Corrigendum: The Defense-Related Isoleucic Acid Differentially Accumulates in Arabidopsis Among Branched-Chain Amino Acid-Related 2-Hydroxy Carboxylic Acids

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A Corrigendum on

The Defense-Related Isoleucic Acid Differentially Accumulates in Arabidopsis Among Branched-Chain Amino Acid-Related 2-Hydroxy Carboxylic Acids

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FIGURE LEGEND

In the original article, there was a mistake in the legend for Figure 6 and Figure 7 as published.

The numbering for Figure 6 was mixed with Figure 7 and not in agreement with the text: this mistake was introduced during proof stage, since the editing changed both legend and figure number. In addition, DC3000 should not be in italics. The correct numbering of Figures 6 and 7 appear below.

FIGURE 6 | BCAA levels after exogenous application of 2-HAs to A. thaliana plants

FIGURE 7 | ILA and LA abundance in response to P. syringae virulent strain infection.

ERROR IN FIGURE

In the original article, there was a mistake in Figures 2–4, and 6 as published. Due to the incorrect calculation of 2-HA level (a factor 50 was missed), all figures showing such measurements have to be replaced with the corrected y axis and limit of detection. The corrected Figures 2–4, and 7 appear below.
In the original article, there was an error in the following section: **Materials and Methods, Determination of ILA, LA and VA by GC-MS, Paragraph 2**. The description of the addition of standards was ambiguous and not correct; this also lead to a wrong calculation of the final 2-HA concentrations, which all have to be multiplied by the factor 50. This error affects the relative content of 2-HA, e.g., in comparison to other plant metabolites like BCAAs or salicylic acid. The correct paragraph 2 into **Materials and Methods, Determination of ILA, LA and VA by GC-MS, appears below:**

Qualitative and quantitative analyses of VA, LA and ILA were performed by gas chromatography-mass spectrometry (GC-MS). Samples were analyzed with a thermo-desorption unit (Gerstel, Mülheim an der Ruhr, Germany) coupled to a GC-MS instrument (GC type: 7890; MS type: 5975C, both Agilent Technologies, Palo Alto, CA, USA). The thermo-desorption unit was used as injector for the conversion of the sample from liquid to gas-phase. GC-MS was run as follows: One µL of sample was injected into the thermo-desorption unit in a dedicated glass tube containing the glass insert for liquid injection (both from Gerstel, Mülheim an der Ruhr, Germany). Prior to each analysis, tubes and inserts were accurately cleaned with acetone, methanol and water, separately used in ultrasonic bath for 30 min each, and kept in hexane solution overnight. Immediately before analysis, tubes were baked out in oven at 400°C for 1 h under ~80 mL min⁻¹ N₂ (5.0 gas purity) flow. Samples were vaporized by quickly rising the temperature from 40 to 270°C at a rate of 360°C min⁻¹ and holding for 0.5 min. The compounds were refocused using a Cryo Injection System (Gerstel, Mülheim an der Ruhr, Germany) at -50°C, then desorbed and injected in splitless mode by rising the temperature to 250°C at a rate of 12°C sec⁻¹ and hold for 1.5 min, followed by ramping at 12°C sec⁻¹ to 275°C and holding for 2 min. Separation was achieved by using the Agilent J&W HP-5ms GC column (30 m x 250 µm x 0.25 µm) with 1 mL min⁻¹ constant flow rate of He, and a temperature program of 90°C for 4 min, followed by ramping at 2°C min⁻¹ to 120°C and holding for 0 min, then 100°C min⁻¹ to 300°C and holding for 5 min. Identification of VA, LA, ILA, and the two IS (2-hydroxyhexanoic acid and 4-nitrophenol) were achieved by spectra and retention time comparison of pure standards. The quantification was obtained by means of a calibration curve obtained from pure standards. MS spectra were parallelly acquired in total ion current and in selective ion monitoring modes. Scans of total ion current were performed in the range of 35-300 mass-to-charge ratios (m/z) (threshold: 150; 7.76 scan sec⁻¹). Selective ion monitoring parameters were as follows, VA: start time: 6.2 min, ion: 145.0 m/z, dwell: 150 ms; LA: start time: 8.5 min, ion: 159.0 m/z, dwell: 150 ms; ILA: start time: 11.5 min, ion: 159.0 m/z, dwell: 150 ms; 2-hydroxyhexanoic acid: start time: 13.9 min, ion: 173.1 m/z, dwell: 100 ms; 4-nitrophenol: start time: 16.0 min, ion: 196.1 m/z, dwell: 25 ms. MS detector was kept off until 6.20 min and switched off after 20.65 min until the end of the run. The calibration was achieved by adding standards into a mixture of Arabidopsis plant extracts, to take into account potentially occurring matrix effects. The respective 2-hydroxy acid standards were directly added into 1 mL of the pooled 70/30% methanol/water plant extract. After all preparative steps, the final standard concentrations of the BSTFA solution used for GCMS injection were of 0, 0.05, 0.1, 0.15, 0.2, 0.25, 0.3, 0.5, 1, 5, 10 ng µL⁻¹. Each concentration of ILA contained the same fix concentration of IS (50 mg L⁻¹). Calibration samples were treated in exactly the same way as the sample preparation explained above (i.e. passing through the...
Data were background corrected using the mean value obtained from measuring the plant extract with the addition of the standard solution lacking ILA to correct for the basal levels of ILA present in the pooled plant material. Standards were prepared independently in triplicate, and each concentration was measured twice. The two technical replicates were averaged and their means were further used for the calculation of response factors. To consider uncertainties of standard preparation, the quantification of 2-HAs were based on three independently created serial dilutions. The resulting MS signal responses were found to be linear ($R^2 > 0.9999$) with an increasing standard concentration. Response factors of VA and LA were calculated based on the matrix-dependent calibration curve of ILA assuming that the matrix effects occurred at the same extent for VA, LA and ILA: serial dilutions of pure standards (0-100 mg L$^{-1}$) of ILA, VA and LA were measured in parallel and the ratios of VA/ILA and LA/ILA were applied to the matrix-dependent response factor of ILA. Data were always normalized to IS values of 4-nitrophenol. Samples showing an inconsistent ratio of the two IS were discarded from the analysis. Limits of detection were calculated using 2 sigma ($\sigma$) and were ranging between 1.35-64.5 (ILA), 2.25-11.45 (VA), 0.6-1.45 (LA) ng g$^{-1}$ dry weight (DW), referred to $A.\ thaliana$ plant material. The limits of quantification (LOQ) were set to three times of their respective limits of detection.

**Issue 2**

In the original article, there was an error in the Results section, Determination of BCAA-related 2-HAs in Plants, Paragraph 1.

The numbers of the limit of detection have to be adapted. The correct paragraph 1 appears below.

To identify and quantify the abundance of valic acid (VA, 2-hydroxy-isovaleric acid), leucic acid (LA, 2-hydroxyisocaproic acid) and isoleucic acid (ILA, 2-hydroxy-3-methyl-valeric acid) in plants, we developed a sensitive method based on derivatization of these molecules by silylation and GC-MS analysis (**Figure 1; Supplementary Data Figure S1**). With this method it was possible to detect low amounts of the BCAA-related compounds. The limits of detection were ranging between 1.35-64.5 (ILA), 2.25-11.45 (VA), 0.6-1.45 (LA) ng g$^{-1}$ DW depending on instrument performance and background noise. To demonstrate the general versatility of the procedure different plant species were examined for
their content in ILA, LA, and VA. ILA could be ubiquitously detected in all examined plant species including monocotyledonous and dicotyledonous plants, herbaceous and woody plants, whereas VA and LA were found only in some species (Figure 2; Supplementary Data Figures S2, S3). All three 2-HAs could be detected in *Populus x canescens*, *Hordeum vulgare* and *Solanum lycopersicum* (Figure 1). In the model plant *A. thaliana* only LA and ILA were present in the extracts (Figure 3, Supplementary Data Figure S3).

**Issue 3**

In the original article, there was an error in the Discussion section, Paragraph 1, as a consequence of the adapted limit of detection. The correct paragraph appears below.

The 2-HA ILA has been discovered in its glucosylated form in *Arabidopsis thaliana*. ILA glucoside formation was dependent on the activity of the small-molecule glucosyltransferase UGT76B1 in planta (von Saint Paul et al., 2011). Exogenous application of ILA activated SA-dependent defense relating it to plant pathogen response as a novel immune-modulating compound (von Saint Paul et al., 2011). This was in line with the in vitro activity of UGT76B1 glucosylating both ILA and SA, whereas ILA inhibited the glucosylation, i.e. inactivation, of SA by UGT76B1 (von Saint Paul et al., 2011; Noutoshi et al., 2012). However, the endogenous level of the aglycon ILA itself could not be assessed by the non-targeted metabolome analysis of von Saint Paul et al. (2011) due to lower instrumental sensitivities for small molecules (<150 a.m.u.). Previously, a targeted approach based on GC-MS has been used to quantify and identify 2-HAs in humans affected by MSUD (Jakobs et al., 1977; Jakobs et al., 1977; Chuang and Shih, 2001). MSUD is due to a genetic disorder of BCAA catabolism leading to the accumulation of 2-keto carboxylic acid catabolites and their reduced 2-HA derivatives ILA, LA and VA (Mamer and Reimer, 1992; Tanaka and Rosenberg, 1983; Chuang and Shih, 2001). These 2-HAs accumulate to high levels of 0.04 – 1.2 mM and even up to 30 mM in MSUD patients’ plasma and urine samples, respectively (Jakobs et al., 1977). Preliminary attempts could not detect ILA in crude plant extracts, indicating that levels of 2-HAs are low in planta. Therefore, we developed a more sensitive approach.
A constitutive UGT76B1 overexpression line led to a lowered ILA concentration. Surprisingly, the isomeric LA was not affected by different UGT76B1 levels in vivo (Figures 3 and 4), albeit LA was a substrate of UGT76B1 in vitro (Table 1). The biochemical ability of the enzyme is reasonable due to the highly similar structure of the aglyca and the frequently broad substrate specificity known of plant UGTs (Bowles et al., 2006); in fact, VA was also shown to be an in vitro substrate of UGT76B1 (von Saint Paul et al., 2011), although VA is not detected in A. thaliana. Thus, the in vitro activity of UGT76B1 towards LA may not be relevant in vivo, UGT76B1 and LA may not occur in the same cells or subcellular compartments, or the level of free LA is controlled to be stable and not influenced by UGT76B1.

The authors apologize for these errors and state that they do not change the scientific conclusions of the article in any way. The original article has been updated.

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