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Metabolic and regulatory responses in citrus rootstocks in response to adverse environmental conditions

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Title: Metabolic and regulatory responses in citrus rootstocks in response to adverse environmental conditions

Running title: Metabolomics of abiotic stress in citrus

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Abstract

In response to adverse environmental conditions, plants modify their metabolism in order to adapt to the new conditions. To differentiate common responses to abiotic stress from specific adaptation to a certain stress condition, two citrus rootstocks (Carrizo citrange and Cleopatra mandarin) with a different ability to tolerate stress were subjected to soil flooding and drought, two water stress conditions. In response to these conditions, both genotypes showed altered root proline and phenylpropanoid levels, especially cinnamic acid that was a common feature to Carrizo and Cleopatra. This was correlated with alterations in the levels of phenylpropanoid derivatives likely involved in lignin biosynthesis. In the regulatory part, levels of both stress hormones ABA and JA decreased in response to soil flooding irrespective of the genotype relative flooding tolerance but, on the contrary, concentration of both metabolites increased in response to drought, showing JA a transient accumulation after a few days and ABA a progressive pattern of increase. These responses are probably associated to different regulatory processes under soil flooding and drought. In addition, alterations in IAA levels in citrus roots seemed to be associated to particular stress tolerance. Moreover, both genotypes exhibited a low degree of overlapping in the metabolites induced under similar stress conditions, indicating a specific mechanism to cope with stress in plant species. Results also indicated a different metabolic basal status in both genotypes that could contribute to stress tolerance.

Keywords: abiotic stress, drought, metabolomics, plant hormones, phenylpropanoids, soil flooding,

Introduction

Environmental variables such as temperature, water availability, irradiance or soil osmolality affect plants in different ways depending on their ability to tolerate a specific adverse situation (Des Marais and Juenger 2010, Qin and others 2011). The most damaging situation is probably water shortage that dramatically affects plant performance and, ultimately, survival. This water deprivation is mainly due to a limitation in the availability of capillary water or liquid water trapped between the soil particles which can be efficiently absorbed by plant roots. Plants respond to this situation by accumulating compatible solutes or soil salts thus decreasing their water potential and closing stomata to avoid dehydration (Munns 2011). A paradigmatic situation is salt stress that has a double component: a first phase of osmotic stress and a second phase of ionic stress that occurs after over-accumulation of toxic ions, such as Na^+ and Cl^-, in photosynthetic organs (Munns 2011).

In citrus, the accumulation of Cl^- in leaves induces down-regulation of the photosynthetic system and reduction in gas exchange parameters, ultimately leading to the overproduction of reactive oxygen species and oxidative stress (Arbona and others 2003, López-Climent and others 2008). As well as in salt stress, the intensity of the response to water deprivation is related to the ability of the plant to regulate water relations (Moya and others 2003). Those genotypes with a lower transpiration rate and a higher ability to rapidly close stomata (for example, Cleopatra mandarin) have improved performance under drought conditions (López-Climent and others 2008). Citrus responses to abiotic stress also include accumulation of jasmonic acid (JA) and abscisic acid (ABA) in roots and leaves (de Ollas and others 2012, Gómez-Cadenas and others 1996). In addition, there is an accumulation of 1-aminocyclopropane-1-carboxylic acid in water-stressed roots that can be eventually transported to the aerial part and oxidized to ethylene triggering leaf and organ drop (Gómez-Cadenas and others 1996).

On the contrary, when subjected to other situations that induce water shortage such as soil flooding, plants develop different strategies to cope with stress (Arbona and others 2008, Arbona and Gómez-Cadenas 2008, Arbona and others 2009b). In citrus, tolerance to soil flooding seems to be associated to higher transpiration rates and root hydraulic conductivity. In a previous work, Arbona and others (2009b) found that under continuous soil flooding Carrizo citrange plants did not show any alteration in gas exchange or chlorophyll fluorescence parameters for thirty days whereas both parameters rapidly decreased in plants of the sensitive genotype Cleopatra mandarin that also exhibited leaf mid-vein yellowing and curling symptoms. Soil flooding tolerance was also correlated to the ability to delay JA and ABA accumulation in leaves. However, no variations in the hormonal profile that could be linked to tolerance were found in roots since both JA and ABA levels importantly decreased right after stress imposition (Arbona and Gómez-Cadenas 2008).

All these results, taken together, indicate that specific hormonal signalling profiles might have evolved associated to particular stress situations. Nevertheless, stressed plants exhibit similar physiological responses. Then, the question whether different signalling events regulate similar biochemical responses or not, seems of particular relevance in this context.

Based on our knowledge, citrus genotypes with varying ability to tolerate different water shortage situations show similar physiological responses (stomatal closure, down-regulation of photosynthesis and production of reactive oxygen species) when subjected to abiotic stress situations (Arbona and others 2003, 2008). However, under identical stress conditions, model plants alter their metabolism in different ways, causing diverse secondary metabolite profiles (Arbona and others 2010). This different response could be associated to a particular basal secondary metabolite composition but also to a different regulation of the metabolism. In a previous publication, Arbona and others (2010) found that even close-related plant genotypes showed very little overlapping in the metabolites altered by a specific stress condition.

Phenolic derivatives constitute the most diverse array of secondary metabolites found in plants. In particular, phenylpropanoids (cinnamic acid, coumaric acid, caffeic acid and ferulic acid) are synthesized from phenylanaline via phenylalanine ammonia lyase (PAL), a enzyme that catalyzes its deamination rendering cinnamic acid, the first precursor of flavonoid and lignin biosynthesis. The increase in PAL activity and phenylpropanoid content under different adverse environmental conditions has been reported (Cabane and others 2012, Moura and others 2010, Vincent and others 2005). Phenylpropanoids are precursors of lignins, which constitute an important stress defense mechanism, especially at the root level where these compounds are involved in cell wall composition and stiffness (Cabane and others 2012, Vincent and others 2005). Besides the structural function of phenylpropanoids, a role as antioxidants has been proposed (Moura and others 2010). As a response to soil flooding, citrus increase their antioxidant capacity in terms of enzyme activity and soluble antioxidants (Arbona and others 2008). Along with this response, flavonoid levels in tolerant genotypes were less affected than in sensitive ones, suggesting that flavonoids might be also part of their tolerance mechanism (Djoukeng and others 2008).

Other secondary metabolites known to have a functional role in response to abiotic stresses are carotenoids and xantophylls. These compounds are lipophilic compounds synthesized in plants from isopentenyl pyrophosphate (IPP) via the plastidial methyl erythritol phosphate (MEP) pathway. Xantophylls are synthesized from β-carotene via its conversion to zeaxanthin and sequentially to violaxanthin by epoxidation. Finally, an arrangement in one epoxy ring of violaxanthin to form an allenic bond forms neoxanthin , the precursor of ABA in plants. In this sense, overexpression of carotenoid biosynthetic genes in transgenic tobacco plants improved osmotic and salt stress tolerance by channelling carotenoid flux to ABA biosynthesis leading to increased levels of this phytohormone (Cidade and others 2012).

To investigate hormonal and secondary metabolite responses and their relationship with abiotic stress tolerance, two stress conditions: progressive drought and soil flooding were assayed in two citrus genotypes used as rootstocks in modern citriculture: Carrizo citrange and Cleopatra mandarin. These rootstocks were chosen because of their different tolerance to the stress conditions assayed. The study focuses on roots as the first organ sensing the stress derived from soil water perturbation. Proline accumulation, hormonal and secondary metabolite profiles were analyzed in the two rootstock species under the two stress conditions mentioned above.

Materials and methods

Plant material, treatments and sample collection

Four-month-old horticulturally true-to-type seedlings of Cleopatra mandarin (*Citrus reshni* Hort. ex Tan.) and Carrizo citrange (*Citrus sinensis* L. Osb. × *Poncirus trifoliata* L. Raf.) were used in soil flooding and drought experiments. Plants were purchased from a commercial nursery and immediately transplanted to 2-L plastic pots with different substrates depending on the kind of experiments (see below). Before the onset of the experiments, all plants were watered three times a week as described in (Arbona and others 2006) and allowed to acclimate for 2 months. During plant acclimation and experiments, plants were kept in a greenhouse under the following conditions: 26 ± 4.0 ºC day temperature, 18 ± 3.0 ºC night temperature, relative humidity between 70 and 90%, and a 16-h photoperiod.

Flooding stress

To carry out flooding experiments a mixture of peat moss:perlite:vermiculite in an 8:1:1 ratio was used as a substrate. At the end of the acclimation period, two groups of 12 plants each were selected based on the uniformity in appearance and state of development: one was set as control and watered three times a week as described in in Arbona and others (2006) and the other group was subjected to soil waterlogging. To impose stress, pots were placed in opaque plastic bags and then into pots of higher capacity (4 L) and filled with tap water until complete saturation of the soil field capacity, adding more water when needed. Root samples of three plants per treatment group were collected after 1, 3, 6 and 8 days of treatment. Young roots were selected and immediately frozen in liquid nitrogen. The samples were stored at -80 °C until analyses.

Drought stress

Before experiments, a total of 30 citrus seedlings were transplanted to plastic pots using perlite as a substrate that allows a tight control of the water content due to its low moisture-retaining capacity. Half of the plants were used as controls and watered three times a week as described in Arbona et al. (2006) and the other was subjected to drought by simply stop watering. The treatment lasted for 14 days when leaf symptoms of dehydration were apparent. Throughout this period, young root samples from three plants per treatment were collected at days 3, 5, 7, 12 and 14, frozen immediately in liquid nitrogen and stored at -80 °C for further analyses.

Proline analysis

Ground frozen leaf tissue (0.05 g) was extracted in 5 ml of 3% sulfosalicylic acid (Panreac, Barcelona, Spain) using a homogenizer (Ultra-Turrax, IKA-Werke, Staufen, Germany), at maximum speed. After centrifugation at 4,000×*g* for 35 min at 4°C, proline was determined as described by Bates and others (1973). Briefly, 1 ml of the supernatant was combined with 2 ml of a mixture of glacial acetic acid and ninhydrin reagent (Panreac) in a 1:1 (v:v) ratio. The reaction mixture was incubated in a water bath at 100 ° C for 1 h and then partitioned against 2 ml of toluene. Absorbance was read in the organic layer at 520 nm. A standard curve was performed with standard proline (Sigma-Aldrich, Madrid, Spain).

Phytohormone analyses

Plant hormone and different phenylpropanoids (cinnamic, caffeic, coumaric and ferulic acids) were extracted and analyzed essentially as described in Durgbanshi and others (2005) with slight modifications. Briefly, 0.5 g of ground frozen plant material was extracted in 5 ml of distilled water after spiking with 100 ng of d_6-ABA, prepared as in Gómez-Cadenas and others (2002); dihydrojasmonic acid (100 ng), synthesized in the laboratory by catalyzed hydrogenation (Kristl and others 2005), and [$^2H_2$]-IAA (10 ng, Sigma-Aldrich) . After centrifugation at 4,000×*g* at 4ºC, supernatants were recovered and pH adjusted to 3.0 with 30% acetic acid. The acidified water extract was partitioned twice against 3 ml of diethyl ether. The organic upper layer was recovered and evaporated under vacuum in a centrifuge concentrator (Speed Vac, Jouan, Saint Herblain Cedex, France). The dry residue was then suspended in a 10% MeOH solution by gentle sonication. The resulting solution was filtered through regenerated cellulose 0.22 µm membrane syringe filters (Albet S.A., Barcelona, Spain) and directly injected into the HPLC system (Waters Alliance 2695, Waters Corp., Milford, MA, USA). Separations were carried out on a C18 column (Kromasil 100 5 µm particle size, 100×2.1 mm, Scharlab, Barcelona, Spain) using a linear gradient of MeOH and H_2O supplemented with 0.01% acetic acid at a flow rate of 300 µl min^{-1}. Hormone and phenylpropanoid fractions were detected with a Quattro LC triple quadrupole mass spectrometer (Micromass, Manchester UK) connected online to the output of the column through an orthogonal Z-spray electrospray ion source. Quantitation of plant hormones and phenylpropanoids was achieved by external calibration with standards of known amount.

Metabolite profiling analyses

1. Extraction and LC conditions

Samples from roots of Cleopatra and Carrizo (0.1 g) were double extracted in 200 µl of a 80% aqueous MeOH solution by gentle ultrasonication, centrifuged at 10,000×*g* at 4ºC for 10 min and filtered through PTFE membrane filters (0.45 µm pore size). Filtered extracts were subjected to RP-HPLC on a C18 column (5 µm particle size, 100×2.1 mm, XTerra™, Waters) with a Waters Alliance 2965 HPLC system using a linear gradient of ultrapure H_2O (A) and HPLC-grade acetonitrile (B) both supplemented with formic acid to a 0.1% (v/v) concentration at a flow rate of 300 µl min^{-1}. The gradient used was: (0-4 min) 95:5 (A:B), (4.01-55 min) 5-95 (B), (55.01-60 min) 95-5 (B) and (60.01-65 min) 95:5 (A:B). Before extraction, samples were spiked with known amounts of standard compounds: kinetin, biochanin A, rutin, *o*-anisic acid, ferulic acid and *N*-(3-indolylacetyl)-L-phenylalanine, all purchased from Sigma-Aldrich.

2. QTOF-MS conditions

Column eluates were introduced into the mass spectrometer, a QTOF I from Micromass Ltd. (Manchester, UK), through an orthogonal electrospray source (ESI) operated in positive mode. Nitrogen was employed as nebulization as well as desolvation gas and working flows were set at 100 and 800 arbitrary units, respectively. Source block temperature was kept at 120 ºC and desolvation gas at 350 ºC. Capillary, cone and extractor voltages were set at 4 kV, 25 eV and 3 eV, respectively. Before analyses, QTOF mass spectrometer was calibrated by infusing a mixture of NaOH and HCOOH at a flow rate of 25 µl min^{-1}. After calibration, the average error was less than 5 ppm. During acquisition, a 1 ppm solution of Leu-enkephalin ($[M+H]^+$=556.2771) was continuously post column infused as a lock mass reference. Data were acquired under continuous mode in the 50-900 amu range, scan duration was set at 1.0 s and inter-scan delay at 0.1 s.

3. Assessment of reproducibility

This test was accomplished following the workflow described in Arbona and others (2010). Annotated chromatographic mass features covering the whole chromatographic run were selected as markers for subsequent linearity assessment. Selected candidates were integrated using MassLynx v. 4.1 throughout extraction and injection sample replicates and variations in retention time and area were collected as markers of stability of the chromatographic system (Supplementary data 1).

4. Assessment of linearity

Plant samples were extracted, diluted and analyzed as in Arbona and others (2010). Afterwards selected features were integrated with Masslynx v. 4.1 and area values represented using MS Excel (Supplementary data 2 and 3). The dilution representing the average point within the dynamic linear range was selected as the optimum dilution to analyze experiments.

5. Analysis of samples

Plant samples were extracted and analyzed as above using the adequate dilutions and spiking samples with known amounts of selected internal standards to assess recovery and stability of the system as indicated in Extraction and LC conditions section. XCMS analysis was carried out essentially as described before after centroidization of files (Arbona and others 2009a, Arbona and others 2010). Before statistical analyses, area values in datasets were appropriately normalized. To extract significantly altered mass signals in response to flooding and drought stress, analyses were performed with maSigPro algorithms as described in Arbona and others (2010). Reference standards for confirmation of annotated metabolites were purchased from Sigma-Aldrich when available.

Data analysis

For analyses, three independent biological replicates (plants) per treatment and date were analyzed. Every biological sample was analyzed in duplicate as technical replicates and the results within a given biological sample averaged. Statistical difference between stressed and control values was assessed by the student's t-test on each sampling date. For metabolomics analyses, significantly altered profiles were assessed using maSigPro algorithms as described above. In order to facilitate visualization of data and interpretation of results, these were expressed as $\log_2$ of the ratio of stress to control values.

Results

In Figure 1, the proline accumulation profile in roots of Cleopatra mandarin (a) and Carrizo citrange (b) plants subjected to soil flooding and progressive drought is shown. To facilitate interpretation, all results were expressed as $\log_2$ of stress/control ratio of each metabolite concentration. In the two genotypes and stress conditions, proline concentrations ranged between 5.1 and 57.6 µmol g^{-1} fw (data not shown). In response to drought, proline levels increased showing very low accumulation ratios in both genotypes, even after 14 days of withholding water supply. In addition, proline levels in roots of Cleopatra showed a progressive accumulation upon drought that could not be clearly identified in Carrizo. In response to soil flooding the resulting picture was different: roots of Carrizo showed a stronger proline accumulation (a maximum 7.7-fold increase in Carrizo after 8 days of flooding versus only a 1.4-fold in Cleopatra after 6 days). At earlier time points only moderate increases could be observed (0.95 to 1.26-fold on average) in roots of both genotypes.

Accumulation of cinnamic and coumaric acids from the phenylpropanoid pathway in citrus roots under abiotic stress is shown in Figure 2. Under the same soil flooding conditions, cinnamic acid accumulated in roots of both genotypes to similar extents. On the contrary, this metabolite showed a moderate and constant accumulation in Carrizo plants (equivalent to a 2-fold increase) from 5 days of stress on in response to drought. However, in roots of Cleopatra plants subjected to the same conditions, cinnamic acid levels did not increase with respect to control plants and kept an erratic profile with an initial increase followed by a transient decrease after 5 days. After these initial variations, levels of the metabolite kept at control levels.

On the other side, coumaric acid levels increased in roots of both genotypes in response to soil flooding, however this increase was more consistent in Cleopatra at the end of the experimental period whereas in Carrizo it was more erratic only showing significant increases on the first and last sampling dates. In response to drought, coumaric acid levels decreased below controls in roots of both genotypes. Levels of the rest of phenylpropanoids (ferulic and caffeic acids) in roots of both genotypes did not exhibit any significant change in response to the stress conditions assayed (data not shown).

The $\log_2$(stress/control) values of JA and ABA concentration in roots of citrus is presented in Figure 3. Both JA and ABA levels followed similar trends in the two citrus species studied when subjected to soil flooding, exhibiting significant and profound decreases (final levels were 5.0 % and 6.3 % of control values for ABA and JA, respectively in Carrizo). Only differences in the degree of reduction were observed when comparing hormone concentration profiles between both genotypes despite their different tolerance to this abiotic stress factor. Drought induced less dramatic changes in hormonal content compared to soil flooding. In previous works (De Ollas and others 2012), when subjected to drought, citrus roots exhibit a transient JA accumulation followed by a progressive ABA build up. In the present work, both Carrizo and Cleopatra showed JA and ABA accumulation patterns similar to those previously reported. Nevertheless, increases of both hormones in response to drought were more pronounced in drought-sensitive Carrizo than tolerant Cleopatra.

Changes in IAA levels in response to soil flooding and drought are shown in Figure 4. IAA levels accumulated always at the end of the experimental period. In Cleopatra roots, an important increase (about 8.0-fold in absolute values) occurred at 6 and 8 d of soil flooding stress. In Carrizo, this increase occurred only after 6 days of soil flooding. When subjected to drought stress, changes in IAA concentration profiles were very similar: starting with reductions below controls and ended with increases at the two last days of sampling.

Metabolite profiling analyses

Root samples of Carrizo citrange and Cleopatra mandarin under control conditions and subjected to drought and soil flooding were analyzed by means of LC/ESI-QTOF-MS. Each experiment was analyzed separately using treatment (control vs stressed) and time point as the two main factors to take into account. Significantly altered mass chromatographic features were grouped into four tendency clusters (Supplementary Data 4 and 5). To validate samples, internal standards added before extraction (see material and methods section) were integrated throughout samples and no significant differences between sample groups were detected (data not shown). Tentative annotations were achieved by searching the most plausible structures in Pubchem (http://pubchem.ncbi.nlm.nih.gov/), Chemspider (http://www.chemspider.com/) or Knapsack (http://www.knapsack.jp) databases.

Soil flooding

Only two out of four tendency clusters obtained in Carrizo plants subjected to soil flooding were considered biologically meaningful: cluster 1 in which 60 mass chromatographic features accounting for 9 putative metabolites showing an accumulating trend over time were grouped and cluster 4 that grouped only 34 mass chromatographic features accounting for 7 putative metabolites that were transiently accumulated after one day of stress but then returned to control values. Clusters 2 and 3 were discarded as biologically meaningful due to their odd behaviour that was attributed to biological variance not related to the stress imposition. After analyzing data from Cleopatra mandarin under the same stress conditions, the four clusters obtained were considered biologically meaningful: cluster 1 was equivalent to cluster 4 in Carrizo, showing 103 mass chromatographic features accounting for 8 metabolites transiently increased after 1 day of stress; cluster 4 with 55 chromatographic mass features, accounting for 13 putative metabolites, was equivalent to cluster 1 in Carrizo. In addition, cluster 2 grouped mass chromatographic features which levels decreased below controls over the entire stress period (64 chromatographic peaks and 12 putative metabolites) and cluster 3 contained mass chromatographic features which levels exhibited a transient increase after one day of stress but decreased below control levels afterwards (the most abundant cluster with 107 peaks accounting for a total of 12 potential metabolites). A total of 329 mass chromatographic features with differential accumulation respect to control plants were found in Cleopatra subjected to soil flooding (Supplementary Data 4). An overlapping test was performed by comparing the differentially expressed mass features in Carrizo and Cleopatra in response to soil flooding (Figure 5). Out of 94 and 329 differential mass chromatographic features in Carrizo and Cleopatra, respectively, only 15 were found to be common, accounting for 4 putative metabolites. Two of the mass chromatographic features that showed a similar behaviour in the two genotypes were annotated as 12-oxophytodienoic acid (OPDA, $_{th}[M+H]^+$ 293.2116, $_{exp}[M+H]^+$ 293.2170, ΔDa 0.0054, identified by comparison to a commercial standard) or a molecule carrying the same OPDA moiety (probably a conjugate, although its identity could not be confirmed). Figure 6, shows OPDA levels below controls in both genotypes thus resembling the behaviour of its derivative JA (see Figure 3). On the contrary, the metabolite carrying the OPDA moiety presented an opposite behaviour increasing upon imposition of soil flooding stress in both genotypes. Another tentatively annotated metabolite was a putative ferulic acid derivative which base peak at *m/z* 251.1329 showed a clear fragment at *m/z* 195.069 ($_{th}[M+H]^+$ 195.0653, ΔDa 0.0037, the mass spectrum of this fragment was compared to that of authentic ferulic acid). The mass difference 56.06 was associated to the existence of four methyl units (C_4H_8 ΔDa 0.0025). The extracted ion chromatograms of the 195.069 ion showed an increasing pattern in response to soil flooding, although in controls it was almost undetectable. In this case, maximum levels were higher in Cleopatra than in Carrizo (Figure 6).

Drought

In response to water stress, a total of 276 and 253 differential mass chromatographic features were found in Carrizo citrange and Cleopatra mandarin roots. As in response to soil flooding, little overlapping was observed, only four mass chromatographic features accounting for one putative metabolite (Figure 5). This metabolite was tentatively annotated as hydroxycinnamyl alcohol glucoside ($_{th}[M+H]^+$ 313.1287 $_{exp}[M+H]^+$ 313.1333 ΔDa 0.005, the identity of the ion was confirmed by the presence of a $[M+Na+CH_3CN]^+$ adduct *m/z* 376.1569, a $[2M+H]^+$ and a $[2M+Na]^+$ ion. Although levels of this metabolite increased in both genotypes in response to drought, this increase occurred earlier and was more consistent in Carrizo. In Cleopatra, only a moderate increase could be observed at 12 days of stress.

Non-stressful conditions

To investigate the relationship of different basal concentrations of metabolites between genotypes on plant tolerance to stress, levels of hormones, phenylpropanoids and proline (Figure 8) were compared in control roots of both genotypes. Results indicated that Cleopatra plants showed higher basal levels of phenylpropanoids (cinnamic, coumaric, caffeic and ferulic acids), proline and the hormones SA and IAA. However, Carrizo showed higher levels of JA and ABA under non-stressful conditions. Non-targeted analysis of metabolites showed more metabolites with higher peak intensity in roots of Cleopatra than in Carrizo. Among these, scopolin, a phenylpropanoid derivative, and the triterpenoid nomilin were tentatively annotated with a fold-change of 1.5 and 2.6 in Cleopatra over Carrizo, respectively (Supplementary material 7).

Discussion

In response to abiotic stress, plants alter their biochemical composition depending on the stress pressure and their relative tolerance to the adverse conditions (Arbona and others 2010, Ballizany and others 2012, Witt and others 2012). In a previous work, it was shown that when subjected to identical stress conditions, model plants *Arabidopsis thaliana* and *Thellungiella halophila* exhibited different amounts of secondary metabolites affected by the stress treatment with a low degree of overlapping among them whereas physiological and regulatory responses were almost identical (Arbona and others 2010). This could be explained in part by the fact that the two species used are not closely related, although both belong to the same family. In this sense, the phytochemical composition, especially secondary metabolism, is highly specific and different compounds might carry out the same protective and/or signaling function in different species (Arbona and others 2009a, Merchant and others 2006).

In the present work, we used two genetically-related citrus genotypes widely used as rootstocks in modern citriculture. On one hand, Cleopatra mandarin with a high tolerance to salt stress due to the reduced Na^+ and Cl^- uptake from roots to shoots associated to a low transpiration (Moya and others 2003). This trait is also associated to a constitutive tolerance to water deprivation due to reduced water uptake requirements. On the contrary, this ability to reduce transpiration seems to be detrimental under soil flooding conditions in which highly vigorous genotypes, such as Carrizo citrange or citrumelo have an advantage (Arbona and others 2008, Arbona and others 2009b). The contrasting physiological traits between Carrizo and Cleopatra that confer tolerance to either soil flooding or drought make these genotypes ideal to identify metabolic traits linked to sensitivity or tolerance to these abiotic constraints.

Under flooding conditions, roots of Carrizo plants (flooding-tolerant) accumulated much more proline than Cleopatra did whereas under drought, proline accumulated earlier and to a higher extent in roots of Cleopatra plants (drought-tolerant). These results could be explained by the fact that roots are the first organ sensing soil-derived adverse conditions and a higher ability to synthesize proline under stress situations could be behind a higher tolerance. However, in leaves, an opposite situation was found. Under similar flooding conditions, Arbona and others (2008) found that the proline concentration ratio between stressed and control plants was smaller in leaves of tolerant citrus genotypes than in sensitive ones. This was also true for model plants *Arabidopsis thaliana* and *Thellungiella halophila* under different stress conditions (Arbona and others 2010). In this scenario, it is likely that the higher proline accumulation in roots could act buffering the damaging effects of stress and, therefore, relieving the pressure exerted on leaves.

The accumulation of phenylpropanoids over time was also studied in these two genotypes in response to the two environmental conditions. An accumulation of cinnamic acid in response to flooding in Cleopatra and in response to both stress treatments in Carrizo was observed. The results suggested an essential involvement of cinnamic acid in the responses of citrus to soil flooding but discarded its involvement in the tolerance mechanisms to this particular stress condition. However, in response to drought, the pattern observed in roots of Carrizo and Cleopatra might suggest its role as a defense compound under stress situations as this compound accumulated only in the sensitive genotype. In this sense, several authors (Dai and others 2012, Sun and others 2012) have recently reported that application of exogenous cinnamic acid to cucumber plants, increased endogenous levels as well as improved heat and drought stress tolerance. The apparent disagreement between data obtained from pretreatment on cucumber with this compound and its endogenous levels in citrus could be explained because the beneficial effect of the exogenous treatment was attributed to an improvement of the antioxidant activity. However, it has been shown that citrus possess an efficient antioxidant system (Arbona and others 2003, Arbona and others 2008). Therefore, it could be suggested that the moderate increase in cinnamic acid levels observed in Carrizo roots upon exposition to drought responds to a higher demand for antioxidant defenses associated to the higher sensitivity of this genotype.

In general, phenylpropanoid levels under non-stressful conditions were higher in Cleopatra than in Carrizo; therefore, it is likely that these higher levels prevent further induction of the phenylpropanoid biosynthetic pathway in the drought-tolerant genotype once the stress has been imposed. In addition, levels of the two metabolites annotated as phenylpropanoid derivatives increased in root tissue upon imposition of stress: a ferulic acid derivative and a hydroxycinnamyl alcohol glycoside synthesized from *p*-coumaric acid and involved in lignin biosynthesis. Lignification is an important stress response and its intensity is parallel to the stress pressure (Cabane and others 2012, Li and others 2012, Moura and others 2010). In a recent report, lignin deposition was reduced in mycorrhizal ryegrass plants subjected to drought compared to non-mycorrhizal plants (Lee and others 2012). In these experiments, mycorrhiza acted as attenuators of the stress pressure on plants, therefore reducing the requirement for lignin biosynthesis. In the present work, the data suggested that phenylpropanoid and lignin biosynthesis were activated in the two citrus genotypes subjected to either soil flooding or drought. However, similar increases in phenylpropanoids (especially cinnamic acid) and in the ferulic acid derivative were found in Carrizo and Cleopatra in response to soil flooding, indicating that this could be a specific response to soil waterlogging common to both genotypes. On the contrary, when subjected to drought only a consistent accumulation of cinnamic acid and hydroxycinnamyl alcohol glycoside was found in Carrizo, suggesting that the build-up of these compounds is dependent on stress pressure which is higher in the sensitive genotype.

In response to soil flooding, the parallel decrease in root ABA and JA concentration seems to be a common trend in the two genotypes independently of their different tolerance to this environmental cue (Arbona and Gómez-Cadenas 2008). However, under control conditions, plants of Carrizo had higher levels of both hormones than Cleopatra (Figure 8) indicating a different ABA and JA status prior to stress imposition. It could be then speculated that this different basal status could be behind the higher tolerance of Carrizo to soil flooding, as suggested in other species and stress conditions (Arbona et al. 2010). The observed reduction in the levels of both hormones has to be considered a stress response rather than a result of O_2 depletion since other metabolites that require oxygen in their biosynthesis were not affected or even upregulated (e.g. see

IAA or lignin precursors). The reduction of JA levels was concomitant with those of its biosynthetic precursor OPDA. Moreover, a molecule bearing an OPDA moiety showed an increasing pattern under flooding stress (Figure 6), suggesting that conjugation of OPDA could be an effective mechanism to reduce the availability of this precursor for JA biosynthesis. Although the identity of this molecule could not be confirmed, other types of OPDA and *dn*-OPDA conjugates have been identified in *Arabidopsis thaliana* (Glauser and others 2008); therefore it is not farfetched that similar compounds exist in other plant species. In response to drought, the pattern followed by JA and ABA was similar to that shown in leaves and roots of citrus in previous publications (Arbona and Gómez-Cadenas 2008, De Ollas and others 2012): a transient JA accumulation preceding ABA buildup. Although in De Ollas and others (2012), both the transient JA and progressive ABA accumulation were more pronounced. This is probably due to the experimental system used: water stress shock in De Ollas and others (2012) versus progressive dehydration in this work. Results of previous research also showed a progressive accumulation of IAA with stress (Arbona and Gómez-Cadenas 2008). The results presented in this work, confirm those previous results and extend the knowledge to different stress conditions. It has been recently described the induction of genes involved in IAA biosynthesis from tryptophan led to enhanced drought resistance (Lee and others 2012). Our results are compatible with the involvement of auxin in plant responses to drought, possibly by promoting root growth.

Secondary metabolite response was more intense in Cleopatra roots subjected to soil flooding than in Carrizo roots under the same conditions suggesting that a stronger induction of defense metabolites was required in this genotype to cope with this stress condition. A similar response was observed in *Arabidopsis thaliana* under simulated drought in comparison with the most tolerant *Thellungiella halophila* (Arbona and others 2010).

A higher number of mass chromatographic features showed only an early induction or repression in roots of Cleopatra plants subjected to drought whereas in Carrizo roots most metabolites accumulated much later (Supplementary Data 4 and 5). These results might indicate that metabolic responses to drought were faster in Cleopatra than in Carrizo. In addition, the lower degree of overlapping between the two genotypes and stress conditions indicates that different plant species have particular responses to stress, especially regarding to the secondary metabolism due to its high specificity (Arbona and others 2009a, Arbona and others 2010).

In addition to this, basal physiological and metabolic status of plants has to be considered an important stress tolerance factor as it is likely a combined process of both stress-induced responses and pre-existent defense mechanisms acting as ‘priming’ against stress (Arbona and others 2010, Mehterov and others 2012). For this reason, the basal metabolite configuration of Cleopatra and Carrizo under non-stressful conditions was investigated. Results from the non-targeted analysis indicated that Cleopatra had more potential metabolites with higher intensity than Carrizo under non-stressful conditions. Among which, the accumulation of a mass chromatographic feature annotated as scopolin, a phenylpropanoid derivative, suggested a redirection of the metabolic flow from cinnamic acid. Moreover, results of targeted analyses showed that, with the exception of ABA and JA, levels of the rest of metabolites (proline, IAA and phenylpropanoids) in roots of non-stressed plants were higher in Cleopatra than in Carrizo. This suggests that higher basal levels of proline, IAA and phenylpropanoids are involved in drought tolerance whereas higher levels of ABA and JA are involved in soil flooding tolerance. Our results are in agreement with previous findings in *Arabidopsis* *thaliana*, where the overexpression of genes involved in ABA signaling resulted in enhanced waterlogging tolerance (Liu and others 2012).

Taken together, results presented in this work suggest the involvement of proline and phenylpropanoids, as well as their derivatives, in the response of citrus to water and flooding stress. The analysis of the hormonal levels revealed a parallel pattern of ABA and JA in response to water stress and confirmed previous observations that showed a strong decrease in their levels in response to soil flooding. To this respect, downregulation of JA biosynthesis under soil flooding stress should be associated to the repression of lipoxygenase and/or the conjugation of the precursor OPDA. Other regulation processes that involve IAA could be oriented towards the production of new roots to cope with the limitation of water availability induced by flooding and drought as suggested by Lee and others (2012). The study of the plant metabolome under stressful conditions indicated a low degree of overlapping in the metabolites altered by the stress treatment in the two genotypes considered, allowing the identification of abiotic stress-responsive metabolites in citrus for the first time, as far as we know. This result was associated with the specificity of the secondary metabolism in citrus, put forward by Arbona and others (2009a), and also the different stress tolerance of Cleopatra and Carrizo. When exploring the metabolite levels under non-stressful conditions scopolin, a phenylpropanoid derivative, nomilin, a triterpenoid, and hydroxycinnamyl alcohol glycoside were identified as potential stress-tolerance markers in Cleopatra and Carrizo, respectively. In addition, the higher concentrations of proline, IAA and phenylpropanoids together with the higher number of putative metabolites found in roots of non-stressed Cleopatra plants could be an effective physiological mechanism preventing or delaying drought-derived damage but not soil flooding.

Instead, higher ABA and JA levels in roots could be behind the higher tolerance of Carrizo to soil flooding.

Acknowledgements

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Conflict of interest

The authors declare that they have no conflict of interest.

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Figure legends

Figure 1. Root proline content (expressed as $\log_2$(stress/control)) in Cleopatra mandarin (a) and Carrizo citrange (b) subjected to soil flooding and water stress. Asterisks denote significant difference at $p \leq 0.05$ between control and stressed samples.

Figure 2. Phenylpropanoid content (expressed as $\log_2$(stress/control)) in roots of Cleopatra mandarin (a) and Carrizo citrange (b) subjected to soil flooding and water stress. Asterisks denote significant difference at $p \leq 0.05$ between control and stressed samples.

Figure 3. Abscisic (ABA) and jasmonic acid (JA) levels (expressed as $\log_2$(stress/control)) in roots of Cleopatra mandarin (a) and Carrizo citrange (b) subjected to soil flooding and water stress. Asterisks denote significant difference at $p \leq 0.05$ between control and stressed samples.

Figure 4. Indole-3-acetic (IAA) levels (expressed as $\log_2$(stress/control)) in roots of Cleopatra mandarin (a) and Carrizo citrange (b) subjected to soil flooding and water stress. Asterisks denote significant difference at $p \leq 0.05$ between control and stressed samples.

Figure 5. Venn diagrams depicting the degree of overlapping between metabolites altered in Carrizo citrange and Cleopatra mandarin subjected to soil flooding (a) and drought (b).

Figure 6. Differential metabolites commonly affected in roots of Cleopatra mandarin and Carrizo citrange subjected to soil flooding (expressed as $\log_2$(stress/control)). Asterisks denote significant difference at $p \leq 0.05$ between control and stressed samples.

Figure 7. Differential metabolites commonly affected in roots of Cleopatra mandarin and Carrizo citrange subjected to water stress (expressed as $\log_2$(stress/control)). Asterisks denote significant difference at $p \leq 0.05$ between control and stressed samples.

Figure 8. Metabolite differences between roots of Cleopatra mandarin (CM) and Carrizo citrange (CC) control plants. Proline, phenylpropanoids and plant hormone contents expressed as $\log_2$ (CM/CC). Asterisks denote significant difference at $p \leq 0.05$ between CM and CC control samples.

Supplementary Data

Supplementary Data 1. Assessment of reproducibility for metabolomics assays

Supplementary Data 2. Assessment of linearity in Carrizo citrange

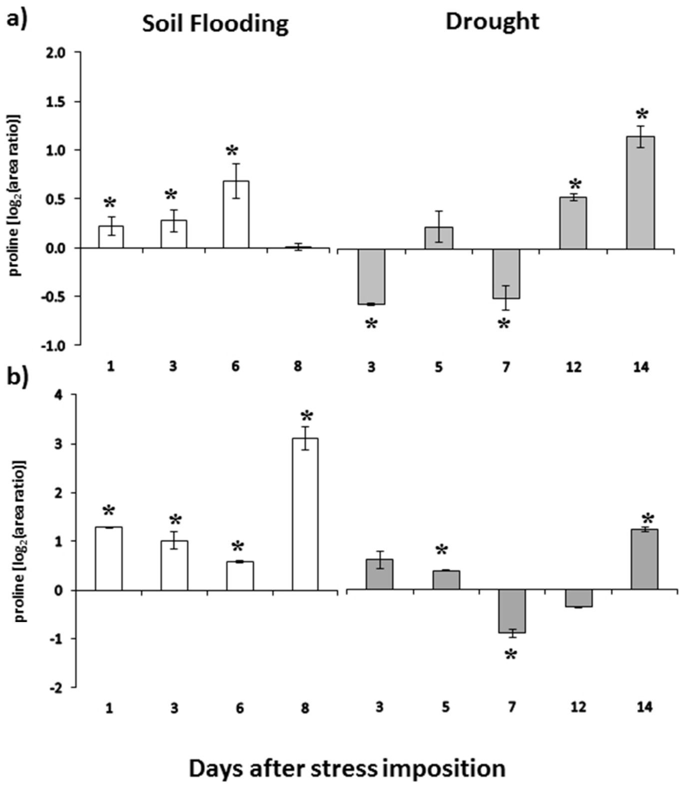
Supplementary Data 3. Assessment of linearity in Cleopatra mandarin

Supplementary Data 4. Differential metabolite profiles in Carrizo citrange (a) and Cleopatra mandarin (b) subjected to soil flooding stress.

Supplementary Data 5. Differential metabolite profiles in Carrizo citrange (a) and Cleopatra mandarin (b) subjected to drought.

Supplementary Data 6. Mass chromatographic features with area values higher in Carrizo citrange than Cleopatra mandarin non-stressed plants.

Supplementary Data 7. Mass chromatographic features with area values higher in Cleopatra mandarin than Carrizo citrange non-stressed plants.



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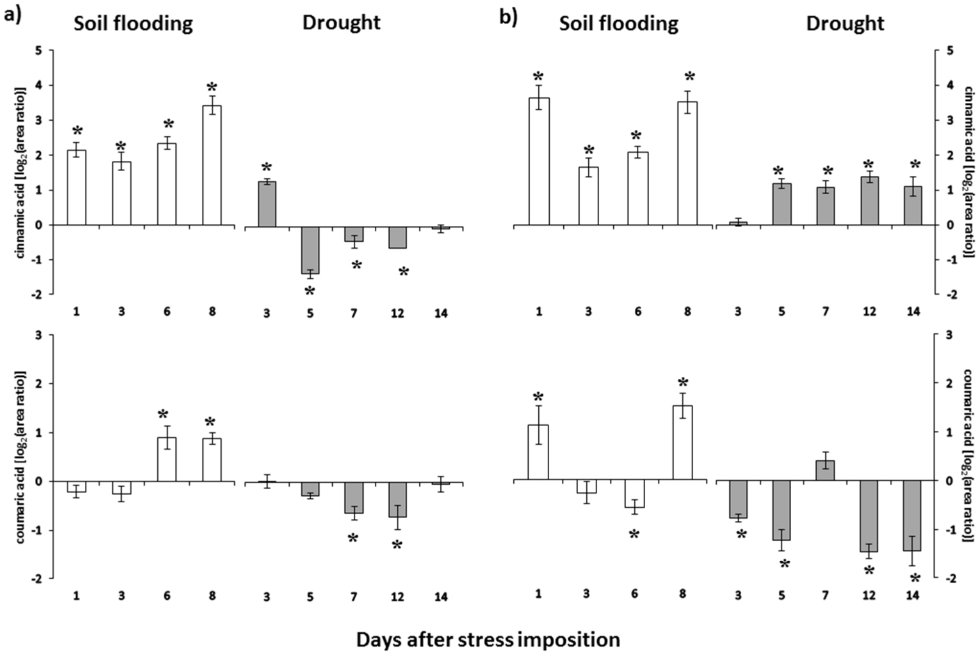

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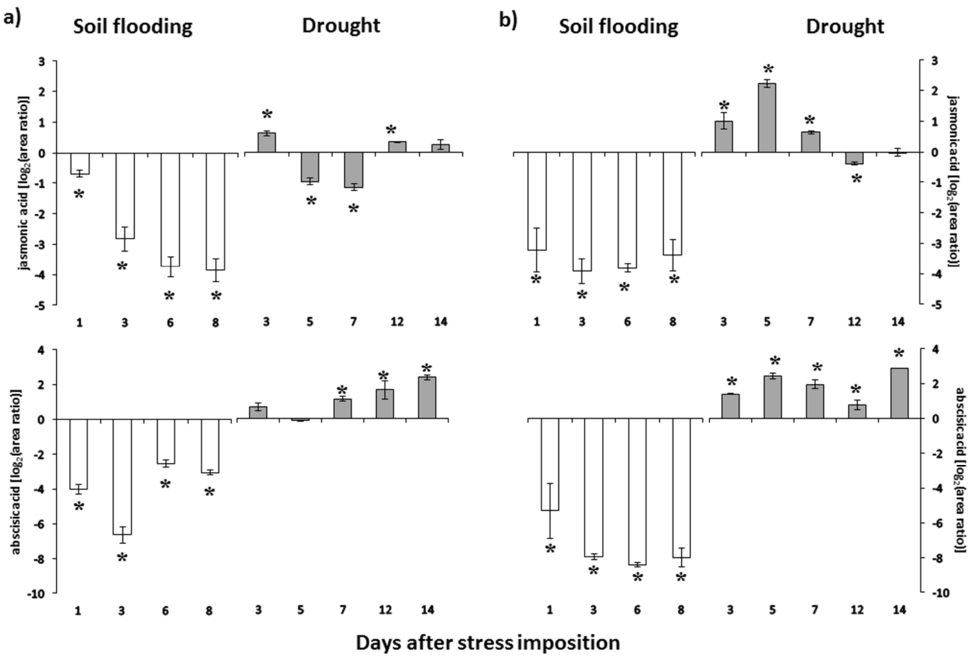
275x190mm (96 x 96 DPI)



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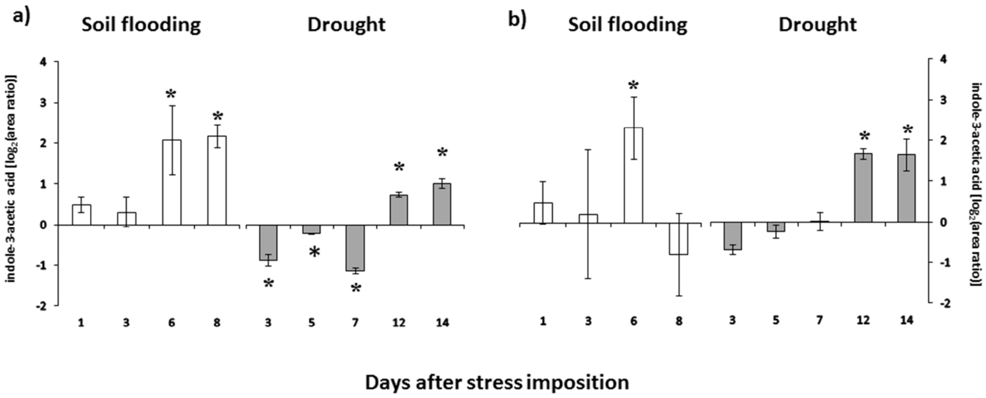

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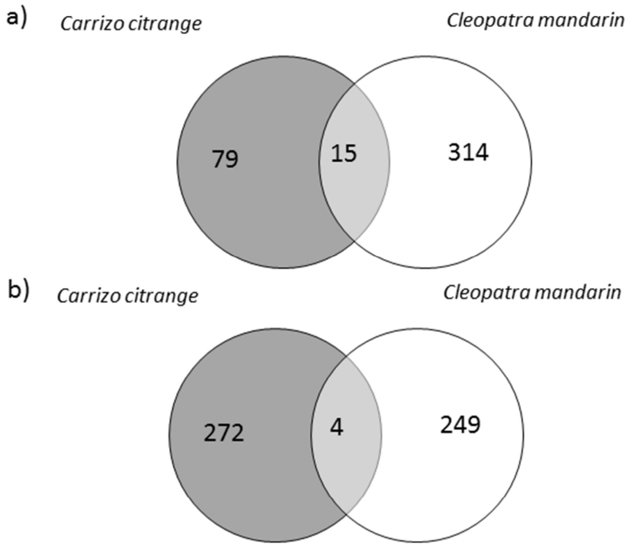

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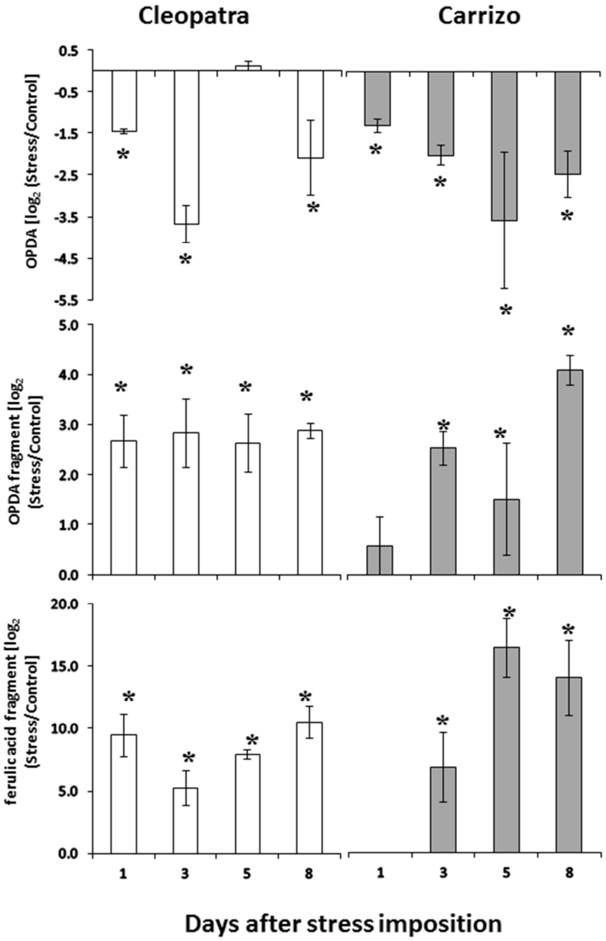
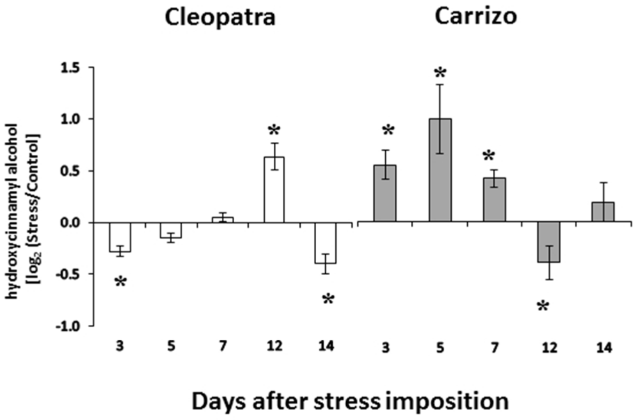


Figure 6. Differential metabolites commonly affected in roots of Cleopatra mandarin and Carrizo citrange subjected to soil flooding (expressed as $\log_2$(stress/control)). Asterisks denote significant difference at $p \leq 0.05$ between control and stressed samples.
190x275mm (96 x 96 DPI)



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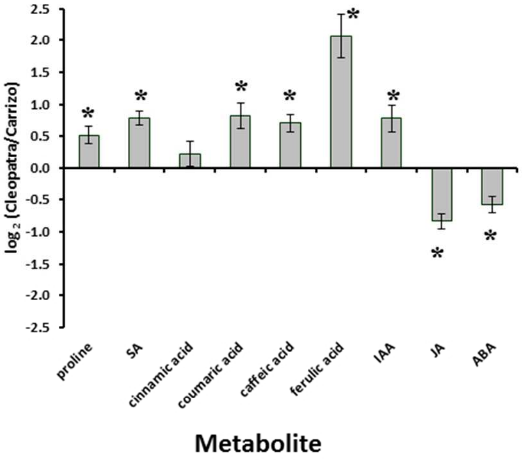
190x275mm (96 x 96 DPI)



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190x275mm (96 x 96 DPI)

Supplementary Data 1. Assessment of reproducibility for metabolomics assays

1. Analysis of Selected Features. Cleopatra mandarin

1.1. Selection of Analytes

No.	*m/z*	Adduct	t_r (min)	[M]
1	501.15195	$[M+H]^+$	10.85	500.14753
2	258.11094	$[M+H-C_6H_{10}O_5]^+$	14.07	419.15854
3	549.21125	$[M+H]^+$	18.11	548.20707
4	286.10803	$[M+H]^+$	21.6	285.10281
5	272.12856	$[2M+H]^+$	25.98	135.56265
6	410.1579	$[M+Na]^+$	30.45	387.17113
7	346.14647	$[M+H]^+$	32.01	345.13956
8	245.09892	$[M+H]^+$	32.6	244.08979
9	310.17995	$[M+H]^+$	33.26	309.17534
10	286.14034	$[2M+H]^+$	34.1	142.56881

1.2. Reproducibility of retention time

No.	extraction rep's (N=16) RSD (%)	injection rep's (n=16) RSD (%)
1	0.4	0.27
2	0.19	0.15
3	0.1	0.13
4	0.14	0.09
5	0.12	0.09
6	0.08	0.05
7	0.09	0.06
8	0.1	0.06
9	0.08	0.04
10	0.08	0.05
average ± sd	*0.14 ± 0.10*	*0.10 ± 0.07*

1.3. Reproducibility of peak intensities

No.	extraction rep's (N=16) RSD (%)	injection rep's (n=16) RSD (%)
1	37.91	19.28
2	11.01	2.74
3	12.81	2.29
4	13.22	4.08
5	16.33	4.48
6	10.81	2.41
7	8.92	4.25
8	3.68	1.75

9	13.12	2.79
10	8.91	5.06
average ± sd	*13.67 ± 9.17*	*4.92 ± 5.17*

1.4. Mass accuracy

No.	$_{\mathrm{exp}}[M]$	Calculated elemental composition	$_{\mathrm{th}}[M]$ (Pubchem)	mass deviation (mDa)
1	500.14753	$C_{30}H_{28}O_3S_2$	500.147978	0.4
2	419.15854	$C_{15}H_{21}N_{11}O_2S$	419.160033	1.5
3	548.20707	$C_{13}H_{37}N_6O_{15}P$	548.205442	1.6
4	285.10281	$C_{13}H_{19}NO_4S$	285.103473	0.7
5	135.56265	-	-	-
6	387.17113	$C_{10}H_{25}N_7O_9$	387.171368	0.2
7	345.13956	$C_{10}H_{23}N_3O_{10}$	345.138338	1.2
8	244.08979			
9	309.17534	$C_{17}H_{27}NO_2S$	309.17624	0.9
10	142.56881			

(-) not calculated

2. Analysis of Selected Features. Carrizo citrange

2.1. Selection of Analytes

analyte	*m/z*	Adduct	t_r (min)	[M]
1	324.15611	$[M+H]^+$	24.13	323.15157
2	326.17072	$[M+H]^+$	27.73	325.16567
3	517.19022	$[M+H]^+$	30.3	516.18667
4	410.15886	$[M+NH_4]^+$	30.44	392.12783
5	284.12665	$[M+H]^+$	31.55	283.12272
6	310.17627	$[M+H]^+$	33.22	309.17438
7	376.15404	$[M+Na]^+$	34.4	353.16346
8	390.16933	$[M+Na]^+$	39.73	367.18189
9	762.4139	$[M+H]^+$	45.57	761.41035
10	444.21327	$[M+Na]^+$	45.58	421.22782

2.2. Reproducibility of retention time

no.	extraction rep's (N=16) RSD (%)	injection rep's (n=16) RSD (%)
1	0.05	0.07
2	0.03	0.07
3	0.06	0.13
4	0.06	0.08
5	0.12	0.14
6	0.04	0.07
7	0.08	0.11
8	0.23	0.15
9	0.06	0.09
10	0.09	0.09
average ± sd	*0.08 ± 0.06*	*0.10 ± 0.03*

2.3. Reproducibility of peak intensities

no.	extraction rep's (N=16) RSD (%)	injection rep's (n=16) RSD (%)
1	13.96	12.84
2	19.37	11.29
3	17.32	10.01
4	6.6	9.63
5	9.31	8.17
6	10.54	11.73
7	3.76	10.46
8	2.15	9.32
9	12.81	10.55
10	3.17	11.46

mitjana ± sd	*9.90 ± 6.00*	*10.55 ± 1.34*

2.4. Mass accuracy

No.	$_{exp}$**[M]**	**Calculated elemental composition**	$_{th}$**[M]** (Pubchem)	**mass deviation (mDa)**
1	323.15157	$C_{20}H_{21}NO_3$	323.152136	0.5
2	325.16567	$C_{12}H_{27}N_3O_5S$	325.167133	1.5
3	516.18667	$C_9H_{24}N_{16}O_{10}$	516.186124	0.5
4	392.12783	$C_7H_{17}N_{14}O_4P$	392.129477	1.6
5	283.12272	$C_9H_{23}N_3O_3P_2$	283.121459	1.3
6	309.17438	$C_{20}H_{23}NO_2$	309.17287	1.5
7	353.16346	$C_{13}H_{29}N_3O_4P_2$	353.163321	0.1
8	367.18189	$C_{12}H_{36}NO_5P_3$	367.180624	1.3
9	761.41035	$C_{18}H_{53}N_{25}O_5P_2$	761.413655	3.3
10	421.22782	$C_{10}H_{28}N_{15}O_2P$	421.228792	1.0

Supplementary Data 2.
Assessment of linearity - Carrizo citrange

Compound 1: 1

	RT	Area	Average	SE
1	24.18	74.551	104.960333	18.3335732
2	24.17	168.694	233.852	38.9779412
3	24.13	337.754	463.782333	69.9616663
4	24.15	714.064	891.655667	105.869764
5	24.15	1298.78	1563.04233	141.388374
6	24.16	2386.893	2706.19933	168.085616
7	24.16	102.422		
8	24.16	229.368		
9	24.15	474.151		
10	24.16	880.596		1:32
11	24.18	1608.018		1:16
12	24.14	2774.798		1:8
13	24.17	137.908		1:4
14	24.18	303.494		1:2
15	24.2	579.442		1:1
16	24.19	1080.307		
17	24.16	1782.329		
18	24.16	2956.907		

Compound 2: 2

	RT	Area	Average	SE
1	27.78	99.541	135.74	22.0032544
2	27.74	217.991	278.142	34.9879469
3	27.74	442.961	550.285	58.6524304
4	27.74	947.072	1137.22333	106.904369
5	27.73	1770.853	2116.62733	178.669486
6	27.77	3777.985	4123.015	181.755409
7	27.77	132.168		
8	27.79	277.252		
9	27.78	562.941		
10	27.81	1147.638		1:32
11	27.78	2211.422		1:16
12	27.77	4196.43		1:8
13	27.79	175.511		1:4
14	27.81	339.183		1:2
15	27.82	644.953		1:1
16	27.82	1316.96		
17	27.77	2367.607		
18	27.75	4394.63		

Compound 3: 3

	RT	Area	Average	SE

1	30.32	16.803	24.3606667	4.7554791
2	30.27	59.138	67.5233333	5.23687522
3	30.27	134.615	155.212667	10.7958816
4	30.29	334.444	338.371333	2.10428613
5	30.27	571.299	580.537667	11.8276033
6	30.34	1057.679	1026.61667	21.092693
7	30.33	23.139		
8	30.37	66.281		
9	30.36	159.903		
10	30.4	339.025		1:32
11	30.38	604.016		1:16
12	30.42	1035.805		1:8
13	30.37	33.14		1:4
14	30.4	77.151		1:2
15	30.41	171.12		1:1
16	30.43	341.645		
17	30.36	566.298		
18	30.34	986.366		

Compound 4: 4

	RT	Area	Average	SE
1	30.45	86.071	128.807	26.3768238
2	30.43	174.96	258.076333	49.9778711
3	30.43	370.578	483.927	63.2016637
4	30.45	709.21	863.190667	83.912099
5	30.44	1084.997	1253.93433	85.0246583
6	30.46	1662.62	1754.90033	46.192503
7	30.48	123.39		
8	30.46	251.55		
9	30.48	492.152		
10	30.5	882.378		1:32
11	30.47	1321.589		1:16
12	30.5	1797.233		1:8
13	30.49	176.96		1:4
14	30.49	347.719		1:2
15	30.53	589.051		1:1
16	30.52	997.984		
17	30.5	1355.217		
18	30.46	1804.848		

Compound 5: 5

	RT	Area	Average	SE
1	31.5	956.477	1173.16533	150.130338
2	31.51	1591.165	1920.79367	201.209096
3	31.5	2517.856	2970.11667	258.582369
4	31.5	4084.958	4589.83933	289.191165
5	31.51	5560.397	6119.74333	282.011897
6	31.54	7271.541	7562.78567	146.655881
7	31.56	1101.504		
8	31.55	1885.698		

9	31.57	2979.015		
10	31.6	4597.912		1:32
11	31.61	6336.64		1:16
12	31.61	7738.512		1:8
13	31.61	1461.515		1:4
14	31.59	2285.518		1:2
15	31.64	3413.479		1:1
16	31.63	5086.648		
17	31.61	6462.193		
18	31.6	7678.304		

Compound 6: 6

	RT	Area	Average	SE
1	33.24	96.023	129.319	19.7835104
2	33.25	192.207	258.02	39.6715652
3	33.25	419.59	524.258667	62.7092584
4	33.25	878.821	1089.23767	114.731274
5	33.23	1741.029	2120.81867	204.543693
6	33.27	3723.473	4201.32767	258.425289
7	33.25	127.455		
8	33.25	252.547		
9	33.27	516.754		
10	33.31	1115.178		1:32
11	33.27	2179.054		1:16
12	33.28	4269.691		1:8
13	33.31	164.479		1:4
14	33.28	329.306		1:2
15	33.32	636.432		1:1
16	33.29	1273.714		
17	33.29	2442.373		
18	33.27	4610.819		

Compound 7: 7

	RT	Area	Average	SE
1	34.38	175.064	213.947667	25.041558
2	34.39	295.381	362.552667	44.4112717
3	34.34	454.569	551.876667	51.2355115
4	34.45	708.883	801.576667	52.1803634
5	34.46	953.088	1048.42833	47.9183056
6	34.34	1195.58	1189.34367	23.7261759
7	34.44	206.053		
8	34.41	345.809		
9	34.44	572.716		
10	34.48	806.399		1:32
11	34.47	1104.534		1:16
12	34.49	1226.964		1:8
13	34.47	260.726		1:4
14	34.5	446.468		1:2
15	34.46	628.345		1:1
16	34.45	889.448		

17	34.47	1087.663
18	34.41	1145.487

Compound 8: 8

	RT	Area	Average	SE
1	39.77	270.965	337.744333	38.7067318
2	39.75	529.406	577.223333	30.385432
3	39.69	800.39	871.342667	37.6318514
4	39.73	1040.308	1175.58367	69.2968947
5	39.74	1379.147	1408.841	15.6636899
6	39.76	1620.677	1585.29633	42.9734785
7	39.75	337.222		
8	39.78	568.652		
9	39.79	885.076		
10	39.84	1269.328		1:32
11	39.88	1415.042		1:16
12	39.95	1635.439		1:8
13	39.84	405.046		1:4
14	39.84	633.612		1:2
15	39.87	928.562		1:1
16	39.87	1217.115		
17	39.82	1432.334		
18	39.89	1499.773		

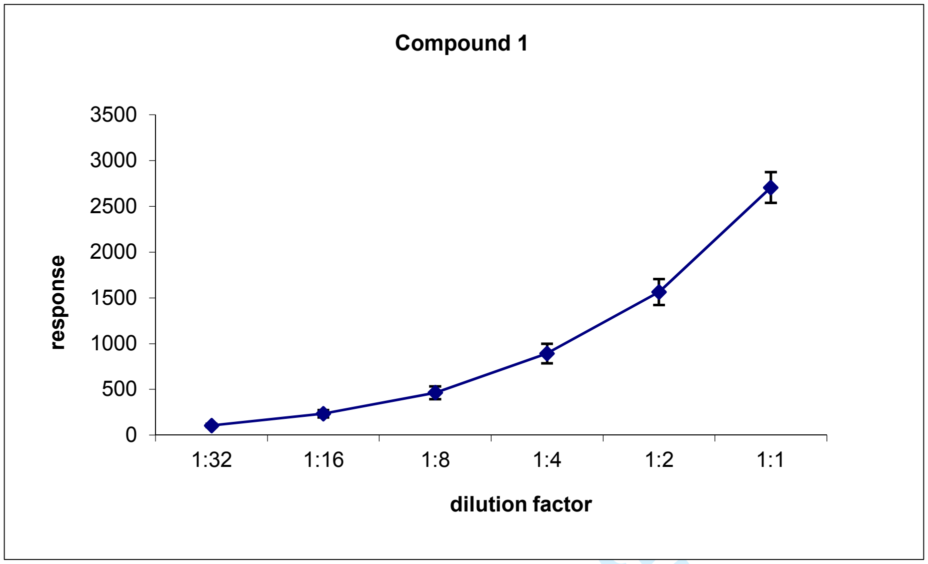
Compound 9: 9

	RT	Area	Average	SE
1	45.61	10.928	17.314	4.99804452
2	45.57	52.688	76.0756667	14.0772188
3	45.59	178.969	235.629	30.2933294
4	45.58	500.531	610.220333	57.0872892
5	45.56	1193.708	1328.207	67.2519709
6	45.59	2561.491	2565.985	117.567103
7	45.63	13.847		
8	45.65	74.195		
9	45.69	245.377		
10	45.69	637.623		1:32
11	45.64	1394.458		1:16
12	45.7	2771.827		1:8
13	45.7	27.167		1:4
14	45.72	101.344		1:2
15	45.7	282.541		1:1
16	45.73	692.507		
17	45.7	1396.455		
18	45.68	2364.637		

Compound 10: 10

	RT	Area	Average	SE
1	45.59	303.754	406.297	64.7972065
2	45.61	667.608	794.941333	74.7289363

3	45.53	1267.042	1401.47833	71.8207116
4	45.56	2115.33	2288.70633	87.4285358
5	45.6	2842.208	3006.536	86.5003693
6	45.69	3884.895	3709.23267	125.503549
7	45.65	388.941		
8	45.65	790.837		
9	45.67	1424.881		
10	45.71	2355.729		1:32
11	45.66	3135.542		1:16
12	45.67	3776.677		1:8
13	45.72	526.196		1:4
14	45.72	926.379		1:2
15	45.7	1512.512		1:1
16	45.69	2395.06		
17	45.68	3041.858		
18	45.62	3466.126		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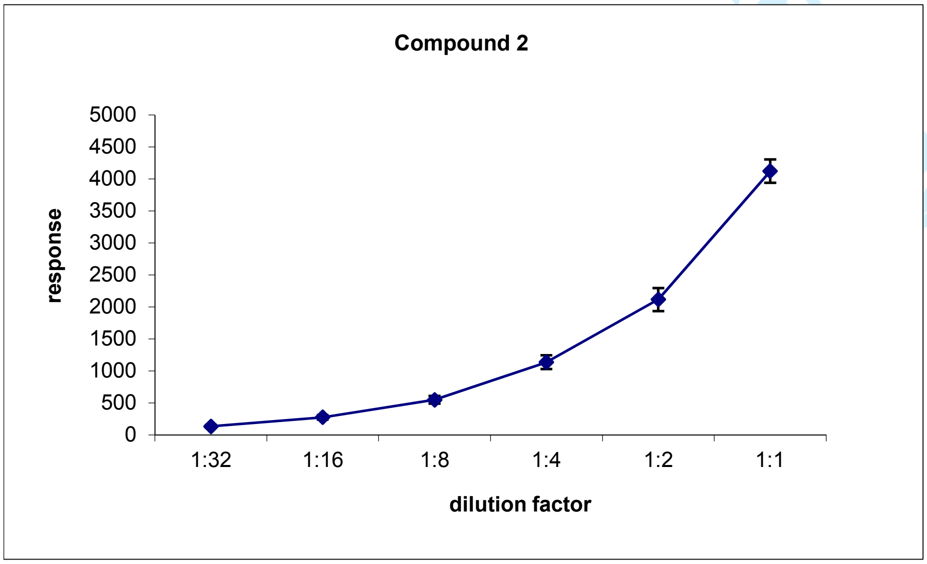
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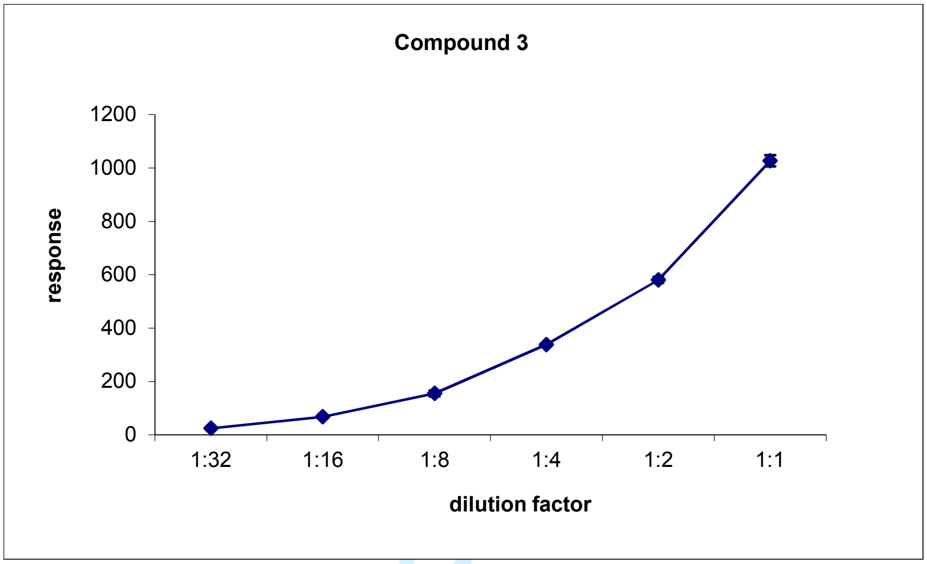
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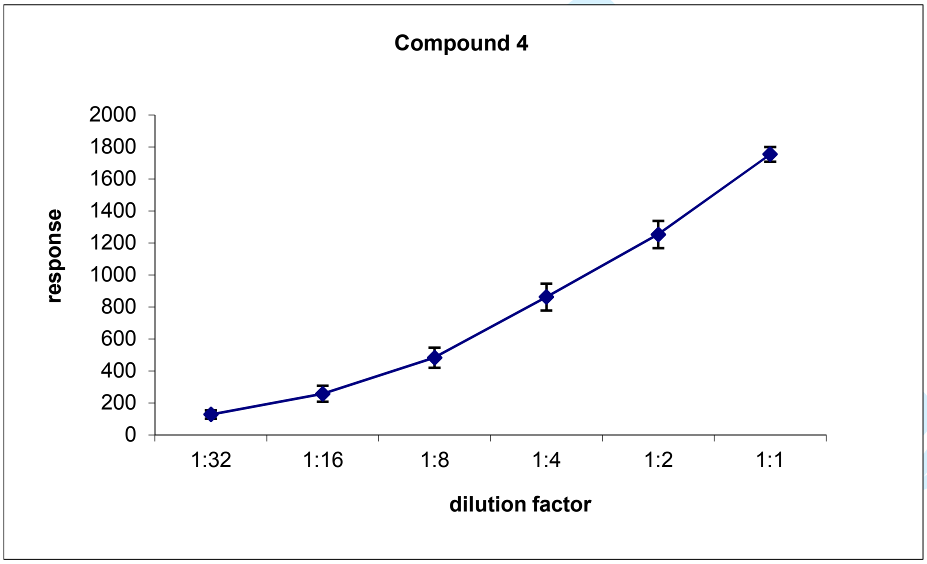
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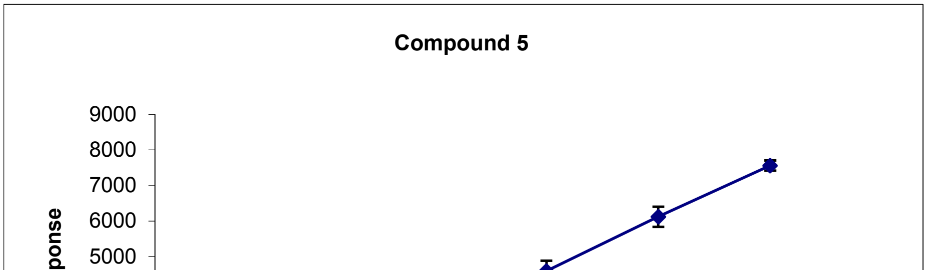
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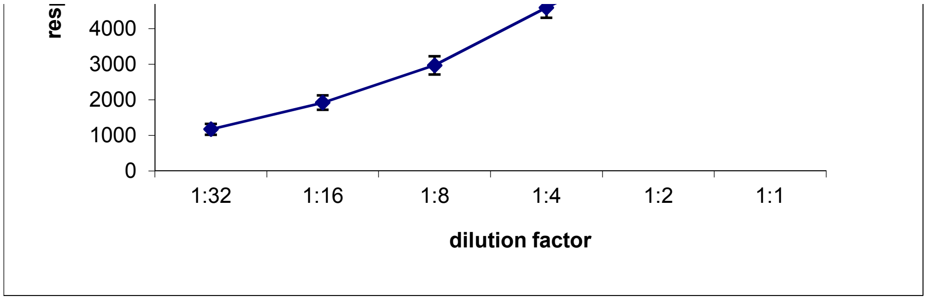

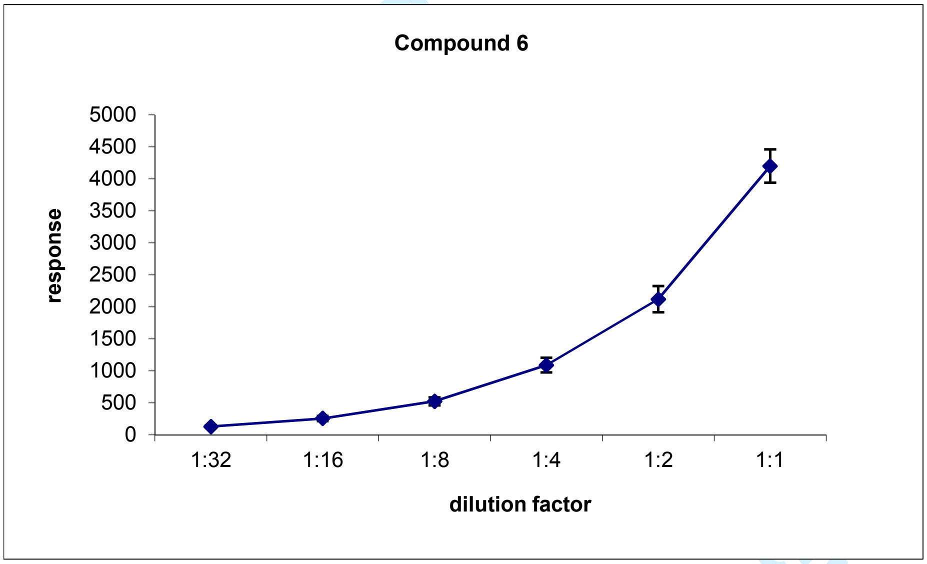
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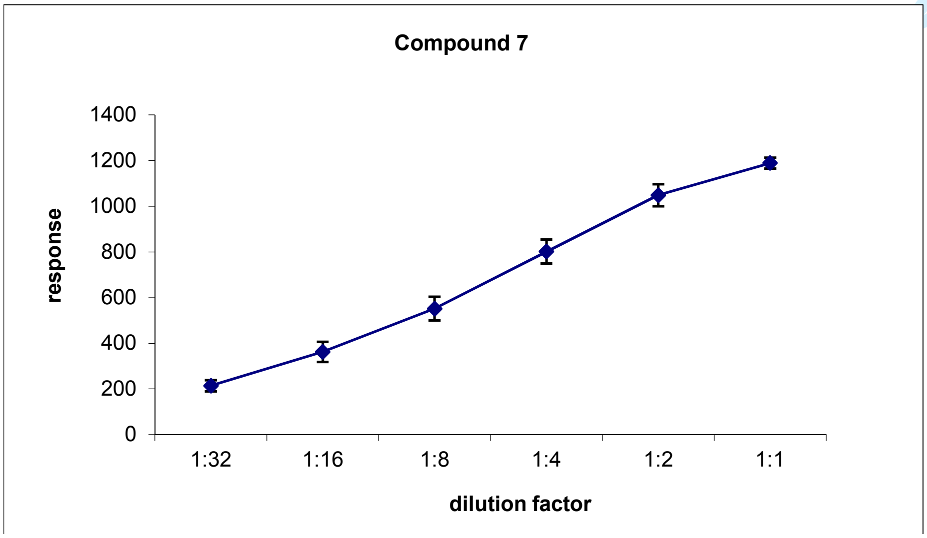
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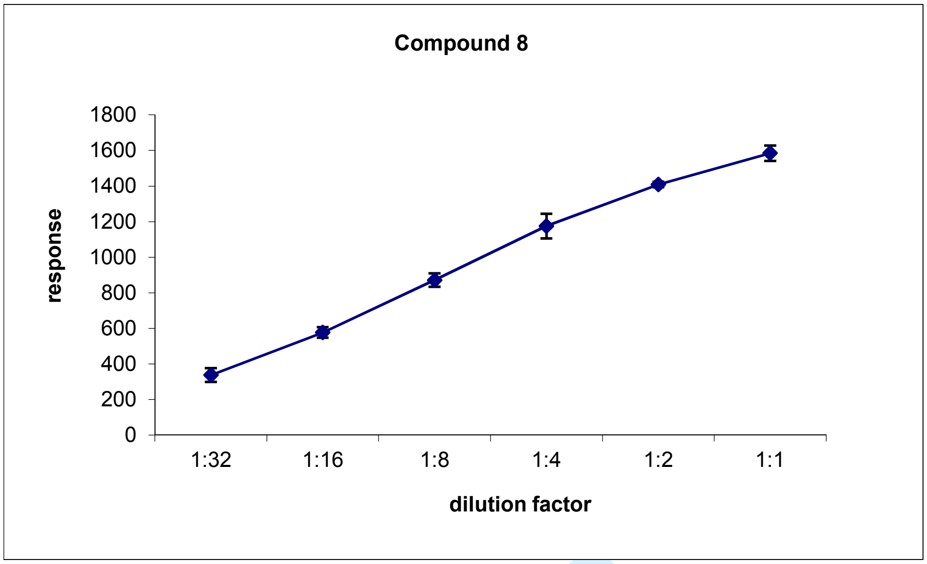
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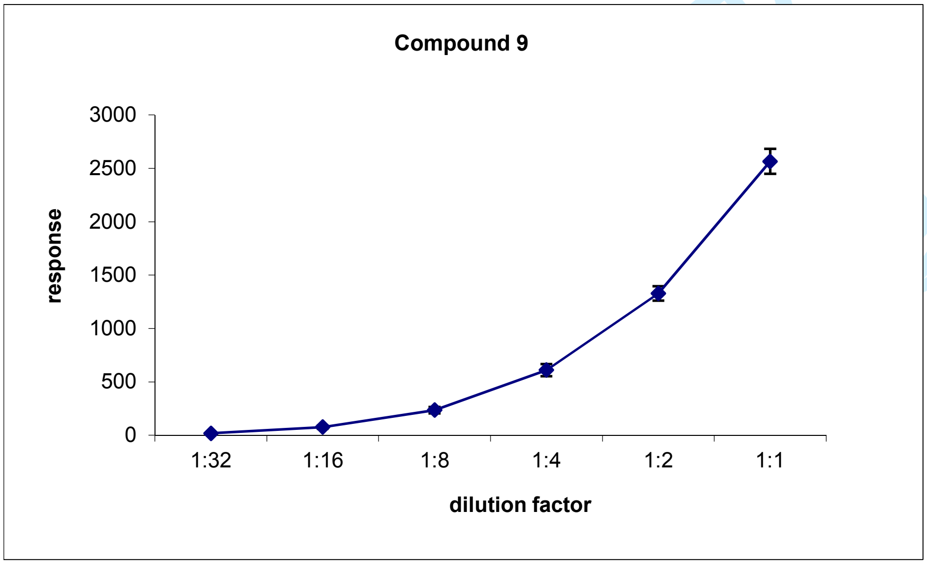
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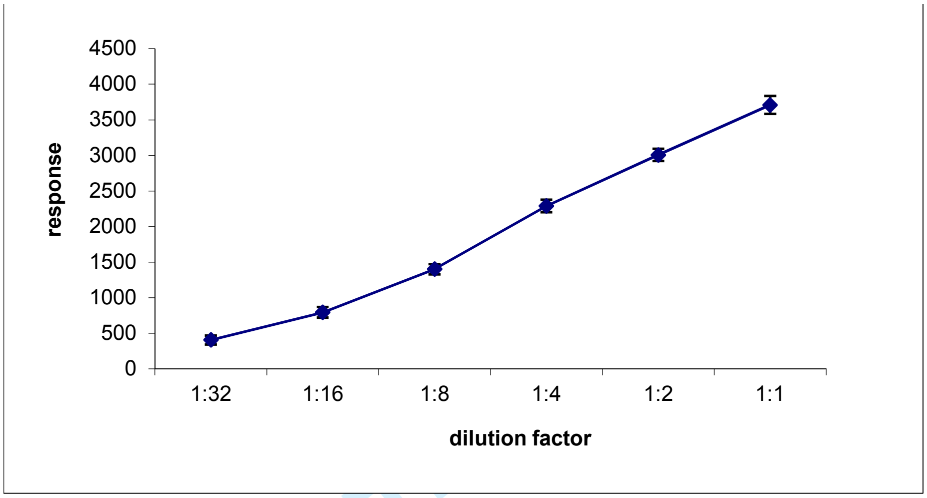
Compound 10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Supplementary Data 3.
Assessment of linearity - Cleopatra mandarin

Compound 1: 1

	RT	Area	Average	SE
1	10.96	17.373	55.0126667	27.8433511
2	10.91	52.319	86.1523333	22.3578713
3	10.89	131.107	250.748667	94.2939156
4	10.88	368.479	641.227333	238.782756
5	10.85	807.574	1264.95	466.874492
6	10.85	3100.715	3326.17367	192.540501
7	10.88	38.291		
8	10.89	77.749		
9	10.89	184.322		
10	10.89	438.105		1:32
11	10.87	788.641		1:16
12	10.87	3168.548		1:8
13	10.86	109.374		1:4
14	10.81	128.389		1:2
15	10.85	436.817		1:1
16	10.9	1117.098		
17	10.9	2198.635		
18	10.9	3709.258		

Compound 2: 2

	RT	Area	Average	SE
1	14.11	36.029	64.9596667	18.3340914
2	14.06	82.932	138.579667	34.9301086
3	14.03	200.656	315.746	78.5363106
4	14.01	496.192	670.704667	118.453473
5	14	967.084	1274.06633	179.799633
6	14.01	1932.176	2272.218	188.480836
7	14.04	59.914		
8	14.04	129.827		
9	14.06	280.719		
10	14.03	619.207		1:32
11	14.05	1265.369		1:16
12	14.05	2301.336		1:8
13	14.04	98.936		1:4
14	14.03	202.98		1:2
15	14.03	465.863		1:1
16	14.07	896.715		
17	14.07	1589.746		
18	14.08	2583.142		

Compound 3: 3

	RT	Area	Average	SE
1	18.15	10.156	21.7116667	7.1406496

2	18.13	28.824	50.355	13.5393135
3	18.1	84.567	127.008333	30.3858719
4	18.09	202.599	272.267333	46.0991345
5	18.11	408.5	489.517333	46.3460039
6	18.13	712.28	781.168333	39.1106885
7	18.11	20.222		
8	18.11	46.899		
9	18.12	110.56		
10	18.1	254.803		1:32
11	18.09	491.026		1:16
12	18.09	783.523		1:8
13	18.11	34.757		1:4
14	18.08	75.342		1:2
15	18.1	185.898		1:1
16	18.13	359.4		
17	18.13	569.026		
18	18.13	847.702		

Compound 4: 4

	RT	Area	Average	SE
1	21.61	61.54	92.9036667	20.9722831
2	21.6	122.389	172.781667	31.1673775
3	21.58	245.081	340.497333	64.6455549
4	21.54	515.044	644.553	90.0481082
5	21.56	941.605	1125.207	111.604444
6	21.6	1644.877	1825.99833	100.262206
7	21.59	84.466		
8	21.59	166.203		
9	21.58	312.648		
10	21.59	600.926		1:32
11	21.58	1107.079		1:16
12	21.59	1842.035		1:8
13	21.6	132.705		1:4
14	21.58	229.753		1:2
15	21.6	463.763		1:1
16	21.6	817.689		
17	21.61	1326.937		
18	21.61	1991.083		

Compound 5: 5

	RT	Area	Average	SE
1	25.91	178.066	248.334	51.2068075
2	25.91	313.709	423.47	69.900961
3	25.89	600.628	793.845333	130.28155
4	25.86	1085.974	1363.017	184.493618
5	25.9	1780.339	2150.107	216.731482
6	25.92	2853.27	3202.81167	192.472331
7	25.9	218.945		
8	25.91	403.365		
9	25.92	739.06		

10	25.95	1290.472		1:32
11	25.9	2139.105		1:16
12	25.92	3237.932		1:8
13	25.93	347.991		1:4
14	25.92	553.336		1:2
15	25.91	1041.848		1:1
16	25.94	1712.605		
17	25.95	2530.877		
18	25.97	3517.233		

Compound 6: 6

	RT	Area	Average	SE
1	30.5	118.64	183.678667	43.2363313
2	30.5	222.359	314.874667	58.272492
3	30.48	396.304	559.770667	105.121709
4	30.46	710.403	903.469667	127.662674
5	30.51	1068.198	1288.66633	130.248764
6	30.44	1435.684	1676.92867	132.164942
7	30.49	166.846		
8	30.47	299.751		
9	30.47	527.002		
10	30.48	855.306		1:32
11	30.45	1278.736		1:16
12	30.47	1703.991		1:8
13	30.48	265.55		1:4
14	30.46	422.514		1:2
15	30.45	756.006		1:1
16	30.48	1144.7		
17	30.47	1519.065		
18	30.46	1891.111		

Compound 7: 7

	RT	Area	Average	SE
1	32.03	8.749	14.15	3.62768618
2	32.04	22.188	30.137	4.87367284
3	32	57.347	78.005	13.843338
4	31.98	138.474	179.87	26.841396
5	32.03	318.916	390.742	39.6826991
6	32.01	724.873	776.961333	27.3343091
7	32.02	12.655		
8	32.01	29.226		
9	32	72.37		
10	32.01	170.968		1:32
11	32.01	397.416		1:16
12	32.01	788.632		1:8
13	32.02	21.046		1:4
14	31.99	38.997		1:2
15	32.01	104.298		1:1
16	32.04	230.168		
17	32.04	455.894		

18	32.04	817.379

Compound 8: 8

	RT	Area	Average	SE
1	32.56	420.83	686.180667	161.440909
2	32.56	877.118	1240.73633	218.671648
3	32.55	1698.738	2409.663	435.238112
4	32.54	3411.708	4276.24133	548.842789
5	32.58	5997.775	6919.35033	491.022785
6	32.56	8910.636	9421.076	275.450367
7	32.57	659.541		
8	32.55	1212.098		
9	32.57	2330.113		
10	32.57	4122.742		1:32
11	32.57	7086.311		1:16
12	32.57	9496.833		1:8
13	32.57	978.171		1:4
14	32.57	1632.993		1:2
15	32.57	3200.138		1:1
16	32.6	5294.274		
17	32.6	7673.965		
18	32.62	9855.759		

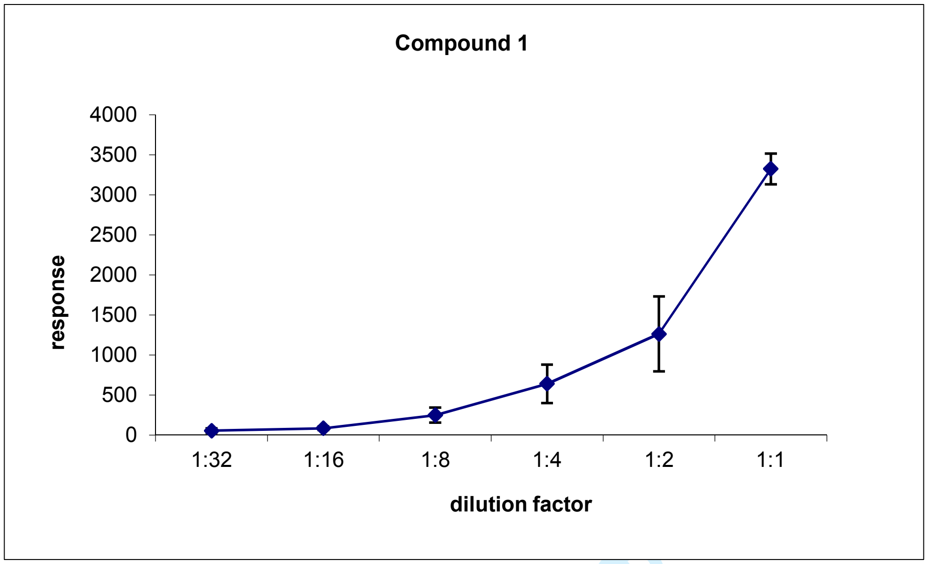
Compound 9: 9

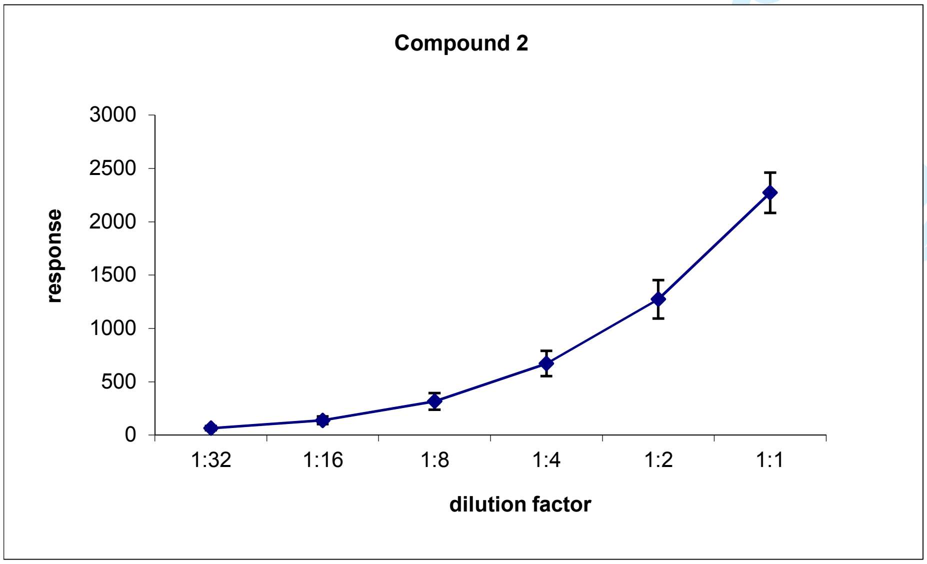
	RT	Area	Average	SE
1	33.29	47.365	79.069	20.1199092
2	33.29	107.984	155.955	30.4653154
3	33.29	224.859	322.539333	63.7615052
4	33.26	503.942	669.413	109.916221
5	33.31	1021.23	1257.58667	139.54641
6	33.27	2080.345	2329.66867	140.176003
7	33.27	73.46		
8	33.27	147.406		
9	33.28	300.383		
10	33.26	626.812		1:32
11	33.26	1247.23		1:16
12	33.26	2343.307		1:8
13	33.28	116.382		1:4
14	33.25	212.475		1:2
15	33.26	442.376		1:1
16	33.28	877.485		
17	33.29	1504.3		
18	33.29	2565.354		

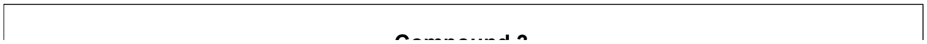
Compound 10: 10

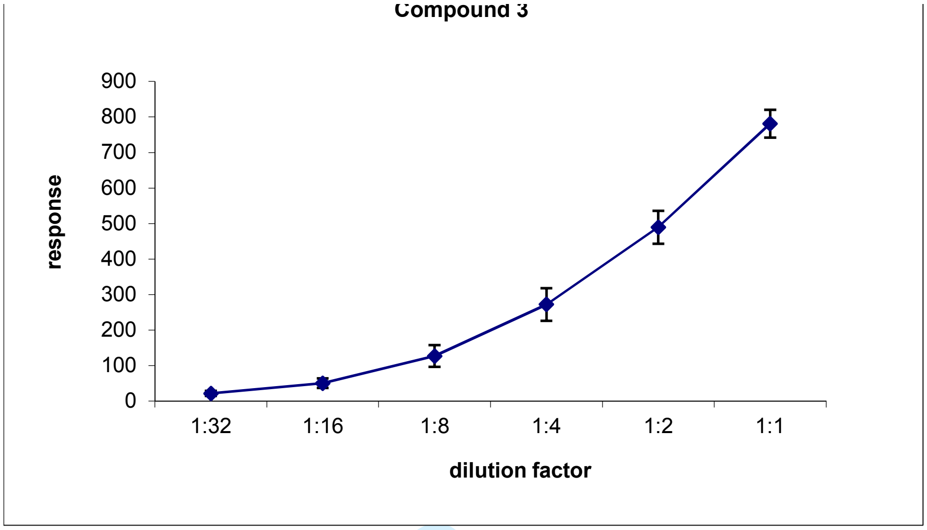
	RT	Area	Average	SE
1	34.08	365.848	486.474	90.1885766
2	34.07	627.232	801.929667	123.902739
3	34.06	1066.674	1413.38867	234.768978

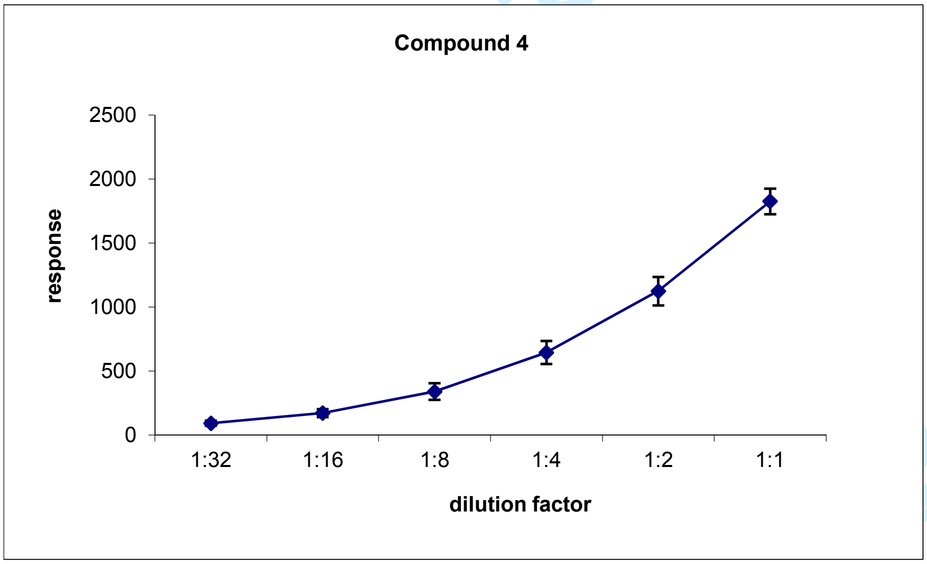
4	34.04	1874.965	2350.174	302.737221
5	34.09	3027.766	3613.29633	336.200904
6	34.07	4664.396	5242.97833	314.015893
7	34.08	430.645		
8	34.06	737.074		
9	34.08	1312.536		
10	34.06	2262.845		1:32
11	34.07	3619.777		1:16
12	34.09	5320.728		1:8
13	34.06	662.929		1:4
14	34.05	1041.483		1:2
15	34.08	1860.956		1:1
16	34.1	2912.712		
17	34.1	4192.346		
18	34.09	5743.811		

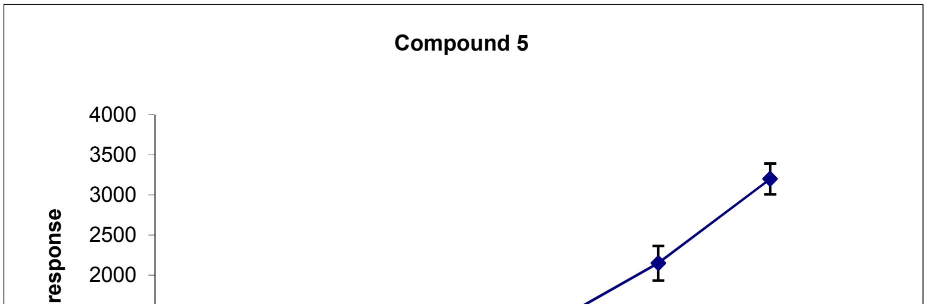

Compound 1
4000
3500
3000
2500
2000
1500
1000
500
0
response
1:32
1:16
1:8
1:4
1:2
1:1
dilution factor

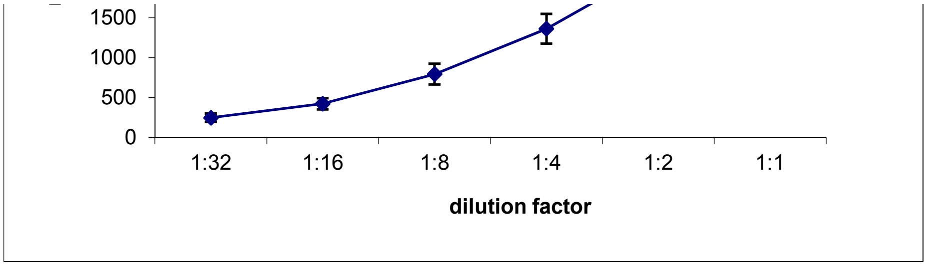

Compound 2
3000
2500
2000
1500
1000
500
0
response
1:32
1:16
1:8
1:4
1:2
1:1
dilution factor

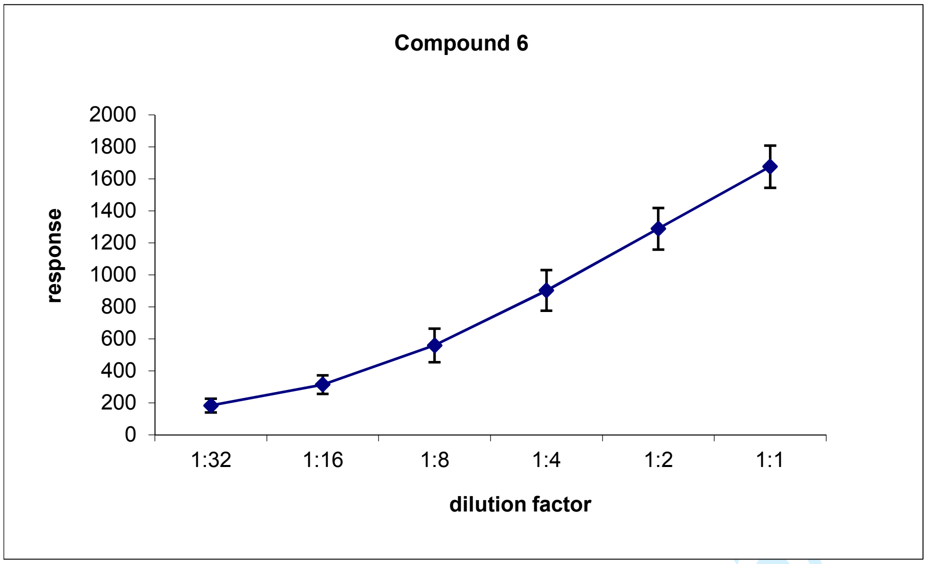

Compound 3

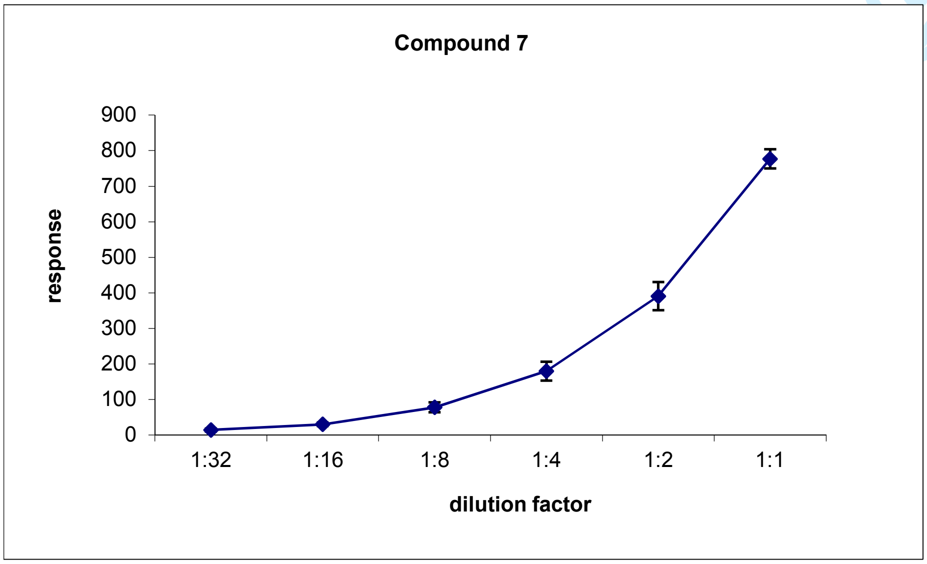

Compound 3
response
900
800
700
600
500
400
300
200
100
0
1:32
1:16
1:8
1:4
1:2
1:1
dilution factor

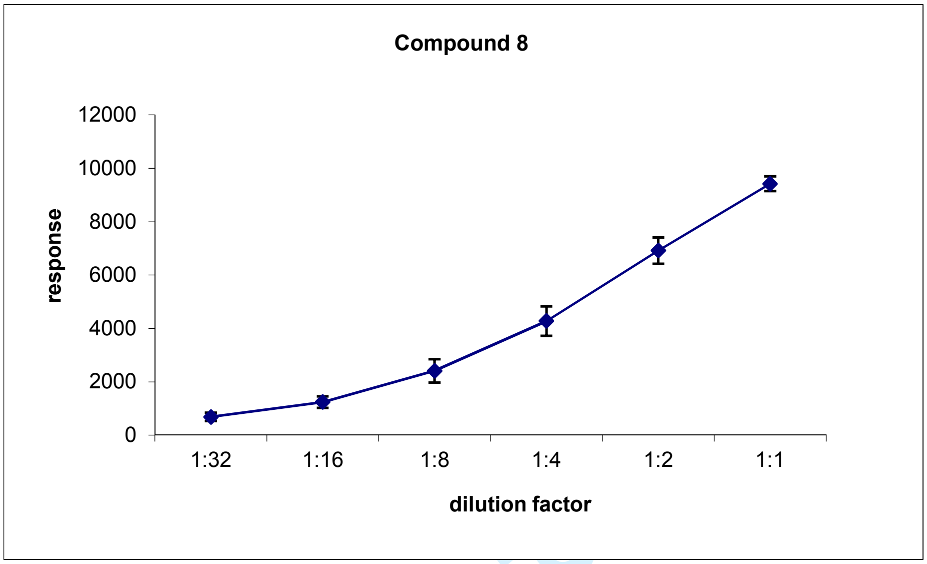

Compound 4
response
2500
2000
1500
1000
500
0
1:32
1:16
1:8
1:4
1:2
1:1
dilution factor

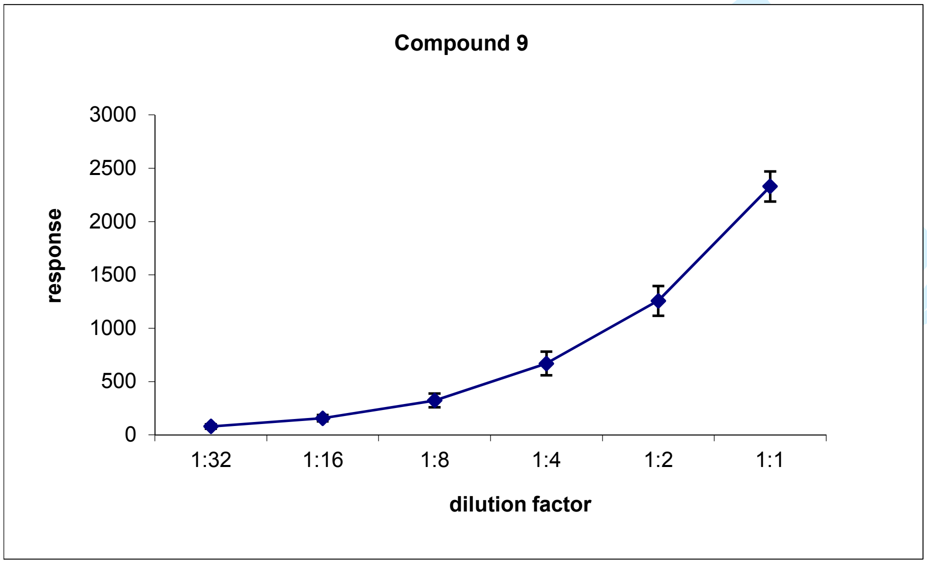

Compound 5
response
4000
3500
3000
2500
2000

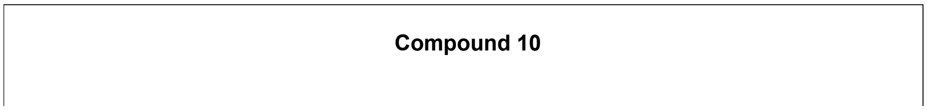

1500
1000
500
0
1:32
1:16
1:8
1:4
1:2
1:1
dilution factor

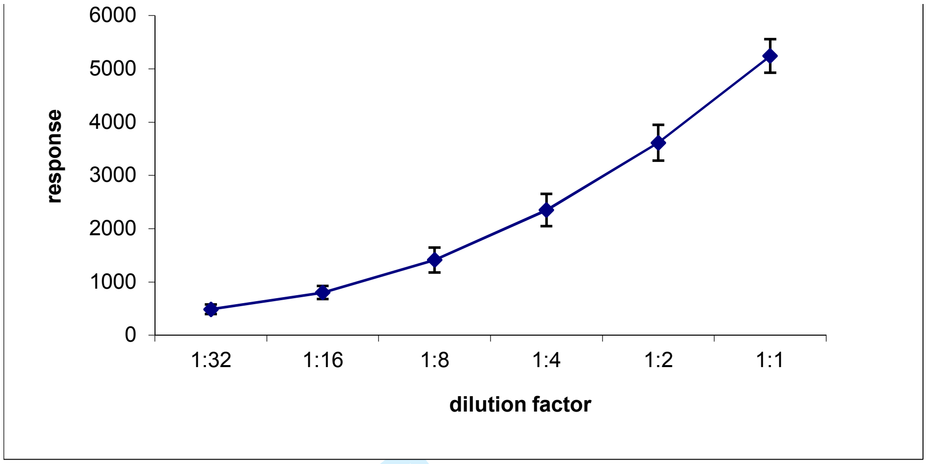

Compound 6
response
2000
1800
1600
1400
1200
1000
800
600
400
200
0
1:32
1:16
1:8
1:4
1:2
1:1
dilution factor


Compound 7
response
900
800
700
600
500
400
300
200
100
0
1:32
1:16
1:8
1:4
1:2
1:1
dilution factor

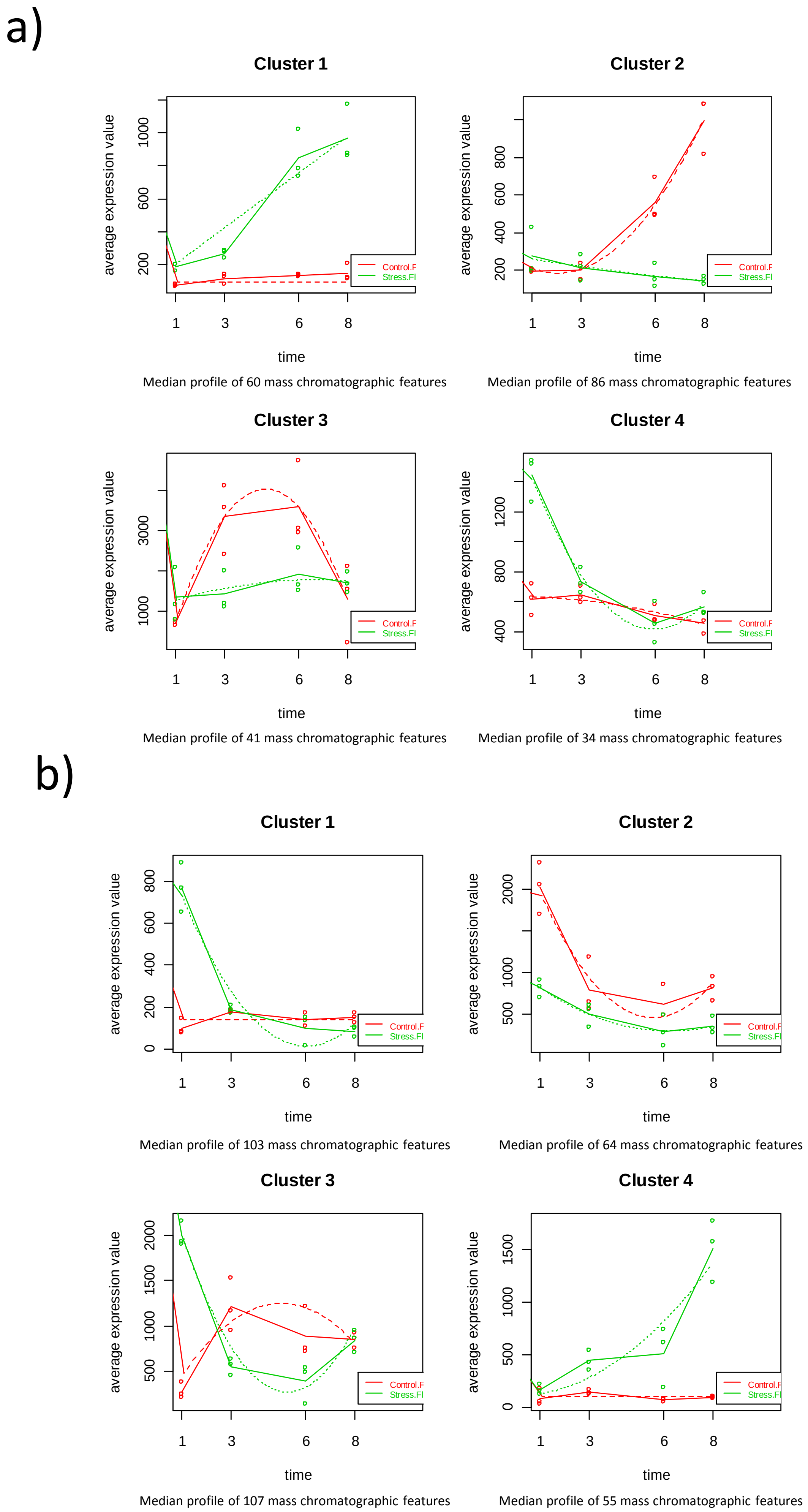

Compound 8
12000
10000
8000
6000
4000
2000
0
response
1:32
1:16
1:8
1:4
1:2
1:1
dilution factor


Compound 9
3000
2500
2000
1500
1000
500
0
response
1:32
1:16
1:8
1:4
1:2
1:1
dilution factor


Compound 10

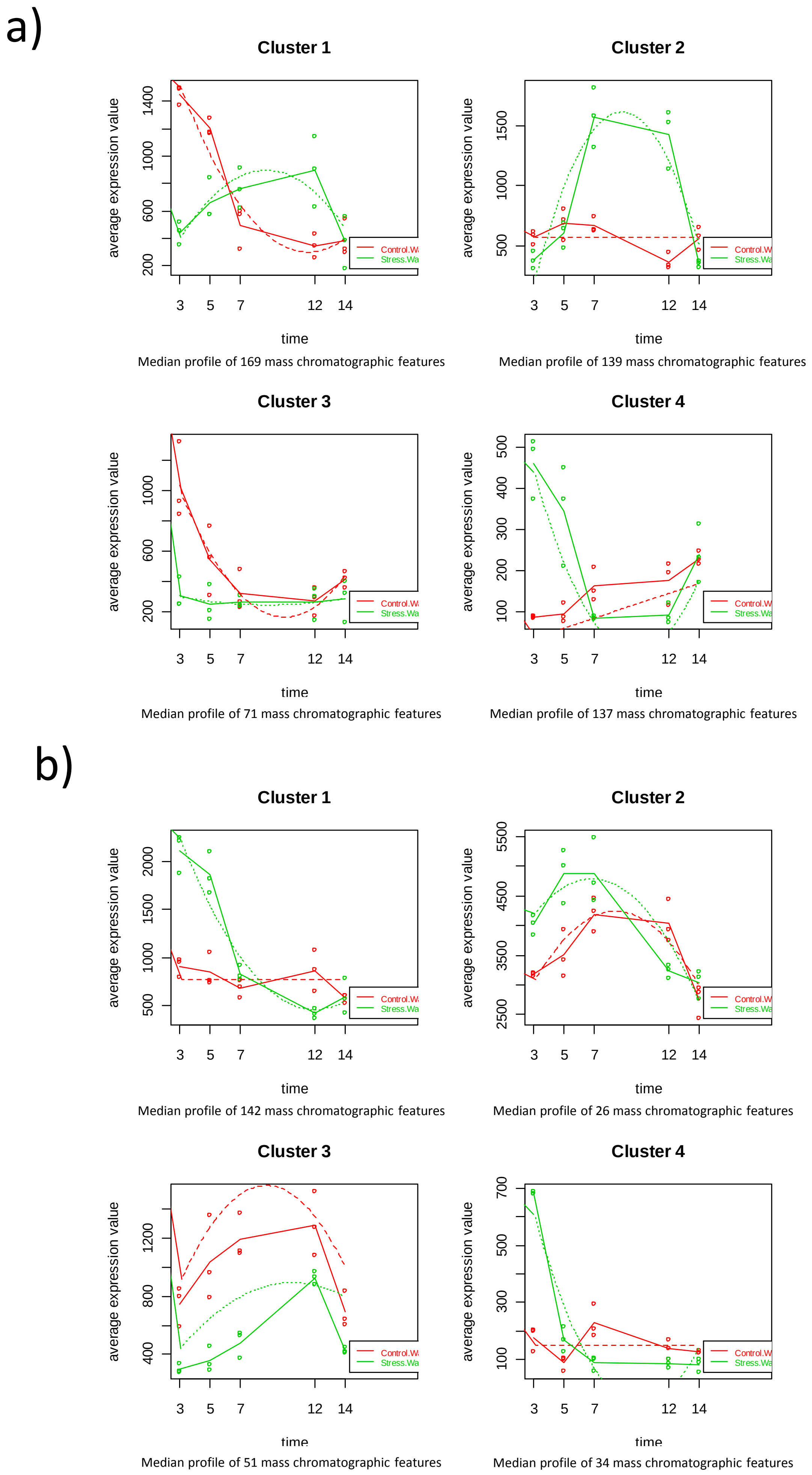

response
6000
5000
4000
3000
2000
1000
0
1:32
1:16
1:8
1:4
1:2
1:1
dilution factor

Supplementary Figure 4



Supplementary Figure 4. Differential metabolite profiles in Carrizo citrange (a) and Cleopatra mandarin (b) subjected to soil flooding stress.

Supplementary Figure 5



Supplementary Figure 5. Differential metabolite profiles in Carrizo citrange (a) and Cleopatra mandarin (b) subjected to drought.

Supplementary Data 6.

Mass chromatographic features with area values

pvalue	*mz*	*rt (s)*	*isotopes*
5.22672E-09	313.1468788	2069.7921	
1.63758E-12	314.1486646	2069.682219	[1][M]+
6.15286E-13	315.1524121	2069.394021	[1][M+1]+
8.54393E-09	376.1570781	2069.478569	[2][M]+
5.98201E-08	377.1626572	2068.444197	[2][M+1]+
4.20722E-07	625.2862184	2068.075967	[3][M]+
4.15608E-05	626.2880626	2069.17181	[3][M+1]+
9.25926E-14	328.1646001	2390.364822	[14][M]+
2.22045E-16	329.1655653	2389.740845	[14][M+1]+
3.9968E-15	330.1751924	2388.720517	[14][M+2]+
8.88178E-16	368.1912706	2389.903487	[15][M]+
0	369.1968911	2388.923696	[15][M+1]+
3.75226E-08	390.1733254	2390.123028	[16][M]+
1.5691E-07	391.1771192	2388.923696	[16][M+1]+
4.37029E-07	653.3185227	2388.720517	[17][M]+
1.06162E-06	654.322455	2388.720517	[17][M+1]+
0.003994468	260.1014206	1826.434064	[21][M]+
0.019818132	261.1068111	1826.586169	[21][M+1]+
0.007577808	300.1264409	1826.434064	[22][M]+
0.002398115	301.1328741	1825.94641	[22][M+1]+
1.42775E-13	313.1418275	2742.121017	[27][M]+
0.000103569	381.2134563	2742.481286	[28][M]+
0	382.2118043	2741.870518	[28][M+1]+
3.4861E-14	314.151054	2741.584455	[27][M+1]+
2.06744E-09	444.2219434	2741.715025	
2.22045E-16	383.216294	2741.715025	[28][M+2]+
4.41145E-10	762.416594	2740.905267	
1.531E-12	315.1538614	2741.281933	[27][M+2]+
1.44605E-10	419.1716396	2741.641049	
0	354.1757737	2741.326506	
0.000101644	258.1065542	2741.001292	
2.26189E-06	403.1952747	2741.258832	
5.99978E-05	278.6200148	2741.441097	
2.37232E-12	384.2257787	2740.938427	[28][M+3]+
1.67875E-06	308.1683092	1671.505414	[48][M]+
1.80416E-07	326.179249	1671.40358	[49][M]+
3.03155E-06	348.1624214	1672.259638	[50][M]+
8.80162E-07	309.1746102	1671.458475	[48][M+1]+
1.41902E-08	327.1827762	1671.197505	[49][M+1]+
3.73766E-07	349.1735807	1672.120694	[50][M+1]+
8.29852E-10	364.138416	1671.586061	
3.86441E-07	350.1733297	1672.101843	[50][M+2]+
4.44089E-16	264.1358706	1921.145078	[60][M]+
2.81464E-12	258.1527211	1922.124795	
0	265.1442719	1921.525893	[60][M+1]+

2.05296E-11	305.1720731	1921.15953	
0.014651845	321.1693713	1921.721262	
4.35318E-12	231.0999318	1721.967116	[62][M]+
5.01209E-09	272.1290451	1722.906013	[63][M]+
1.06581E-14	232.1092284	1721.197485	[62][M+1]+
4.00188E-10	273.1361768	1721.560939	[63][M+1]+
1.15695E-10	233.1125381	1720.572456	[62][M+2]+
2.26861E-05	548.2089466	1091.70815	[64][M]+
2.01323E-05	549.2187797	1091.70815	[64][M+1]+
2.87621E-08	570.1974478	1091.785586	[65][M]+
2.17864E-06	571.2019639	1091.732883	[65][M+1]+
5.10882E-09	310.182849	2000.341207	[66][M]+
0.003071409	244.1103332	2002.774216	
2.16364E-08	311.1882177	2000.286668	[66][M+1]+
0.002198696	242.1220082	2000.622518	
1.40675E-05	332.1661945	2001.070012	
1.0508E-05	351.2154818	2000.277004	
6.80335E-06	312.1932443	2000.277004	[66][M+2]+
3.79461E-06	231.0699965	1088.217064	[109][M]+
2.71372E-05	272.0966672	1088.171893	[110][M]+
3.52599E-05	232.0741416	1088.027702	[109][M+1]+
0.000279277	273.1062782	1086.845614	[110][M+1]+
0.000196877	218.1229754	1520.277359	
3.92608E-10	473.2207663	1522.561706	[111][M]+
4.2705E-11	490.2468165	1521.878499	
0.007574759	474.2271077	1522.974235	[111][M+1]+
6.11977E-12	536.2273197	1520.621652	

higher in Carrizo citrange than Cleopatra mandarin non-stressed p

adduct	*Carrizo citrange*	*Cleopatra mandarin*	*fold change*
[M+H]+312.13	290184.0208	23908.24764	12.14
	85505.08195	3865.860407	
	10638.48459	258.6482332	
[M+Na+ACN]+353.166	12892.57719	397.8099814	
	1916.406354	52.3790692	
[2M+H]+312.14	4861.67793	11.5790206	
	1622.005022	7.054174825	
[M+H]+ 327.16	186778.211	1969.812091	94.82
	20019.34932	104.7335221	
	1832.439992	3.324694453	
[M+ACN]+ 327.16	11214.75131	7.475435945	
	1504.509133	2.271053048	
[M+Na+ACN]+ 327.16	16166.09755	77.10016911	
	2498.772399	13.53596688	
	17945.29399	4.726566201	
	5566.518071	4.675706559	
[M-C3H4]+ 299.12	94076.36495	62780.35866	1.50
	9996.135234	7663.635389	
[M+H]+ 299.12	53064.05615	36420.29187	1.46
	8610.43796	5877.710911	
[M-C3H4N2]+	308909.793	7965.682322	38.78
[M+H]+ 380.207	233584.7679	14443.4521	
	130960.5368	2672.148282	
	47686.67399	1134.73395	
	35576.48538	1553.089874	
	16112.18599	176.2863332	
	11915.49538	2.684322767	
	5179.249081	48.89602021	
[M+K]+ 380.207	4540.259868	83.59180512	
	2800.738759	6.595742735	
	2780.013625	7.875899558	
[M+Na]+ 380.207	2648.982829	77.01910739	
	2626.925459	2.637831669	
	837.1135461	3.27224688	
[M+H-H20]+ 325.174	68458.19485	14798.45544	4.63
[M+H]+ 325.174	56966.12599	9375.724894	
[M+Na]+ 325.174	31408.942	7330.142756	
	13148.85016	2913.035956	
	10961.55686	1502.04594	
	6810.927018	1357.883302	
[M+K]+ 325.174	3400.106326	348.8370896	
	554.5114275	158.072588	
[M+H]+ 263.13	148350.7466	15.03034001	9870.09
	32549.72879	8451.17319	
	25504.56771	460.6893242	

[M+ACN]+ 263.13	869.7572447	18.53779447	
	283.9420179	174.9197311	
[M+H]+ 230.09	152102.9009	54337.27383	2.80
[M+ACN]+ 230.09	28357.59972	13964.05822	
	17890.71071	5635.789139	
	3958.791388	1049.540779	
	1671.251573	498.0187971	
[M+H]+ 547.204	79535.84179	39495.1967	2.01
	18995.14586	9366.461777	
[M+Na]+ 547.204	16632.70894	8438.976455	
	3167.260537	1517.859274	
[M+H]+ 309.176	63711.5784	37734.76948	1.69
	15289.6609	9095.195676	
	12111.11292	7501.631374	
	10177.35936	7445.505253	
[M+Na]+ 309.176	6401.723536	2884.040591	
	1188.822668	560.9396299	
	806.0839477	420.6298443	
[M+H]+	21859.3092	10707.45453	2.04
[M+ACN]+	13058.07999	4381.571537	
	2473.9932	1129.149933	
	2208.172882	894.927201	
[M+H]+ 217.119	22496.26032	6839.958659	3.29
[M+H-NH3]+ 489.239	19985.70579	118.8699554	
[M+H]+ 489.239	8424.239977	22.96569662	
	4420.331499	2698.315243	
	668.3688678	22.20534603	

lants.

hydroxycinnamyl alcohol glycoside

Supplementary Data 7.

Mass chromatographic features with area values l

pvalue	*mz*	*rt (s)*	*isotopes*
9.80E-04	282.264433	2777.089954	
3.54E-04	283.2855498	2776.680982	[5][M]+
2.86E-03	323.3094487	2777.080931	[6][M]+
1.29E-04	265.257675	2776.841276	[4][M]+
3.19E-03	563.5572439	2776.83698	
8.93E-05	247.2474115	2776.755472	
2.00E-04	284.2940806	2776.606811	[5][M+1]+
3.87E-03	324.3144327	2776.765285	[6][M+1]+
1.83E-04	266.2625381	2776.515146	[4][M+1]+
1.50E-04	259.0984867	1906.000845	
0.00E+00	260.1057441	1905.829779	[7][M]+
3.45E-11	300.1266018	1906.666895	[8][M]+
6.94E-06	259.4670555	1906.071538	
1.68E-09	557.2696479	1907.75895	
2.41E-08	517.1931607	1907.693864	
0.00E+00	322.1102053	1907.80293	[9][M]+
0.00E+00	261.1046813	1907.439723	[7][M+1]+
1.19E-12	301.1325032	1907.187266	[8][M+1]+
0.00E+00	539.1759875	1908.524679	[11][M]+
0.00E+00	556.6744066	1907.836455	[12][M]+
2.22E-16	528.1729001	1906.49247	[10][M]2+
4.46E-10	657.2160183	1906.49247	
5.27E-11	665.2128359	1907.901058	[13][M]2+
4.38E-10	665.7123527	1907.72807	[13][M+1]2+
4.44E-16	549.1828804	1905.502942	
2.22E-15	528.6734256	1906.294882	[10][M+1]2+
0.00E+00	323.1133731	1907.78808	[9][M+1]+
2.70E-10	536.163264	1906.592086	
0.00E+00	540.1771036	1907.836455	[11][M+1]+
2.59E-07	544.1540877	1907.81778	
5.57E-14	529.1754832	1906.773627	[10][M+2]2+
1.62E-11	521.1772495	1903.482825	
3.35E-14	557.6826402	1907.984266	[12][M+1]+
7.66E-04	457.1712724	1735.914695	[18][M]+
1.98E-03	468.156872	1736.477701	
1.68E-06	590.1834097	1737.923629	
8.93E-04	458.1759674	1735.975807	[18][M+1]+
2.05E-02	378.1142715	1736.60855	
5.22E-12	245.1043824	2048.249023	[24][M+]+
2.14E-09	286.1458768	2048.071294	[25][M]+
0.00E+00	246.1214227	2048.095801	[24][M+1]+
4.13E-10	287.1529635	2048.095801	[25][M+1]+
0.00E+00	247.1284897	2048.095801	[24][M+2]+
2.88E-06	489.2338413	2047.643557	
4.51E-11	288.1562985	2047.566656	[25][M+2]+

4.44E-16	308.169005	1772.875798	[29][M]+
5.55E-15	309.1736901	1772.93815	[29][M+1]+
1.39E-09	193.1031453	1774.016844	
4.64E-05	231.0965088	1561.800315	[31][M]+
1.04E-03	272.1302097	1561.537083	[32][M]+
2.16E-06	216.0701393	1561.878427	[30][M]+
2.24E-05	232.1077652	1561.878427	[31][M+1]+
3.32E-04	273.1367925	1561.718629	[32][M+1]+
4.16E-06	217.0769333	1561.752874	[30][M+1]+
1.15E-03	175.0460425	1561.439806	
2.05E-07	224.1019082	1448.156258	[33][M]+
2.98E-07	225.112756	1447.66845	[33][M+1]+
6.27E-07	265.1386393	1448.635079	
5.44E-06	226.1191194	1447.66845	[33][M+2]+
3.27E-04	105.0434649	1447.43803	
5.74E-09	245.1159735	1958.475151	[34][M]+
1.02E-05	286.1406358	1958.188211	[35][M]+
2.12E-06	230.0895316	1959.132922	
1.36E-08	246.1231605	1958.458816	[34][M+1]+
4.68E-06	287.1504612	1957.820256	[35][M+1]+
1.40E-08	247.1265824	1958.6303	[34][M+2]+
4.59E-07	189.0612502	1958.652742	
3.73E-14	245.0808304	1512.70364	[36][M]+
2.16E-11	246.0888173	1513.063872	[36][M+1]+
9.89E-08	286.1127624	1512.70364	
2.14E-12	247.0941728	1513.423453	[36][M+2]+
6.26E-11	308.1023736	1513.053574	
6.66E-16	227.1672336	1817.138624	
1.68E-06	195.1400606	1818.924988	
0.00E+00	249.1504795	1817.176626	
2.13E-14	306.1153218	1898.092942	[43][M]+
1.44E-08	281.0657846	1898.2441	
1.03E-12	265.0900209	1898.130858	
1.02E-14	307.1212824	1897.576604	[43][M+1]+
1.65E-13	533.1863373	1897.598001	
5.24E-14	261.1125803	1307.080831	[M]+
1.17E-08	262.1149881	1305.459417	[M+1]+
2.96E-09	302.1425513	1306.613596	[51][M]+
1.33E-02	263.1071071	1304.575495	[M+2]+
2.48E-09	303.1466126	1307.525899	[51][M+1]+
7.51E-03	225.1453602	1686.865482	[52][M]+
1.71E-05	266.1787386	1686.931744	[53][M]+
4.30E-04	226.1562533	1686.67035	[52][M+1]+
1.36E-08	210.1174399	1686.984823	
8.20E-08	234.1531421	1687.014686	
1.09E-06	267.1832447	1686.69904	[53][M+1]+
3.54E-05	227.1579162	1686.539025	[52][M+2]+
2.14E-08	211.1293976	1686.822934	
4.82E-04	235.1475353	1685.340052	
4.79E-08	223.0624263	1021.801644	[55][M]+

1.59E-08	224.0677965	1021.715512	[55][M+1]+
3.56E-07	225.0765657	1022.112998	[55][M+2]+
1.46E-08	309.122711	1020.529389	
1.85E-03	245.0799603	1303.248494	[56][M]+
8.96E-08	267.0672697	1303.850353	[57][M]+
1.56E-04	246.0888778	1303.026233	[56][M+1]+
5.75E-06	308.0946227	1303.553662	[59][M]+
1.39E-08	268.0744185	1303.745658	[57][M+1]+
1.72E-05	309.0974046	1302.964643	[59][M+1]+
9.39E-06	237.0799463	1151.07355	[61][M]+
5.53E-13	293.1064854	1152.159883	
1.98E-05	238.0847699	1150.934575	[61][M+1]+
1.30E-03	278.1127998	1153.116238	
2.54E-04	300.0914065	1151.171647	
2.39E-11	329.1384115	1832.177331	[67][M]+
1.20E-04	347.1526412	1831.370275	[68][M]+
1.47E-05	410.1615116	1830.664165	[73][M]+
5.25E-07	715.2765116	1831.031487	[74][M]+
2.51E-12	330.1459459	1832.20026	[67][M+1]+
2.79E-07	369.1356852	1830.359678	[70][M]+
9.77E-07	385.1108287	1831.105301	[72][M]+
8.07E-09	716.2834353	1831.006556	[74][M+1]+
6.96E-05	348.1569645	1831.330608	[68][M+1]+
4.05E-06	411.1672473	1831.079565	[73][M+1]+
5.92E-08	379.1810379	1831.693027	[71][M]+
2.25E-05	388.1822671	1831.642124	
2.44E-08	370.1410139	1830.133763	[70][M+1]+
2.09E-09	331.1504048	1831.676713	[67][M+2]+
8.10E-06	386.115981	1830.979651	[72][M+1]+
1.77E-05	380.185505	1831.97623	[71][M+1]+
9.62E-06	412.1732428	1829.935667	[73][M+2]+
1.71E-09	224.1092715	1794.545851	[75][M]+
1.03E-09	225.1217874	1794.647183	[75][M+1]+
1.35E-07	265.1391286	1794.782586	
1.17E-03	355.1038989	663.4085103	[78][M]+
1.37E-03	356.1109769	663.1219429	[78][M+1]+
2.73E-03	411.218581	1697.329612	
3.22E-04	393.2100445	1697.545514	
1.62E-02	437.2036237	1697.405528	
4.80E-04	297.2931433	2710.649083	[86][M]+
3.44E-04	298.2996515	2710.736262	[86][M+1]+
2.73E-03	256.270041	2710.641451	[85][M]+
4.16E-03	257.2741232	2710.647738	[85][M+1]+
1.76E-08	515.228536	1220.651216	[91][M]+
2.83E-09	516.2347258	1220.293959	[91][M+1]+
3.14E-10	455.2118822	1221.622279	[90][M]+
3.60E-07	471.2416474	1219.743618	
1.78E-08	517.2372739	1220.474615	[91][M+2]+
5.84E-09	456.2134189	1220.610322	[90][M+1]+

higher in Cleopatra mandarin than Carrizo citrange non-stressed p

adduct	*Carrizo citrange*	*Cleopatra mandarin*	*fold change*
[M+H]+ 281.264	374504.3098	482457.8463	1.28825713
	77873.62817	122034.0111	
	29616.5264	53107.76225	
[M+H-NH3]+ 281.264	15865.28319	28801.41417	
[2M+H]+ 281.264	8915.712117	19066.50631	
	5601.698334	11119.59738	
	6728.67628	10499.22138	
	5338.318385	9488.495519	
	2339.569332	4680.089996	
[M+H]+ 258.098	57917.30935	274585.8598	4.74099821
	11798.18674	199598.8449	
[M+ACN]+ 258.098	2873.04505	115819.6709	
	0.027302946	72582.64021	
	485.3981055	25763.45665	
[2M+H]+ 258.098	44.60420569	23479.25944	
[M+Na+ACN]+ 258.098	68.22574465	19321.76623	
	889.0319223	17617.86652	
	212.8192158	17463.64988	
[2M+Na]+ 258.098	19.06911034	8018.889042	
	33.29466123	6373.399498	
	21.17773324	6078.900338	
	4.874408221	6075.009248	
	0.933524186	5362.077042	
	0.172876234	4133.540336	
	6.299312062	3968.442212	
	3.996801306	3834.110792	
	6.201457766	2375.396246	
	9.748552816	1875.380666	
	6.151756514	1824.203565	
	3.156869095	1737.438006	
	8.849406736	1526.278104	
[M+H-CO]+ 548.173	34.22624497	1470.072602	
	5.443295413	937.7502821	
	14273.14579	33483.91573	2.34593805
[M+Na]+ 445.172	7505.1339	15325.31055	
	4477.086871	13383.06821	
	2610.80365	6902.739174	
	694.3880712	1182.124367	
[M+H]+ 244.101	232161.121	499186.0885	2.15017091
[M+ACN]+ 244.101	38308.70834	145667.3419	
	37179.07425	105417.163	
	6098.451998	22972.29535	
	3923.333215	10555.89938	
[2M+H]+ 244.101	112.2384382	1896.704333	
	286.1864438	1880.690709	

	3893.079251	12605.25648	3.2378628
	621.6363947	2279.731898	
	62.02274217	1027.810449	
[M+H]+ 230.088	184626.7995	279221.6519	1.5123571
[M+ACN]+ 230.088	43411.02213	77536.14672	
[M+H-CH3]+ 230.088	22630.67383	44363.04623	
	24753.83629	41861.54418	
	6735.611467	12443.21176	
	2755.692972	5660.699224	
[M-C3H6N]+ 230.088	339.6423767	980.9973961	
[M+H]+ 223.095	163035.4046	318905.1202	1.95604826
	25076.28813	57675.39669	
[M+ACN]+ 223.095	2422.514681	7402.354795	
	2521.885514	5230.867874	
[M-C3H7N2O3]+ 223.095	652.1120904	1526.848592	
[M+H]+ 244.108	41539.03396	190119.2431	4.57688167
[M+ACN]+ 244.108	9102.527179	39388.90736	
[M+H-CH3]+ 244.108	14124.26869	33732.67456	
	5721.742955	28858.96445	
	1096.450379	6298.880204	
	383.4765063	2782.297786	
	176.7644056	2001.287187	
[M+H]+ 244.08	59778.75991	200427.9848	3.35282942
	9096.319033	28288.90244	
[M+ACN]+ 244.08	4849.203991	21692.62277	
	699.1236096	3199.740179	
[M+Na+ACN]+ 244.08	230.6469073	1045.247284	
[M+H]+ 226.16	32390.83067	56622.89799	1.74811503
	7059.076979	11439.26226	
[M+Na]+ 226.16	1279.924137	4981.612981	
[M+Na]+ 283.123	7482.58695	14726.1417	1.96805487
[M+2K]2+ 484.197	1393.361651	2290.52497	
[M+2Na]2+ 484.197	756.6920101	1821.365313	
	631.6201329	1749.406011	
	175.9438447	1973.493261	
[M+H]+ 260.10	20790.58814	153706.3618	7.39307425
	18301.8863	32801.6492	
[M+ACN]+ 260.10	1527.239529	15504.14404	
	6384.564971	7878.714149	
	96.78046349	2268.464573	
[M+H]+ 224.137	176816.9186	234304.6096	1.32512551
[M+ACN]+ 224.137	33251.15317	47775.70615	
	23320.91151	33010.40826	
[M+H-CH3]+ 224.137	7320.716647	14084.01083	
	3894.798965	7390.104951	
	4581.939603	6954.373117	
	2200.570187	3645.525415	
[M+H-CH2]+ 224.137	1449.394416	3156.797217	
	743.77066	1318.805928	
	16306.27451	104907.6073	6.43357299

	1675.702212	11832.07435	
	215.1901782	1567.48386	
	77.7463572	1494.464951	
[M+H]+ 244.074	108502.2651	133896.207	1.23404066
[M+Na]+ 244.074	6794.368997	22029.22622	
	14407.56955	18855.60679	
[M+Na+ACN]+ 244.074	2035.634002	9226.318689	
	535.0651045	2632.098824	
	146.3806251	938.5281094	
[M+H]+ 236.08	5666.360476	68674.50128	12.1196845
	143.8813739	9322.240125	
	448.7287595	7848.110475	
[M+ACN]+ 236.08	2066.649958	6232.656559	
[M+Na+ACN]+ 236.08	88.62928488	3640.929051	
[M+H-H2O]+ 346.146	72529.72303	134031.4212	1.84795165
[M+H]+ 346.146	36870.98285	51546.92268	
[M+Na+ACN]+ 346.146	23239.00407	44426.37211	
[2M+Na]+ 346.146	10054.19005	31250.47302	
	12225.37175	22672.75677	
[M+Na]+ 346.146	10394.07255	18345.70189	
[M+K]+ 346.146	7554.754381	13212.23134	
	2670.380729	10230.92482	
	5851.49438	8734.568673	
	4283.767177	8472.937837	
	4497.051155	8376.88151	
[M+H]+ 387.173	2734.40225	4383.072678	
	1246.536735	2510.714754	
	1125.032599	2498.138286	
	909.2433118	1577.525311	
	504.0203151	969.677241	
	333.9611173	662.8653118	
[M+H]+ 223.10	31869.29641	112426.7645	3.52774542
	6129.13081	19419.09349	
[M+ACN]+ 223.10	1856.446515	9151.218936	
	22248.34503	33444.45315	1.5032333
	2717.727976	4135.941742	
[M-CO2]+ 454.206	2760.846963	4586.649889	1.66131986
[M-2xH2O]+ 454.206	1071.266604	1959.535232	
[M-H20]+ 454.206	961.5262687	1449.809324	
[M+ACN]+ 255.27	46460.92437	84232.9917	1.8129857
	7832.215808	14528.45626	
[M+H]+ 255.27	3442.228957	6469.734062	
	250.4109619	673.855499	
[M+H]+ 514.222	25233.78416	64843.65124	2.5697157
	5678.017965	15924.53301	
[M-CH4]+ 470.234	2410.606859	7187.401679	
[M-CO2]+ 514.222	1277.036429	3666.003794	
	438.8613518	2073.046038	
	275.3852543	1251.59415	

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