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A genome-scale metabolic network reconstruction of tomato (Solanum lycopersicum L.) and its application to photorespiratory metabolism

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SUMMARY

Tomato (Solanum lycopersicum L.) has been studied extensively due to its high economic value in the market, and high content in health-promoting antioxidant compounds. Tomato is also considered as an excellent model organism for studying the development and metabolism of fleshy fruits. However, the growth, yield and fruit quality of tomatoes can be affected by drought stress, a common abiotic stress for tomato. To investigate the potential metabolic response of tomato plants to drought, we reconstructed iHY3410, a genome-scale metabolic model of tomato leaf, and used this metabolic network to simulate tomato leaf metabolism. The resulting model includes 3410 genes and 2143 biochemical and transport reactions distributed across five intracellular organelles including cytosol, plastid, mitochondrion, peroxisome and vacuole. The model successfully described the known metabolic behaviour of tomato leaf under heterotrophic and phototrophic conditions. The in silico investigation of the metabolic characteristics for photorespiration and other relevant metabolic processes under drought stress suggested that: (i) the flux distributions through the mevalonate (MVA) pathway under drought were distinct from that under normal conditions; and (ii) the changes in fluxes through core metabolic pathways with varying flux ratio of RubisCO carboxylase to oxygenase may contribute to the adaptive stress response of plants. In addition, we improved on previous studies of reaction essentiality analysis for leaf metabolism by including potential alternative routes for compensating reaction knockouts. Altogether, the genome-scale model provides a sound framework for investigating tomato metabolism and gives valuable insights into the functional consequences of abiotic stresses.

Keywords: genome-scale metabolic model, flux balance analysis, photorespiration, drought, tomato, reaction essentiality, Solanum lycopersicum L.

INTRODUCTION

Tomato is one of the most important vegetable crops grown in the world. An estimated worldwide production is 161.8 million tons fresh weight on 4.8 million hectares each year (FAO, 2014). In addition to its high economic value in the market, tomato has always drawn much attention due to its high content in health-promoting antioxidant compounds such as ascorbic acid (vitamin C), carotenoids, and flavonoids (Dixon, 2005; Story et al., 2010). Among the plentiful plant species bearing fleshy fruits, tomato serves as an excellent model system for addressing the fruit development and metabolism because of its ease of cultivation, and short life cycles (Klee and Giovannoni, 2011). Of particular interest is that the tomato fruit undergoes a shift from partially photosynthetic to
truly heterotrophic metabolism during development (Kahlau and Bock, 2008; Lytovchenko et al., 2011). Therefore, tomato is considered to be an ideal model plant for both basic and applied studies.

The growth, yield and fruit quality of tomatoes can be affected by several environmental factors, among which drought is a widespread abiotic stress, limiting the growth and yield of crop plants (Haupt-Herting et al., 2001; Pinheiro and Chaves, 2011; Nakashima et al., 2014). Under drought conditions, photosynthesis is inhibited mainly by stomatal limitation (Cornic, 2000; Flexas et al., 2007; Chaves et al., 2009). Moreover, the occurrence of photorespiration dramatically increases under drought stress as the leaf stomata are closed to prevent water loss, resulting in reduced CO₂ concentration in leaves (Bauwe et al., 2012). Photorespiration is a process in which O₂ competes with CO₂ to react with ribulose-1,5-bisphosphate (RuBP), thereby producing 3-phosphoglycerate (3-PGA) and 2-phosphoglycolate (2-PG). Although this process represents a potential limitation on photosynthetic efficiency and, ultimately, crop production, it has also been suggested that photorespiration plays an important role in other metabolic processes such as energy dissipation under stress conditions (Wingler et al., 2000).

In the past two decades, much effort has been made towards understanding the metabolic properties of tomato, ranging from physiology and biochemistry to molecular and genetics by conventional experimental techniques (Haupt-Herting et al., 2001; Lieberman et al., 2004; Liu et al., 2004). However, its metabolic behaviour and the interactions between different metabolic pathways across the whole system are still not completely understood. A recent emerging modelling approach, namely constraint-based modelling, has shown to be particularly promising as it offers a powerful tool to systematize our current knowledge of the complex metabolic network (Bordbar et al., 2014). Flux balance analysis (FBA), a constraint-based modelling approach, is able to predict the metabolic fluxes with stoichiometric metabolic models, some of which are at genome-scale (Orth et al., 2010). Genome-scale metabolism models are constructed by extracting the metabolic reactions encoded by the genomes of the target organisms. Large-scale metabolic models have been reconstructed for a variety of plants thus far, including Arabidopsis (Poolman et al., 2009; de Oliveira Dal’Molin et al., 2010a; Cheung et al., 2013; Arnold and Nikoloski, 2014), barley (Grafahrend-Belau et al., 2009, 2013), rice (Poolman et al., 2013), rapeseed (Hay and Schwender, 2011), and C₃ plants (de Oliveira Dal’Molin et al., 2010b; Saha et al., 2011; Simons et al., 2014). Most recently, a stoichiometric model was developed to describe the developing process of tomato fruit by modelling central metabolism (Colombie et al., 2015), but at a medium-scale level. Nevertheless, no large-scale model of tomato metabolism has been published to date. With the recent completion of the tomato genome in 2012 (Tomato Genome Consortium, 2012), it is timely to expand the scope to a genome-scale model of tomato metabolism for better understanding its metabolic behaviour by integrating biochemical, physiological, and proteomic data derived from the literature and databases.

Most modelling contributions have been made in the area of plant growth and primary metabolism with very little work focusing on changes in metabolism in response to abiotic stress such as drought. However, the effect of drought stress via water deficit cannot be directly modelled using FBA. Considering the changes in biomass composition and growth rates caused by drought (Boyer, 1982), we introduced the condition-specific growth rate that accounts for biomass accumulation in drought conditions to mimics the effect of drought. Simultaneously, we set the flux ratio of carboxylation to oxygenation of Rubisco (Vc/Vo) to a value of 1 to represent drought stress (Jordan and Ogren, 1984). In this study, we describe the reconstruction of a compartmentalized genome-scale metabolic model for tomato, we then used this model to simulate tomato leaf metabolism. We evaluated the model by simulating several well studied metabolic scenarios and showed that the simulation results were in good agreement with the main biochemical properties of plant metabolism as described in the literature. Subsequently, we applied the model to investigate the cellular metabolic characteristics, in particular the interplay of photorespiration with other pathways, under drought stress by identifying changes in reaction fluxes. The model predicted that the flux distributions through the mevalonate (MVA) pathway under drought conditions significantly differed from that under normal conditions, which gave hints to possible adaptive metabolic responses to drought stresses.

RESULTS AND DISCUSSION
General model properties

In this study, we aimed to build a genome-scale metabolic network for tomato by making use of the recent availability of the tomato genome (Tomato Genome Consortium, 2012). The metabolic reconstruction was based on information in LycoCyc (http://pathway.plantcollaborative.org/lycocyc.html), published literature and known biochemical knowledge in plants from textbook. The resulting model, HY3410, comprises 3410 genes, 1998 metabolites and 2143 reactions, of which 1211 could carry a non-zero flux given the defined nutrients input and the specified biomass components. Among the 2143 reactions, 1885 represent biochemical conversions and 258 represent metabolite transporter processes indicating the high interconnectivity among the intracellular compartments (i.e. cytosol, mitochondria, plastid, peroxisome, and vacuole) and with the environment. There were 11 exchange
reactions with the environment, of which O₂ and CO₂ can freely exchange with the environment. Biomass synthesis was represented by a set of independent transporters, one for each representative of biomass components: protein (amino acids), sugars, nucleotides, soluble metabolites, cell wall (cellulose, xylan and lignin), fatty acids and pigments (chlorophyll a and chlorophyll b). The model was able to simulate biomass synthesis of leaf using starch or CO₂, NO₃⁻ and/or NH₄⁺, SO₄²⁻, and Pi as the C, N, S, and P sources, respectively. A graphical overview of the reconstructed network is shown in Figure 1.

The definition of the photosynthetic light reactions in iHY3410 was considerably expanded over previous models of other plant species (de Oliveira Dal’Molin et al., 2010a; Lakshmanan et al., 2013b; Poolman et al., 2013). Rather than using aggregate reactions for the photosynthetic electron transport, we provided sufficient detail in iHY3410 to define all individual electron transport reactions including photosystem II (PSII), cytochrome b₆f complex, photosystem I (PSI), and ferredoxin NADP⁺ reductase (FNR) as components of the linear electron chain, the plastidic ATP synthase and the cyclic electron transfer ferredoxin plastoquinone reductase (FQR) reaction around PSI. The reconstruction was provided in SBML (System Biology Markup Language; Hucka et al., 2003) format, ScrumPy format and Excel format Data S1, S2 and S3, respectively. The inclusion of gene-protein-reaction (GPR) associations enables iHY3410 to be used for the integration and contextualization of proteomic and transcriptomic data.

Comparisons with existing plant models

The model properties of iHY3410 were compared with genome-scale metabolic models of two plant species, Arabidopsis (Cheung et al., 2013) and rice (Poolman et al., 2013). Our tomato model contained more unique reactions and metabolites than the rice model but fewer than the Arabidopsis model. One possible reason for the higher number of reactions and metabolites in the Arabidopsis model is that the biochemical databases from which the construction of the Arabidopsis model is based on is more comprehensively annotated. Of all the unique reactions and metabolites in iHY3410, 1417 reactions (72% overall) and 1344 metabolites (81% overall) were also present in Arabidopsis model (Figure 2a). Similarly, we systematically compared iHY3410 to a rice model (Figure 2b). Our tomato model shared 1155 reactions (64% overall) and 1043 metabolites (63% overall) with the rice model. This overlap was smaller than that with the Arabidopsis model, which reflects the divergence between eudicots, including Arabidopsis and tomato, and monocots such as rice (Considine et al., 2002; Tomato Genome Consortium, 2012). Irrespective of the Arabidopsis or rice models, a total of 269 reactions and 240 metabolites are specific to our tomato model. These reactions were predominately involved in secondary metabolism such as volatiles, alkaloids and phytoalexins biosynthesis. For example in iHY3410, reactions ‘RXN-6721’, ‘2.1.1.146-RXN’, ‘RXN-6741’ and ‘RXN-6742’ described the biosynthesis of volatile ester, which has no counterpart in Arabidopsis or rice. While iHY3410 was applied to model a growing tomato leaf cell in this study, the inclusion of reactions that are dedicated to the tomato fruits enables future investigations and characterization of fruit metabolism using our model. Phytoalexins have been considered as plant antibiotics in the plant defense systems, which are induced and accumulated in plants in response to microbial infection or abiotic stress (Kuc, 1995). Our tomato model contains a sequence of four reactions, ‘RXN-4843’, ‘RXN-4823’, ‘RXN-4844’ and

![Figure 1. Schematic overview of iHY3410. Different compartments are indicated along with the number of associated reactions. Grey arrows denote transporters across membranes or as biomass drains, with the number of reactions indicated.](© 2015 The Authors. The Plant Journal published by Society for Experimental Biology and John Wiley & Sons Ltd., The Plant Journal, (2016), 85, 289–304)
Heterotrophic conditions. During the dark period, cellular prediction. that only a subset of reactions were active in our model considered in the biomass constraints, it is not surprising respectively. Given that secondary metabolites were not and *Salmonella typhimurium* predicted that starch was degraded to form maltose and sole carbon source in the dark. As expected, the model tomato leaf was simulated by considering starch as the period. In this study, the metabolic behaviour of a growing stored compounds that are accumulated during the light metabolism of leaves is supported by the catabolism of recent reactions of the Calvin cycle. ‘RXN-4848’, which are responsible for sesquiterpenoid phytoalexins biosynthesis. Although phytoalexins are not synthesized in healthy plants (*Back et al.*, 1998), the inclusion of these reactions provides a platform for the future *in silico* investigation of disease states in tomato.

Model validation

The functional capabilities of *HY3410* were examined by predicting cell growth under heterotrophic, phototrophic and photosynthetic conditions using FBA with minimization of total flux as the objective function subjecting to mass-balance constraints for all internal metabolites and to the flux constraints of biomass production and ATP maintenance cost (see Experimental procedures). In total, between 359 and 371 (16.8–17.3%) of all reactions carried a non-zero flux in the examined conditions, while the majority of the reactions were not used in the simulations. Our observation did not stand alone as similar observations had also been reported in previous studies. For example, only 232 of 1406 reactions (16.5%), 309 of 1736 reactions (17.8%), and 248 out of 1097 reactions (22.6%) were used for biomass synthesis in a genome-scale model of Arabidopsis (*Poolman et al.*, 2009), rice (*Poolman et al.*, 2013), and *Salmonella typhimurium* (*Hartman et al.*, 2014), respectively. Given that secondary metabolites were not considered in the biomass constraints, it is not surprising that only a subset of reactions were active in our model prediction.

Heterotrophic conditions. During the dark period, cellular metabolism of leaves is supported by the catabolism of stored compounds that are accumulated during the light period. In this study, the metabolic behaviour of a growing tomato leaf was simulated by considering starch as the sole carbon source in the dark. As expected, the model predicted that starch was degraded to form maltose and glucose-1-phosphate (G1P). The glucose-6-phosphate (G6P) generated from G1P was predicted to be oxidized via the oxidative pentose phosphate (OPP) pathway, which predominately provides reducing power for the production of biomass, in particular for fatty acid synthesis (Figure 3a). The model also predicted that the mitochondrial pyruvate dehydrogenase (PDH) is active in darkened leaves, providing acetyl-CoA for fatty acid biosynthesis and as a substrate for the TCA cycle. Thereby, the TCA reactions operate in a conventional cyclic manner. The TCA cycle functions not only to generate ATP and reducing equivalents but also to provide carbon skeletons for the biosynthesis of amino acids. The operation of a complete cyclic flux through the TCA cycle was thought to be favorable to provide efficient energy in the dark, which is in line with the previous reports (*Tcherkez et al.*, 2005).

Phototrophic conditions. In photoautotrophic growth conditions, biomass synthesis is supported by the assimilation of CO₂ by RubisCO using light as the energy source. Under this imposed mass balance and with the fixed biomass, minimization of total flux predicted a photon influx of 9.84 mmol g⁻¹ DW h⁻¹. The production of ATP and reducing power by the photosynthetic light reaction liberates OPP pathway from supplying reducing power. The flux map obtained under illumination therefore exhibited a completely different flux distribution compared with heterotrophic conditions (Figure 3b). Not surprisingly, the pentose phosphate pathway shifted to the reductive mode as ribulose-5-phosphate kinase (PRK) and RubisCO became active in the presence of light, which then drove the subsequent reactions of the Calvin cycle.

Compared with the cyclic mode of the TCA cycle in heterotrophic conditions, the TCA cycle reactions shifted to a noncyclic mode in phototrophic conditions in our prediction, where the reactions from α-ketoglutarate (2-OG) to...
Figure 3. Flux distribution of the tomato central metabolism under (a) dark conditions; and (b) light conditions. The solid lines indicate reactions with non-zero fluxes, the grey lines indicate reactions with zero fluxes, and the dashed lines indicate reactions with zero or non-zero fluxes based on FVA solutions.
malate carry no flux. The enzyme 2-oxoglutarate dehydrogenase (OGDH) plays an essential role in overall metabolic activity, in particular nitrogen assimilation (Bunik and Fernie, 2009). Nevertheless, the genetic manipulation of OGDH in tomato plants revealed little impact on the photosynthetic capacity (Araujo et al., 2012b). Given the fact that multiple cytosolic isoforms exist for the mitochondrial TCA reactions, it would not be surprising that some mitochondrial TCA isoforms were not used in our model prediction (Sweetlove et al., 2010), e.g. fumarase operates in the form of cytosolic bypass in the prediction. Succinate dehydrogenase (SDH, also known as Complex II) has a dual function as a component of the TCA cycle and the electron transport chain (ETC). As yet, the role of SDH has been studied in tomato plants employing mutagenic approach, showing that inhibition of the iron-sulphur subunit of Complex II had a favorable effect on the photosynthesis (Araujo et al., 2011). Thus, it is not unexpected that in our prediction, Complex II was not used under light conditions (Figure 3b). The operation of an incomplete TCA cycle in the light by our tomato model is supported by a study in French bean leaves (Phaseolus vulgaris), which indicated that the TCA cycle was almost completely inhibited in the light (Tcherkez et al., 2005). Similarly, Poolman et al. (2013) reported that the TCA cycle did not function as a conventional cycle at high light conditions in rice.

Photorespiratory conditions. To investigate specific features of photorespiratory metabolism in tomato plants, we simulated the reconstructed model by setting a 3:1 carboxylation to oxygenation ratio (Vc/Vo = 3) based on experimental evidence (Leegood, 2007), with other constraints including biomass synthesis unchanged. Not surprisingly, the photon input was 10.89 mmol g⁻¹ DW h⁻¹, higher than that of non-photorespiratory conditions (9.84 mmol g⁻¹ DW h⁻¹) as photorespiration is generally thought of as a process which reduces the efficiency of carbon fixation (Douce and Neuburger, 1999). The resulting flux distribution is depicted in Figure 4.

The photorespiratory pathway spanned four subcellular compartments in wildtype including the plastid, the peroxisome, the mitochondrial and the cytosol. The associated transporters between different compartments such as glycolate and glycerate transporters were also included in the model. It has been established that the mitochondrial enzyme glycine decarboxylase (GDC), which catalyzes the tetrahydrofolate-dependent catabolism of glycine, has essential roles in metabolic processes. This enzyme functions in the photorespiratory pathway of all photosynthetic tissues of C₃ plants. In addition, it contributes to one-carbon metabolism in association with serine hydroxymethyltransferase (SHMT), which forms an obligatory route for C1 metabolism (Mouillon et al., 1999; Hanson and Roje, 2001; Engel et al., 2007). In our model prediction, the cytosolic SHMT was in operation with GDC (reaction 7, Figure 4).

During photorespiration, ammonia is generated in the mitochondria due to the oxidation of glycine by GDC. This is re-assimilated by the glutamine synthetase/glutamate synthase (GS/GOGAT) system to scavenge the cytotoxic metabolite. The mechanism of NH₃ re-assimilation has been a subject of controversy to date. GOGAT, either in the form of Fd-GOGAT or NADH-GOGAT, is exclusively present in the plastid to produce glutamate (Coschigano

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**Figure 4.** Flux distribution of photorespiratory metabolism under normal conditions (Vc/Vo = 3). The solid lines indicate reactions with non-zero fluxes, the dashed lines indicate reactions with zero or non-zero fluxes based on FVA solutions. Numbered reactions are catalyzed by the following enzymes: 1, RubisCO oxygenase; 2, Phosphoglycolate phosphatase; 3, Glycolate oxidase; 4, Catalase; 5, Glutamate:glyoxylate aminotransferase; 6, Glycine decarboxylase; 7, Serine hydroxymethyltransferase; 8, Glutamine synthetase; 9, Serine:glyoxylate aminotransferase; 10, Hydroxypropruvate reductase; 11, Glycerate kinase; 12, Glutamate synthase; 13, Nitrate/nitrite reductase; ETC, electron transport chain.
et al., 1998). Therefore, it was assumed that the photorespiratory NH3 is transported to plastids, which is then re-assimilated into glutamate by GS and GOGAT (Wingler et al., 2000). In contrast, an isoform of GS was found to be dual targeted to the chloroplast and the mitochondria in Arabidopsis leaves (Taira et al., 2004). Based on this, Linka and Weber (2005) hypothesized a possible metabolite route where the NH3 is assimilated by the mitochondrial GS, which does not require more energy than the plastidic GS route. Our model prediction supports the hypothesis that NH3 generated by GDC during photorespiration could be re-assimilated by the mitochondrial GS, rather than being transported to the chloroplast.

For further confirmation, flux variability analysis (FVA) was performed to examine the flux capacity for each reaction under the simulated conditions (Data S4). As we expected, light-dependent reactions such as photosystem II and photosystem I in the photosynthetic light reactions, PRK and Rubisco from the Calvin cycle, nitrate reductase (NR), nitrite reductase (NiR), plastidic GS andFd-GOGAT involved in nitrogen assimilation were activated by light. These reactions characterize the metabolic response that must be appropriately regulated in order to adapt to the available energy/carbon source.

Study of photorespiratory metabolism under stressed conditions

As previously mentioned, drought is a common abiotic stress that limits tomato growth, yield and fruit quality. To elucidate the metabolic response of tomato plants to water-limited conditions, we modelled the drought stress using our reconstructed model by incorporating the drought-specific growth rate of tomato (2.84 mg g−1 DW h−1) based on the work of Sánchez-Rodríguez et al. (2010) while constraining the ratio of Vc/Vo to a value of 1 (i.e. Vc/Vo = 1), which results from the stomata closure in response to drought stress that leads to a reduced CO2 concentration in leaves (Bauwe et al., 2012; Lakshmanan et al., 2013a). We then compared the metabolic characteristics of drought conditions with that of normal conditions with a growth rate of 3.75 mg g−1 DW h−1 (Sánchez-Rodríguez et al., 2010), and the ratio of Vc/Vo was set to 3 (i.e. Vc/Vo = 3). In total, 376 and 371 reactions were found to be active under drought and normal conditions, respectively. A set of 358 active reactions were common between the two flux solutions. It is expected that the photon uptake rate under stressed conditions (13.32 mmol g−1 DW h−1) was higher than that under normal conditions (10.88 mmol g−1 DW h−1) as more energy is required for the recycling of higher amounts of 2-PG produced under drought stress. Differences in metabolically active reactions between normal and drought conditions. We identified sets of reactions which were only used either in normal or in drought conditions according to results from FVA, and we investigated these reactions in more detail. In total, 10 reactions were identified to carry non-zero flux only under normal conditions, which implies these reactions were always inactive under drought conditions. Among these reactions, eight reactions (EC 2.2.1.7; 1.1.1.267; 2.7.7.60; 2.7.1.148; 4.6.1.12; 1.17.1.2; 1.17.7.1) are involved in the 2C-methyl-erythritol-4-phosphate pathway or MEP pathway (Table 1). Conversely, 14 reactions were found to be active under drought conditions, of which seven (EC 6.2.1.16; 1.1.1.34; 2.7.1.36; 2.7.4.2; 4.1.1.33; 2.3.1.9; 4.1.3.4) belong to MVA pathway, whilst, three are the related metabolite transporters ('CPD-499_Pero_tx', 'DELTA3-IPP_Pero_tx' and 'DELTA3-IPP_Plas_tx'). In plants, both the MEP pathway and the MVA pathway are predominantly used to synthe-

| Identifier | Description |
|------------|-------------|
| Vc/Vo = 3 | EC 2.2.1.7; MEP pathway |
| DXS-RXN_Pl | | |
| DXPREDISOM-RXN_Pl | | |
| 2.7.7.60-RXN_Pl | | |
| 2.7.1.148-RXN_Pl | | |
| RXN0-302_Pl | | |
| RXN0-884-(NADP)_Pl | | |
| ISPH2-RXN-(NADP)_Pl | | |
| CPKIN-RXN_Pl | | |
| CMPKI-RXN_Pl | | |
| ACETOACETATE—COA-LIGASE-RXN_Cyto | | |
| ATP-CITRATE-PRO-S—LYASE-RXN_Cyto | | |
| 1.1.1.34-RXN_Cyto | | |
| MEVALONATE—KINASE-RXN_Cyto | | |
| PHOSPHOHEMVALONATE—KINASE-RXN_Pero | | |
| IPPISOM-RXN_Pl | | |
| DIPHOSPHOHEMALONATE—DECARBOXYLASE-RXN_Pero | | |
| ACETYL-ACOA—ACETYLTRANSFER—RXN_Cyto | | |
| HYDROXYMETHYLGLOTHARYL—COA—LYASE-RXN_Cyto | | |
| CPD-499_Pero_tx | Peroxiosomal phosphohemvalonate transporter |
| DELTA3-IPP_Plas_tx | Plastidic isothenylpyrophosphate transporter |
| DELTA3-IPP_Pero_tx | Peroxiosomal isothenylpyrophosphate transporter |
| CO2_Pero_tx | Peroxiosomal CO2 transporter |
| ATP_ADG_Pero_tx | Peroxiosomal ATP transporter |
size isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP), which can be further utilized for the biosynthesis of isoprenoids (Vranova et al., 2013). Isoprenoids, also called terpenoids, are required for the production of a variety of important compounds such as chlorophylls, carotenoids, and plant hormones. The MEP pathway utilizes pyruvate and d-glyceraldehyde-3-phosphate (GAP) for IPP production, which takes place in the plastid. Isoprenoid biosynthesis through the MEP pathway depends on the availability of reduced carbon, ATP and NADPH, which are contributed by photosynthesis in unstressed plants (Loreto and Schnitzler, 2010). In contrast, the MVA pathway in the cytosol and the peroxisome uses acetyl-CoA as the precursor for IPP synthesis. Ultimately, IPP generated either via the MEP pathway or the MVA pathway was used for the biosynthesis of chlorophylls in our model (Figure 5).

Compartmentalization of IPP-generating pathways would enable plants to optimize isoprenoid biosynthesis and regulation according to fixed carbon, available ATP and reducing power in diverse environmental conditions. Previous studies have shown that chlorophylls and carotenoid-related pigments are derived from the plastidic MEP pathway (Lichtenthaler et al., 1997), while MVA-derived precursors can be transported into the plastid and used for the isoprenoids biosynthesis in the plastid (Wang et al., 2003; Rodriguez-Concepcion et al., 2004). Under normal conditions, our model predicted that IPP was synthesised via the chloroplastic MEP pathway, which supports previous findings that isoprenoid is produced primarily using carbon skeletons directly derived from the Calvin cycle via the photosynthesis-dependent MEP pathway under stress-free conditions (Affek and Yakir, 2002; Fortunati et al., 2008). However, under drought conditions, our model predicted that the MVA pathway predominated over the MEP pathway for producing IPP for chlorophyll biosynthesis. The precursor for IPP biosynthesis through the MVA pathway is cytosolic acetyl-CoA. A detailed investigation of the flux solution under drought conditions revealed a metabolic route that produces acetyl-CoA from primary photo-synthates via phosphoenolpyruvate carboxylase (PEPC) and threonine aldolase (Figure 5). For each molecule of 3-PGA converted into oxaloacetate (OAA) via phosphoenolpyruvate (PEP), one molecule of CO$_2$ is fixed by PEPC. OAA is then used as carbon skeleton to form threonine. Threonine aldolase splits threonine into acetaldehyde, which is converted into acetyl-CoA after oxidation to acetate, and glycine, which is recycled through the photorespiratory pathway to produce 3-PGA. For each molecule of glycine recycled, 0.5 molecule of CO$_2$ is released and 0.5 molecule of 3-PGA is produced. In summary, the carbon of acetyl-CoA comes from 0.5 molecule of 3-PGA and 0.5 molecule of CO$_2$ of which one molecule is fixed by PEPC and 0.5 molecule is released from the photorespiratory pathway. While this route appears to be inefficient in normal conditions, it could become favorable under

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**Figure 5.** Subcellular compartmentalization of the MVA and MEP pathways and ABA biosynthesis. Red lines indicate the corresponding reactions are only active under normal conditions ($Vc/Vo = 3$); blue lines indicate reactions are only active under drought conditions ($Vc/Vo = 1$); black solid lines indicate reactions are active both under normal and drought conditions; dashed lines indicate those reactions are not active in the prediction. 3-PGA, 3-phosphoglycerate; PEP, phosphoenol pyruvate; OAA, oxaloacetic acid; GAP, glyceraldehyde-3-phosphate; MEP, 2C-methyl-D-erythritol-4-phosphate; IPP, isopentenyl pyrophosphate; MVA, mevalonate; MVA-5P, mevalonate-5-phosphate; ABA, abscisic acid.
drought condition when the leaf internal CO₂ concentration is so low that the cost of fixing CO₂ by RubisCO coupled with a high level of photorespiration becomes higher than the cost of the PEPC-threonine aldolase route in which some of the carbon for IPP synthesis is fixed by PEPC. The PEPC-threonine aldolase route has also been proposed as a possible route for producing acetyl-CoA for citrate synthesis in leaves growing under constant illumination (Cheung et al., 2014).

It is evident that drought stress leads to a substantial reduction in the rate of photosynthetic CO₂ assimilation, resulting in limited photosynthesize accumulation and an increased supply of photosynthetic energy and reducing power to non-photosynthetic carbon reduction sinks such as photorespiration. It has been proposed that the MEP pathway competes with other sinks such as photorespiration for the reducing power, which is not invested in carbon assimilation for primary metabolism (Dani et al., 2014; Morfopoulos et al., 2014). However, the reducing power requirement by the MEP pathway is relatively small compared to that of photorespiration (Sharkey et al., 2008; Dani et al., 2014). When photosynthesize is limiting, alternate carbon sources such as intermediates from the MVA pathway and photorespiration could directly contribute to isoprenoid production in plants (Flügge and Gao, 2005; Jardine et al., 2014). Isoprenoid emissions can even continue in the complete absence of photosynthesis under severe drought conditions (Pegoraro et al., 2004; Fortunati et al., 2008). Furthermore, it has been suggested that genes encoding in the MVA pathway are activated and highly expressed in response to oxidative stress (Vranova et al., 2013), which also supports our model prediction that the MVA pathway was used for IPP biosynthesis under drought conditions.

Given that minimization of total flux was used as objective function in this study, it can be inferred that the MEP pathway is more efficient in terms of amount of flux for the biosynthesis of IPP than the MVA pathway under normal conditions, and vice versa for drought conditions. To confirm this, we simulated four scenarios, in which IPP can only be produced either via MEP pathway or MVA pathway. The four scenarios were denoted as MEP:Normal, MVA:Normal, MEP:Drought, and MVA:Drought. As expected, the sum of total flux for MEP:Normal scenario was lower than that for MVA:Normal scenario, which were 65.85 and 65.95 mmol g⁻¹ DW h⁻¹, respectively. Likewise, the total flux of MVA:Drought scenario was lower than that of MEP:Drought scenario, which were 77.86 and 77.89 mmol g⁻¹ DW h⁻¹, respectively. Regarding the photon influx, MEP:Normal scenario required less photons than that for MVA:Normal scenario (10.89 and 9.90 mmol g⁻¹ DW h⁻¹, respectively), despite a higher total RubisCO flux with the MEP pathway (0.320 and 0.318 mmol g⁻¹ DW h⁻¹, respectively). By contrast, MEP:Drought scenario required more photons than that for the MVA:Drought scenario (13.34 and 13.32 mmol g⁻¹ DW h⁻¹, respectively) because the photon requirement for the net fixation of CO₂ was higher under drought conditions (total RubisCO flux was 0.604 and 0.600 mmol g⁻¹ DW h⁻¹ for the MEP: Drought scenario and the MVA:Drought scenario, respectively).

It is noteworthy that abscisic acid (ABA), belonging to isoprenoids, derives from the intermediate IPP (Figure 5). A wealth of evidence supports the hypothesis that ABA is a regulator triggering plant responses to numerous abiotic stresses such as drought (Cornish and Zeevaart, 1985; Harris and Outlaw, 1991). During drought stress, the synthesis of ABA was induced rapidly, whereas the increased amounts of ABA promoted stomatal closure to reduce water loss (Bray, 1988). ABA in higher plants is derived from carotenoid precursors through an oxidative cleavage reaction in plastid, followed by a two-step conversion of the xanthoxin to ABA via ABA-aldehyde (Xiong and Zhu, 2003). However, because carotenoids are not in the output constraints of the model, this ABA biosynthetic route was not active in our model prediction, whilst, the observations that reactions in the MVA pathway were activated for biomass synthesis may relate to an increase in ABA biosynthesis in response to drought stress.

As sessile organism, plants have evolved several mechanisms that allow them to cope with drought stress, for example, increased accumulation of secondary metabolites such as phenols and isoprenoids (Wilhelm and Selmar, 2011). It has been documented that isoprenoids can provide protection from abiotic stresses, e.g. through quenching the accumulation of reactive oxygen species (ROS), which result in oxidative damage (Loreto and Velikova, 2001). One of the inevitable consequences caused by drought stress is the over accumulation of ROS (Smirnoff, 1993). The switch from the MEP pathway to the MVA pathway could also be due to isoprenoid production, which allows plants to protect against the deleterious effects caused by drought stress.

To test if the operation of the MVA pathway under drought conditions is related to the choice of the objective function, we calculated flux solutions using minimization of photon uptake as the objective function as well (Data S5). Minimization of photon uptake gave similar flux solutions to those from minimization of total flux in regard to IPP biosynthesis, which strengthens our hypothesis that IPP is produced via the MEP pathway under normal conditions, but switched to the MVA pathway in drought conditions.

**Effect of drought stress on the core metabolic pathways.** Photorespiratory metabolism affects many parts of cellular metabolism, including the Calvin cycle, the TCA cycle as well as nitrogen assimilation (Foyer et al., 2009).
To understand the coordinated function of photorespiration with other metabolic pathways, we investigated the change in fluxes between normal and drought conditions for reactions involved in several important metabolic processes (Table 2). With exposure to drought stress, sharp increases in fluxes through the Calvin cycle and photorespiration were observed to support the biomass synthesis. Growth reduction under water-deficit conditions has been well documented (Sánchez-Rodríguez et al., 2010; Pinheiro and Chaves, 2011). In the model flux solutions, reactions involved in ammonia assimilation (the mitochondrial GS and Fd-GOGAT) showed significant differences in fluxes between normal and drought conditions. Large amounts of ammonia released during photorespiration via GDC in the mitochondria, were re-assimilated via mitochondrial GS. Similar to the flux distribution under non-photorespiratory light conditions (Figure 3b), reactions through the OPP pathway were inactive, and the TCA cycle exhibited a non-cyclic flux mode under stressed conditions. Haupt-Herting et al. (2001) have showed that the rate of mitochondrial respiration in the light is reduced under drought stress in tomato mature leaves, while, we modelled a growing leaf cell. Though a growing body of evidence from tomato and Arabidopsis suggests that the activity of the TCA cycle was considerably higher in the light than reported by Tcherkez et al. (2005), the degree of inhibition of the TCA cycle in the light is still controversial (Araujo et al., 2012a). Compared with other TCA reactions, the reaction catalyzed by malate dehydrogenase (MDH) carried a relatively high flux in both normal and drought conditions in our prediction. This could be because MDH functions in redox perturbation but also acts as a component of the malate-aspartate and malate-oxaloacetate shuttles for the exchange of reducing equivalents between the mitochondria and the cytosol.

Evaluation of reaction deletions

A genome-scale metabolic model is a powerful tool to assess the metabolic capabilities of an organism. Aiming to examine the phenotypes of genetic manipulations with the model, we performed a reaction essentiality analysis for normal ($V_c/V_o = 3$) and drought-stressed conditions ($V_c/V_o = 1$). To investigate the essentiality of each reaction, we added an additional constraint by setting each reaction except the biomass transporters to carry zero flux in addition to the general constraints described in Experimental procedures.

In total, 146 and 149 lethal reaction knockouts were identified for normal and drought conditions, respectively, indicating that these reactions must carry non-zero fluxes to support biomass synthesis under the simulated conditions (Table S6). All 146 essential reactions identified for normal conditions were also indispensable for drought conditions. With respect to the ability to produce biomass, approximately 93% of the reactions in the model were non-essential, reflecting that the metabolic network was robust to random knockout mutations. We selected a number of key enzymes mainly from the Calvin cycle and the photorespiratory pathway, and compared their essentiality in models of tomato, rice, Arabidopsis and maize for further verification (Table 3). In general, these predictions are supported by the existing experimental evidence available in other plants such as Arabidopsis and barley.

Although a few of the essential reactions are common across all four plants, such as RubisCO, ribulose-5-phosphate epimerase and PGLP, our predictions showed significant difference from that of rice, Arabidopsis and maize (Table 3). The essential reactions of HY3410 listed in Table 3 were identified as essential in rice as well (Lakashman et al., 2013a). However, most of photorespiratory reactions including glutamate:glyoxylate aminotransferase (GGT), serine:glyoxylate aminotransferase (SGT), SHMT, hydroxy pyruvate reductase (HPR), glycerate kinase (GLYK) and catalase (CAT) can be bypassed by other isoforms or pathways in HY3410, but were regarded as essential in the core rice model (Lakashman et al., 2013a). Importantly, the predictions of essentiality are critically dependent on the coverage and completeness of the model. In the case of rice, these simulation results were obtained from a core metabolic network with 417 reactions and only three sub-cellular compartments (cytosol, chloroplast, and mitochondrion) without the peroxisome. In comparison, our tomato model was not only constructed at the genome-scale level but also more compartmentalized. It is likely that the addition of compartmentation in a model will give rise to parallel and alternative pathways (Sweetlove and Ratcliffe, 2011). For example, glyoxylate oxidized from glycolate by GOX is subsequently metabolized by aminotransferases which redistributes the nitrogen to a range of amino acids, which then are used for synthesis of the other amino acids. The assignment of the reactions involved in photosynthetic metabolism in our tomato model and the rice model were illustrated in Figures 4 and S1, respectively, and were summarized in Table S7. Though the directionality of these reactions was consistent between the two models, the assigned compartments were different for most of the reactions. In the peroxisome of HY3410, there were three aminotransferases involved in the redistribution of amino group in the photosynthetic pathway, namely SGT, GGT and alanine:glyoxylate aminotransferase (AGT). As a result, unlike other photosynthetic mutants (e.g. PGLP), mutants defective in SGT, GGT or AGT activity did not result in a lethal phenotype in our predictions from HY3410. By contrast, SGT was classified as essential in the core rice model in which AGT was not included. Likewise, our simulations showed that knockout of RPI ($V_c/V_o = 3$) and HPR can be complemented by alternative routes (Figures S2 and S3). These examples illustrate that the
Table 2 Predicted flux levels (μmol g⁻¹ DW h⁻¹) of the main metabolic reactions under normal (Vc/Vo = 3) and drought conditions (Vc/Vo = 1)

| Enzyme name                  | EC no. | Biochemical reaction                                  | Vc/Vo = 3 | Vc/Vo = 1 |
|------------------------------|--------|-------------------------------------------------------|-----------|-----------|
| Calvin cycle                 |        |                                                       |           |           |
| RubisCO carboxylase          | 4.1.1.39 | RuBP + CO₂ → 2-3PGA                                     | 239.67    | 300.11    |
| PGK                          | 2.7.2.3 | 3-PGA + ATP ↔ DPG + ADP                                 | 576.23    | 1037.85   |
| GAPDH                        | 1.2.1.13 | DPG + NADPH ↔ GAP + NADP + P₁                          | 576.23    | 1039.23   |
| TPI                          | 5.3.1.1 | GAP ↔ DHAP                                             | 232.72    | 404.30    |
| Aldolase                     | 4.1.2.13 | GAP + DHAP ↔ FBP                                       | -110.01   | -202.65   |
| FBPass                       | 3.1.3.11 | FBP → F6P + P₁                                         | 110.01    | 202.65    |
| TKT                          | 2.2.1.1 | GAP + F6P ↔ E4P + XSP                                  | -110.01   | -202.65   |
| Aldolase                     | 4.1.2.13 | DHAP + E4P → SBP                                       | 108.69    | 201.65    |
| SBPass                       | 3.1.3.37 | SBP → S7P + P₁                                         | 108.69    | 201.65    |
| TKT                          | 2.2.1.1 | GAP + S7P ↔ RSP + XSP                                  | -108.69   | -201.65   |
| RPE                          | 5.1.3.1 | XSP ↔ Ru5P                                            | -218.75   | -404.34   |
| RPI                          | 5.3.1.6 | Ru5P ↔ RuSP                                           | 101.07    | 195.88    |
| PRK                          | 2.7.1.19 | Ru5P + ATP ↔ RuBP + ADP                                | 319.82    | 600.22    |
| Photosynthesis               |        |                                                       |           |           |
| RubisCO oxygenase            | 4.1.1.39 | RuBP + O₂ → 3-PGA + 2-PG                               | 79.96     | 300.11    |
| PLGP                         | 3.1.3.18 | 2-PG → Glit + P₁                                       | 79.96     | 300.11    |
| GOX                          | 1.1.3.15 | Glit + O₂ → Gox + H₂O₂                                  | 79.96     | 300.11    |
| CAT                          | 1.11.1.6 | 2 H₂O₂ + 2 H₂O → O₂                                     | 39.97     | 150.05    |
| GTR                          | 2.6.1.4 | Glu + Gox → Gly + 2-OG                                  | -46.54    | -153.95   |
| AGT                          | 2.6.1.44 | Ala + Gox → Gly + Pyr                                   | -         | -         |
| GDC                          | 1.4.4.2; 2.1.2.10; 1.8.1.4 | Gly + THF + NAD⁺ → CO₂ + NH₃ + NADH + M-THF              | 45.55     | 155.35    |
| SHMT                         | 2.1.2.1 | Gly + M-THF ↔ Ser + THF                                 | -         | -         |
| STG                          | 2.6.1.45 | Ser + Gox → OH-PYR + Gly                               | 33.51     | 146.23    |
| HPR                          | 1.1.1.29 | OH-PYR + NADH → Glyerate + NAD                         | 33.51     | 146.23    |
| GLYK                         | 2.7.1.31 | Glyceraldehyde + ATP → 3-PGA + ADP                      | 33.51     | 146.23    |
| TCA                          |        |                                                       |           |           |
| PDH                          | -      | Pyr + NAD⁺ → CoA → AcCoA → NADH + H⁺ + CO₂             | -         | -         |
| CIT                          | 2.3.1.1 | OAA + AcCoA + H₂O → Cit + CoA                          | -         | -         |
| Aconitase                    | 4.2.1.3 | Cit ↔ Isocit                                          | 3.77      | 2.84      |
| IDH                          | 11.1.1.41 | IsoCit + NAD⁺ → 2-OG + NADH + H⁺ + CO₂                  | 3.77      | 2.84      |
| OGDH                         | 1.2.4.2; 1.8.1.4 | 2-OG + NAD⁺ + CoA → SucCoA + NADH + H⁺ + CO₂          | -         | -         |
| STK                          | 6.2.1.5 | SucCoA + P₁ + ADP + H⁺ → Suc + ATP + CoA               | -         | -         |
| Complex II                   | 13.99.1; 1.3.5.1 | Suc + Q → Fum + OH₂                                  | -         | -         |
| Fumarase                     | 4.2.1.2 | Fum + H₂O → Mal                                        | -         | -         |
| MDH                          | 11.1.37 | Mal + NAD⁺ ↔ OAA + NADH + H⁺                           | 1969.48   | 2192.19   |
| OPPP                         |        |                                                       |           |           |
| G6PD                         | 1.1.1.49 | G6P + NAD⁺ → 6PGL + NADPH + H⁺                          | -         | -         |
| 6PGL                         | 3.1.3.31 | 6PGL + H₂O → 6PG                                       | -         | -         |
| 6PGD                         | 1.1.1.44 | 6PG + NAD(P)⁺ ↔ Ru5P + NAD(P)H + CO₂ + H⁺              | -         | -         |
| ETC                          |        |                                                       |           |           |
| Complex I                    | 1.6.5.3 | NADH + Q → 5 H⁺ → NAD⁺ + OH₂ + 4 H⁺                    | 2018.80   | 2079.37   |
| Complex II                   | 1.10.2.2 | OH₂ + 2Cy₇red + 2 H⁺ → Q + 2Cy₇red + 4 H⁺               | 2021.11   | 2081.13   |
| Complex IV                   | 1.9.3.1 | 4Cy₇red + O₂ + 8 H⁺ → 4 Cy₇red + 2H₂O + 4 H⁺          | 1010.59   | 1040.59   |
| Complex V                    | 3.6.3.14 | ADP + P₁ + 4 H⁺ ↔ ATP + H₂O + 4 H⁺                    | 1557.50   | 1612.28   |
| Nitrogen assimilation        |        |                                                       |           |           |
| Nir                          | 1.7.7.1 | NO₃ + NADH → NO₂ + NAD⁺                               | 17.62     | 13.34     |
| NR                           | 1.7.1.2 | Fd₇red + NO₂ → Fd₇ox + NH₄⁺                            | 17.62     | 13.34     |
| GS_Plus                      | 6.3.1.2 | NH₃ + Glu + ATP → Gln + ADP + P₁ + H⁺                  | 35.10     | 26.56     |
| GS_Mito                      |        |                                                       | 45.55     | 155.35    |
| GOGAT                        | 1.4.1.14 | Glu + 2-OG + NADH + H⁺ → 2 Glu + NAD⁺                  | -         | -         |
|                             | 1.4.7.1 | Glu + 2-OG + 2Fd₇red + H⁺ → 2 Glu + 2Fd₇ox            | 70.01     | 173.86    |

*‘-‘ indicates no flux. ‘GS_Plus’ indicates the plastidic GS; ‘GS_Mito’ indicates the mitochondrial GS.*
application of essentiality analysis on a core metabolic network could give an overestimation of essential reactions as potential alternative routes that may compensate the reaction deletion were not included in the core model.

The model predictions on reaction essentiality may also differ depending on the conditions simulated. For example, in Arabidopsis and maize, 1,4-alpha-glucan branching enzyme (EC 2.4.1.18) was essential when optimizing biomass production, while in the same networks, its knockout had no lethal effect regarding CO₂ fixation (Wang et al., 2012). A handful of photorespiratory mutants in C₃ species have been generated, notably Arabidopsis and barley, but a very limited set has been generated for tomato (Foyer et al., 2009). Thus, the majority of the in silico photorespiratory mutants predicted from our tomato model remain to be validated experimentally.

CONCLUSIONS

In this study, we reconstructed a genome-scale metabolic network for tomato and used it to model tomato leaf metabolism. iHY3410 allowed us to simulate the metabolic behaviour under various environmental conditions, including normal and drought conditions using FBA. Our model simulations predicted that the flux distributions through the MVA pathway under drought-stressed conditions were distinct from that under normal conditions. In addition, the change in fluxes through the core metabolic pathways between normal and drought conditions reflected the plant adaptive response to drought stress. The application of genome-scale networks to the abiotic stresses in plants is still in its infancy, and we believe our tomato model can serve as a starting point for studying the functional consequences of abiotic stresses. While iHY3410 was used for analysing the behaviour of leaves in this study, it could also be used for exploring the metabolic behaviour of tomato fruit in future studies. Altogether, our genome-scale metabolic model of tomato is a valuable tool for the applied and fundamental research of tomato, as well as the study of large-scale metabolic systems of plants.

EXPERIMENTAL PROCEDURES

Metabolic reconstruction

A genome-scale model of tomato was constructed using the ScrumPy metabolic modelling package (Poolman, 2006). An initial draft model was generated from a tomato metabolic pathway

### Table 3 Comparisons of essential reactions with other models

| Enzyme    | EC       | Pathway          | iHY3410 | Rice Lakshmanan et al. (2013a) | Arabidopsis | Maize | References                        |
|-----------|----------|------------------|---------|--------------------------------|-------------|-------|-----------------------------------|
| Rubisco   | 4.1.1.39 | Calvin cycle     | +       | +                              | +           | +     | Quick et al. (1991)               |
| PRK       | 2.7.1.19 | Calvin cycle     | +       | +                              | +           | +     | Moll and Levine (1970)            |
| RPE       | 5.1.3.1  | Calvin cycle/PPP | +       | +                              | +           | +     | Xiong et al. (2009)               |
| RPI       | 5.3.1.6  | Calvin cycle/PPP | #       | +                              | +           | +     |                                   |
| TKT       | 2.2.1.1  | Calvin cycle/PPP | +       | +                              | +           | +     |                                   |
| SBPase    | 3.1.3.37 | Calvin cycle     | –       | +                              | Unknown     | Unknown |                                   |
| PEPC      | 4.1.1.31 | C4 photosynthesis| –       | –                              | +           | +     | Rademacher et al. (2002)          |
| PPDK      | 2.7.9.1  | C4 photosynthesis| –       | Unknown                        | –           | –     | Mechin et al. (2007)              |
| PLGP      | 3.1.3.18 | Photorespiration | +       | +                              | –           | –     | Schwarte and Bauwe (2007)         |
| GOX       | 1.1.3.15 | Photorespiration | +       | +                              | +           | +     | Xu et al. (2009)                  |
| GGT       | 2.6.1.4  | Photorespiration | –       | Unknown                        | Unknown     | Unknown | Liepmann and Olsen (2001)         |
| SGt       | 2.6.1.45 | Photorespiration | –       | +                              | Unknown     | Unknown |                                   |
| GDC       | 1.4.4.2; | Photorespiration | #       | +                              | Unknown     | Unknown | Engel et al. (2007)               |
|           | 2.1.2.10;|                  |         |                                |             |       |                                   |
|           | 1.8.1.4  |                  |         |                                |             |       |                                   |
| SHMT      | 2.1.2.1  | Photorespiration | –       | #                              | Unknown     | Unknown |                                   |
| HPR       | 1.1.1.29 | Photorespiration | –       | Unknown                        | Unknown     | Unknown |                                   |
| GLYK      | 2.7.1.31 | Photorespiration | –       | +                              | Unknown     | Unknown |                                   |
| CAT       | 1.11.1.6 | Photorespiration | –       | +                              | Unknown     | Unknown |                                   |
| GS        | 63.1.2   | GS/GOGAT cycle   | –       | +                              | Unknown     | Unknown |                                   |
| Fd-GOGAT  | 1.4.7.1  | GS/GOGAT cycle   | –       | +                              | Unknown     | Unknown |                                   |
| GOGAT     | 2.4.1.18 | Starch biosynthesis | +   | +                              | +           | +     | Hernández et al. (2008)           |
| GLBE      | 2.4.1.18 | Starch biosynthesis | +   | +                              | +           | +     |                                   |
| TAL       | 2.2.1.2  | PPP               | –       | Unknown                        | –           | –     | Carrari et al. (2003)             |
| Aconitate | 4.2.1.3  | TCA               | –       | Unknown                        | +           | +     |                                   |

‘+’, essential; ‘-’, non-essential; ‘Unknown’, not reported; ‘#’, essential only under stressed conditions.
components (Tables S1).

The draft model was manually curated with regard to atomic balance, energy conservation, reaction stoichiometry, reversibility, and subsequently assigned to respective subcellular localization. Mass conservation was achieved by checking the atomic balance of each reaction for which the empirical formulas of all metabolites are known, and by identifying unconserved metabolites through stoichiometric consistency analysis (Gevorgyan et al., 2008). All reactions were atomically balanced with respect to carbon (C), nitrogen (N), phosphorus (P), and sulphur (S), but not hydrogen (H) and oxygen (O). The inconsistency regarding H and O was assumed to have minimal effect on the model predictions as water and stoichiometric protons were defined as external metabolites as in Poolman et al. (2009) and Cheung et al. (2013).

Information on reaction directionality was primarily extracted from the MetaCyc database (http://metacyc.org) wherever possible as it contains comprehensive metabolic pathways and enzymes which have been experimentally determined and reported in the literature (Casi et al., 2008). For the remaining reactions, the directionality data was adopted from the LycoCyc database. Intracellular compartmentalization was determined according to a published genome-scale model of Arabidopsis (Cheung et al., 2013). Reactions were distributed in five intracellular compartments, cytosol (‘Cyto’), plastid (‘Plas’), mitochondrion (‘Mito’), peroxisome (‘Pero’) and vacuole (‘Vacu’). Transport reactions were also defined based on the model of Cheung et al. (2013). Transporters were represented using the subscripts ‘Cyto’, ‘Plas’, ‘Mito’, ‘Pero’ and ‘Vacu’. Transport reactions were also defined based on the model of Cheung et al. (2013). Transporters were represented using the subscripts ‘Cyto’, ‘Plas’, ‘Mito’, ‘Pero’ and ‘Vacu’. Transport reactions were also defined based on the model of Cheung et al. (2013). Transporters were represented using the subscripts ‘Cyto’, ‘Plas’, ‘Mito’, ‘Pero’ and ‘Vacu’. Transport reactions were also defined based on the model of Cheung et al. (2013). Transporters were represented using the subscripts ‘Cyto’, ‘Plas’, ‘Mito’, ‘Pero’ and ‘Vacu’.

Model analysis

In this study, FBA was carried out using the Gnu linear programming kit (GLPK, http://www.gnu.org/software/glpk) and the ScrumPy metabolic modelling package (Poolman, 2006). The objective function of total flux minimization was used in this study as a proxy for minimizing the total enzyme costs (Holzhutter, 2004), subject to several different types of constraints: (i) steady state, (ii) the requirement for production of individual biomass components (Tables S1–S4), and (iii) the energy demand for cell maintenance. Mathematically, the linear programming problem can be represented as follows: Minimize: $\nu^T v$

Subject to $Sv = 0$ (i)

$v_i = f(i)$ (ii)

$V_{ATP_{ase}} = m$ (iii)

where $S$ is the stoichiometric matrix, $v$ is a vector of all reaction fluxes in the system, $i$ are the transporters of the biomass components, $t$ is the corresponding flux that each biomass transporter carries, $V_{ATP_{ase}}$ represents the flux that a generic ATPase reaction carries to sustain cellular functions that are not associated with biomass accumulation and growth, and $m$ is the flux value that the generic ATPase reaction carries. It has been shown that a large amount of cellular ATP is used for cellular maintenance in plant cells, for example, more than 85% of cellular ATP was estimated for the non-growth associated maintenance in Arabidopsis (Masakapalli et al., 2010). Since no information is available regarding the ATP maintenance energy for tomato, the ATP maintenance of 7.1 mmol g$^{-1}$ DW h$^{-1}$ reported in an Arabidopsis model has been exploited (Poolman et al., 2009). As most plants uptake nitrogen in the form of nitrate and ammonium, nitrogen input was set to be 50% nitrate and 50% ammonium for simulating the photosynthetic scenario. Photosynthetic metabolism was simulated by additionally setting the flux ratio of carbonic to oxygenic RubisCO (Vc/Vo), which is three under ambient conditions, and one under drought-stressed conditions. For the simulations of drought conditions, we constrained the biomass outputs using the drought-specific growth rate (Tables S1-S4). Due to lack of information on ATP maintenance for drought conditions, we kept ATP maintenance constant for all the simulations. Other constraints for simulating the photosynthetic scenario are listed in Table S5. FBA uses these constraints to identify a flux distribution which optimizes a defined objective function (Orth et al., 2010), such as total flux minimization.

Flux balance analysis was also applied to test the production of each biomass component independently, while minimizing total flux to simulate growth. To characterize the optimal flux solution space, flux variability analysis (FVA) was used to determine a possible flux range for each reaction while giving rise to the same optimal value for the objective function (Mahadevan and Schilling, 2003). This range denotes the minimum and maximum flux values each reaction can take among all alternative optimal solutions.

Deletion simulation

Large-scale metabolic models serve as platforms for rapidly predicting growth phenotypes in response to reaction knockouts in silico. In order to simulate reaction deletions, the flux through the corresponding reaction was constrained to zero in addition to the constraints on biomass production, ATP maintenance, nitrogen input and Vc/Vo. With the constraints of biomass synthesis, a reaction was categorized as essential if its deletion resulted in no feasible steady-state solution. In contrast, reactions were defined as non-essential if a feasible solution could be found.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article.

Figure S1. Assignment of the reactions involved in the photosynthetic metabolism in the rice core model by Lakshmanan et al. (2013a).

Figure S2. The bypass of RPI knockout under normal conditions (Vc/Vo = 3) in JHY3410.

Figure S3. The bypass of HPR knockout in JHY3410.
Tables S1. Detailed information on the biomass composition of tomato leaves.
Table S2. Cellulose and lignin precursors.
Table S3. Hemicellulose precursors.
Table S4. Condition-specific growth rates of tomato leaves.
Table S5. Boundary constraints for simulating the photoautotrophic scenario.
Table S6. List of essential reactions of tomato leaves for normal and drought conditions.
Table S7. Comparisons of photorespiratory reactions between HY3410 and a rice core model.

Data S1. Genome-scale metabolic model of HY3410 in SBML format.
Data S2. Genome-scale metabolic model of HY3410 in ScrumPy format.
Data S3. Genome-scale metabolic model of HY3410 in Microsoft Excel format.
Data S4. FBA and FVA results of dark, light scenarios, and photorespiratory scenarios in normal and drought conditions for minimizing total flux.
Data S5. FBA and FVA results of reactions involved in the MEP and MVA pathways in normal and drought conditions for minimizing photon uptake.

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