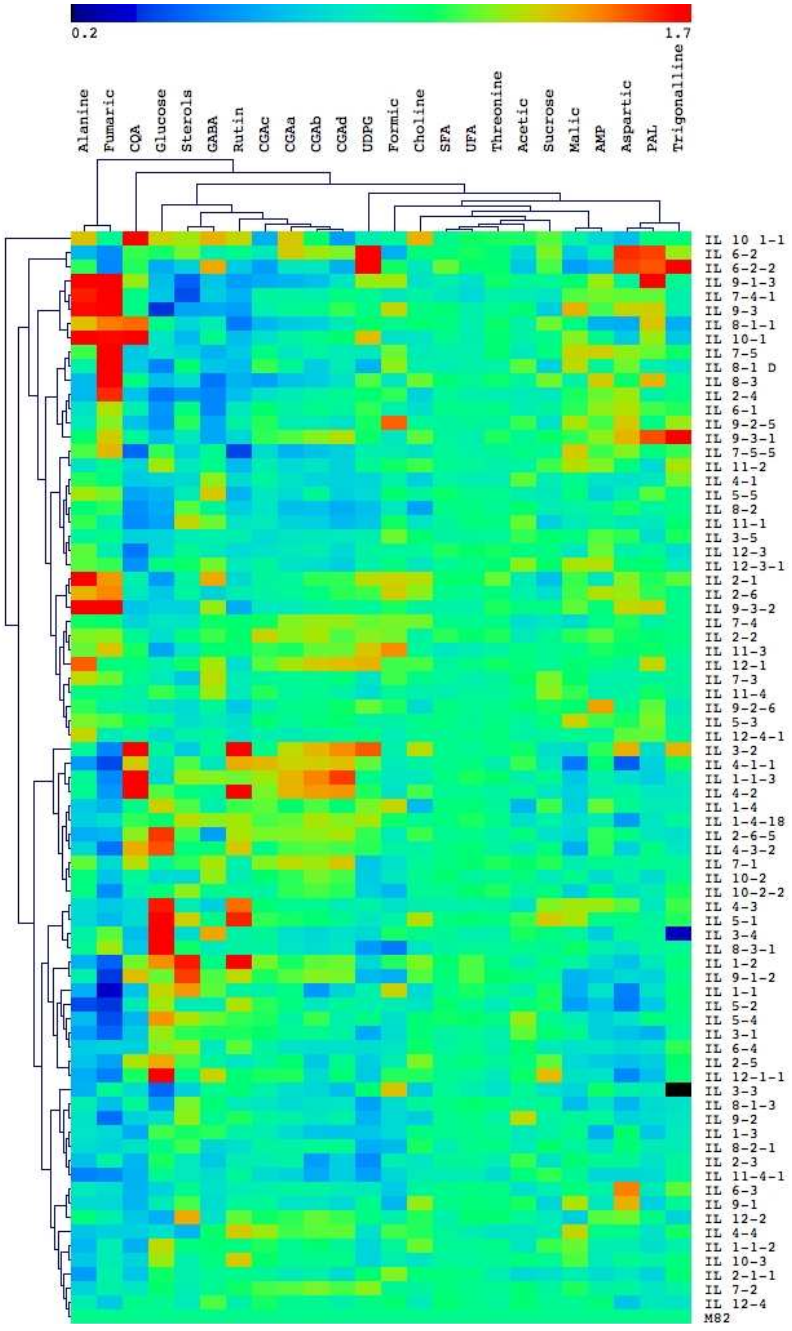

Figure 6. Genetic map of QTL for catechol-like phenolics in the *Solanum pennellii* x *lycopersicum* introgression population. Thrips damage has been included to facilitate co-localization analysis. Bars on the left side of each chromosome match QTL for increased levels of phenolics while those on the right side correspond to QTL for decreased levels relative to the parent *S. lycopersicum* M82.