Supplementary materials (Wright et al. 2014)

Supplementary figure legends

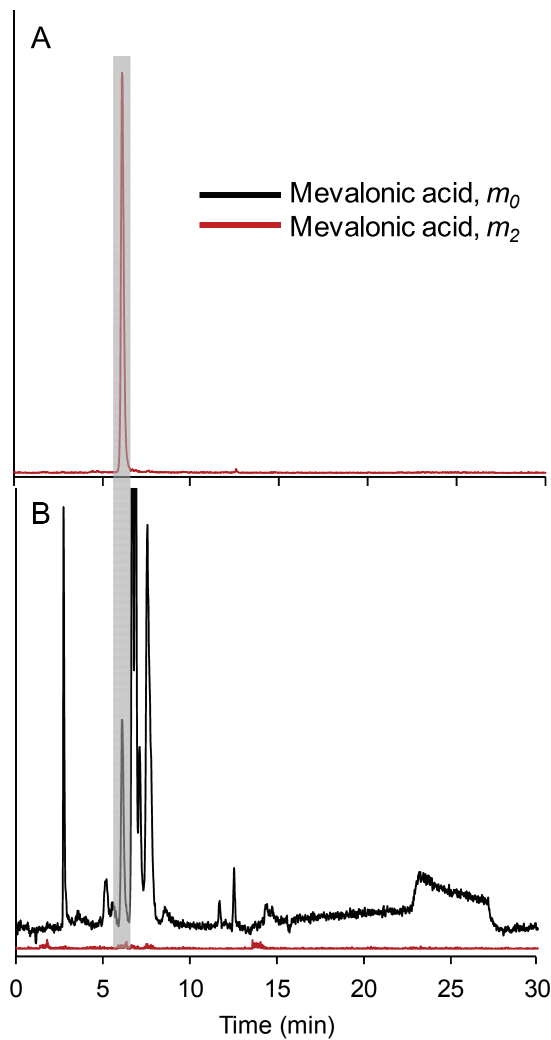
Suppl. figure 1. Incorporation of $^{13}CO_2$ label into mevalonate, a principal intermediate of the cytosolic isoprenoid biosynthetic pathway, as measured by LC-MS/MS analysis operating in negative ionization mode. A, The [1,2]^{13}C-mevalonic acid standard (m_2) peak eluting at 6.1 min; B, Multiple reaction monitoring of the m_2 labeled (red; *m/z* 149→60.8) and m_0 unlabeled (black; *m/z* 147→58.8) mevalonic acid isotopologues in Arabidopsis extracts following 45 min of *in vivo* labeling with $^{13}CO_2$ at 380 ppm. No m_2 or higher order isotopologue was detected.

Suppl figure 2. DXS enzyme assay optimization. (A) Effect of pH on DXS activity. The reactions were done in triplicate using the described DXS assay conditions, with the exception that the buffer consisted of 100 mM MES, 100 mM HEPES, 100 mM TRIS, 100 mM CAPSO and 100 mM CAPS. The buffer was adjusted to the correct pH with either HCl or NaOH. Shown are the relative amounts of DXP produced after 2 hours reaction time at 25 °C, expressed as a percentage relative to assays at pH 8.0. (B) Effect of temperature on DXS activity. The reactions were incubated at different temperatures in triplicate using the described DXS assay conditions. Shown are the amounts of DXP produced after 2 hours reaction time at different temperatures, expressed as a percentage relative to assays at 35 °C. (C) Linearity of DXS activity over time. The reactions were performed in triplicate using the described DXS assay conditions. Shown are the amounts of DXP produced after different reaction times at 25 °C, expressed as a percentage relative to the maximum amount of DXP produced after 4 hours. (D) Linearity between plant material and DXS activity. Enzymes were extracted from 2, 3, 4 and 5 mg of lyophilized plant material and used to initiate DXS enzyme reactions in triplicate. Shown are the amounts of DXP produced after 2 hours of incubation at 25 °C as a percentage of the DXP produced using enzymes extracted from 5 mg DW plant material.

Suppl figure 3. DXS activity in total protein extracts of wild type, transgenic 35S:DXS, and *dxs-3* mutant lines. DXS activity was determined by direct detection of DXP produced from

pyruvate and glyceraldehyde 3-phosphate in *in vitro* assays by LC-MS/MS operating in negative mode using MRM (*m/z* 212.9→138.9). Assays were performed according to the conditions described in Suppl. figure 2. Absolute quantification of products was determined by a [3,4,5-^{13}C]DXP internal standard. Means of n > 10 ± SE are shown.

Suppl figure 4. Elasticity of 1-deoxy-D-xylulose-5-phosphate synthase (DXS) for dimethylallyl diphosphate (DMADP). Total protein extracts of wild type Arabidopsis were assayed in the presence of variable concentrations of DMADP spanning a physiological range to test its potential for negative feedback inhibition of DXS. The elasticity (ε) of DMADP for DXS is obtained from the slope of relative DXS activity as a function of DMADP concentration. Assays were conducted at physiologically relevant concentrations of the substrates pyruvate (30 μM) and glyceraldehyde 3-phosphate (70 μM). Shown are means of three assays (n=3) and SEM.



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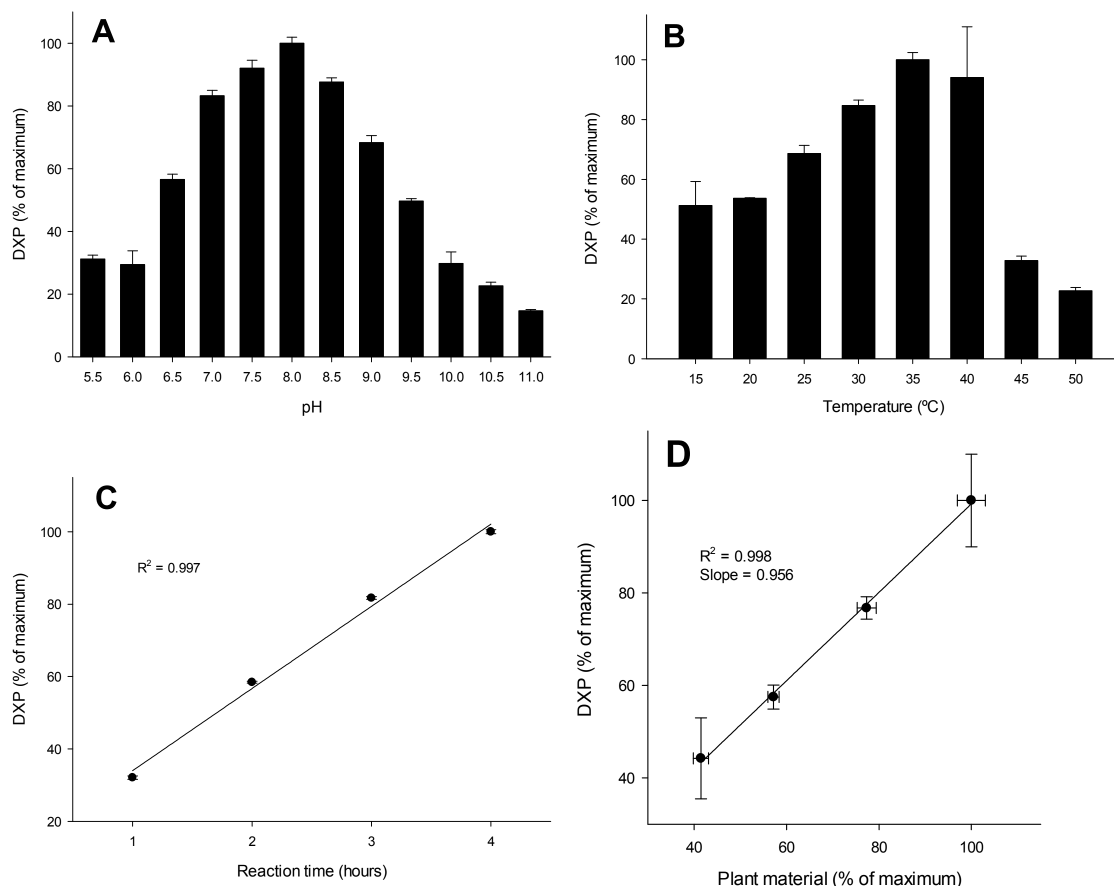

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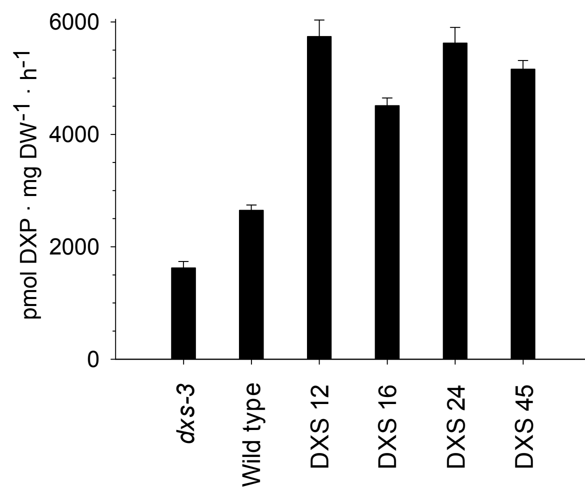

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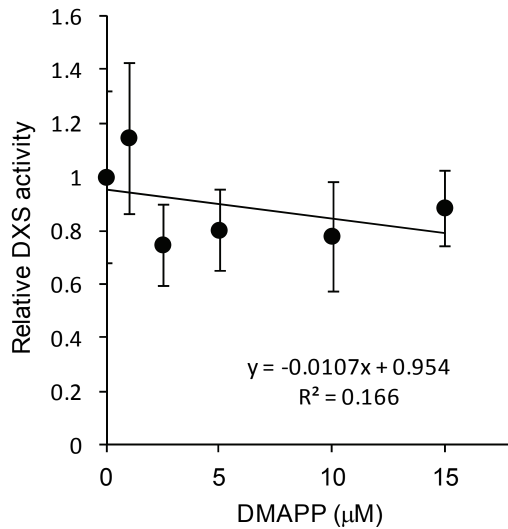

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Suppl. table I. Calculation of deviation indices in DXS-altered lines relative to a wild type reference state

Line	DXS activity[a]	Flux[b]	Slope	Deviation index[c]
dxs-3	1624	2.72	0.00071	0.42
Wild type	2648	3.44		Reference state
35S:DXS16	4510	6.20	0.00148	1.08
35S:DXS45	5159	6.36	0.00116	0.94
35S:DXS24	5623	6.68	0.00109	0.92

[a] pmol DXP·mg^{-1} DW·h^{-1}
[b] pmol DXP·mg^{-1} DW·min^{-1}
[c] As described in Small and Kacser (1993)

Suppl. table II. Quantitative PCR primer sequences used in this study

Designation	Sequence
RP2lsF	GAAGGCAAAGGAAGGCAGAATCAG
RP2lsR	GCAATACTCCACGGAACACCAAG
APT1F	GTTGCAGGTGTTGAAGCTAGAGGT
APT1R	TGGCACCAATAGCCAACGCAATAG
DXSF	TCGCAAAGGGTATGACAAAG
DXSR	CAGTCCCGCTTATCATTCC
DXRF	AGTAGCGGATGCGTTGAAGC
DXRR	GCGGATGAATGACAATCTCTATATCG
CMSF	TTCTGATTCGCTTGTGGTG
CMSR	AACTGGATGCTTGAGGTATTC
CMKF	TCGGTGGTGGAAGTAGTAATG
CMKR	AGGAAGGTCTTGGACAATCTC
MDSF	CATCGTTTAGAGCCAGGGTATCC
MDSR	TGAAGTAACACATCGCCATCGG
HDSF	CAGAATGCGTAACACTAAGAC
HDSR	GAGAACCACCTACATATCCG
HDRF	TCGTGCGGGAGAATCATC
HDRR	TCTTACGGAACACCTTGGC

Supplementary methods

Calculation of exact label incorporation into DMADP

While positive chemical ionization gas chromatography mass spectrometry (PCI-GC-MS) reduces fragmentation of the isoprene molecular ion and allows accurate measurement of ^{13}C incorporation, a significant amount of fragmentation still occurs due to hydride abstraction and charge exchange, leading to $[M - H]^+$ and $[M]^+$ ions, respectively, in addition to the predominantly formed $[M+H]^+$ ion. The isoprene peak formed from the acid hydrolysis of a DMADP standard will thus consists of 4 ions, namely: (i) *m/z* 67 due to the hydride abstraction $[M\text{-}H]^+$ ion of $^{13}C_0$ isoprene ($^{13}C_0[M\text{-}H]^+$), (ii) *m/z* 68 consisting of the $[M]^+$ ion of $^{13}C_0$ isoprene ($^{13}C_0[M]^+$) and the $[M\text{-}H]^+$ ion of $^{13}C_1$ isoprene ($^{13}C_1[M\text{-}H]^+$), (iii) *m/z* 69 consisting predominantly of the $[M+H]^+$ of $^{13}C_0$ isoprene ion ($^{13}C_0[M+H]^+$) but also containing a small amount of the M^+ ion of $^{13}C_1$ isoprene ($^{13}C_1[M]^+$) due to natural ^{13}C abundance, and (iv) *m/z* 70 consisting of the $[M+H]^+$ ion of $^{13}C_1$ isoprene ($^{13}C_1[M+H]^+$). The sensitivity of the method did not allow any detection of natural $^{13}C_2$ isoprene ions. To deconvolute the different molecular ions of isoprene and determine the exact isotopologue distribution in a given labeled sample, the following constants were calculated from unlabeled spectra:

$$k_1 = {}^{13}C_0[M]^+ / {}^{13}C_0[M+H]^+ \quad (1)$$

$$k_2 = {}^{13}C_0[M\text{-}H]^+ / {}^{13}C_0[M+H]^+ \quad (2)$$

These fractions were calculated from the experimental data obtained from the acid hydrolysis of 200 ng DMADP standard. These data will also contain the natural abundant isotope ^{13}C, so that the total ions of the isoprene standard measured will consist of both $^{13}C_0$ isoprene ($^{13}C_0M$) and $^{13}C_1$ isoprene ($^{13}C_1M$). Knowing the natural abundance of ^{13}C, the following equations can be deduced:

$$^{13}C_0M = 0.945M^{Total} \quad (3)$$

$$^{13}C_1M = 0.055M^{Total} \quad (4)$$

$$M^{Total} = {}^{13}C_0M + {}^{13}C_1M = m/z\ 67 + m/z\ 68 + m/z\ 69 + m/z\ 70 \quad (5)$$

In equation (5) *m/z* 67, *m/z* 68, *m/z* 69 and *m/z* 70 represent the integrated peak areas of the respective ion masses. The following equations can be deduced based on the assumption that the fragmentation of $^{13}C_0M$ and $^{13}C_1M$ is similar:

$$^{13}C_0[M\text{-}H]^+ / {}^{13}C_0M = {}^{13}C_1[M\text{-}H]^+ / {}^{13}C_1M \quad (6)$$

$$^{13}C_0[M]^+ / {}^{13}C_0M = {}^{13}C_1[M]^+ / {}^{13}C_1M \quad (7)$$

Using these equations, k_1 and k_2 can now be calculated as follows:

$$^{13}C_0[M]^+ = m/z\ 68 - {}^{13}C_1[M\text{-}H]^+ \quad (8)$$

$$^{13}C_1[M\text{-}H]^+ = ({}^{13}C_0[M\text{-}H]^+)({}^{13}C_1M) / {}^{13}C_0M \quad (9)$$

$$^{13}C_0[M\text{-}H]^+ = m/z\ 67 \quad (10)$$

$$^{13}C_1[M]^+ = ({}^{13}C_0[M]^+)({}^{13}C_1M) / {}^{13}C_0M \quad (11)$$

$$^{13}C_0[M\text{+}H]^+ = m/z\ 69 - {}^{13}C_1[M]^+ \quad (12)$$

Using equations (1) and (2), as well as the fact that each isoprene mass consists of three fragment ions, namely $[M\text{-}H]^+$, $[M]^+$ and $[M\text{+}H]^+$, the absolute amounts of the different isoprene masses can be calculated as follows:

$$^{13}C_5\text{isoprene} = (m/z\ 74)(1 + k_1 + k_2) \quad (13)$$

$$^{13}C_4\text{ isoprene} = A(1 + k_1 + k_2) \quad (14)$$

$$^{13}C_3\text{isoprene} = B(1 + k_1 + k_2) \quad (15)$$

$$^{13}C_2\text{isoprene} = C(1 + k_1 + k_2) \quad (16)$$

$$^{13}C_1\text{isoprene} = D(1 + k_1 + k_2) \quad (17)$$

$$^{13}C_0\text{isoprene} = E(1 + k_1 + k_2) \quad (18)$$

where

$$A = (m/z\ 73) - k_1(m/z\ 74) \quad (19)$$

$$B = (m/z\ 72) - k_1A - k_2(m/z\ 74) \quad (20)$$

$$C = (m/z\ 71) - k_1B - k_2A \quad (21)$$

$$D = (m/z\ 70) - k_1 C - k_2 B \tag{22}$$

$$E = (m/z\ 69) - k_1 D - k_2 C \tag{23}$$

To determine the absolute quantity of DMADP, each plant sample was analyzed a second time by adding 100 ng DMADP standard to the lyophilized plant material directly after the phosphoric acid was added during the acid hydrolysis procedure. Labeled samples consisted of ions with *m/z* values ranging from *m/z* 67 up to *m/z* 74. The DMADP standard, containing only natural ^{13}C isotopic label, consists of ions from *m/z* 67 to 70. The amounts of the higher mass peaks (*m/z* 72 to *m/z* 74) of the sample with added standard can be used to determine the amounts of *m/z* 67 to *m/z* 70 originating from the plant material, when compared to the values obtained for the sample not containing any added standard. In this way, the signal originating from the added standard and that from the plant material can be determined, thereby using the added DMADP standard as internal standard to quantify the absolute quantities of DMADP. Any matrix effect on the acid hydrolysis of DMADP to isoprene, as well as any ion suppression effects in the mass spectrometer, could be accounted for in this manner.