SPECIAL GUEST EDITOR SECTION

# Overaccumulation of Higher Polyamines in Ripening Transgenic Tomato Fruit Revives Metabolic Memory, Upregulates Anabolism-Related Genes, and Positively Impacts Nutritional Quality

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**Vegetables and fruits are essential components of the human diet as they are sources of vitamins, minerals, and fiber and provide antioxidants that prevent chronic diseases. Our goal is to improve durable nutritional quality of tomato fruit. We developed transgenic tomatoes expressing yeast *S*-adenosylmethionine decarboxylase (ySAMdc) gene driven by a fruit-specific E8 promoter to investigate the role of polyamines in fruit metabolism. Stable integration of E8-ySAMdc chimeric gene in tomato genome led to ripening-specific accumulation of polyamines, spermidine (Spd) and spermine (Spm), which in turn affected higher accumulation of glutamine, asparagine, and organic acids in the red fruit with significant decrease in the contents of valine, aspartate, sucrose, and glucose. The metabolite profiling analysis suggests that Spd/Spm are perceived as "signaling" organic-N metabolites by the fruit cells, resulting in the stimulation of carbon sequestration; enhanced synthesis of biomolecules; increased acid to sugar ratio, a good attribute for the fruit flavor; and in the accumulation of another "vital amine," choline, which is an essential micronutrient for brain development. A limited transcriptome analysis of the transgenic fruit that accumulate higher polyamines revealed a large number of differentially expressed genes, about 55% of which represented discrete functional categories, and the remaining 45% were novel, unknown, or unclassified: amino acid biosynthesis, carotenoid biosynthesis, cell wall metabolism, chaperone family, flavonoid biosynthesis, fruit ripening, isoprenoid biosynthesis, polyamine biosynthesis, signal transduction, stress/defense-related, transcription, translation, and vacuolar function. There was a good correspondence between some gene transcripts and their protein products, but not in the case of the tonoplast intrinsic protein, which showed post-transcriptional regulation. Higher metabolic activity of the transgenic fruit is reflected in higher respiratory activity, and upregulation of chaperones and mitochondrial cytochrome oxidase transcripts compared to the control. These transgenic plants are a new resource to understand the role of Spd/Spm in fruit biology. Transcriptome analysis and metabolic profiles of Spd/Spm accumulating, transgenic fruit suggest the presence of an intricate regulation and interconnection between certain metabolic pathways that are revived when Spd and Spm likely reach a certain threshold. Thus, polyamines act as antiapoptotic regulatory molecules and are able to revive metabolic memory in the tomato fruit.**

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Vegetables and fruits are essential components of the human diet and provide vitamins, antioxidants, and minerals. It is increasingly recognized that these nutritional molecules when consumed as part of a human diet

have a great potential to prevent chronic diseases, including epithelial cancers, cardiovascular diseases, digestive disorders, and immune deficiency (1–3). Commonly, in the daily diet several servings of vegetables and fruits are recommended because the nutrient levels in them are much below the recommended daily allowance (RDA). Advances in crop variety development using traditional breeding strategies in conjunction with integrated pest management practices led in the 20th century to the Green Revolution, with higher-yielding crop varieties that also were more resistant to diseases. Further sustenance of the production yields with lesser chemical input, including pesticides and without negatively affecting soil fertility and ecosystem, is becoming a major challenge. It is not enough to optimize production of staple crops but also to develop strategies for meeting the nutritional needs of the world's growing population (4). Nutritional quality of crops such as tomato depends on the available cultivars and is influenced by production, preharvest, and postharvest factors (5). Although traditional breeding techniques have succeeded in significantly improving macronutrients such as proteins, carbohydrates, and oil quality in various crops, the same cannot be said about essential micronutrients, such as vitamins, antioxidants, minerals, amino acids, flavonoids, and carotenoids.

The integration of transgenic research into modern biotechnology can advance variety development with particular emphasis on nutritional enhancement and achieve results not possible with traditional breeding techniques and yet within a much shorter time frame. Already, the development of first-generation biotech crops that are resistant to insects and diseases have shown great potential in improved yields, increased profits, and reduced use of herbicides and insecticides, thus bringing positive effects on human health and environment (6–8).

Improvements in the micronutrient levels will come from precise redesigning of the weakest link(s) in the biosynthetic pathway (metabolic engineering) and using innovation to override the endogenous regulation for each micronutrient. Therefore, it is imperative to develop a fundamental knowledge base and a clear understanding of the metabolite fluxes, reactions limiting the endogenous accumulation pathway, and genetic regulation for each micronutrient whose level requires substantial enhancement.

Tomato is our model plant for improvement. It is a member of Solanaceae family and represents other family members, viz., potato, tobacco, eggplant, and pepper. Tomato is one of the most consumed and widely grown vegetable crops in the world, valued at $5–6 billion (9). One medium-sized tomato provides 57% of the RDA of vitamin C, 25% RDA of vitamin A, and 8% RDA of iron, yet with only 35 calories. The red ripe color of tomato fruit is largely due to the carotenoid lycopene. It is a biologically important, natural antioxidant whose physical quenching rate constant with singlet oxygen is more than that of vitamin E and β-carotene (10, 11). This characteristic may be responsible for lycopene's ability to mitigate epithelial prostate and breast cancers, and coronary heart disease (12–15). Giovannucci et al. (16) reported that the risk of prostate cancer was reduced nearly 45% among men who ate at least 10 servings a week of tomato-based foods. In addition, lycopene intake is beneficial for cardiovascular health, and for anti-inflammatory and antimutagenic activities (17).

Tomato fruit is classified as a climacteric fruit in which the onset of ripening is accompanied by an increase in both respiration and ethylene production. The plant hormone ethylene plays a central role in fruit ripening and plant senescence, in the initiation of early events during ripening and the integration of subsequent changes, a subject that has been heavily reviewed (18–26). The fruit and its processed products are the principal dietary sources of lycopene, other antioxidants, vitamins, and minerals. There is a considerable interest in elevating the levels of carotenoids in tomato fruit by genetic manipulation and, thereby, improve its nutritional quality (27–29). Earlier, Fray et al. (30) had shown that the constitutive expression of phytoene synthase 1 (*Psy*-1) in transgenic tomato resulted in a number of pleiotropic effects, including dwarfism due to diversion of geranygeranyl diphosphate away from gibberellin biosynthesis. Subsequently, it was demonstrated that the use of regulatable promoters with heterologous genes could override the endogenous regulation and enable accumulation of higher levels of carotenoids in a fruit-specific manner (28, 29). Fraser et al. (28) transformed tomato var. Ailsa Craig with a bacterial phytoene synthase fused with the tomato polygalacturonase (PG) promoter. The red ripe fruit of transgenics thus obtained had significantly higher levels of phytoene, lycopene, β-carotene, and lutein. Interestingly, the levels of related isoprenoids—tocopherols, plastoquinone, and ubiquinone—were not altered. Mehta et al. (29) developed transgenic tomatoes by introducing yeast *S*-adenosylmethionine (SAM) decarboxylase fused to the ripening-inducible promoter E8, and the transgenic fruits accumulated polyamines spermidine (Spd) and spermine (Spm) at the cost of the diamine putrescine. The independent, homozygous transgenic lines accumulated lycopene levels that were 2 to 3 times higher than the nontransformed or azygous control, had longer vine life, and were enriched by 50% higher fruit juice quality. Together, these findings emphasized the power of new genetic engineering approaches to increase phytonutrient levels in plants. Thus, pathway engineering of secondary metabolites has led to the production of novel polyphenolic flavonoids in tomato fruit (31) while overexpression of Arabidopsis *p*-hydroxyphenylpyruvate dioxygenase and homogentisate phytyltransferase genes resulted in a substantial increase in the level of α-tocopherol (vitamin E) in potato tubers, although the magnitude of this increase was more in leaves and roots (32).

Mehta et al. (29) invoked chloroplast-chromoplast metabolism as one site where higher polyamines could affect higher accumulation of lycopene. The differentiation of chloroplasts into chromoplasts is a developmental process accompanied by redirected and regulated gene transcription and translation that involves genes controlling carotenoid

biosynthesis. Interestingly, Lu et al. (33) identified *Or* gene from cauliflower as a DnaJ cysteine-rich domain-containing protein associated with differentiation of proplastids into chromoplasts and showed that controlling the formation of chromoplasts is one of the mechanisms by which plants regulate carotenoid accumulation.

The development of unique transgenic plants provides an applied angle, in making available highly nutritious "speciality crops," and also adds to the genetic resources that can be used to develop insightful knowledge base about genetic, biochemical, and physiological regulation of various metabolic pathways and functional metabolites. The transgenic tomatoes that accumulate higher polyamines, Spd and Spm, during ripening are akin to a "gain of function" genotype, and we are using them to address the questions on the role of polyamines in fruit metabolism, in particular, their crosstalks with other functional molecules to enable higher nutritional quality of vegetables and fruits.

Polyamines are ubiquitous aliphatic nitrogenous compounds with essential functions in living organisms (34–36). Several laboratories have altered endogenous polyamines in animals and plant cells by overexpression and knockout of genes of polyamine biosynthesis for the purpose of studying their role(s) (29, 37–43). In our case, a fruit ripening-specific promoter was used to drive the expression of yeast SAM decarboxylase gene, with the result that the introduced gene was not active during the early growth and development of the plant but became active along with the normal ripening process of the fruit (29). For our studies, we harvested mature green fruit of these transgenics and ripened them on the bench, thus allowing evaluation of the effects of higher polyamines (Spd/Spm) in the absence of any contribution from the parent plant. The fruit tissues at different stages of ripening were sampled and analyzed for gene transcripts (transcriptome using macroarrays), metabolite levels [nuclear magnetic resonance (NMR) spectrometry], and nutritional attributes. The array data were validated by Northern analysis.

## Higher Polyamines Upregulate Global Gene Expression in Transgenic Tomato Fruit

Homozygous progenies of 2 independent transgenic lines designated 556HO and 579HO that overexpress yeast SAMdc gene were compared with azygous (556AZ) nontransgenic parental line for differential transcript profiles using a custom-made tomato complementary deoxyribonucleic acid (cDNA) array (44). Among 1066 unigene cDNAs present on this array, 710 (66%) displayed quantifiable signal intensities for the transgenic and the control lines. About 22% of these unigenes were found to be differentially expressed ($P < 0.05$) in the transgenic compared to the control wild-type fruits. More than half of the differentially expressed genes (about 55%) represented discrete functional categories, and the remaining 45% were novel, unknown, or unclassified: amino acid biosynthesis, carotenoid biosynthesis, cell wall metabolism, chaperone family, flavonoid biosynthesis, fruit ripening, isoprenoid biosynthesis, polyamine biosynthesis, signal transduction, stress/defense-related, transcription, translation, and vacuolar function (Figure 1; 44). The downregulated genes in the transgenics included genes involved in transport and ethylene signaling, which decreased by 2-fold. The effect of increased Spd/Spm on transcriptional regulation is uniform for unigenes representing all functional, novel, unclassified, and unknown categories, with 17–28% of the unique expressed sequence tags (ESTs) present in each of these categories being differentially regulated (44).

A higher percent of genes from the stress/defense and transcription categories was >2-fold. More than 50% of defense/stress and transcription-related ESTs were upregulated in high Spd/Spm tomatoes, whereas the expression of only 8% of the defense/stress and none of the transcription-related ESTs declined during the same ripening period. Similar patterns were observed for the metabolism and protein synthesis/degradation-related ESTs. Upregulated genes were 4 times as much of those downregulated. This ratio was 6 for the expression of ESTs in the protein synthesis and degradation category and 2 for ESTs from the signal transduction category. Ethylene response pathway was represented by only 8 ESTs in the array, which mostly showed a downward trend in the transgenic fruits. Genes in the transport functional category were represented on the array by 44 unique ESTs, but only a small number of them were differentially regulated between the wild-type and transgenic fruits. These results suggest that higher levels of Spd/Spm cause a marked shift in gene expression, especially for genes involved in metabolism, energy, transcription and protein synthesis, signal transduction, and defense/stress responses in tomato fruit.

Thus, genes whose products contribute to anabolic pathways are upregulated in Spd/Spm-accumulating fruits. The control fruit showed a general downward trend of gene expression during fruit ripening. This downward trend was characteristically prevented in the high Spd/Spm fruits, which is consistent with their known role as antisenescence effectors. Figure 1 summarizes these data, showing the diverse categories of genes impacted in the high Spd/Spm transgenic fruit, most of which represent cellular metabolism and signaling pathways, and, therefore, illustrates polyamines as anabolic growth regulators.

## Northern Analysis of Selected Genes and Immunoblots of Proteins Are in Concurrence with Spd/Spm Mediated Regulation of Fruit Metabolism

We performed Northern and immunoblot analyses of select genes and proteins to validate the macroarray data and check coordination between the expression of gene transcripts and their corresponding proteins at the pink and red ripe stage of the transgenic versus control, azygous fruit. Among the "fruit ripening" gene category, transcripts for 1-aminocyclopropane-1-carboxylate (ACC) synthase (ACS2), ACC oxidase (ACO1), and pectin methylesterase (PME2) peaked at the pink stage and declined thereafter in the red fruit of the

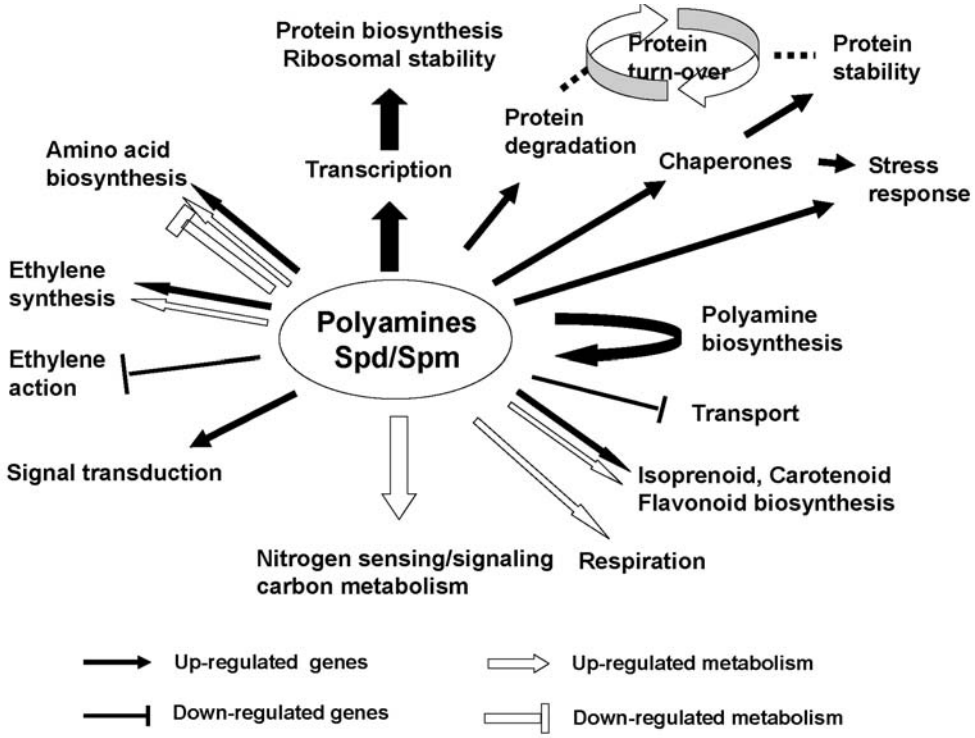


**Figure 1. Polyamines regulate gene expression in ripening tomato fruits. Shown are various categories to which these genes belong and the diversity of the processes regulated. Adapted from Srivastava et al. (ref. 44).**

azygous control plant. On the other hand, the transcripts of MADS-box transcription factor (MADS-RIN; 45) gene continued to accumulate in the red ripe azygous fruit (Figure 2). In the 556HO line, ACS2 and MADS-RIN transcripts were slightly higher in the pink fruit as compared to the azygous control, and both showed an increasing trend from pink to the red stage, but the ACO1 and PME2 transcript levels registered a decline. In the second transgenic line, 579HO, the decreased expression of the transgene [yeast SAM decarboxylase (ySAMdc)] at the red stage of the fruit was accompanied by decreases in ACS2, PME2, and MADS-RIN transcripts, but not in ACO1 whose levels were lower than either the azygous fruit or the 556HO line. The "chaperone" category genes, small heat shock protein gene (Hsp17.6) and cyclophilin (CyP; 44), were at more or less similar and low levels in the pink and red azygous fruit, but registered higher levels and an increasing trend from the pink to red stage fruit of 556HO line (Figure 2). Although the levels of these chaperones were markedly higher in the pink 579HO fruit, they decreased in the red 579HO line fruit in parallel with the decrease seen in the transgene during ripening. In the "signaling" category, G-protein β-subunit (ArcA1) and tonoplast intrinsic protein (TIP; 48, 49) gene transcripts declined at the red fruit stage in the azygous plant. In the 556HO line, ArcA1 transcript was at a higher level and remained so during late ripening, but in the 579HO line the ArcA1 and TIP transcripts and their trends were similar to the control line. Eukaryotic translation initiation factor 5A2 (eIF5A2; 46) transcripts were at similar levels in the pink fruit of azygous and both the transgenic plants. This level increased in the red control fruit but declined in both the transgenic lines.

Interestingly, the transcript levels for mitochondrial cytochrome oxidase (CoxI; 47) showed an increasing trend during ripening of fruit from all 3 lines: azygous control, 556HO, and 579HO. However, it is clear that both the transgenics registered higher levels than the control, indicating that this mitochondrial gene is upregulated by higher polyamines (Figure 2). These results are consistent with the higher respiration rates found in the fruit from both the transgenic plants late into ripening compared to the control, azygous fruit (50).

In order to ascertain if the trends seen above for selected transcripts are also reflected in the pattern of corresponding protein products, immunoblot analyses were carried out. These results are presented in Figure 3 for ACC oxidase, PG, SAM synthetase, heat shock proteins (Hsp17.6, Hsp90), and γ and δ subunits of the TIP. Protein levels and patterns in pink versus red fruit for ACC oxidase and Hsp17.6 closely paralleled those for their transcripts (compare Figure 3 with Figure 2). There is a significant deviation between the TIP transcript expression and the levels of γ and δ subunits of the TIP. At both the stages of ripeness, both the transgenics showed a higher level of γ-TIP than the control, although the levels at the pink stage were considerably higher than at the red stage (Figure 3, γ-TIP). On the other hand, the levels of δ-TIP subunit were highest in the pink 579HO line fruit, but those in the red 579HO fruit were still higher than either the azygous control or 556HO fruit. These data suggest that polyamines may regulate TIP posttranscriptionally.

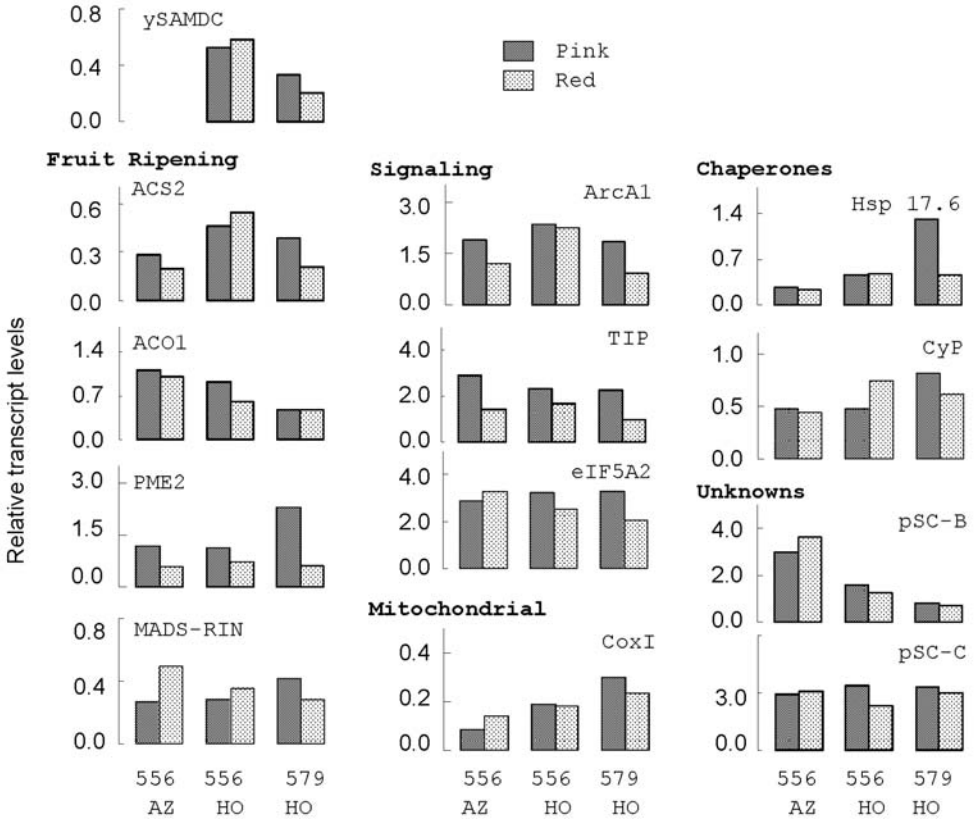


**Figure 2. Quantification of select class of genes analyzed by Northerns showing comparative changes in transcript abundance in ripening fruits between the azygous control (556AZ) and 2 independent, homozygous transgenic lines (556HO and 579HO) that accumulate higher polyamines, spermidine and spermine. Shown are genes that are fruit ripening-specific or implicated in signaling and chaperone activities. Also shown are changes in the abundance of a mitochondrial cytochrome oxidase I (CoxI) and other unidentified genes. AZ and HO represent lines azygous (a segregant of transgenic line with no transgene) and homozygous for the introduced yeast SAM decarboxylase gene, respectively. Details about yeast SAM decarboxylase, LeHsp17.6, LeArcA1, LeACO1, LeCyP, LeTIP, and LePME2 are provided in ref. 44. EIF5A2 (ref. 46), MADS-RIN (ref. 45), and CoxI (ref. 47) sequences from tomato ribonucleic acid were amplified by polymerase chain reaction using the information from the indicated references. The signal intensities were normalized to actin signals.**

Specificity of these data is revealed by relatively little change in the SAM synthetase protein between the lines at either fruit ripening stage. PG levels increased during ripening, and the differences were inconsistent among the 3 lines, the level of PG in red 556HO line being lower than the azygous line fruit, while in red 579HO fruit the level was higher than either of them.

## High Spd/Spm Fruits Are Nutritionally Enriched

An analysis of the principal, soluble constituents of wild-type and Spd/Spm-accumulating transgenic tomato, generated using high-resolution NMR spectroscopic methods, showed that the same metabolites were present in wild-type/azygous control tomatoes as in the transgenic tomato fruit. However, the latter conspicuously revealed differential metabolite content as compared to the controls (50). The red transgenic fruit were characterized by higher accumulation of the amino acids glutamine and asparagine; micronutrient choline; the organic acids citrate, fumarate, and malate; and an unidentified compound A. Compared to the control, wild-type fruit, the levels of valine, aspartic acid, sucrose, and glucose in the transgenic red fruit were reduced. These changes reflected specific alteration of metabolism, since the levels of isoleucine, glutamic acid, γ-aminobutyric acid, phenylalanine, and fructose remained similar in the nontransgenic and transgenic fruits. Consequently, the transgenic red fruit have significantly higher fructose/glucose and acid [citrate+malate]/sugar [glucose+fructose+sucrose] ratios (50), consistent with higher fruit juice and nutritional quality reported in the 2 transgenics (29), attributes favorably considered as higher quality in tomato breeding programs.

The essential micronutrient choline, one of the cellular methyl donors significantly accumulated and showed a profile during ripening similar to that of asparagine, glutamine, and compound A (50). An unidentified, singlet compound, compound C, possibly a choline derivative, was also higher in one of the transgenics. Choline has been classified as a "vital amine," with an essential role as a micronutrient required for

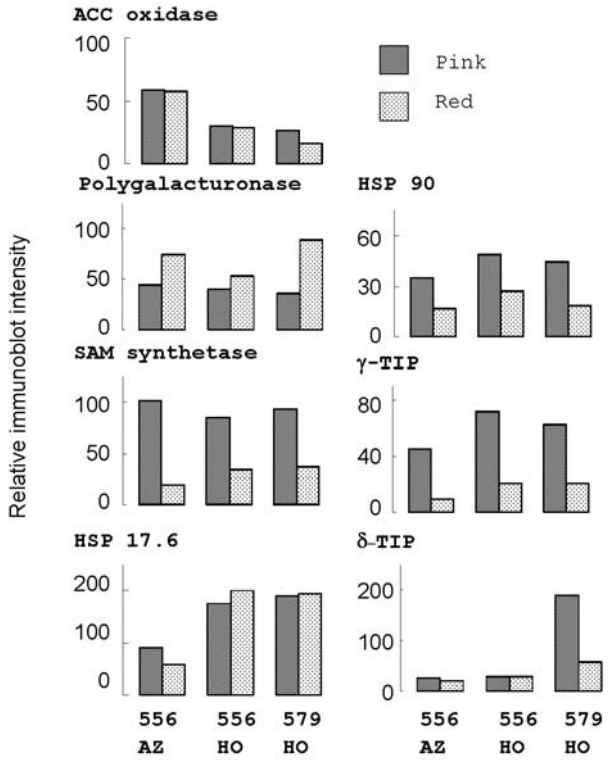


**Figure 3. Concurrence of transcript abundance with respective protein is exemplified by immunoblot analysis of selected fruit proteins. Except for γ and δ tonoplast intrinsic protein (TIP) subunits, which seem to be posttranscriptionally regulated, the other examples indicate coordinated regulation of transcript and protein abundance by higher polyamines.**

brain development (51, 52). An osmoprotectant glycine betaine is synthesized from choline (53, 54), however not in tomato (55), and is known to confer plant tolerance to environmental stresses such as salinity and drought (53, 56). Transgenic rice plants accumulating higher levels of Spd/Spm exhibit drought stress response (57). These results suggest that polyamine-regulated stress responses may occur via an effect on the biosynthesis of choline in other plants (54). It is likely, therefore, that Spd/Spm crosstalk with the aspartate family of amino acids and choline signaling networks.

The metabolic pathways that link the identified metabolites are illustrated in Figure 4. Mattoo et al. (50) suggested that the pathways involved in the nitrogen sensing/signaling and carbon metabolism are targeted when higher levels of Spd/Spm accumulate in the fruit. These results also demonstrated that fruit cells retain the "metabolic memory" late in fruit ripening, which can be tapped to activate various metabolic pathways independent of the parent plant. Spd/Spm may be sensed as "regulatory" amines/organic-N which, in turn, signal carbon metabolism. Thus, a reproductive organ, such as the transgenic fruit in this case, houses and maintains an organic-N (here Spd/Spm) sensing machinery late into ripening, as do roots and leaves. Further, the skeletons and moieties for most of the building blocks of biomolecules are more available in the transgenic fruit as compared to the control fruits, indicating a higher anabolic activity in these transgenics. The nature of the polyamine circuitry that integrates with transgene-activated N:C signaling responses remains to be determined. Thus, these results provided for the first time an insight into the profound effects that Spd and Spm have on cellular metabolism, likely via regulation of distinct biochemical pathways.

## Polyamines Revive Fruit's Metabolic Memory

Generally, fruit ripening was considered an irreversible, degradative process and an end point in the life of the plant development by leading to seed development and its dispersal once the whole fruit has degenerated (58). In recent years, molecular inroads into the ripening process have demonstrated the involvement of intricate regulation involving hormonal signaling and interconnected networks together that influence the ripening behavior (21, 22, 24, 26). The decisive evidence for ethylene as a plant hormone, identification of hormone receptors and their downstream signaling pathways, the demonstration of on/off responses to the availability or the lack of ethylene, and the success in controlling ripening behavior have enormously opened up the field of fruit ripening and our understanding of this process at the molecular level. It is becoming clear that fruits respond to endogenous and exogenous cues even at late ripening stages of development. Such an advance predicates that it will be possible in not-too-distant future to modulate ripening and influence nutrient content in fruits and vegetables by rational design of genes with precision-based and ripening stage-specific promoters.

Synthesis of information derived from transcriptome analysis and metabolic profiles of Spd/Spm accumulating, transgenic fruit suggests the presence of an intricate regulation and interconnection between certain metabolic pathways that respond to "signaling" molecules such as Spd and Spm. For instance, it would seem that tomato fruit senses Spd and Spm at certain threshold levels, which correspondingly leads to increases in N forms such as glutamine and asparagine. Glutamic acid, glutamine, and asparagine are the major N-forms in plant leaves (59–61). Asparagine to glutamine ratio (62), glutamine (60), and other amino acids (61) have been suggested as sensors of nitrogen status in plant cells.

The high Spd/Spm transgenic fruit follow N regulatory aspects similar to roots or leaves (59, 63), to optimize C-N budgets. This is revealed by the observations that the level of glucose decreased while that of citrate, malate, and fumarate remained higher in the red ripe transgenic fruit (Figure 4; 50). Glucose can feed into the tricarboxylic acid (TCA) cycle, which can generate more 2-oxoglutarate (2-OG) that, in turn, becomes a substrate to produce the glutamate family of amino acids. The higher content of citrate, malate, and fumarate in red ripe transgenic tomatoes in comparison to nontransgenic fruit suggests a more active metabolic status of the transgenic fruit. This is consistent with the increased respiratory activity (50) and upregulation of the mitochondrial

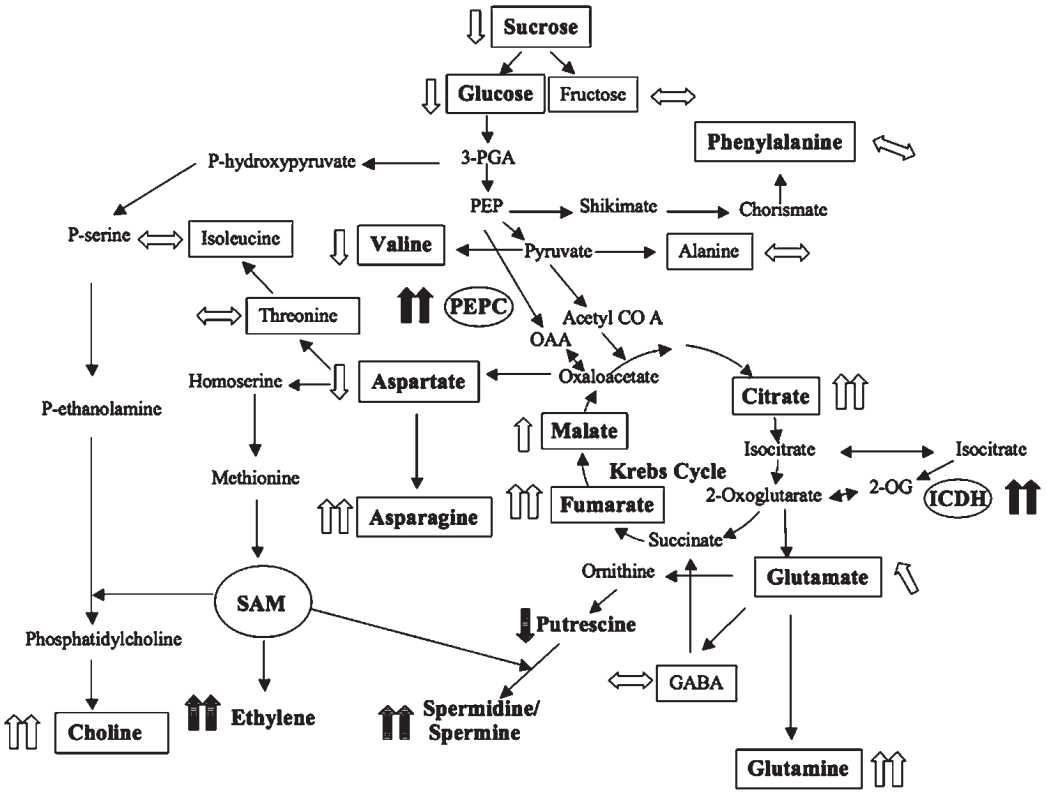


**Figure 4. An illustration of metabolic pathways for the biosynthesis of the identified metabolites, pinpointing linkages between nitrogen and carbon metabolism in the transgenic tomato fruits. Open arrows represent high (⇑), higher (⇑⇑), lower (⇓), or no change (⇔) in the indicated metabolite levels in the transgenic, higher polyamines accumulating red fruit compared to wild-type/azygous fruit. Light-dark, striped arrows indicate metabolites—spermidine, spermine, and ethylene—that were higher, and putrescine, which was lower, in the transgenics than the controls (from ref. 29). Dark arrows (⬆⬆) indicate the sites of the reaction of the corresponding transcripts of PEPC and ICDHc, whose levels were higher in the transgenic fruit than the control fruit. Adapted from Mattoo et al. (ref. 50).**

cytochrome oxidase transcripts (Figure 2) in the transgenic tomatoes as compared to azygous tomatoes. These data reveal an in vivo role of polyamines in mitochondrial metabolic regulation, as previously proposed for Spm function in rat liver mitochondria (64).

Further support for crosstalk between C metabolism and N sensing in the Spd/Spm accumulating, transgenic tomatoes was provided by the data showing upregulation of phospho*enol*pyruvate carboxylase (PEPC) and cytosolic isocitrate dehydrogenase (ICDHc) transcripts (50). These gene transcripts are generally seen activated in response to N assimilation (65, 66). Overexpression of PEPC in transgenic potato plants showed increased flux of soluble sugars and starch into production of organic acids such as malate and amino acids glutamate and glutamine (67). Thus, the sensing/signaling mechanism and gene players involved in N assimilation and carbon metabolism in different organs of a plant are conserved. By inference, therefore, N assimilation signaling in plants may also be linked to a certain threshold of higher polyamines, Spd/Spm.

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