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Effect of deficit irrigation and methyl jasmonate application on the composition of strawberry (*Fragaria* x *ananassa*) fruit and leaves

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Abstract

Drought stress is among the most severe environmental risks threatening strawberry production. In the present study, the effect of deficit irrigation (DI; 50 mL/day) and/or elicitation with methyl jasmonate (MeJA; 0.1 mM) on the composition of secondary fruit and leaves from three strawberry pre-commercial cultivars (253/29, 279/4 and 279/5) was investigated and compared to plants kept at or near field capacity (200 mL/day). For certain cultivars (253/29), DI applied at green stage of fruit development resulted in a considerable reduction in berry size (1.7-fold). In other cultivars (279/4 and 279/5), fruit size was comparable in DI-treated and fully irrigated plants. Changes in the major sugars and organic acids of strawberry leaves and fruit were cultivar and organ dependent and were associated to an osmotic adjustment strategy within the plant to counteract the effects of drought. Overall, elicitation with MeJA had a minimal effect on plant growth and morphological traits. Nevertheless, MeJA increased fructose content of DI-treated leaves and palliated the differences in glucose content of fruit from different water treatments. The most pronounced effect of MeJA was related to an enchanced synthesis and accumulation of pelargonidin-3-glucoside (nearly 2-fold) in red-ripe fruit from cultivar 279/5.

Keywords: anthocyanins, berry size, drought, organic acids, sugars.

1. Introduction

Berries have long been recognised to play an important role in human nutrition, providing health-benefits against a wide range of diseases, mainly due to their elevated content in certain bioactives including ascorbate, anthocyanins, phenolic acids, carotenoids, etc. (Giné Bordonaba and Terry, 2011a; Manganaris et al., 2013). Most bioactive compounds within the plants are secondary metabolites whose synthesis can be triggered in response to biotic and abiotic stresses, such as UV radiation, drought, wounding as well as infections (Terry and Joyce, 2004; Terry et al., 2007a; Jahangir et al., 2009). In the particular case of strawberries, several studies have demonstrated the effect that certain preharvest treatments or cultivation practices have on strawberry fruit biochemistry (Terry et al., 2007a; Keutgen and Pawelzik, 2008; Crespo et al., 2010; Giné Bordonaba and Terry, 2010), including the effect on the concentration of certain taste- and health-related compounds. For instance, earlier works demonstrated that besides the positive environmental effects (i.e. water savings), deficit irrigation (DI) in strawberry plants resulted in berries with higher concentrations of anthocyanins and antioxidant capacity (Terry et al., 2007a) as well as other markers of strawberry fruit quality. Later studies revealed that such effects were cultivar dependent (Giné Bordonaba and Terry, 2010). Nonetheless, DI applied to strawberry plants has been linked with a significant reduction in fruit size and yield (Blatt, 1984; Serrano et al., 1992; Krüger et al., 1999; Liu et al., 2007; Terry et al., 2007a) which also seems to be cultivar dependent (Giné Bordonaba and Terry, 2010).

Jasmonic acid (JA) and its methyl ester methyl jasmonate (MeJA) are naturally occurring plant hormones which have been shown to regulate a wide range of physiological and biological processes (Cheong and Choi, 2003; Rohwer and Erwin, 2008), including responses to drought stress. Given the capacity of MeJA to act as an elicitor and considering that this

compound is already classified as Generally Recognise As Safe (GRAS) substance by the U.S. Food and Drug Administration (Wang et al., 2009) it may have a potential for enhancing the synthesis of bioactive compounds (Perez-Balibrea et al., 2011) and increase fruit quality whilst palliating the negative effects of DI. In this context, Wang (1999) reported that preharvest application of MeJA (0.01-0.1 mM) resulted in changes in plant metabolism that rendered strawberry leaves to better withstand in vitro water stress. Preharvest application of MeJA seems to alter stomatal opening in strawberry (Wang, 1999) and other crops (Horton, 1991) resulting in better transpiration control and hence potentially improving water stress tolerance. In soybean, MeJA has been shown to ameliorate the damaging effects of drought stress by modifying endogenous phytohormones and polyamines (Hassanein et al., 2009). Moreover, both preharvest and postharvest application of MeJA has been associated with greater antioxidant capacity in Chinese red bayberry (Wang et al., 2009) and strawberry (Ayala-Zavala et al., 2005) as well as enhanced anthocyanin synthesis in apples (Rudell and Mattheis, 2008) or other berries (Wang, 2003; Wang et al., 2008).

This study was conducted to further understand the response mechanisms of strawberry plants to water stress conditions and to elucidate whether or not elicitation with MeJA may be a suitable alternative to minimise the negative effects of DI on berry weight of three different pre-commercial strawberry cultivars whilst maintaining/enhancing the taste- and health-related composition of the fruit. Special attention was given to quantifying sugar and organic acids in both fruit and leaves as major respiratory substrates, and anthocyanins and ascorbic acid concentrations in fruit.

2. Materials and methods

2.1 Plant materials and experimental design

Three different maiden year cold-stored strawberry pre-commercial cultivars (*viz.* 253/29, 279/4 and 279/5) were supplied by Redeva (Surrey, UK) and grown in a glasshouse (April to July) in 1 L capacity pots containing commercial standard compost. Cultivars were selected by Redeva breeders to assess their potential adaptation to dry climates and drought. A completely randomised design was adopted considering cultivar, water treatments (50 or 200 ml day^{-1}) and MeJA treatments (none or 0.1 mM) as the principal sources of variation. Prior to commencing water treatments plants were kept at or near field capacity (*ca*. 0.7 m^3 of water per m^3 of soil; conductivity ca. 850 mV) for approximately three weeks following the methodology described in earlier works (Terry et al., 2007a; Giné Bordonaba and Terry, 2010). Water treatments started once the majority of primary fruit from the primary truss were at green I stage of development (prior to the second fruit expansion growth phase; Terry et al., 2004). Then, plants were irrigated daily (*ca*. 09:00 h) with either 50 or 200 ml day^{-1} over an eight-week period. Methyl jasmonate (Sigma, Dorset, UK) treatments at 0.1 mM + 0.05% Tween-20 (Wang, 1999) were applied as a foliar spray to incipient runoff every 72h. MeJA treatments started when the majority of the primary fruit from the primary truss were at white stage of development. Similarly, control plants were sprayed with a 0.05% Tween-20 solution.

2.2 Soil moisture content and environmental monitoring

Soil moisture content, recorded as the conductivity from the growing media (mV), was measured daily (*ca*. 16.00h) by time-domain-reflectometry (TDR) using a Thetaprobe (ThetaKit type TK3, Delta-T devices, Cambs., UK). Hourly temperatures within the

glasshouse were recorded by means of a Tiny Tag Ultra 2 data logger (Gemini Data Logger, Sussex, UK), shielded from solar radiation. Mean temperature inside the glasshouse through the growing period was 21 ºC.

2.3 Fruit, leaf and runner sampling

From each plant, all fruit from the primary truss were harvested at red stage. Four fully expanded leaves of similar size and age per plant were excised towards the end of the trial (30 days after initiation of water treatments and when all experimental fruit had been harvested) and the length and surface area of the leave recorded. On the last day of the experiment, following leaf sampling, the length as well as the total runner density (g) was determined for each plant. After harvest or excision, objective colour of fruit and leaves was measured using a Minolta CR-400 colorimeter and a DP-400 data processor (Minolta Co. Ltd., Japan) with an 8 mm light-path aperture, respectively (Terry et al., 2007b). Berry and leave weight was measured and recorded and thereafter immediately snap-frozen in liquid nitrogen and stored briefly at -40°C before being freeze-dried in an Edwards Modulyo freeze drier (W. Sussex, UK) for 6 and 4 days at 0.015kPa, respectively. Lyophilized samples were subsequently ground in a pestle and mortar, weighed and returned to the freezer until use. All reagents were purchased from Sigma (Dorset, UK) unless otherwise stated.

2.4 Extraction and quantification of sugars and organic acids

Sugars from both freeze-dried berries and leaves were extracted using 62.5% (v/v) aqueous methanol as described elsewhere (Terry et al. 2007a). Sugar content was determined using an Agilent 1200 series HPLC binary pump system (Agilent, Berks., UK), equipped with an Agilent refractive index detector (RID) G1362A. Strawberry extracts (20 µL) were diluted

138 (1:10), and injected into a Rezex RCM monosaccharide Ca+ (8%) column of 300 mm x 7.8
139 mm diameter (Phenomenex, CA, USA; Part no. 00H-0130-K0) with a Carbo-Ca^{2+} guard
140 column of 4 mm x 3 mm diameter (Phenomenex,; Part no. AJ0-4493). Column and oven
141 temperature as well as the mobile phase conditions were those reported earlier (Giné
142 Bordonaba and Terry, 2010). Extracts for organic acids determination were prepared as
143 described elsewhere from both berry or leave freeze-dried samples (Giné Bordonaba and
144 Terry, 2010). L-ascorbic, citric, and malic acid contents in extracts were detected at 210 nm
145 using the same HPLC system as described above equipped with an Agilent DAD
146 G1315B/G1365B photodiode array with multiple wavelength detector. The mobile phase (1.0
147 mL min^{-1}) was analytical grade degassed 0.2% (w/v) metaphosphoric acid in H_2O (Giné
148 Bordonaba and Terry, 2009). The presence and abundance of individual sugars or organic
149 acids were automatically calculated by comparing sample peak area to standards (0.025-2.5
150 mg mL^{-1}) using ChemStation Rev. B.02.01.

151 **2.5 Antioxidant capacity of strawberry leaves**

152 Antioxidant capacity from strawberry leaves was measured using the FRAP assay as
153 described in earlier works (Terry et al., 2007a; Giné Bordonaba and Terry, 2012) with some
154 modifications. A 50 µL aliquot of diluted sample extract (1:9; v/v) or Fe^{2+} ($FeSO_4$. $7H_20$)
155 standards (0 – 5.0 mM) was added to 3.6 mL of freshly prepared FRAP working solution (*viz*.
156 5 mL of 10 mM TPTZ (2,4,6-tripyridyl-2-triazine) in 40 mM HCl + 5 mL of 10 mM $FeCl_3$
157 in 50 mL of 300 mM acetate buffer). The reaction mixture was incubated at 37°C for 10 min
158 and absorbance measured spectrophotometrically at 593 nm using a Camspec M501 UV/Vis
159 spectrophotometer. Antioxidant capacity was expressed as the concentration of antioxidants
160 having a ferric reducing ability (mmols Fe^{2+} g^{-1} DW).

2.6 Analysis of individual anthocyanins

Individual anthocyanins were extracted using the methodology described in earlier works (Giné Bordonaba and Terry, 2011b) by mixing 150 mg of freeze-dried fruit sample with 3 ml of 70% (v/v) methanol and 0.5% (v/v) HCl in HPLC-grade water. The slurry obtained was held at 35 ºC in a water bath with constant shaking for 1.5 h; mixing the samples every 15 min. Finally, the flocculate obtained was filtered through a 0.2 μm Millex-GV syringe driven filter unit (Millipore Corporation, MA) and the clear extract analyzed by HPLC coupled to a Diode Array Detector (DAD), using the same equipment as described for sugars and organic acids. The anthocyanin profile of strawberry fruit was determined according to the method described by Gine Bordonaba et al. (2011).

2.7 Data analysis

All statistical analysis were carried out using Genstat for Windows, Version 10 (VSN International Ltd., Herts., UK). Data were subjected to analysis of variance (ANOVA) tests based on a completely randomised design. Least significant difference values (LSD; $P = 0.05$) were calculated for mean separation using critical values of t for two-tailed tests.

3. Results and Discussion

3.1 Morphological and physiological changes in response to drought or elicitation

In agreement with earlier works (Terry et al., 2007a; Giné Bordonaba and Terry, 2009; Grant et al., 2010) soil water content differed between treatments but also between cultivars according to the water stress conditions (**Figure 1**). In all cultivars, deficit irrigation resulted, in average, in 2-fold lower water content in the growing media if compared to fully irrigated plants. Soil water content for DI-treated plants declined following similar water-soil dynamics to that previously reported (Liu et al., 2007; Terry et al., 2007a; Savić et al., 2008; Giné Bordonaba and Terry, 2010; Grant et al., 2010). This said, greater differences between normal (200 mL day^{-1}) or deficit (50 mL day^{-1}) irrigated plants where encountered for cultivar 253/29 (less drought-tolerant cultivar; **Figure 1**). Accompanying the observed changes in plant water uptake, changes in berry weight of secondary strawberry fruit, from the primary truss, in response to different irrigation conditions were cultivar depended and therefore in agreement with earlier findings from Giné Bordonaba and Terry (2010) and Grant et al. (2010, 2012). Whereas no significant changes were observed in fruit weight of cvs. 279/4 and 279/5 subjected or not to DI conditions, cv. 253/29 held under drought conditions produced nearly 1.7-fold smaller fruit (**Figure 2**). This result together with the observed soil water conductivity values suggest that both cvs. 279/4 and 279/5 were more drought-tolerant than cv. 253/29.In comparison with other studies (Liu et al., 2007; Terry et al., 2007a; Savić et al., 2008; Giné Bordonaba and Terry, 2010) the lesser reduction of berry weight in fruit from cvs. 279/4 and 279/5 grown under DI conditions may be related to not only the capacity of these cultivars to better withstand water stress conditions but also may be associated to initiating water stress at later fruit developmental stages (i.e. green stage instead of flower initiation as reported earlier (Terry et al., 2007a; Giné Bordonaba and Terry, 2010)).

In contrast to the clear effect of water treatments on soil water content, plants from different cultivars responded differently to foliar treatment with MeJA. Whereas preharvest application of MeJA did not have an effect on the amount of water extracted from the growing media in cv. 253/29, 1.5-fold lower soil water content was observed in MeJA-treated plants from cv. 279/4, regardless of the irrigation treatment (**Figure 1**). In cultivar 279/5, MeJA increased (1.6-fold) and reduced (1.2-fold) soil water content for DI-treated and non-water stressed plants, respectively. Differences in the amount of water extracted by the plant from the growing media may be related to the direct effect of MeJA on root growth (Staswick et al., 1992), which seems to be cultivar dependent, as well as a direct effect on root hydraulic conductivity (Savić et al., 2008). For instance, in *Arabidopsis thaliana*, primary root growth was inhibited by 50% when seedlings were grown on medium containing MeJA (Staswick et al., 1992). Accordingly, visual inspection of root development at the end of the trial for each plant indicated a positive correlation between the capacity of the plant to extract water and root growth (data not shown) but supported only partially the associations between MeJA and root development found by others (Staswick et al., 1992; Maksymiec and Krupa, 2007). Differences between this and early studies may also be the result of the different species being investigated as well as different methodologies used in the application of MeJA. In addition, in the present study, MeJA was applied at an advanced fruit phenological stage when most of the root development has already occurred. Elicitation with MeJA had a minimal effect on berry weight and was cultivar dependent. In fruit from cv. 279/4 treatment with MeJA resulted in a slight reduction of fruit weight regardless of the water treatment whereas no effect was noticed in fruit from the rest of cultivars (**Figure 2**).

Fruit dry matter as a proportion of fresh weight was not significantly affected by the conditions imposed in this study (**Figure 2**). In contrast, earlier works have shown that DI

applied at earlier fruit developmental stages resulted in fruit with greater dry matter content (Serrano et al., 1992; Krüger et al., 1999; Liu et al., 2007; Terry et al., 2007a; Giné Bordonaba and Terry, 2010). Dry matter content from leaves was neither affected by water treatments and hence it may be plausible to speculate that the concentration effect reported in earlier works (Terry et al., 2007a; Giné Bordonaba and Terry, 2010) is likely to have been related to the limitation of water uptake from the plants rather than to an enhanced import of solutes into the fruit from other parts of the plant (i.e. leaves).

As expected, greater water supplied to the plant resulted in greater runner biomass in all except in plants from the less drought-tolerant cv. 253/29. Accordingly, it is well accepted that greater water supply to strawberry plants generally resulted in higher vegetative growth, including runners fresh mass (Grant et al., 2010 and 2012). No other morphological traits (leaves length, foliar density) except some differences in objective colour of leaves were altered by the different treatments applied (data not shown).

3.2 Leaf biochemical changes

Fructose content of leaves was significantly different between cultivars, water treatments and the interaction between water treatments and MeJA (**Table 1**). Excised leaves from cultivar 279/5 had the greatest fructose content (20.26 mg g^{-1} FW) followed by cv. 279/4 (15.41 mg g^{-1} FW) and cv. 253/29 (13.24 mg g^{-1} FW) and regardless of the water treatments. Under DI conditions, leaves had on average, 1.2-fold greater fructose content than leaves from plants kept at or near field capacity. Similar trends were observed for glucose and hence these results suggest that hexose sugars accumulate in response to drought as also reported for tomato plants submitted to other abiotic stress (Khelil et al., 2007). This specific behaviour may represent a beneficial plant response by avoiding metabolic inhibitions while

concomitantly contributing to an osmotic adjustment (solute potential) within the plant (Balibrea et al., 2000). Elicitation with MeJA in combination with DI resulted in all cases in higher glucose content as compared to non-elicitated plants (**Table 1**). Nor sucrose or the total sugar content was affected by the conditions imposed in this study rather than genotypic differences. AsA concentrations were significantly different between cultivars, irrigation regimes but were not affected by MeJA. Specifically, AsA was greater in leaves from plants grown under reduced irrigation (1.53 mg g^{-1} DW) as compared to plants kept at or near field capacity (1.15 mg g^{-1} DW). Greater AsA content in DI-treated leaves may be a plant defence strategy to scavenge hydroxyl radicals and detoxify the accumulation of H_2O_2 resulting from water stress. The greatest increase (2-fold) in AsA as a result of drought was observed in leaves from cv. 279/5. Contrasting results were found by Wang et al (1999) where leaves of drought-stressed strawberry plants had lower AsA content if compared to leaves from control plants. However, different cultivars and experimental conditions were tested in each study. Malic acid content in leaves was affected by the interaction between water treatments and genotypes. Except for cv. 279/5, where malic acid was lower (1.7-fold) in leaves from DI-treated plants, reduced water resulted in greater (1.15-fold in average) amounts of malic acid in leaves from cultivars 279/4 and 253/29. Increased malic acid content in leaves, as also observed for certain sugars, may result from lowered respiration and hence confirm the osmotic adjustment (solute potential) strategies within different plant tissues or organs to counteract the effects of drought. Citric acid was the only acid affected by the interaction between cultivar, water treatments and elicitation with MeJA (**Table 1**). The differential osmotic adjustments observed among cultivars warrants further research aiming to obtain new strawberry cultivars with increase resistance to drought.

In terms of the leaves antioxidant capacity (AC), cultivar was the main source of variation, with cultivar 253/29 having the greatest AC values (133.5 mg Fe^{2+} g^{-1} DW) followed by cvs. 279/5 (131.8 mg Fe^{2+} g^{-1} DW) and 279/4 (111.2 mg Fe^{2+} g^{-1} DW), respectively. Whereas none of the treatments alone had a significant effect on leaf AC, the interaction between water treatment and MeJA was significant. In plants kept at or near field capacity, elicitation with MeJA resulted in fairly similar AC values, whereas greater values were observed in plants subjected to DI conditions (**Figure 3**). This result is of particular interest since MeJA may partly alleviate drought stress by increasing the capacity of the plant to scavenge free radicals and thus limiting the oxidative stress.

3.3 Fruit biochemical changes

High variability in the sugar concentration was observed between fruit from different cultivars but overall values were in the range of those reported by others (Terry et al., 2007a; Giné Bordonaba and Terry, 2009; Giné Bordonaba and Terry, 2010; Crespo et al., 2010). Fruit from 279/4 and 279/5 had *ca*. 1.2-fold greater glucose and fructose concentrations than that of 253/29. Greater fructose or glucose content in these cultivars may be related to greater activity of some sugar mobilising enzymes (Khelil et al., 2007) or may be just a characteric trait associated to greater drought tolerance. Sucrose concentrations were greater in cultivar 279/5 (254.6 mg g^{-1} DW) followed by 253/29 (240.4 mg g^{-1} DW) and 279/4 (203.9 mg g^{-1} DW). None of the treatments applied had a marked effect on fruit sugar concentration. A significant interaction between MeJA, irrigation treatments and cultivar was only observed for glucose concentrations whereby DI resulted in lower glucose content only for fruit from cv. 279/5. MeJA minimised the differences in glucose content of fruit receiving different amounts of water. In agreement with these results, the response of strawberry plants to DI conditions

298 applied at earlier development stages resulted in different sugar concentrations depending on
299 the cultivar (Giné Bordonaba and Terry, 2010). In other fruit, it has already been
300 demonstrated that DI, unless applied during the major sugar-accumulation period, does not
301 alter final sugar content (Barry et al., 2004). Therefore, the greater extent of DI treatments on
302 strawberry sugars as described in earlier works (Terry et al., 2007a; Giné Bordonaba and
303 Terry, 2010) may either be genotypically regulated or associated to initiating drought
304 conditions at earlier fruit developmental stages (**Table 2**). Future studies should clarify the
305 specific effect of applying DI conditions at different phenological stages on the final fruit
306 composition of a range of cultivars. Wang et al. (2008) found that preharvest application of
307 MeJA resulted in higher soluble solid content and lower titratable acidity of three blackberry
308 cultivars and similar findings were earlier reported by Wang and Zeng (2005) on raspberries.
309 Despite sugar content not always being well correlated with soluble solids measurements in
310 strawberry fruit (Giné Bordonaba and Terry, 2009), the results presented herein demonstrate
311 the differential effect of preharvest application of MeJA combined with full or deficit
312 irrigation on sugar concentrations of strawberry fruit (**Table 2**).

313 Fruit AsA content was not affected by the conditions imposed in this study except for
314 some cultivar differences. In agreement, earlier works (Giné Bordonaba and Terry, 2010)
315 found AsA to be either higher, lower or not affected in plants submitted to drought stress
316 depending on the cultivar. Besides, high variability in the AsA content among different
317 strawberry cultivars has been extensively described in the literature (Tulipani et al., 2008;
318 Giné Bordonaba and Terry, 2009; Giné Bordonaba and Terry, 2010; Crespo et al., 2010). AsA
319 concentration in fruit from cultivar 253/29 (17.1 mg g^{-1} DW) was 2.3- or 1.5-fold higher than
320 that for fruit from cvs. 279/4 (7.3 mg g^{-1} DW) and 279/5 (11.7 mg g^{-1} DW), respectively.
321 Elicitation with MeJA did not induce significant changes in fruit AsA concentrations and

322 hence is in agreement with studies conducted on other horticultural crops (Pérez-Balibrea et
323 al., 2011). In contrast, citric acid was quite similar among cultivars (56 mg g^{-1} DW) but
324 significantly affected by deficit irrigation or MeJA. Plants subjected to drought stress resulted
325 in either higher or lower citric acid content depending on the cultivar which corroborates the
326 genotype-specific response to reduced water supply reported by Giné Bordonaba and Terry
327 (2010). Preharvest foliar application of MeJA resulted in fruit with greater citric acid content
328 (60.2 mg g^{-1} DW) if compared to fruit from non-treated plants (51.1 mg g^{-1} DW) whilst
329 opposite results were observed by Wang and Zheng (2005) in raspberries. The concentration
330 of malic acid was affected by differences in genotypes as well as the interactions between
331 irrigation and cultivar or between the different preharvest treatments applied fruit (**Table 2**),
332 yet no clear conclusions may be drawn.

333 Four different anthocyanins, namely cyanidin-3-glucoside (Cya-3-gluc), pelargonidin-
334 3-glucoside (Pg-3-gluc) and two pg-derivatives, were identified in the present study according
335 to their retention time, UV spectra and comparison with standards (Giné Bordonaba et al.,
336 2011), although their concentration strongly depended on the cultivar and treatment applied.
337 Fruit from cultivar 253/29 were characterised by the presence of these four pigments whereas
338 fruit from cultivars 279/4 and 279/5 lacked the pg-derivative 2 (**Table 3**). Significant
339 differences between cultivars were observed for each individual anthocyanin hence
340 corroborating the strong influence of genetic background in determining anthocyanin content
341 of strawberry fruit (Carbone et al., 2009; Crespo et al., 2010). In detail, fruit from cultivar
342 279/5 had *ca*. 2-fold greater pg-3-gluc concentrations than that of fruit from cultivars 279/4
343 and 253/29. DI had little effect on the anthocyanin concentrations of the different cultivars
344 investigated. In contrast, earlier works (Terry et al., 2007) found anthocyanin concentrations
345 to be greater in fruit from plants (cv. Elsanta) that received less water. In the above-

346 mentioned study deficit irrigation was applied at flower initiation and hence it may be
347 possible to speculate that even though anthocyanin accumulation occurs during later
348 developmental stages (Carbone et al., 2009) the enhanced synthesis of these pigments as a
349 result of drought stress may be up-regulated or triggered at much earlier development stages
350 (i.e. from flower initiation up to green-stage). In addition, results from the present study
351 revealed the differential effect that water deficit irrigation may have on strawberry
352 anthocyanins of different cultivars as also observed for other metabolites herein and
353 elsewhere (Giné Bordonaba and Terry, 2010). Preharvest elicitation with MeJA has been
354 shown to enhance anthocyanin concentrations in berries (Pérez et al., 1997; Wang and Zheng,
355 2005; Wang et al., 2009) and other fruit (Rudell et al., 2008). In a recent study using *Fragaria*
356 *Chiloensis* ripened *in vitro*, MeJA (10 and 100 µM) was shown to induce ripening through its
357 involvement of anthocyanin accumulation, cell wall degradation and the biosynthesis of
358 ethylene (Concha et al., 2013). In the present study, anthocyanin concentration of the fruit
359 was also positively influenced by preharvest treatment with MeJA; this was especially
360 noticeable in fruit from cv. 279/5 where the concentration of cya-3-gluc, Pg-3-gluc and Pg-
361 derivatives was 1.8, 2.7 and 1.8-fold greater, respectively, in MeJA-treated plants if compared
362 to control. The enhanced synthesis of anthocyanins following elicitation with MeJA has been
363 related to the direct effect of this hormone on plant secondary metabolism and especially on
364 the activation of the enzyme phenylalanine ammonia lyase (Gundlach et al., 1992; Concha et
365 al., 2013).

366 **CONCLUSIONS**

367 Plants have developed different mechanisms to survive periods of drought. The results
368 from this study suggest that osmotic adjustment involving changes in sugar and acid

metabolism is one mechanism which in turn depends not only on the cultivar but also on the plant organ (namely leaf and/or fruit). Accordingly, this is the first report describing changes in the major sugar and organic acids in both fruit and leaves of strawberry plants subjected to deficit irrigation. Elicitation with MeJA, under the conditions described in this study, had a minimal effect on plant growth and some morphological traits (fruit weight, runners biomass, dry matter of leaves and fruit, etc.) which in turn was cultivar dependent. Nonetheless, MeJA resulted in increased fructose content of DI-treated leaves and minimised the differences in glucose content between fruit from different water treatments. Under the conditions investigated, MeJA did not alter fruit ripening (sugar and acid accumulation) but had a pronounced effect on the plant secondary metabolism by increasing the synthesis and accumulation of the main strawberry anthocyanin (Pg-3-gluc) in cultivar 279/5. In less drought-tolerant cultivars (namely 253/29), the negative effects of DI on fruit size were not mitigated and anthocyanin accumulation was not enhanced by elicitation with MeJA. Further studies are encouraged to investigate greater doses of this natural hormone or more continued applications aiming to further understand the relationship between drought resistance and MeJA in strawberry plants.

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522 **Table captions:**

523 **Table 1**: Sugar concentration (mg g^{-1} DW) in strawberry secondary fruit and leaves of three
524 different cultivars in response to drought and/or elicitation with 0 (-MJ) or 0.1 mM MeJA
525 (+MJ).

526 **Table 2**: Organic acid concentration (mg g^{-1} DW) in strawberry secondary fruit and leaves of
527 three different cultivars in response to drought and/or elicitation with 0 (-MJ) or 0.1 mM
528 MeJA (+MJ).

529 **Table 3**: Concentration of individual anthocyanins (cyanidin-3-glucoside (Cya-3-gluc),
530 pelargonidin-3-glucoside (Pg-3-gluc) and pelargonidin derivatives (Pg-deriv 1 and 2) in
531 strawberry secondary fruit from three different cultivars in response to drought and/or
532 elicitation with 0 (-MJ) or 0.1 mM MeJA (+MJ).

533

534 **Figure captions:**

535 **Figure 1**: Average water conductivity of the growing media in plants from three strawberry
536 pre-commercial cultivars (A: 253/29; B: 279/4; C: 279/5) grown under deficit irrigation (DI;
537 50 mL day^{-1}) or full irrigation (CT; 200 mL day^{-1}) and treated with 0 or 0.1 mM foliar
538 application of MeJA. (D) Dynamic changes in soil water content (expressed as soil
539 conductivity) of the growing media grown under deficit irrigation (50 mL day^{-1}; empty

symbols) or full irrigation (200 mL day^{-1}; full symbols) and treated with 0 (round) or 0.1 mM (triangle) foliar application of MeJA. Error bar depicts LSD values ($p<0.05$) for the interaction between water treatment*MeJA (A, B and C) or water treatment*MeJA*day (D).

Figure 2: Runners biomass (g $plant^{-1}$) and weight characteristics of fruit and leaves from three strawberry pre-commercial cultivars grown under deficit irrigation (DI; 50 mL day^{-1}) or full irrigation (CI; 200 mL day^{-1}) and treated with 0 (MJ-) or 0.1 mM foliar application of MeJA(+MJ). Error bars depict standard deviation for n=3.

Figure 3: Antioxidant capacity (mg Fe^{2+} g^{-1} DW) of strawberry leaves from three strawberry pre-commercial cultivars grown under deficit irrigation (DI; 50 mL day^{-1}) or full irrigation (CI; 200 mL day^{-1}) and treated with 0 (MJ-) or 0.1 mM foliar application of MeJA (+MJ). Error bars depict standard deviation for n=3.

Table 1: Sugar and organic acid concentration (mg g^{-1} DW) in leaves of strawberry plants from three different pre-commercial cultivars in response to drought and/or elicitation with 0 (-MJ) or 0.1 mM MeJA (+MJ).

		Fructose		Glucose		Sucrose		Ascorbic		Citric		Malic	
Cv	Irrigation	-MJ	+MJ	-MJ	+MJ	-MJ	+MJ	-MJ	+MJ	-MJ	+MJ	-MJ	+MJ
253/29	50	35.5	42.3	41.3	48.7	59.3	64.3	1.7	1.5	11.3	10.5	27.2	25.5
	200	36.9	33	51.9	41.2	65.1	48.5	1.3	1.1	8.4	8.0	24.6	15.8
279/4	50	45.1	55.9	51.8	65.2	82.4	75.9	1.1	1.4	10.9	7.6	28.0	32.9
	200	32.4	36.1	41.4	45.1	66.8	75.6	1.0	1.0	9.0	10.8	26.3	20.1
279/5	50	51.4	69	53.4	70.3	84.7	87.6	2.8	2.0	12.2	16.0	16.6	19.5
	200	52.8	50.3	56.3	56.4	82.3	87.2	1.2	1.3	16.4	12.4	28.9	27.1
LSD_{cv}		7.53		3.61		10.22		0.39		2.51		7.50	
$LSD_{cv*irrigation}$		10.64		10.56		14.46		0.55		3.56		10.61	
$LSD_{cv*irrigation*MeJA}$		15.05		15.00		20.45		0.78		5.04		14.00	

Table 2: Sugar and organic acid concentration (mg g^{-1} DW) in strawberry secondary fruit of three different pre-commercial cultivars in response to drought and/or elicitation with 0 (-MJ) or 0.1 mM MeJA (+MJ).

		Fructose		Glucose		Sucrose		Ascorbic		Citric		Malic	
Cv	Irrigation	-MJ	+MJ	-MJ	+MJ	-MJ	+MJ	-MJ	+MJ	-MJ	+MJ	-MJ	+MJ
253/29	50	189.5	191.2	183.6	190.2	213.5	262.4	15.6	25.5	62.4	82.4	8.3	14.1
	200	178.9	191.3	174.4	185.3	264.3	221.4	16.6	10.5	56.6	44.7	13.1	10.5
279/4	50	209.2	201.3	213.9	194.8	210.7	193.1	5.4	5.0	42.7	50.1	6.1	6.6
	200	204.2	211.4	206.7	210.2	210	201.9	8.5	10.5	54.9	60.9	13.9	8.5
279/5	50	248	226.4	181.5	228.8	249.8	232.7	12.5	15.0	59.4	68.1	16.9	13.0
	200	216.5	209.4	218.9	204.4	284.3	251.6	5.5	13.9	34.8	54.9	13.6	11.6
LSD_{cv}		24.14		15.34		25.61		5.14		9.54		2.59	
$LSD_{cv*irrigation}$		30.13		21.78		36.22		7.26		13.49		3.67	
$LSD_{cv*irrigation*MeJA}$		38.27		30.68		51.22		10.27		19.07		5.19	

Table 3: Concentration (µg g^{-1} DW) of individual anthocyanins (cyaniding-3-glucoside (Cya-3-gluc), pelargonidin-3-glucoside (Pg-3-gluc) and pelargonidin derivatives (Pg-deriv 1 and 2) in strawberry secondary fruit from three different cultivars in response to drought and/or elicitation with 0 or 0.1M MeJA.

		Cya-3-gluc		Pg-3-gluc		Pg-deriv. 1		Pg-deriv. 2	
Cv	Irrigation	-MJ	+MJ	-MJ	+MJ	-MJ	+MJ	-MJ	+MJ
253/29	50	44.2	33.5	1559.2	880.2	148.3	89.8	377.7	227.2
	200	41.4	33.3	776	874	68.1	94.8	166.6	237.2
279/4	50	20.1	30.8	764	587	47.5	42.6	-	-
	200	23.2	27.9	1252	1054	59.7	58.8	-	-
279/5	50	26	48.3	1174	3198	72.4	132.3	-	-
	200	22.9	31.5	890	2782	57.6	123.6	-	-
LSD_{cv}		5.11		356.27		15.49		-	
$LSD_{cv*irrigation}$		7.23		503.82		21.91		75.84	
$LSD_{cv*irrigation*MeJA}$		10.22		712.51		30.99		102.13	

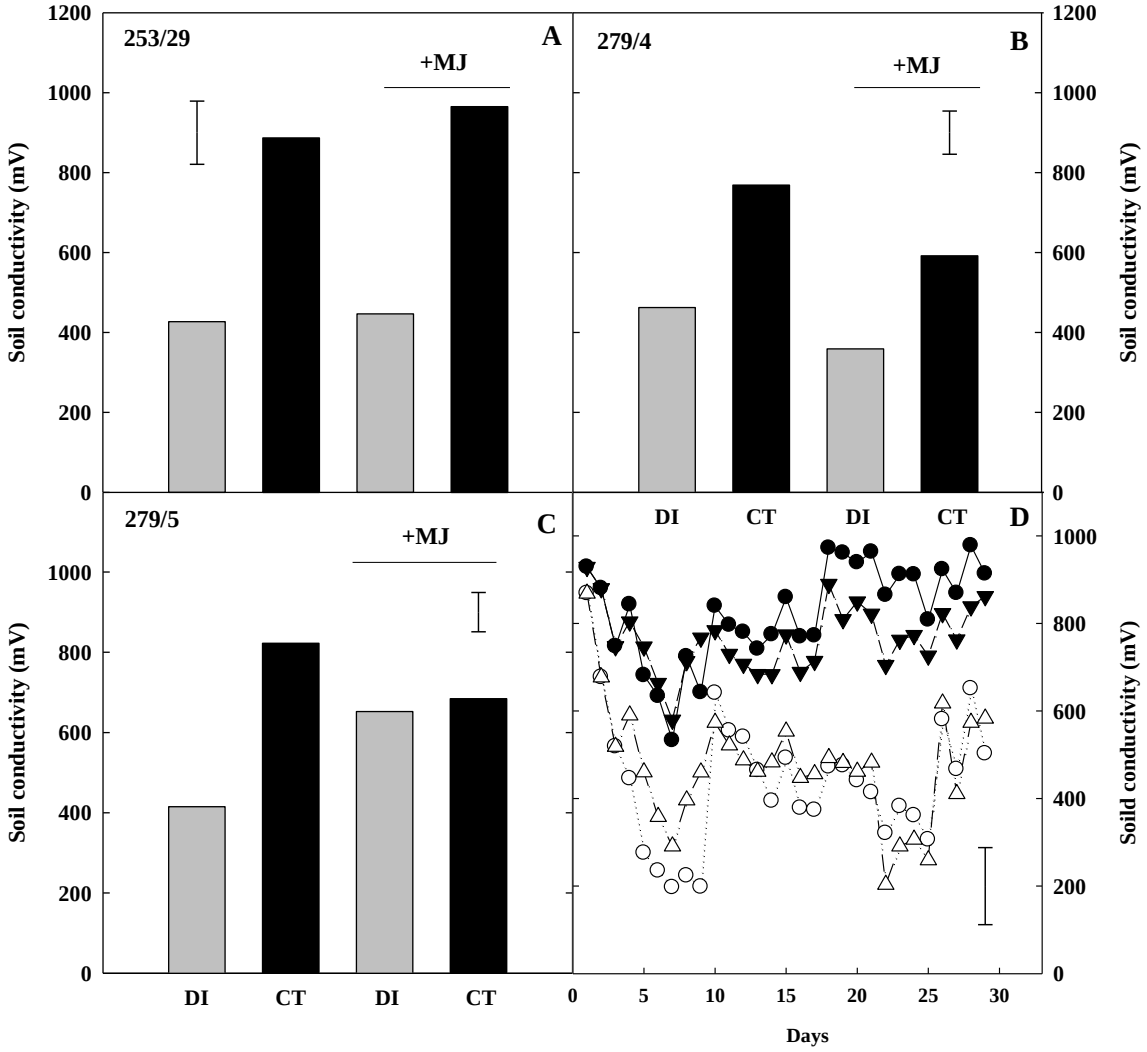

Figure 1: Giné Bordonaba and Terry

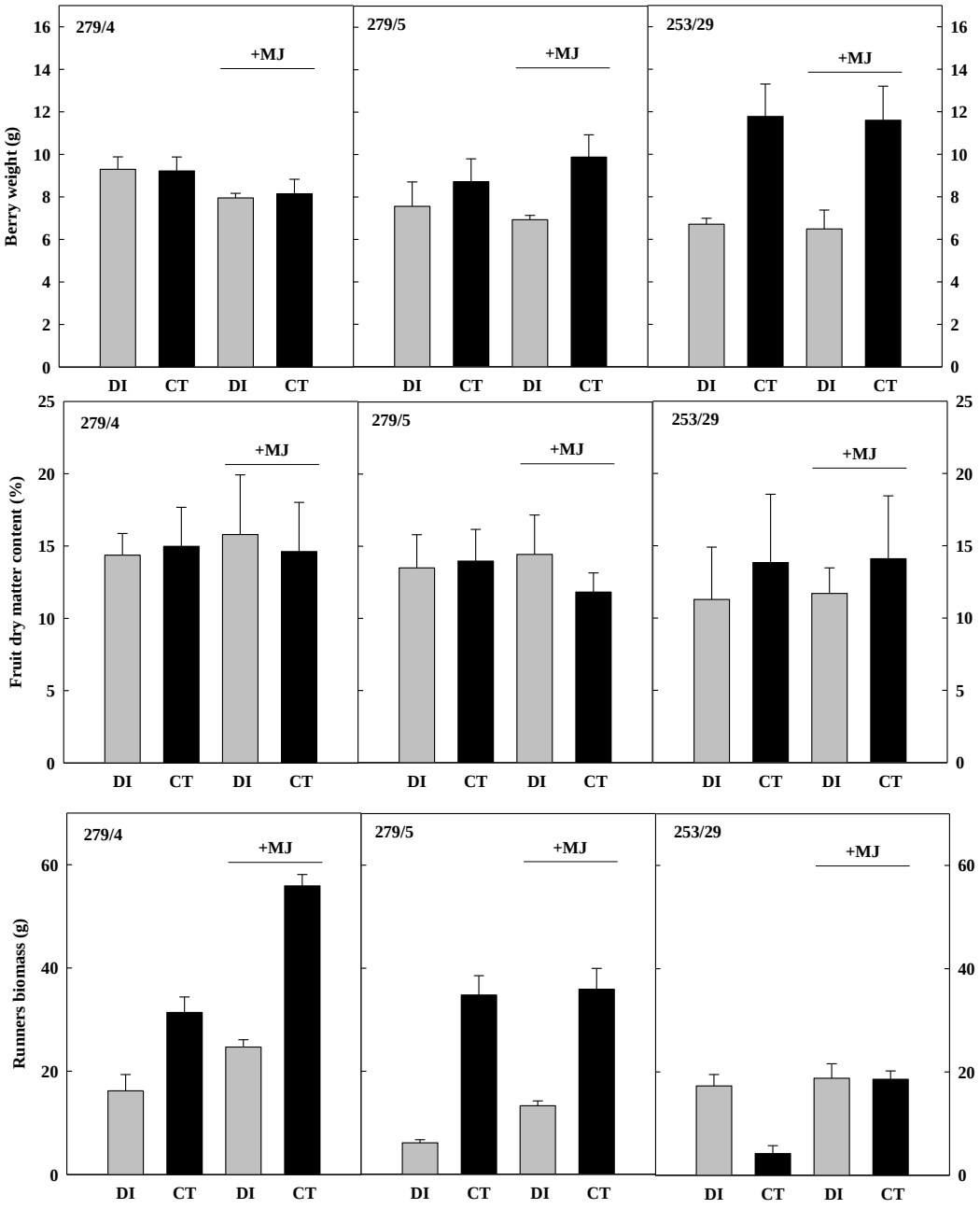

Figure 2: Giné Bordonaba and Terry

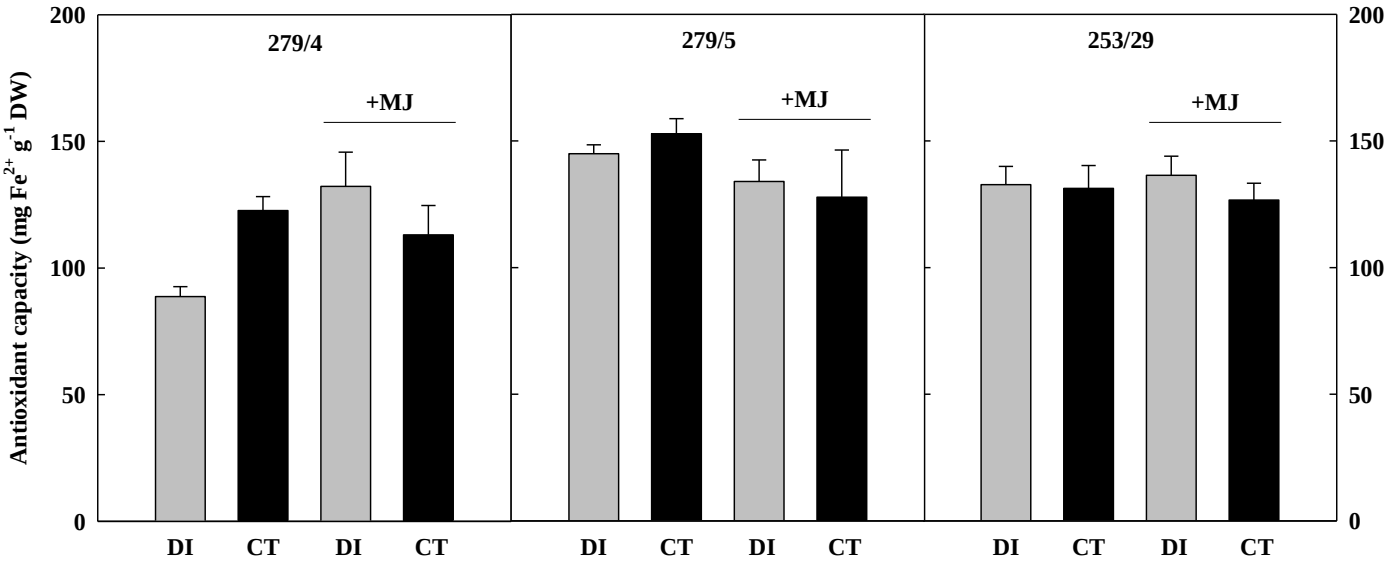

Figure 3: Giné Bordonaba and Terry

RESEARCH HIGHLIGHTS

- The effects of deficit irrigation from green stage to ripe fruit on strawberry fruit size and quality are cultivar dependent.
- Changes in sugar and organic acids in response to drought were differently regulated in fruit than in leaves.
- Elicitation with MeJA had a minimal effect on plant growth.
- MeJA increased the concentration of pelargonidin-3-glucoside in ripe red fruit from one cultivar.

Graphical Abstract