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ORIGINAL ARTICLE

Methyl jasmonate deficiency alters cellular metabolome, including the aminome of tomato (*Solanum lycopersicum* L.) fruit

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Abstract Exogenous treatment with jasmonates (JA) has been shown to reduce the levels of polyamines in many plants. But the role of endogenous JA on polyamine biosynthesis or other cellular metabolites has thus far remained uninvestigated. We developed transgenic tomato (*Solanum lycopersicum* L.) having severely reduced methyl JA levels by silencing a fruit ripening-associated lipoxygenase (LOX), *SlLoxB*, using a truncated LOX gene under the control of the constitutive CaMV35S promoter. The LOX suppressed and MeJA-deficient fruits had lowered polyamine levels. Thus, these transgenic fruits were used as a plant model to evaluate the effects of reduced endogenous MeJA on cellular metabolites in ripening tomato fruits using NMR spectroscopy. During on-shelf ripening, transgenic fruits were significantly reduced in the content of 19 out of 30 metabolites examined, including Ile, Val, Ala, Thr, Asn Tyr, Glu, Gln, His, Phe, Trp, GABA, citrate, succinate, myo-inositol, unidentified compound B, nucleic acid compound Nucl1, choline, and trigonelline as compared to the wild-type azygous counterparts. A significant increase in β-glucose levels in transgenic fruits was observed at the pink stage. The transgenic fruits were equivalent to the wild type in lycopene level and chlorophyll degradation rates. Taken together, these results show that intracellular MeJA significantly regulates overall primary metabolism, especially aminome (amino acids and polyamines) of ripening fruits.

Kurt D. Kausch and Anatoly P. Sobolev contributed equally to this paper.

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Keywords Aminome · Fruit metabolism · Lipoxygenase · Oxylipins · Tomato · Transgenics

Abbreviations

JA	Jasmonates
MeJA	Methyl jasmonate
SAM	S-adenosylmethionine
dab	Days after breaker

Introduction

Biogenic amines putrescine (Put), spermidine (Spd), and spermine (Spm) are ubiquitous biological constituents that are essential for cell division and viability, influencing a myriad of growth and development processes in most living cells (Nambeesan et al. 2008; Mattoo and Handa 2008; Pegg 2009; Handa and Mattoo 2010). Put is a major diamine in plants and the substrate for triamine Spd and tetraamine Spm (Handa and Mattoo 2010). Put synthesis initiates from either arginine (Arg) by Arg decarboxylase (ADC) or ornithine (Orn) by Orn decarboxylase (ODC). Spd is then synthesized from Put and decarboxylated S-adenosylmethionine (dcSAM) by Spd Synthase, while Spm synthesis is catalyzed by Spm synthase that uses Spd and dcSAM as substrates. The production of dcSAM from S-adenosylmethionine (SAM) is a committed and rate-limiting step in polyamine pathway (Handa and Mattoo 2010). Back conversion of Spm to Spd and Spd to Put by spermidine/spermine N-1-acetyltransferase (SSAT) and polyamine oxidase (PAO) plays a role in regulating intracellular levels of different polyamines (Kamada-Nobusada et al. 2008; Fincato et al. 2010). Catabolism of PAs by diamine oxidases (DAO) and PAO can potentially regulate polyamine levels and also generate signaling molecule hydrogen peroxide (Nambeesan et al. 2008). In spite of significant knowledge on polyamine biosynthesis, catabolism and action that has emerged in recent years we still know very little about the mechanisms present in a living cell which regulate their intracellular levels and how that in turn impacts cellular metabolome.

A number of in vitro studies have implicated jasmonate (JA) family of plant hormones to interact with regulating polyamine biosynthesis. For instance, exogenous application of JA was found to increase the levels of free polyamines in tobacco (Biondi et al. 2001), barley (Walters et al. 2002), and wheat (Haggag and Abd-El-Kareem 2009) and, in some cases, their conjugates (Biondi et al. 2001; Walters et al. 2002). In this context, it is interesting that treatment with JA increased also transcripts of arginine decarboxylase, ornithine decarboxylase, SAM synthetase, SAM decarboxylase, polyamine oxidase, and diamine oxidase (Biondi et al. 2001; Perez-Amador et al. 2002; Biondi et al. 2003; Chen et al. 2006; Jung et al. 2007; Walia et al. 2007; Ziosi et al. 2009). However, JA effects on rice (Peremarti et al. 2010) were exactly opposite of the latter reports. Thus, the effects of exogenous application of JA on plant polyamine biosynthesis and genes involved need to be ascertained and confirmed through in vivo studies. JA and its derivatives are metabolic products of octadecanoid, oxylipin pathway, and ubiquitous plant growth regulators involved in myriad plant responses including biotic and abiotic stresses (Creelman and Mullet 1997; Pauwels et al. 2009). Lipoxygenase (LOX) is a key enzyme in the oxylipin pathway responsible for generating signaling molecules such as JA and its derivatives (Bell et al. 1995; Pauwels et al. 2009). We hypothesized that silencing of LOX would create a deficiency of JA in the transgenic plants, particularly in the fruit, and the LOX-silenced fruits would provide a good resource to explore the role of endogenous JA in plant processes.

We engineered a truncated form of a ripening-associated LOX gene under the control of CaMV35S promoter and introduced it into tomato. This way, transgenic tomato plants with severe reductions in LOX transcripts and protein in ripening fruits were developed. We show here that these LOX-silenced fruits exhibit 60–90% reduction in methyl jasmonate (MeJA) compared with wild-type azygous fruits. These MeJA fruits were profiled for changes in the metabolome that included aminome (amino acids and polyamines). We report here that reduction in endogenous MeJA significantly affects overall primary metabolism and dramatically impacts the levels of cellular metabolites including polyamines.

Materials and methods

Development of *SlLoxB* transgenic tomato plants

A 2.4-kb *EcoR*I fragment, representing nucleotides 158–2,598 of the full-length lipoxygenase gene Leu13681 (accession # U13681), was excised and cloned in sense orientation into an intermediate vector pTZ35rbcS, and the resultant chimeric construct was designated as pTZSL (Kausch and Handa 1995; Handa and Kausch 2002). The vector pTZ35rbcS was developed by ligating the 1.7-kb *EcoR*I-*Cla*I cassette containing the Cauliflower Mosaic Virus (CaMV) 35S promoter and the small subunit of the ribulose-1, 5-bisphosphate carboxylase/oxygenase (rbcS) 3′ terminator from pKYLX7 (Schardl et al. 1987) into *Kpn*I and *BamH*I sites of pTZ18U (Handa and Kausch 2002). The 4.1-kb DNA fragments containing the CaMV 35S promoter, 2,440 bp LOX DNA fragment in sense orientation, and rbcS 3′ terminator were obtained from pTZSL and

cloned between the *EcoR*I and *Sma*I sites in PMLJ1 (an *Agrobacterium*-based transformation vector) to obtain PMLSL. The chimeric gene construct pMLSL was mobilized into *Agrobacterium tumefaciens* strain pGV3850 using standard tri-parental mating techniques with a helper plasmid pGJ23 (Van Haute et al. 1983) and used to transform tomato (*Solanum lycopersicum* cv. Ohio 8245) cotyledons as described previously (Tieman et al. 1992). Two primary transformants, 650-1 and 650-2, obtained after independent transformation events, were selected for further evaluation. Southerns were used to confirm the presence of the introduced transgene.

Plant material

Wild-type tomato (*Solanum lycopersicum* cv. Ohio 8245) and transgenic plants were grown in greenhouse as described previously (Biggs and Handa 1989) for evaluating the expression patterns of the *SlLoxB* in developing fruits. For metabolic profiling, the T5 generation seeds were grown in a temperature-controlled greenhouse at the USDA-ARS, Beltsville Agricultural Research Center, Beltsville, MD, USA. Fruits were collected at the mature green (M) stage and transferred to the laboratory where they were monitored. Fruits at the following stages were collected for analysis: Mature green (G), breaker (B), turning (T), pink (P), and red-ripe (R) stages as defined previously (Mehta et al. 2002). The pericarp tissue was peeled, weighed, and frozen in liquid nitrogen before lyophilizing (Mattoo et al. 2006).

Northern blot analysis

Total RNA from fruit pericarp was extracted, 5 μg of which was separated by electrophoresis through a denaturing formaldehyde gel, blotted onto a Hybond N (Amersham Life Sciences, IL) nylon membrane. The filters were probed with ^{32}P-labeled *SlLoxB* cDNA as described earlier (Kausch and Handa 1997). The ^{32}P-labeled probe was prepared using an Ambion (Austin, TX) DECAprime II DNA labeling kit. Polygalacturonase transcripts and ribosomal RNAs were also detected similarly using corresponding ^{32}P-labeled probes (Goldsbrough and Cullis 1981; Biggs and Handa 1989).

Total and membrane protein extraction, SDS–PAGE, and immunoblotting

Frozen tomato fruit pericarp was homogenized in an isolation medium containing 50 mM Tris–Cl (1:1 w/v), pH 7.4, 10% (v/v) glycerol, 50 mM NaCl, 20 mM $MgSO_4$, 1 mM EDTA, 5 mM 2-mercaptoethanol, 0.5 mM phenylmethylsulfonylfluoride, 10μM leupeptin, and pepstatin (1 μg ml^{-1}; Mehta et al. 1992). During extraction, insoluble polyvinylpolypyrrolidone (20 mg g^{-1} FW) was added and mixed thoroughly with the slurry. The homogenate was filtered through two layers of cheesecloth and then Mira cloth. The filtrate was centrifuged at 500*g* for 2 min to remove debris and unbroken cells. The supernatant was then centrifuged at 25,000*g* for 30 min to pellet the membrane fraction. The pellet was washed twice with isolation medium containing 0.2 M NaCl. Membrane proteins were solubilized by stirring the pellet in the isolation medium and Triton X-100 (made to 1%, v/v) on ice for 1 h. The mixture was clarified by centrifugation to collect the solubilized membrane proteins. Proteins were electrophoresed on 12% SDS–polyacrylamide gels (Mehta et al. 1992). Either the gel was stained with Coomassie blue R-250 or proteins were electrotransferred onto a nitrocellulose membrane (0.1μ, Schleicher & Schull, Germany) in 25 mM Tris base, 192 mM glycine, 20% methanol, and 0.02% SDS. LOX protein was detected with an anti-LOX antibody and alkaline phosphatase-conjugated 2° anti-rabbit IgG as previously described (Kausch and Handa 1997).

Quantification of intracellular polyamines and methyl jasmonate levels

Intracellular levels of putrescine, spermidine, and spermine in pericarp tissue of fruit from wild-type and transgenic lines were determined as described previously (Mehta et al. 2002). Heptanediamine was added to the supernatants as an internal standard to determine recovery after dansylation of polyamines. For methyl jasmonate quantification, $HgCl_2$ (1.35 mg g^{-1} FW) was added to frozen pericarp tissue powdered in liquid nitrogen and allowed to thaw on ice. The thawed mixture was clarified by centrifugation at 10,000*g* for 5 min. Aliquots (3 ml) were transferred to 4-ml vials and capped with Teflon-lined septa. Samples were frozen at −80°C until analyzed. A solid-phase microextraction (SPME; Supelco Co., Bellefonte, PA, USA) fiber coated with polydimethylsiloxane (PDMS; 1 cm long, 100 μm thickness) was used to collect jasmonates and other compounds by virtue of their adsorption characteristics (Pawliszyn 1997). Jasmonate adsorption to the fiber was carried out for 20 min. The fiber was briefly rinsed in distilled water to remove any adhering extract, and the jasmonates were desorbed from the fiber at 250°C for 2 min directly into a glass-lined, splitless injection port of a gas chromatograph (GC, model 6890, Agilent Technologies, Rockville, MD, USA) equipped with a quadruple mass spectrometer (MS, model 5973, Agilent Technologies, Rockville, MD, USA) using 70 eV electron impact ionization. Jasmonates were separated from other compounds using a capillary column (HP 5, 11 m × 0.1 mm id., 0.34 μm coating thickness). Helium (6.0 research

grade) at a flow velocity of 52 cm s^{-1} was used as the carrier gas. Temperature program was isothermal for 2 min at 40°C and then raised at 10°C min^{-1} to 250°C, and held for 3 min. Injector and transfer line temperatures were 250 and 280°C, respectively. Mass spectra were collected over the range of *m/z* 40–240. Standards were used to compare mass spectra and identify jasmonates. Peak areas (from extracts and standard solutions of known concentration) were integrated based on *m/z* 156 ion counts as opposed to total ion counts. Exploratory experiments indicated that quantification of jasmonates by SPME techniques gave similar results to that obtained by direct injection of sample extracts and standards, even though the SPME fiber adsorbed only a small fraction of the jasmonates in tomato extracts during the 20-min adsorption period.

Ethylene production and contents of lycopene and chlorophyll

Rates of ethylene production by fruits were quantified by gas chromatography as described previously (Biggs et al. 1988). For measurements of lycopene and chlorophyll contents, 1.5 g of frozen pericarp tissue was ground in liquid nitrogen followed by homogenization with a Tissuemizer (Tekmar, Cincinnati, Ohio). The extracts were incubated overnight at 4°C with equal volume of 5:4 (v/v) hexane:acetone, and the organic phase analyzed for lycopene and chlorophyll contents by spectroscopic methods as described previously (Handa et al. 1985).

NMR spectroscopy for metabolic profiling

Dry powder (25 mg) from each fruit sample was rapidly dissolved in 1 ml of 0.4 M sodium phosphate buffer prepared in D_2O containing known amounts of an authentic standard, 3-(trimethylsilyl)-1-propionic-2,2,3,3-d_4 acid sodium salt (TSP), pH 6.5, and EDTA (0.01 mM). The solution was centrifuged at 11,000*g* for 7 min and the supernatant filtered to remove any insoluble material. NMR spectra of extracts were recorded at 300 K on a Bruker AVANCE AQS600 spectrometer operating at the proton frequency of 600.13 MHz. Proton spectra were referenced to the TSP signal ($\delta = 0.00$ ppm). Proton signals were acquired by co-adding 512 transients with a recycle delay of 3 s. The strong water signal was suppressed by using a NOESY-presat scheme with solvent pre-saturation during relaxation delay and mixing time (Braun et al. 1998). The one-dimensional spectra were run using 45° flip angle pulses of 6 µs, 32 K data points. After Fourier-transformation and manual phase correction, the baseline correction was performed using automatic cubic-spline correction with 25 points distributed over the spectrum. The assignment of ^{1}H NMR spectra was performed as previously published (Sobolev et al. 2003; Mattoo et al. 2006). Additionally, the signals of adenosine, adenosine monophosphate (AMP), tyrosine, histidine, and tryptophan were assigned using literature data (Fan 1996) and by addition of the corresponding standard compounds. The singlet at 8.531 ppm was attributed to both adenosine triphosphate (ATP) and adenosine diphosphate (ADP). The intensities of three unassigned signals denoted as B, Nucl1, and Nucl2, whose identity was not established, were also used in the statistical analysis. The intensity of 30 selected resonances attributed to 30 metabolites (Online Resource 1) was referenced to the intensity of the internal standard, TSP at 0.00 ppm. The spectra of 3–5 fruits were analyzed for each ripening stage. Data of ^{1}H-NMR in solution were subjected to statistical analysis by using Statistica software package for Windows (1997; edition by Statsoft) to determine whether and to what extent the selected variables were able to distinguish between the different tomatoes and their ripening stages. ANOVA and PCA were used to treat the data (Martens and Martens 2001).

Results

Tomato transformation with a truncated *SlLoxB* cDNA impairs expression of the endogenous *SlLoxB* in ripening fruits

A chimeric construct pMLSL (Fig. 1a) containing a truncated LoxB cDNA under the control of a constitutive promoter CaMV 35S in the sense orientation was introduced into the tomato genome using *Agrobacterium tumefaciens*-mediated transformation. Two independent transgenic lines, 650-1 and 650-2, found to have transcriptional silencing of *LoxB* gene, were selected for further characterization. The steady state levels of *LoxB* transcripts in the fruit [7 days after breaker (dab) stage of ripening] from the two transgenic lines fruits were about 40–50% of the level in the corresponding fruits from non-transformed wild-type line (Fig. 1b). The reduction in the LOX transcript levels was accompanied by a similar reduction in the LOX protein that cross-reacted with anti-TomloxB-specific antibodies (Fig. 1e). Such a decrease was not seen in the steady state levels of ripening-regulated polygalacturonase gene (Fig. 1c). These results indicated that the introduction of a truncated *SlLoxB* transgene resulted in homology-dependent gene silencing.

To evaluate inheritance of the introduced transgene and select lines homozygous for the transgene, the primary (T_0) transgenic lines, 650-1 and 650-2, were selfed and segregating T_0 seeds were collected. The T_1 seeds were collected from a number of independent segregating lines, and T2 seedlings from these seeds were scored for the

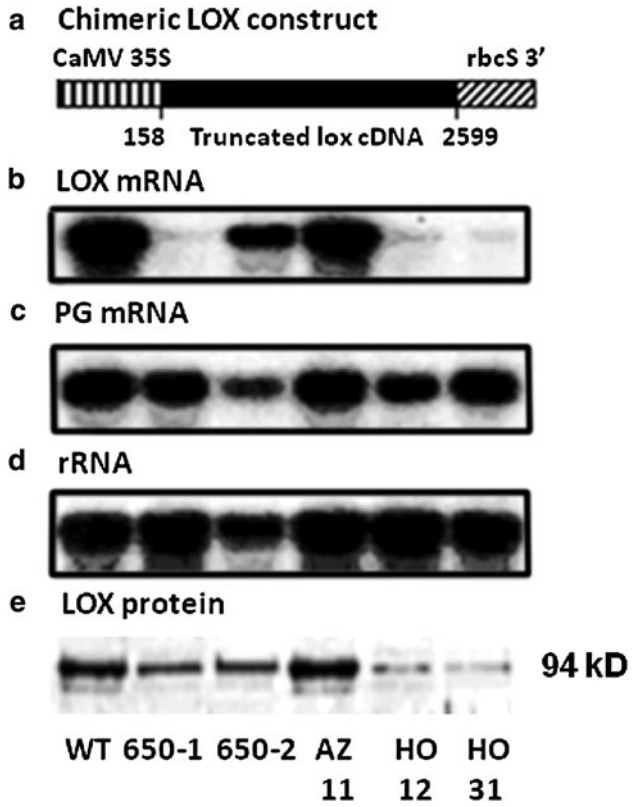

Fig. 1 Expression of a truncated *TomLoxB* under the CaMV 35S promoter causes homology-dependent gene silencing of the endogenous *SiLoxB* gene. **a** Chimeric gene construct pMLSL containing nucleotides 158–2,499 (accession U13681) of the fruit ripening-regulated lipoxygenase in sense orientation under the control of the CaMV 35S promoter and the rbcS 3′ terminator in Agrobacterium transformation vector pKYLX7. The pMLS construct also houses the selectable marker for kanamycin resistance behind the nopaline synthase promoter (not shown). **b** Northern bolt analysis showing steady state levels of LOX transcripts in ripening (7 days post breaker stage) fruits from wild-type Ohio 8245 (WT), two independent primary transformants 650-1 and 650-2, and segregating azygous (AZ)) and two homozygous (HO12 and HO31) progenies of transformant 650-1. **c** Steady state levels of polygalacturonase (*PG*) transcript using the same RNA preparations as in **b**. **d** Hybridization intensity of total RNA to radiolabeled rRNA to show loading of total RNA from different tissue samples. **e** Levels of immuno-reactive LOX protein (94-kD) in the ripe pericarp of the indicated genotypes. Ten micrograms of total protein extracted from the same frozen pericarp samples, portions of which were used for RNA isolation, were separated on 10% SDS–PAGE. Proteins were transferred to supported nitrocellulose and detected with rabbit antiserum to purified fruit LOX followed by alkaline phosphatase-linked goat anti-rabbit antiserum as described (Kausch and Handa 1997)

transgene by PCR. Among 13 independent T1 plants from 650-1 analyzed, 2 were homozygous, 9 heterozygous, and 2 lacked (azygous) the transgene gene, which indicated a normal Mendelian segregation for the introduced chimeric *LoxB* transgene. Red-ripe fruits from two homozygous lines, 650-1-12 (650-12HO) and 650-1-31, and azygous line 650-1-11 (650-1AZ) were analyzed for TomloxB transcript and protein levels. Fruits from both the homozygous lines were found to have highly reduced levels of TomloxB transcripts, which correlated with equally lower levels of immuno-cross-reactive LOX protein on immunoblots (Fig. 1b, e; compare AZ11 and WT lanes with HO12 and HO31 lanes). The fruit from segregating azygous lines and wild type had similar levels of *LoxB* transcript and protein (Fig. 1b, e; WT and AZ lanes), suggesting that the reduction in *LoxB* mRNA and protein in homozygous line was due to gene silencing. The reduced *LoxB* expression in line 650-12HO is stable and maintained in the subsequent 5 generations evaluated for the transgene (data not shown) and the protein (see Fig. 3).

Impaired expression of *SlLoxB* does not affect major fruit ripening characteristics

Changes in chlorophyll degradation, lycopene accumulation, and ethylene biosynthesis were investigated to determine whether reduced *SlLoxB* expression affected some of the fruit ripening processes. Slightly higher levels of chlorophyll were present in the 1 dab fruits from line 650-12HO, but significant differences in ripening-associated chlorophyll degradation compared with azygous wild-type fruits were not evident (Fig. 2). Lycopene accumulation in 650-12HO fruits was also similar to the wild-type fruits (Fig. 2). Although a consistent but slight reduction in ethylene levels throughout fruit ripening was evident in transgenic fruit compared with wild-type fruits, statistical treatment of the data did not reveal these to be significant (Fig. 2).

SlLoxB silencing resulted in reduced methyl jasmonate accumulation in ripening fruits

LOX is a key enzyme in the octadecanoid pathway that generates signaling molecules such as jasmonic acid (Bell et al. 1995; Pauwels et al. 2009). We hypothesized that silencing of a fruit-specific LOX would create a deficiency of jasmonates in the transgenic plants, particularly in the fruit. Therefore, levels of methyl jasmonate were quantified in the fruit at different ripening stages from azygous 650-1AZ and the *SlLoxB*-silenced 650-12HO plants (Fig. 3). Indeed, silencing of *SlLoxB* resulted in a dramatic decrease in the content of methyl jasmonate in transgenic tomato fruit, which were lowered three to ninefold compared with the 650-1AZ fruits. Interestingly, marked and significant decrease in methyl jasmonate was also seen in mature green (G) fruits. Since *SlLoxB* is primarily expressed in ripening fruits, these results suggest that the introduced truncated *SlLoxB* transgene also down-regulated the expression of other LOXs responsible for the production of methyl jasmonate in developing fruit.

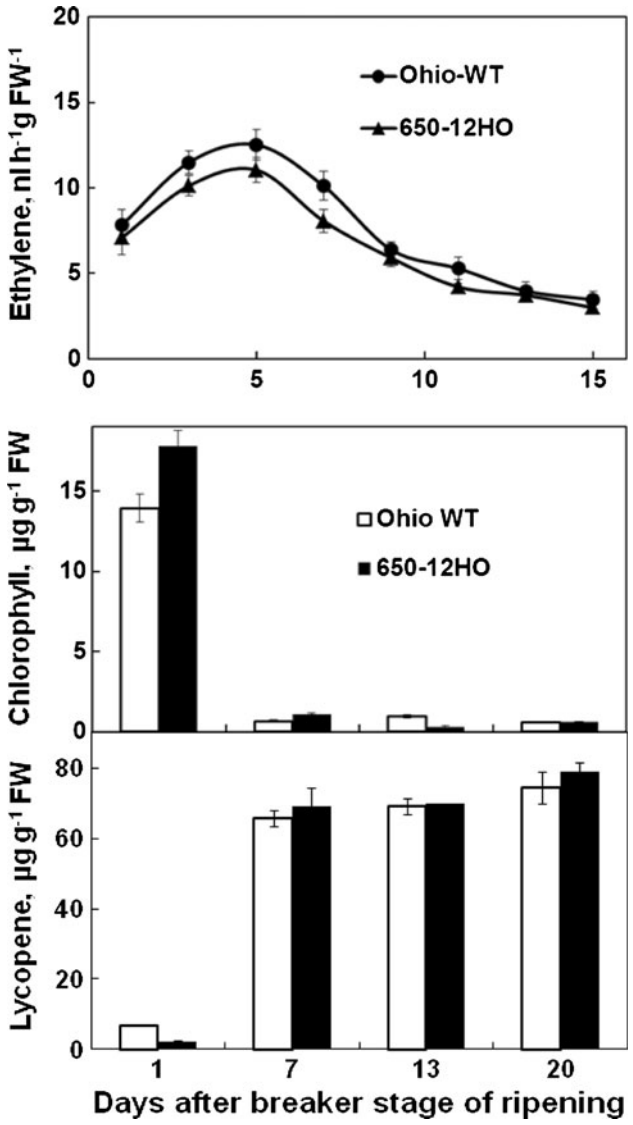

Fig. 2 Effect of silencing ripening-associated lipoxygenase on the rate of ethylene evolution, chlorophyll loss, and lycopene accumulation in ripening tomato fruit

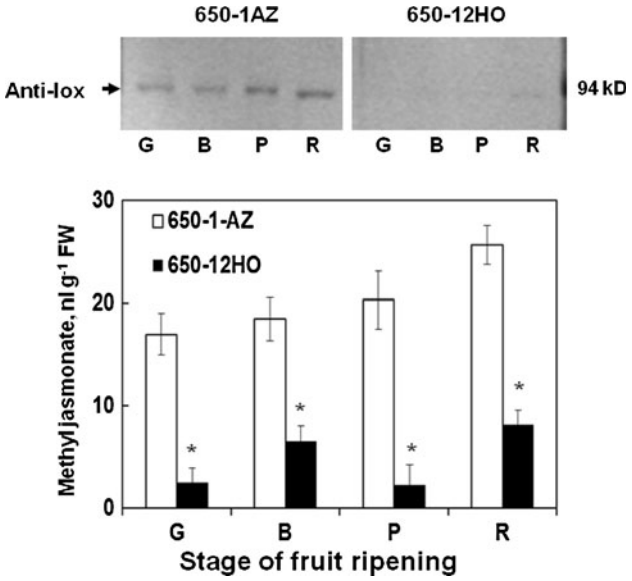

Fig. 3 Down regulation of methyl jasmonate levels in LOX-silenced tomato fruit. The upper panel (immunoblot) shows LOX protein levels in the T_5 generation of LOX-silenced 650-12HO fruits in comparison to the azygous control (650-1AZ). The lower panel shows reduced methyl jasmonate levels during ripening of LOX-silenced homozygous fruits (650-12HO) as compared to the azygous control line (650-1-AZ). Stages of ripening: green (*G*), breaker (*B*), pink (*P*), and red ripe (*R*)

Methyl jasmonate regulates polyamine levels and metabolic profiles of tomato fruit

As described in the Introduction, exogenous treatment of some plants with JA increases the levels of Put, Spd, and Spm. We, therefore, quantified polyamine levels in ripening tomato fruits, shown in Fig. 4. The pattern of changes in Put, Spd, and Spm during ripening of wild-type fruit was similar to those previously reported (Mehta et al. 2002). However, MeJA-deficient fruit had over 50% reduction in the content of polyamines at most stages of fruit ripening as compared to the non-transgenic fruits (Fig. 4). To determine the magnitude of the effects on cellular metabolism associated with MeJA-regulated polyamine levels, we studied metabolic profiles of fruits at different ripening stages in 650-1AZ and 650-12HO lines using ^{1}H NMR spectroscopy as previously described (Mattoo et al. 2006) with slight modifications as indicated in Materials and Methods.

Principal Component Analysis (PCA) was performed first since, as an explorative analysis, it gives an overall picture of data variability and sample agglomeration (Fig. 5). The PCA score plot showed that a majority (13 out of 17) of 650-1AZ tomato samples had positive PC1 scores, while most LOX-silenced 650-12HO samples had negative PC1 scores. It is noteworthy that the distribution of samples along the PC2 axes reflects the fruit ripeness, the lowest and the highest PC2 scores corresponded to the green and red fruits, respectively. This tendency is clearly seen for the 650-1AZ fruit samples, where all four ripening stages are well separated along the PC2 axis, but is less evident in the case of fruits from 650-12HO, transgenic line (Fig. 5). This provided the first indication that reduced MeJA resulting from the introduction of *SlLoxB* transgene negatively influenced ripening-associated cellular metabolism. The PCA data showed clear separation between the two lines, particularly as the fruit ripened, i.e., the separation was good in breaker (B) fruits but highly distinct at the pink (P) stage.

Considering the PCA results, two principal factors influenced the metabolic profile of tomato samples: genetic factor (transgene factor) and ripening process, the

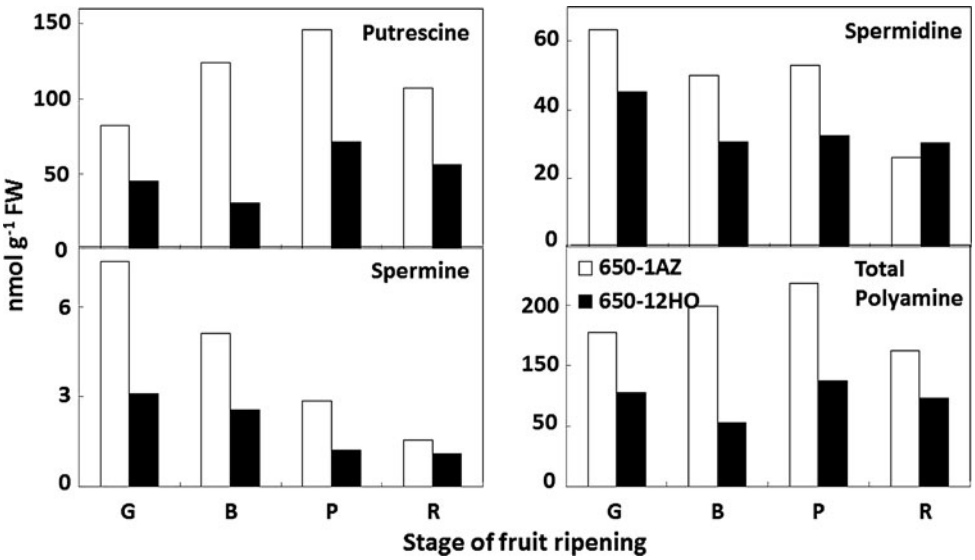

Fig. 4 Changes in putrescine, spermidine, and spermine levels in T_5 generation of LOX-silenced 650-12HO fruits in comparison to the azygous control (650-1AZ) during ripening. Stages of ripening are same as in the legend to Fig. 3

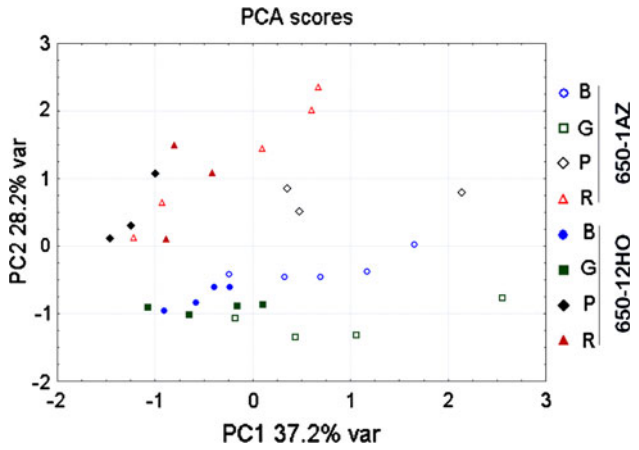

Fig. 5 Principal component analysis (*PCA*) map of 31 tomato fruit samples. Stages of fruit ripeness listed are G (*green*), B (breaker), P (*pink*), and R (*red*). *Open symbols* represent samples from azygous 650-1AZ control line, while the *closed symbols* represent corresponding fruit samples from the LOX-silenced 650-12HO line

transgene factor being the most important one (37.2% of variability). In previous studies with a different transgenic event, we had found that the ripening process masked the contribution of genetic modification on tomato metabolome using PCA scores (Mattoo et al. 2006). A more prevalent influence of transgene factor on the metabolome relative to the ripening process per se is clearer in the present instance. PC1 and PC2 are linear combinations of original variables, and the contribution of single metabolites in the PCA loadings clearly indicate that Ile, Val, Thr, Gln, Asn, Tyr, Phe, and succinate are responsible for the separation of 650-1AZ and 650-12HO lines, while adenosine, AMP, Glu, Asp, His, Nucl1, and Nucl2 mostly contribute to the separation of samples according to ripening stage (see plot of loadings in Online Resource 2).

Aminome

MeJA not only regulates polyamine levels but also affects the steady state levels of aminome belonging to the aspartate family (Asn, Thr, Ile), glutamate family (Glu, Gln, GABA), and those generated via the chorismate pathway (Tyr, Phe, Trp), the statistically significant changes are marked with asterisk(s) (Fig. 6a). Also, changes in Ala and Val were apparent (Fig. 6a). It is worth noting here that these amino acids are derived from oxaloacetate, 2-oxoglutarate, phosphoenolpyruvate, and pyruvate, respectively.

The levels of other amino acids—Asp, Trp, and His—increased during ripening in the fruit from both lines, reaching their highest levels at the red-ripe stage, but the levels were higher in the azygous control compared with the LOX-silenced line except for Asp at red stage. The decreases in His and Trp in the LOX-silenced fruit were significant in the breaker stage, and breaker and pink stages, respectively (Fig. 6a). Noticeable decreases in Ile, Val, Thr, Gln, Asn, Tyr, and Phe in LOX-silenced line relative to the control azygous line were observed mostly in breaker (B) and pink (P) ripening stages, which represent the onset of the ripening program (Fig. 6a). Moreover, this tendency was observed also for other amino acids (Glu, His, Trp) but to a lesser extent.

Organic acids, carbohydrates, and choline

Malate and fumarate levels were highest at the green stage of the fruit and decreased with the ripening process in both the azygous and the transgenic 650-12HO lines but with no significant differences between the lines (Fig. 6b). Citrate and succinate levels in fruit from both lines remain steady throughout ripening but the levels were lower in the LOX-silenced fruit, the difference for citrate being highly

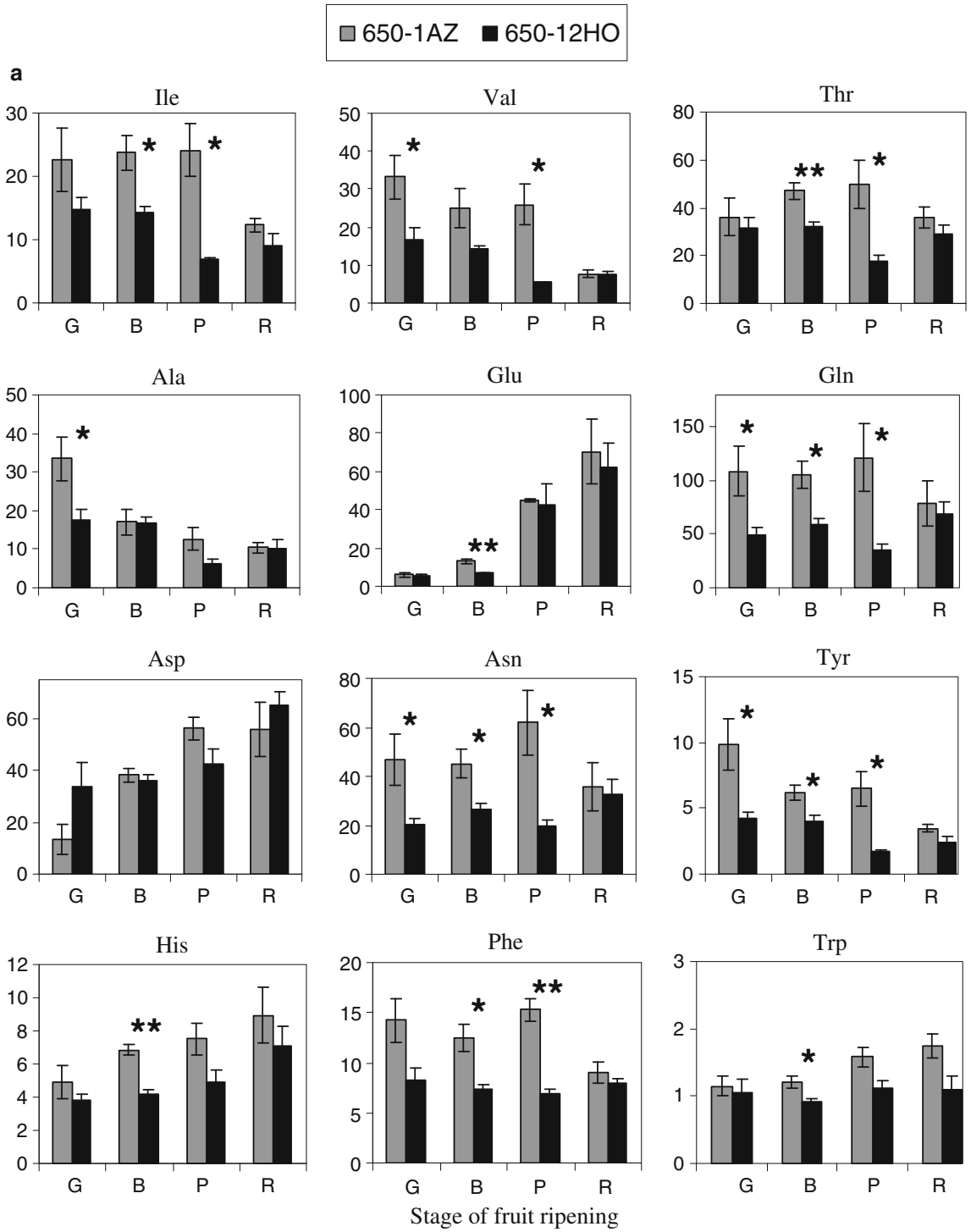

Fig. 6 Relative molecular abundance of 30 metabolites in fruit at different stages of ripening (postharvest) from transgenic (650-12HO) and non-transgenic, azygous (650-1AZ) plants analyzed by NMR spectroscopy. **a** Aminome. **b** Organic acids, sugars and miscellaneous metabolites. **c** Nucleotides, nucleosides, and trigonelline. Data shown are means ± SE ($n = 3$–5). *Asterisks* denote the significant difference between 650-12HO and 650-1AZ: $0.05 > P$ level > 0.01 (*single asterisk*), and P level < 0.01 (*two asterisks*)

significant at the pink stage and for succinate at the earlier stages, i.e., green and breaker (Fig. 6b).

Among the carbohydrate class of metabolites analyzed, β-glucose and myo-inositol showed opposite trends. There was a slight but consistent increase in β-glucose levels in the LOX-silenced (650-12HO) fruit during ripening compared with that in the control 650-1AZ line: the increase was significant at the pink stage (Fig. 6b). Myo-inositol levels were

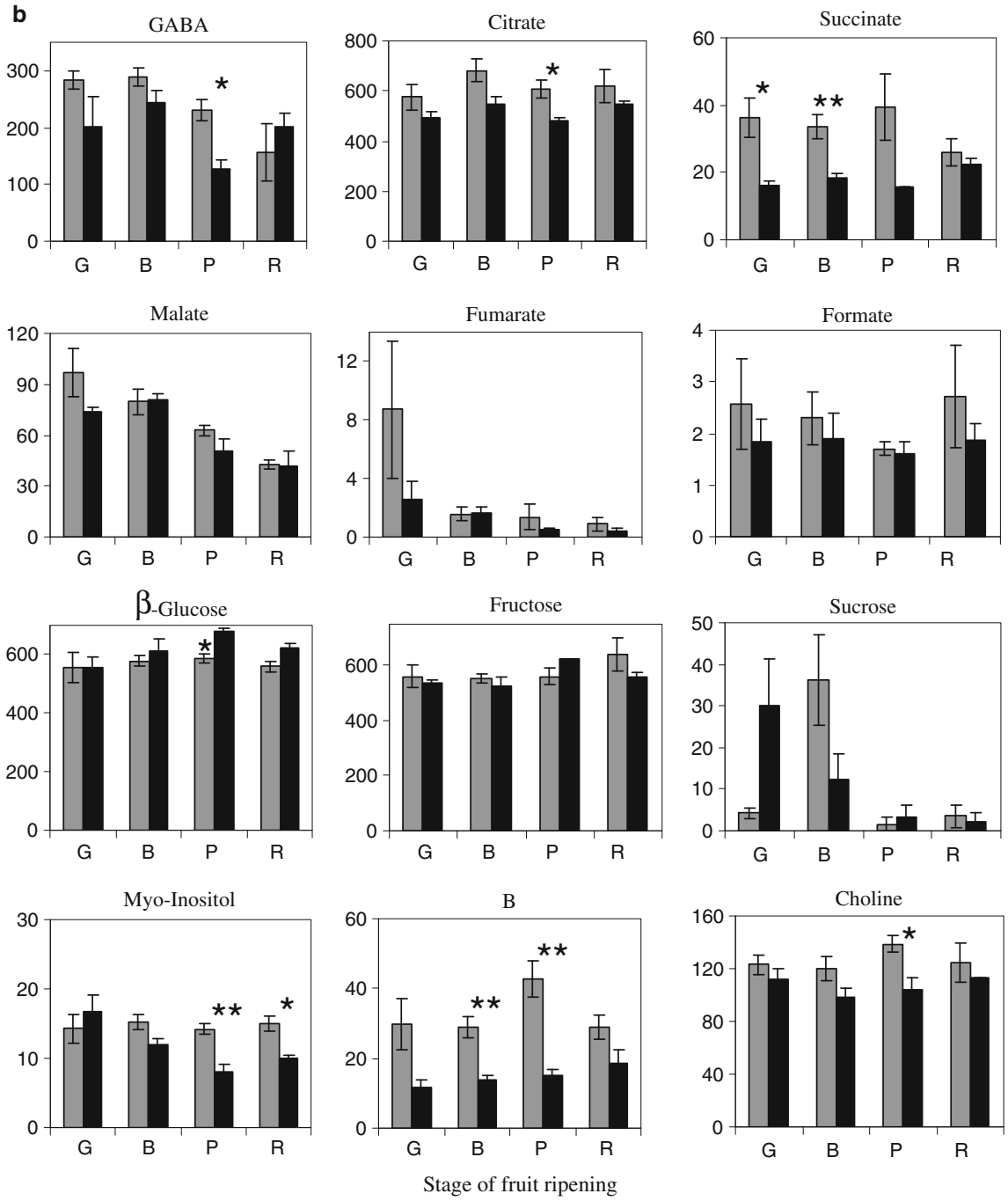

Fig. 6 continued

higher in the 650-1AZ control fruit at later stages of ripening as compared to the 650-12HO fruit, and the differences were significant at the breaker and pink stages of ripening (Fig. 6b). However, the patterns of fructose levels remained high and consistent throughout ripening of fruit from both the control azygous and the transgenic lines, while the effects on sucrose were differential and opposite at the green versus breaker stage. The levels of Compound B were also impacted in the fruit from line 650-12HO being most significantly reduced at the breaker and pink stages (Fig. 6b). Choline levels were also lower in the 650-12HO fruit but the difference with the control fruit was statistically significant at the pink stage of ripening.

Nucleosides, nucleotides and trigonelline

Adenosine and AMP progressively increased during ripening, while two nucleic acid-related signals (Nucl1 and

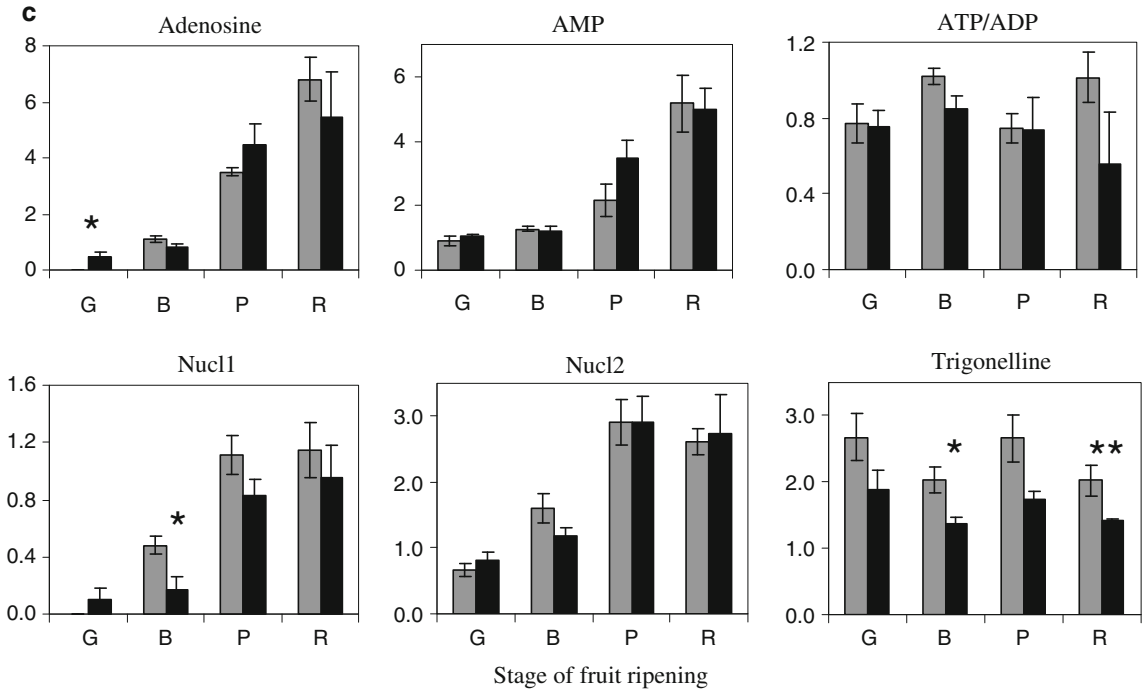

Fig. 6 continued

Nucl2) that also increased reaching a plateau in red-ripe fruits for both lines (see Fig. 6c). However, the amplitude of changes (from minimum to maximum levels) for these metabolites during ripening was higher in the control fruit (650-1AZ line) compared with the transgenic LOX-silenced (650-12HO) line, changes in adenosine being significant at green (G) stage and in Nucl1 at breaker (B) stage (Fig. 6c). The ATP/ADP levels varied but not significantly.

Trigonelline is N-methyl-nicotinate, a vitamin B6 derivative with several therapeutic and anti-carcinogenic properties, whose levels vary in plants and in response to stressors (http://ntp.niehs.nih.gov/ntp/htdocs/Chem_Background/ExSumPdf/Trigonelline.pdf). Its levels were significantly reduced at the breaker and red stages of the fruit from the 650-12HO line as compared to the control 650-1AZ line fruit (Fig. 6c). Since His is synthesized from purines, such a correlation may have physiological consequences.

ANOVA confirmed the PCA results relative to metabolites that were most affected due to the silencing of LOX. According to ANOVA results, out of 30 metabolites analyzed, the levels of 7 metabolites (Val, Ala, Gln, Asn, Tyr, succinate, and adenosine), 13 metabolites (Ile, Thr, Glu, Gln, Asn, Tyr, His, Phe, Trp, succinate, B, Nucl1, and trigonelline), 13 metabolites (Ile, Val, Thr, Asn, Tyr, Phe, GABA, citrate, β-glucose, myo-inositol, B, and choline), and 2 metabolites (myo-inositol, and trigonelline) were significantly different between 650-1AZ and LOX-silenced (650-12HO) fruits, respectively, at green, breaker, pink and red-ripe stages of ripening (see also Online Resource 3). These data confirmed and highlighted the interaction of transgene expression with the early stages of fruit ripening.

Discussion

We demonstrate that silencing of *SlLOXB*, a ripening-induced lipoxygenase (LOX), impaired endogenous production of MeJA and had profound effects on the levels of polyamines and metabolic profile of a number of primary metabolites. The 31 fruit metabolites profiled encompass diverse cellular molecules including different classes of amino acids, organic acids, carbohydrates, and other signaling molecules. A majority of these metabolites showed differential accumulation (mostly negative) in *SlLOXB*-silenced transgenic fruit as compared to the wild-type (azygous) line. Maximal differences occurred in the beaker and pink stages of the ripening process, which were coincident with the loss of LOX transcript and protein and marked reduction in MeJA and polyamine content. Both MeJA (Imanishi and Nagata 2003) and polyamines (Srivastava et al. 2007) are known to alter the expression of a large number of genes in tomato fruit, which have consequences on the cellular metabolism. Earlier, we showed that Spd and Spm are positive regulators of cellular amino acid metabolism, whereas putrescine exerts negative effects (Handa and Mattoo 2010; Mattoo et al. 2010). Here,

we demonstrate that MeJA deficiency has mostly a negative effect on the accumulation of aminome and other cellular metabolites.

Free amino acids are known to add to flavor and taste of a fruit and increase during fruit ripening (Carrari et al. 2006; Mounet et al. 2007; Sorrequieta et al. 2010). Transgenic fruits with silenced LOX and greatly reduced levels of MeJA accumulated lower levels of several amino acids during ripening. These include the Glu and Asp family of amino acids, as well as those generated from chorismate, oxaloacetate, 2-oxoglutarate, phosphoenolpyruvate and pyruvate, suggesting a metabolic interaction with the glycolytic and Krebs cycle pathways. Interestingly, succinate and citrate levels decreased in the LOX-silenced fruits but the levels of organic acids—malate, fumarate and formate, and signaling molecules such as adenosine, AMP and ATP/ADP remained unchanged. The molecular signals regulating amino acid metabolism (including activities of peptidases and biosynthesizing enzymes) and their interaction with the above-mentioned pathways in fruit during postharvest ripening are not yet known. Metabolic network seems to be complex and highly regulated during tomato fruit development (Carrari et al. 2006). It is known that a concurrence exists between metabolic pathways and tomato fruit transcriptome in response to endogenous levels of polyamines, spermidine, and spermine (Srivastava et al. 2007; Mattoo et al. 2010). The latter study also pinpointed the potential gene targets in glycolysis, amino acid biosynthesis pathways, Krebs cycle, methylation reactions, ethylene biosynthesis and perception and transcriptional factors that may coordinate or play a role in metabolic interconnections during tomato fruit ripening. In as much as LOX silencing impacted methyl jasmonate content in the ripening fruit, it may not be far fetched to implicate methyl jasmonate in regulating the levels of some or most of the affected metabolites in the *SlLOXB*-silenced fruit during early stages of ripening.

The levels of free amino acids increase significantly during ripening of both climacteric and non-climacteric fruits (Burroughs 1970; Tadeo et al. 1988; Carrari et al. 2006; Mattoo et al. 2006; Mounet et al. 2007; Sorrequieta et al. 2010). This increase in free amino acids correlates with decreased total protein content in a fruit during ripening, and it is thought that this is partly due to increased proteolytic activity (Mehta et al. 1996; Sorrequieta et al. 2010). Therefore, one route for amino acids to accumulate in a ripening fruit is the proteolysis factor. Early work showed that among amino acids, the levels of Gln, Glu, and Asp are highest in the ripening mango fruit (Mattoo et al. 1975). In tomato, Glu was found to be the most abundant amino acid (Grierson et al. 1985; Gallardo et al. 1993; Baxter et al. 2005). Glu is responsible for the characteristic "umami" flavor in tomato (Bellisle 1999; Pratta et al. 2004). We have, however, found variations in the aminome as a function of ripening (Mattoo et al. 2006), the other amino acids considered at levels similar to Glu include Asp, Gln, and Asn (Fig. 6a). Asp-family network constitutes a set of highly coordinated genes that have been suggested to be linked with genome-wide gene expression networks (Less et al. 2010). This network may determine the steady state level of Thr, Ile, Lys, Glu, and Met. Met serves as a branch point for the synthesis of S-adenosylmethionine, a precursor of hormone ethylene and glucosinolates, which are associated with plant defense against herbivores (Caroline et al. 2007; Yan and Chen 2007). Highly significant lowered levels of Asp-family aminome in LOX-silenced line, particularly as the ripening commences, reinforce the concept that jasmonates regulate shifts in cellular metabolome accompanying fruit ripening. Aromatic aminome including Tyr, Trp, and Phe is a source of precursors for secondary metabolism that generate flavonoids, alkaloids, and lignins (Tzin and Galili 2010). Notably, these pigment and aroma generating amino acids are present at significantly low levels in LOX-silenced transgenic tomato compared with the azygous control fruit suggesting that jasmonates/polyamines are a factor in regulating their levels. This conclusion is consistent with the observation in tobacco where these amino acids were found to increase upon treatment with methyl jasmonate (Hanik et al. 2010).

The increase in Glu content was not affected in tomato mutant "green flesh", suggesting that the increase is independent of chloroplast-chromoplast transition (Bortolotti et al. 2003; Carrari et al. 2006). Associated changes at the onset of ripening include the increase in the activities of the Glu metabolizing enzymes, glutamate dehydrogenase (GDH; Mattoo et al. 1975; Sorrequieta et al. 2010), 2-oxoglutaric aspartate transaminase (Mattoo et al. 1975), and 2-oxoglutarate-dependent γ-aminobutyrate transaminase (GABA-T; Sorrequieta et al. 2010). Also, the activity of c-GTase, which catalyzes Glu liberation, increases during ripening and can sustain release of Glu from endogenous substrates such as glutathione (Martin et al. 1995; Sorrequieta et al. 2010). Fruits attached to the parent plant can accumulate free Glu via the tomato phloem sap (Valle et al. 1998); however, this is not relevant in the case of detached fruits, which were the subject of metabolic profiling in the present investigation.

Exogenously applied jasmonates have been shown to have different effects on the biosynthesis and/or action of ethylene, a key plant hormone that promotes fruit ripening. Low concentrations of exogenous jasmonate stimulate activities of ethylene biosynthesis enzymes, ACC synthase and ACC oxidase, in apple and tomato (Fan et al. 1998). In a different study, exogenous methyl jasmonate was found to alter expression of ripening-related genes in ripening

peach fruits, namely, down regulation of ACC oxidase, polygalacturonase, and transcriptional modulator IAA7 and up regulation of stress-related genes including those involved in jasmonic acid biosynthesis (Ziosi et al. 2008). However, application of a synthetic derivative of methyl jasmonate, N-propyl dihydrojasmonate (PDJ), to peach fruits reduced ethylene production and fruit softening while enhancing the accumulation of polyamines (Ziosi et al. 2009). In apples, methyl jasmonates were found to influence volatile production that was fruit growth-dependent and associated with endogenous ethylene levels (Kondo et al. 2005). The negative effect of methyl jasmonate deficiency on select aminome seen here with *SlLOXB*-silenced tomato fruit is consistent with these findings. From these observations, it is apparent that jasmonates play a role in the cellular metabolism of a ripening fruit. Among other scenarios, this modulation of processes by jasmonates during fruit ripening, perhaps more intensified in postharvest ripening, may or may not occur through effects on ethylene biosynthesis and/or action in climacteric fruits (Fan et al. 1998; Srivastava and Handa 2005; Ziosi et al. 2009).

The 26S proteasome-dependent degradation pathway is connected with signaling pathways of many phytohormones including jasmonic acid, auxin, and gibberellins (Smalle and Vierstra 2004). Ubiquitin/26S proteasome has been implicated in the degradation of two family members of ethylene receptors, *LeETR4* and *LeETR6*, which then results in tomato fruit ripening (Kevany et al. 2007). If the 26S proteasome pathway is prevalent in a fruit ripened on the shelf, one can expect the reduction in the endogenous levels of methyl jasmonate to impair degradation of ethylene receptor protein and thereby reduce ripening-associated increases in metabolites as analyzed here. Alternatively, it is possible that changes in amino acid profiles may be a result of protein degradation set into motion by methyl jasmonate-activated proteases. In this regard, since we did not see any significant changes in either the rate of ethylene production or ripening-associated pigment accumulation/degradation in the LOX-silenced fruits, it is likely that LOX-regulated jasmonates act independent of ethylene in altering metabolic profiles.

We propose that jasmonate may also act via altering intracellular levels of polyamines. This hypothesis is supported by evidence that exogenous application of MeJA alters intracellular levels of polyamines in peach (Ziosi et al. 2009), and we show here that reducing endogenous MeJA levels also negatively impacts polyamine levels. Based on transcriptome, limited proteome, and metabolome analyses of transgenic tomato overexpressing yeast SAM decaboxylase, we have suggested earlier that polyamines act as "surrogate messengers" and nudge other signaling molecules to activate a vast genetic network to regulate growth, development, and senescence (Mattoo and Handa 2008). However, it is conceivable that both ethylene and methyl jasmonate act via independent signaling mechanisms that cross talk during ripening. Since increases in amino acids, carbohydrates, organic acids, and jasmonates occur in both climacteric and non-climacteric fruits, some of the ripening-associated metabolic changes may be regulated by jasmonates, independent of ethylene action.

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References

Baxter CJ, Carrari F, Bauke A, Overy S, Hill SA, Quick PW, Fernie AR, Sweetlove LJ (2005) Fruit carbohydrate metabolism in an introgression line of tomato with increased fruit soluble solids. Plant Cell Physiol 46:425–437

Bell E, Creelman RA, Mullet JE (1995) A chloroplast lipoxygenase is required for wound-induced jasmonic acid accumulation in *Arabidopsis*. Proc Natl Acad Sci USA 92:8675–8679

Bellisle F (1999) Glutamate and the UMAMI taste: sensory, metabolic, nutritional and behavioural considerations. A review of the literature published in the last 10 years. Neurosci Biobehav Rev 23:423–438

Biggs MS, Handa AK (1989) Temporal regulation of polygalacturonase gene expression in fruits from normal mutant and heterozygous tomato genotypes. Plant Physiol 89:117–125

Biggs MS, Woodson WR, Handa AK (1988) Biochemical basis of high temperature inhibition of ethylene biosynthesis in ripening tomato fruits. Physiol Plant 72:572–578

Biondi S, Scaramagli S, Capitani F, Altamura MM, Torrigiani P (2001) Methyl jasmonate upregulates biosynthetic gene expression, oxidation and conjugation of polyamines, and inhibits shoot formation in tobacco thin layers. J Exp Bot 52:231–242

Biondi S, Scoccianti V, Scaramagli S, Ziosi V, Torrigiani P (2003) Auxin and cytokinin modify methyl jasmonate effects on polyamine metabolism and ethylene biosynthesis in tobacco leaf discs. Plant Sci 165:95–101

Bortolotti S, Boggio SB, Delgado L, Orellano EG, Valle EM (2003) Different induction patterns of glutamate metabolising enzymes in ripening fruits of the tomato mutant green flesh. Physiol Plant 119:384–391

Braun S, Kalinowski HO, Berger S (1998) 150 and more basic NMR experiments, 2nd edn. Wiley, Weinheim

Burroughs LF (1970) Amino acids. In: Hulme AC (ed) The biochemistry of fruits and their products, vol 1. Academic Press, London, pp 119–146

Caroline C, von Dahl, Baldwin IT (2007) Deciphering the role of ethylene in plant—herbivore interactions. J Plant Growth Regul 26:201–209

Carrari F, Baxter C, Usadel B, Urbanczyk-Wochniak E, Zanor MI, Nunes-Nesi A, Nikiforova V, Centero D, Ratzka A, Pauly M, Sweetlove LJ, Fernie AR (2006) Integrated analysis of metabolite and transcript levels reveals the metabolic shifts that underlie tomato fruit development and highlight regulatory aspects of metabolic network behavior. Plant Physiol 142:1380–1396

Chen H, Jones AD, Howe GA (2006) Constitutive activation of the jasmonate signaling pathway enhances the production of secondary metabolites in tomato. FEBS Lett 580:2540–2546

Creelman RA, Mullet JE (1997) Biosynthesis and action of jasmonates in plants. Annu Rev Plant Physiol Plant Mol Biol 48:355–381

Fan TWM (1996) Metabolite profiling by one- and two-dimensional NMR analysis of complex mixtures. Prog Nucl Magn Reson Spectrosc 28:161–219

Fan X, Mattheis JP, Fellman JK (1998) A role for jasmonates in climacteric fruit ripening. Planta 204:444–449

Fincato P, Moschou PN, Spedaletti V, Tavazza R, Angelini R, Federico R, Roubelakis-Angelakis KA, Tavladoraki P (2010) Functional diversity inside the *Arabidopsis* polyamine oxidase gene family. J Exp Bot 62:1155–1168

Gallardo F, Canton FR, Garcia-Gutierrez A, Canovas FM (1993) Changes in photorespiratory enzymes and glutamate synthases in ripening tomatoes. Plant Physiol Biochem 31:189–196

Goldsbrough PH, Cullis CA (1981) Characterization of the genes for ribosomal RNA in flax. Nucl Acids Res 9:1301–1309

Grierson D, Slater A, Maunders M, Crookes P, Tucker GA, Schuch W, Edwards K (1985) Control of ethylene synthesis and ripening by sense and antisense genes in transgenic plants. In: Roberts JA, Tucker GA (eds) Ethylene and plant development. Butterworth, London, pp 147–161

Haggag WM, Abd-El-Kareem F (2009) Methyl jasmonate stimulates polyamines biosynthesis and resistance against leaf rust in wheat plants. Arch Phytopath Plant Protec 42:16-31

Handa AK, Kausch KD (2002) Improvement of fruit quality by inhibiting production of lipoxygenase in fruits. United States Patent Number 6355862, Issued on March 12, 2002

Handa AK, Mattoo AK (2010) Differential and functional interactions emphasize the multiple roles of polyamines in plants. Plant Physiol Biochem 48:540–546

Handa AK, Singh NK, Biggs MS (1985) Effect of tunicamycin on in vitro ripening of tomato pericarp tissue. Physiol Plant 63:417–424

Hanik N, Gomez S, Best M, Schueller M, Orians CM, Ferrieri RA (2010) Partitioning of new carbon as ^{11}C in Nicotiana tabacum reveals insight into methyl jasmonate induced changes in metabolism. J Chem Ecol 36:1058–1067

Imanishi S, Nagata M (2003) The effect of methyl jasmonate on expression of the genes involved in ethylene biosynthesis in tomato fruits. Abst. 169. American Society of Plant Biologists, Plant Biology 2003, Honolulu

Jung C, Lyou S, Yeu S, Kim M, Rhee S, Kim M, Lee J, Choi Y, Cheong J–J (2007) Microarray-based screening of JA-responsive genes in Arabidopsis thaliana. Plant Cell Rep 26:1053–1063

Kamada-Nobusada T, Hayashi M, Fukazawa M, Sakakibara H, Nishimura M (2008) A putative peroxisomal polyamine oxidase, $AtPAO_4$, is involved in polyamine catabolism in *Arabidopsis thaliana*. Plant Cell Physiol 49:1272–1282

Kausch KD, Handa AK (1995) Molecular cloning and nucleotide sequence of a lipoxygenase cDNA from ripening tomato fruit. Plant Physiol 107:669–670

Kausch KD, Handa AK (1997) Molecular cloning of a ripening specific lipoxygenase and its expression during wild-type and mutant tomato fruit development. Plant Physiol 113:1041–1050

Kevany BM, Tieman DM, Taylor MG, Cin VD, Klee HJ (2007) Ethylene receptor degradation controls the timing of ripening in tomato fruit. Plant J 51:458–467

Kondo S, Setha S, Rudell DR, Buchanan DA, Mattheis JP (2005) Aroma volatile biosynthesis in apple affected by 1-MCP and methyljasmonate. Postharvest Biol Technol 36:61–68

Less H, Angelovici R, Tzin V, Galili G (2010) Principal transcriptional regulation and genome-wide system interactions of the Asp-family and aromatic amino acid networks of amino acid metabolism in plants. Amino Acids 39:1023–1028

Martens H, Martens M (2001) Multivariate analysis of quality. Wiley, Chichester

Martin MN, Cohen JD, Saftner RA (1995) A new 1-aminocyclopropane-1-carboxylic acid-conjugating activity in tomato fruit. Plant Physiol 109:917–926

Mattoo AK, Handa AK (2008) Higher polyamines restore and enhance metabolic memory in ripening fruit. Plant Sci 174:386–393

Mattoo AK, Murata T, Pantastico EB, Chachin K, Ogata K, Phan CT (1975) Chemical changes during ripening and senescence. In: Pantastico EB (ed) Postharvest physiology, handling and utilization of tropical and subtropical fruits and vegetables. The AVI Publishing Co, USA, pp 104–127

Mattoo AK, Sobolev AP, Neelam A, Goyal RK, Handa AK, Segre AL (2006) Nuclear magnetic resonance spectroscopy-based metabolite profiling of transgenic tomato fruit engineered to accumulate spermidine and spermine reveals enhanced anabolic and nitrogen-carbon interactions. Plant Physiol 142:1759–1770

Mattoo AK, Minocha SC, Minocha R, Handa AK (2010) Polyamines and cellular metabolism in plants: transgenic approaches reveal different responses to diamine putrescine versus higher polyamines spermidine and spermine. Amino Acids 38:405–413

Mehta RA, Fawcett TW, Porath D, Mattoo AK (1992) Oxidative stress causes rapid membrane translocation and in vivo degradation of ribulose-1, 5-bisphosphate carboxylase/oxygenase. J Biol Chem 267:2810–2816

Mehta RA, Warmbardt RD, Mattoo AK (1996) Tomato fruit carboxypeptidase: properties, induction upon wounding, and immunocytochemical localization. Plant Physiol 110:883–892

Mehta RA, Cassol T, Li N, Ali N, Handa AK, Mattoo AK (2002) Engineered polyamine accumulation in tomato enhances phytonutrient content, juice quality and vine life. Nature Biotech 20:613–618

Mounet F, Lemaire-Chamley M, Maucourt M, Cabasson C, Giraudel J-L, Deborde C, Lessire R, Gallusci P, Bertrand A, Gaudille‘re M, Rothan C, Rolin D, Moing A (2007) Quantitative metabolic profiles of tomato flesh and seeds during fruit development: complementary analysis with ANN and PCA. Metabolomics 3:273–288

Nambeesan S, Handa AK, Mattoo AK (2008) Polyamines and regulation of ripening and senescence. In: Paliyath G, Murr DP, Handa AK, Lurie S (eds) Postharvest biology and technology of fruits, vegetables and flowers. Blackwell, Oxford, pp 319–340

Pauwels L, Inzé D, Goossens A (2009) Jasmonate-inducible gene: what does it mean? Trends Plant Sci 14:87–91

Pawliszyn J (1997) Solid phase microextraction: theory and practice. Wiley, New York

Pegg AE (2009) Mammalian polyamine metabolism and function. IUBMB Life 61:880–894

Peremarti A, Bassie L, Yuan D, Pelacho A, Christou P, Capell T (2010) Transcriptional regulation of the rice *arginine decarboxylase* (*ADC1*) and *S-adenosylmethionine decarboxylase* (*Samdc*) genes by methyl jas monate. Plant Physiol Biochem 48:553–559

Perez-Amador MA, Leon J, Green PJ, Carbonell J (2002) Induction of the arginine decarboxylase *ADC2* gene provides evidence for the involvement of polyamines in the wound response in *Arabidopsis*. Plant Physiol 130:1454–1463

Pratta G, Zorzoli R, Boggio SB, Picardi LA, Valle EM (2004) Glutamine and glutamate levels and related metabolizing enzymes in tomato fruits with different shelf-life. Sci Hortic 100:341–347

Schardl CL, Byrd AD, Benzion G, Altschuler MA, Hildebrand DF, Hunt AG (1987) Design and construction of a versatile system for the expression of foreign genes in plants. Gene 61:1–11

Smalle J, Vierstra RD (2004) The ubiquitin 26S proteasome proteolytic pathway. Annu Rev Plant Biol 55:555–590

Sobolev AP, Segre AL, Lamanna R (2003) Proton high-field NMR study of tomato juice. Magn Reson Chem 41:237–245

Sorrequieta A, Ferraro G, Boggio SB, Valle EM (2010) Free amino acid production during tomato fruit ripening: a focus on L-glutamate. Amino Acids 38:1523–1532

Srivastava A, Handa AK (2005) Hormonal regulation of tomato fruit development: a molecular perspective. J Plant Growth Reg 24:67–82

Srivastava A, Chung SH, Fatima T, Datsenka T, Handa AK, Mattoo AK (2007) Polyamines as anabolic growth regulators revealed by transcriptome analysis and metabolite profiles of tomato fruits engineered to accumulate spermidine and spermine. Plant Biotechnol 24:57–70

Tadeo JL, Ortiz JM, Martin B, Estellés A (1988) Changes in the nitrogen content and amino acid composition of navel oranges during ripening. J Sci Food Agric 43:201–209

Tieman DM, Harriman RW, Ramamohan G, Handa AK (1992) An antisense pectin methylesterase gene alters pectin chemistry and soluble solids in tomato fruit. Plant Cell 4:667–679

Tzin V, Galili G (2010) New insights into the shikimate and aromatic amino acids biosynthesis pathways in plants. Mol Plant 3:956–972

Valle EM, Boggio SB, Heldt HW (1998) Free amino acids content of phloem sap and fruits in *Lycopersicon esculentum*. Plant Cell Physiol 39:458–461

Van Haute E, Joos H, Maes M, Warren G, van Montagu M, Schell J (1983) Intergenic transfer and exchange recombination of restriction fragments cloned in pBR322: a novel strategy for the reversed genetics of the Ti plasmids of *Agrobacterium tumefaciens*. EMBO 2:411–417

Walia H, Wilson C, Condamine P, Liu X, Ismail AM, Close TJ (2007) Large-scale expression profiling and physiological characterization of jasmonic acid-mediated adaptation of barley to salinity stress. Plant Cel Environ 30:410–421

Walters D, Cowley T, Mitchel A (2002) Methyl jasmonate alters polyamine metabolism and induces systemic protection against powdery mildew infection in barley seedlings. J Exp Bot 53:747–756

Yan X, Chen S (2007) Regulation of plant glucosinolate metabolism. Planta 226:1343–1352

Ziosi V, Bonghi C, Bregoli AM, Trainotti L, Biondi S, Sutthiwal S, Kondo S, Costa G, Torrigiani P (2008) Jasmonate-induced transcriptional changes suggest a negative interference with the ripening syndrome in peach fruit. J Exp Bot 59:563–573

Ziosi V, Bregoli AM, Fregola F, Costa G, Torrigiani P (2009) Jasmonate induced ripening delay is associated with up-regulation of polyamine levels in peach fruit. J Plant Physiol 166:938–946