Short Communication

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Correlation between water stress and phenolic compounds of hydroSOStainable almonds

Leontina Lipan,[a] Marina Cano-Lamadrid,[a] Jacinta Collado-González,[b] Aneta Wojdyło,[c] David López-Lluch,[d] Alfonso Moriana[e,f] and Ángel A Carbonell-Barrachina[a*]

Abstract

BACKGROUND: Water scarcity is currently affecting many areas of the world, reaching worrying levels in drought areas such as southern Spain. To cope with this issue, researchers in the agricultural sector have implemented deficit irrigation strategies intended to reduce water consumption by increasing fruit quality. Almond is among the most popular tree nuts worldwide and also the most nut cultivated in Spain. Almond consumption, together with other nuts, has been widely associated with improvements in cardiovascular health, metabolic syndrome and diabetes owing to their bioactive compounds such as polyphenols. Water deficit strategies generate hydroSOStainable almonds, raised under water stress conditions, with high content of bioactive compounds. The aim of this work was to study the relationship between water stress, color and polyphenols in hydroSOStainable almonds. For this, instrumental color, total phenolic content and phenolic compounds were measured and correlated using Pearson's correlation.

RESULTS: The results showed a strong relationship between water stress, color and polyphenols of almonds, showing that increasing water stress in plants up to ∼100 MPa × day values of stress integral increase the polyphenols in almonds, leading to a reddish color.

CONCLUSION: Finally, this research demonstrated that implementing water-saving strategies help to improve the phenolic content and color of hydroSOStainable almonds and also that isorhamnetin-3-*O*-rutinoside, isorhamnetin-3-*O*-glucoside and kaempferol-3-*O*-glucoside could be important markers of hydroSOStainable almonds (cv. Vairo). Besides, hydroSOStainable almonds could be an important source of phenols, providing 25% of the estimated total polyphenolic daily intake.

Keywords: *Prunus dulcis*; deficit irrigation; *a** color coordinate; isorhamnetin-3-*O*-rutinoside; isorhamnetin-3-*O*-glucoside; kaempferol-3-*O*-glucoside

INTRODUCTION

A growing water scarcity is occurring in many parts of the world as a result of climate change and its effect on temperature and rainfall, leading to a substantial increase in irrigation water demand.[1] In addition, population growth leads to an expansion in intensive food production, further contributing to water scarcity.[2] Plants subjected to environmental stress suffer alteration in their growth, metabolism and production, water stress being one of the most adverse factors affecting cultivar growth and yield.[3] However, appropriate water management practices such as deficit irrigation (DI) can help to ensure the sustainability of water resources, reduce production costs, increase bioactive compounds and minimize the leaching of nutrients and pesticides into groundwater.[4,5]

Almond (*Prunus dulcis* (Mill.) D.A. Webb) is the most cultivated tree nut in Spain, and climate change in this crop can provoke phenological variations in fruit, which is likely to affect final yield, quality and marketability.[6,7] However, almond is a well-known drought-tolerant species, for which DI conditions can achieve satisfactory tree development, minimize yield losses and adjust irrigation inputs to water resource availability.[5,7] Consequently,

* Correspondence to: ÁA Carbonell-Barrachina, Department of Agro-Food Technology, Research Group 'Food Quality and Safety, CSA', Universidad Miguel Hernández de Elche (UMH), Escuela Politécnica Superior de Orihuela (EPSO), Carretera de Beniel, km 3.2, 03312 Orihuela, Alicante, Spain. E-mail: angel.carbonell@umh.es

a Department of Agro-Food Technology, Research Group 'Food Quality and Safety, CSA', Universidad Miguel Hernández de Elche (UMH), Escuela Politécnica Superior de Orihuela (EPSO), Orihuela, Spain

b Research Group on Quality, Safety and Bioactivity of Plant Foods, Department of Food Science and Technology, CEBAS (CSIC), University Campus of Espinardo, Murcia, Spain

c Department of Fruit, Vegetable and Plant Nutraceutical Technology, Wrocław University of Environmental and Life Sciences, Wrocław, Poland

d Department of Agroenvironmental Economics, EPSO, UMH, Orihuela, Spain

e Departamento de Ciencias Agroforestales, ETSIA, Universidad de Sevilla, Sevilla, Spain

f Unidad Asociada al CSIC de Uso sostenible del suelo y el agua en la agricultura (US-IRNAS), Seville, Spain

using DI strategies hydroSOStainable almonds are generated with a greater amount of bioactive compounds and higher sensory properties in the fruits.[8]

Almonds are considered a healthy product owing to their functional composition (polyphenols, mono- and polyunsaturated fatty acids, tocopherols, etc.) and are a tasty snack because of their characteristic flavor.[9] Thus they are widely appreciated and consumed in the Mediterranean diet as a snack or as an ingredient in confectionery (*turrón*) and bakery products.[10] The average intake of almond consumption in 2018 in Spain was estimated at around 0.68 g per person per day, with an average cost of 3.07 euros per person per year, according to Ministery of Agriculture, Fishery and Food in Spain.

HydroSOStainable almonds, as previously stated, grow under water stress conditions, suffering changes in their metabolism. Higher values of color coordinates, together with higher values of total phenolic content, were previously reported in hydroSOStainable almonds as a consequence of water stress.[11–13] Polyphenols significantly contribute to the color of the almond seed coat due to flavonoids, which play a key role in yellow pigmentation, and to those compounds in the high-molecular-weight fraction that contribute to brown pigmentation.[14]

Consequently, the aim of this study was to check the above-mentioned tendency exploring the possible relationship between water stress, color coordinates and phenolic compounds in hydroSOStainable almonds. In addition, the total polyphenolic daily intake from almonds was also estimated.

MATERIALS AND METHODS

Irrigation treatments

The experiment was performed during the 2017 season in a 7-year-old commercial orchard ('La Florida', 37.23° N, −5.91° W, Dos Hermanas, Seville, Spain) under the same conditions as previously reported by Lipan *et al.*[12] The threshold values of midday stem water potential (SWP) were measured with a pressure chamber (PMS Instrument Co., Albany, OR, USA) and were used to calculate the plant stress integral from the following equation:

$$\mathrm{SI} = \left| \sum [\psi_{stem} - (-0.2)] \times n \right| \quad (1)$$

where SI is the stress integral, ψ_{stem} is the average minimum stem water potential for any interval and n is the number of days in the interval.[15]

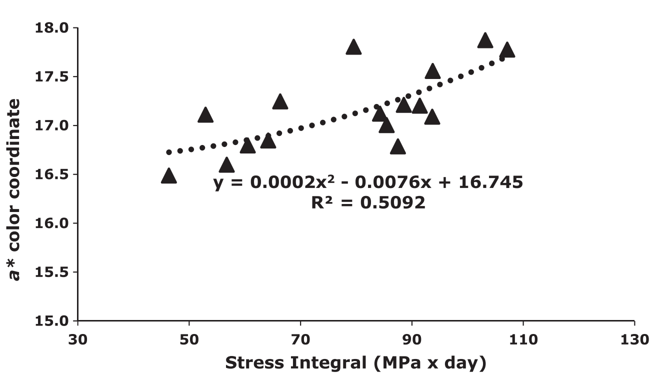

Figure 1. Quadratic correlation between *a** color coordinate and stress integral (SI). Four repetitions per treatment were used for the correlations.

The irrigation treatments applied to the experimental crops consisted of: (i) full irrigation (T1), irrigated to assure crop requirements (433 mm water was applied during the whole growing cycle); (ii) moderate regulated deficit irrigation (RDI) (T2), in which the water stress was imposed during the kernel filling period; thus almonds were irrigated when SWP < -1.5 MPa, and for the rest of the time trees were irrigated to keep SWP as the baseline proposed by McCutchan and Shackel (148 mm);[16] (iii) severe RDI (T3) (103 mm); the same as T2 except that trees were irrigated when SWP < -2.0 MPa during kernel filling; and (iv) SDI (T4); the same as T3, but tree water status was not considered. Irrigation was applied at a constant daily rate of around 1–2 mm per day, creating a gradual stress during the growing cycle, instead of creating a stress only in the kernel filling phase as in T3 (114 mm). The main difference between both strategies (T3 and T4) was that T4 limited postharvest irrigation more than T3.

Almonds were harvested, dried in sunlight (below 5% moisture content) and delivered to Miguel Hernández University for analysis.

Instrumental color

A colorimeter (model CR-300, Minolta, Osaka, Japan) was used for instrumental color determination at 25 ± 1 °C. Exterior color was directly measured on the skin of 100 individual almond kernels per treatment. CIE $L^*a^*b^*$ color coordinates describe the color in a three-dimensional space as follows: L^* for lightness ($L^* = 0$, black; $L^* = 100$, white); a^* for green–red (a^* = red; $-a^*$ = green); b^* for blue–yellow components (b^* = yellow; $-b^*$ = blue).

Total phenolic content (TPC) and phenolic compound identification and quantification

TPC was determined as previously described.[12] The results were calculated from the gallic acid calibration curve and expressed as gallic acid equivalents (g GAE kg^{-1}). The measurements were completed in a UV–visible spectrophotometer (Helios Gamma model UVG 1002E, Helios, Cambridge, UK).

Polyphenol identification and quantification were analyzed as previously described by Lipan *et al.*[17] Phenolic compound separation was performed using an ultra-performance liquid chromatograph coupled with a photodiode detector (Waters, Milford, MA, USA) quadrupole and tandem time-of-flight mass spectrometer (Waters, Manchester, UK), equipped with an electrospray ionization source. All analyses were performed in triplicate.

Statistical analysis

Pearson's correlation test was run using XLSTAT Premium 2016 and statistically significant correlations were considered when $P < 0.05$. Moreover, a nonlinear (quadratic) correlation was carried out to check at which point the compounds started to decrease. For preparation of figures, Sigma Plot 11 was used.

RESULTS

Relationship between stress integral with color coordinates, phenolic compounds and total phenolic content

The correlation between SI with a^* color coordinate was studied. Figure 1 shows that this correlation produced a quadratic relationship, in which it can be highlighted that higher stress in the plant (higher values of SI) generates higher values of a^* color coordinates, which means redder color notes in hydroSOStainable almonds.

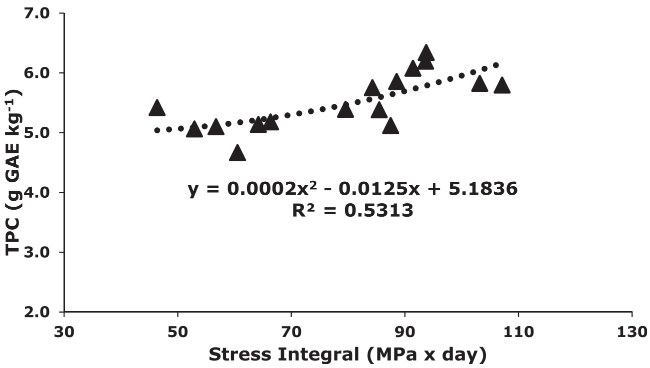

Figure 2. Quadratic correlation between total phenolic content (TPC) and stress integral (SI). Four repetitions per treatment were used for the correlation.

TPC was also correlated with SI, and a quadratic relationship was also found between them. As seen in Fig. 2, TPC started to increase with increasing stress integral.

Finally, Pearson's correlation coefficient (*R*) was also determined between SI and the phenolic compounds, and the results are presented in Table 1. A significantly positive correlation was observed between SI and isorhamnetin-3-*O*-rutinoside ($R = 0.64$; $P < 0.01$), isorhamnetin-3-*O*-glucoside ($R = 0.53$; $P < 0.05$) and kaempferol-3-*O*-glucoside ($R = 0.50$; $P < 0.05$). Thus an increase in water stress in plants helps to increase these three phenolic compounds, which could be important markers of hydroSOStainable almond cv. Vairo. It is well known that each almond cultivar possesses its own phenolic profile; thus the present findings open a further research possibility to check the behavior of phenolic compounds in other cultivars subjected to water deficit.

In order to check the relationship between color coordinates, polyphenols and water stress in almonds, a Pearson's correlation was carried out (Fig. 3). As stated before, a significant and positive correlation between SI and isorhamnetin-3-*O*-rutinoside was shown and, in addition, this phenolic compound was also correlated with a^* color coordinate ($R = 0.53$; $P < 0.05$). Moreover, a significant and positive correlation was also shown between the TPC in almonds with the SI ($R = 0.72$; $P < 0.01$) and between TPC with a^* color coordinate ($R = 0.51$; $P < 0.05$), which might explain the relationship between water stress, total phenolic content and color. Finally, L^* color coordinate was significantly positively correlated with two phenolic compounds (procyanidin B-type trimer and naringenin-7-*O*-glucoside), showing that almonds containing these compounds in higher amounts tend to have a lighter color.

DISCUSSION

The nonlinear (quadratic) equation between a^* color coordinate with SI and between TPC with SI was determined to check the evolution of these parameters with water stress. For instance, in previous studies on olive *cv*. Arbequina, a quadratic relationship was reported between water stress (stem water potential) and TPC, by increasing the TPC up to −4 MPa, at which it began to decrease.[18] In the present study the nonlinear equation showed that higher water stress levels in almond trees led to reddish hydroSOStainable almonds with a greater amount of phenolic content. Moreover, at these stress levels no decrease in a^* color coordinate and TPC was observed,

Table 1. Pearson's correlation coefficients (*R*) between stress integral and phenolic compounds

	SI	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11	PC12	PC13	PC14	ΣPC
SI	**1.00**															
PC1	0.43	**1.00**														
PC2	−0.40	−0.32	**1.00**													
PC3	−0.28	−0.56*	−0.12	**1.00**												
PC4	−0.31	−0.34	−0.05	0.72**	**1.00**											
PC5	−0.05	−0.27	−0.09	0.25	0.46	**1.00**										
PC6	−0.16	−0.48	−0.11	0.86***	0.71**	0.34	**1.00**									
PC7	0.40	0.31	−0.31	−0.49	−0.31	0.11	−0.26	**1.00**								
PC8	−0.04	−0.61*	−0.13	0.80***	0.71**	0.46	0.89***	−0.06	**1.00**							
PC9	−0.37	−0.64**	−0.04	0.86***	0.75***	0.41	0.85***	−0.31	0.87***	**1.00**						
PC10	0.64**	0.20	−0.34	−0.30	−0.33	0.05	−0.13	0.81***	0.10	−0.21	**1.00**					
PC11	0.11	0.06	−0.12	0.56*	0.67**	0.27	0.54*	−0.46	0.48	0.49	−0.24	**1.00**				
PC12	0.23	−0.07	−0.15	0.55*	0.67**	0.35	0.54*	−0.38	0.57*	0.52*	−0.11	0.96***	**1.00**			
PC13	0.53*	0.11	−0.12	0.01	0.09	0.05	−0.12	−0.36	−0.01	−0.04	−0.11	0.51*	0.61***	**1.00**		
PC14	0.50*	0.18	−0.30	0.35	0.44	0.30	0.37	−0.09	0.48	0.32	0.21	0.86***	0.89***	0.62*	**1.00**	
ΣPC	0.33	−0.07	−0.39	0.45	0.59*	0.54*	0.60*	0.34	0.76***	0.56*	0.44	0.58*	0.67**	0.14	0.74***	**1.00**

*, **, ***, significant at $P < 0.05$, 0.01, and 0.001, respectively.
Abbreviations: PC1, *p*-hydroxybenzoic acid; PC2, vanillic acid; PC3, procyanidin B-type dimer; PC4, (−)-epicatechin; PC5, procyanidin B-type dimer; PC6, procyanidin B-type trimer; PC7, procyanidin B-type tetramer; PC8, naringenin-7-*O*-glucoside; PC9, kaempferol-3-*O*-rutinoside (isomer 1); PC10, isorhamnetin-3-*O*-rutinoside; PC11, kaempferol-3-*O*-rutinoside (isomer 2); PC12, kaempferol-3-*O*-galactoside; PC13, isorhamnetin-3-*O*-glucoside; PC14, kaempferol-3-*O*-glucoside; SI, stress integral.

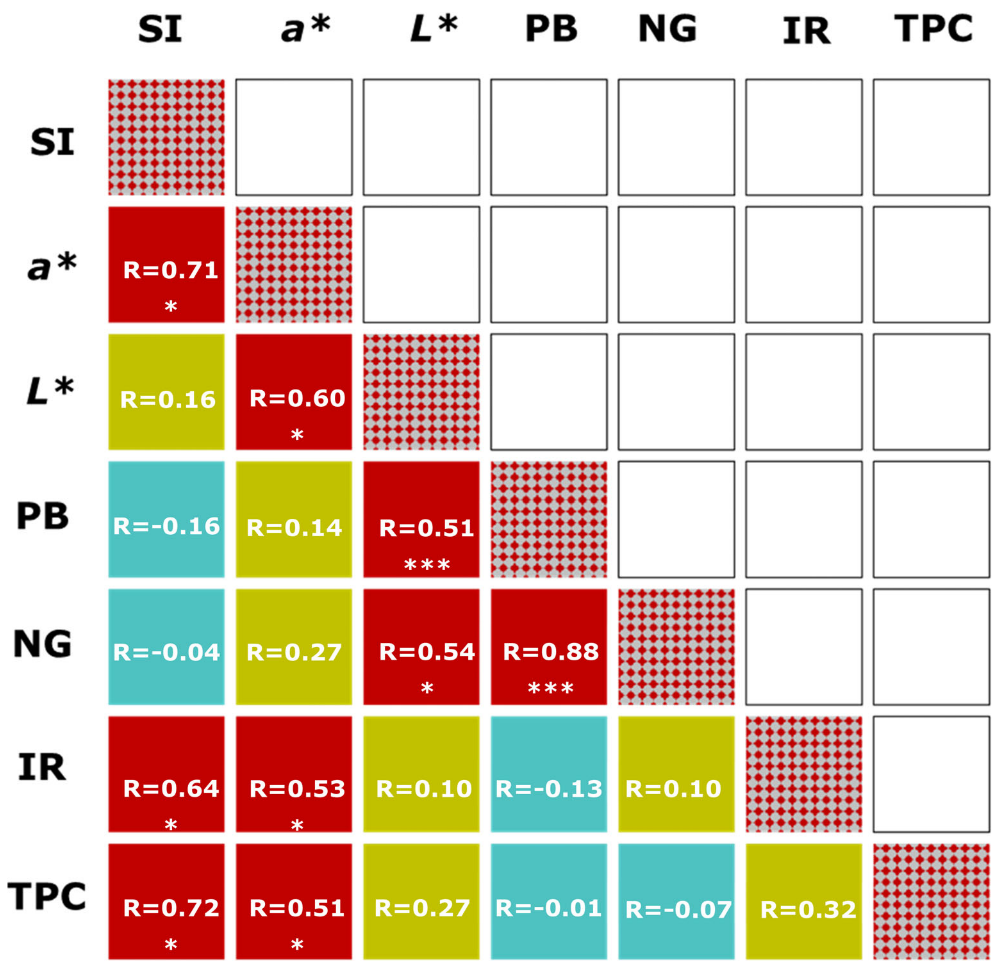

Figure 3. Heat map of correlation matrix of color parameters, phenolic compounds and stress integral. Each square indicates Pearson's correlation coefficient for a pair of data and the color represents the positive and negative correlation as follows: $R = 1$; significant ($P < 0.05$) positive correlation; positive but not correlated ($P > 0.05$); negative but not correlated values. Note: SI = stress integral; *a** and *L** = color coordinates; PB = procyanidin B-type trimer; NG = naringenin-7-*O*-glucoside; IR = isorhamnetin-3-*O*-rutinoside; TPC = total phenolic compounds.

which means that up to 107 MPa × day SI (stem water potential = −2.2 MPa) values the plant was still able to produce phenolic compounds. Horner[19] reported a nonlinearity of phenolic compound concentration with water deficit. The demonstration that water stress in the tree produces an increase in phenolic compound precursors (free phenylalanine) and their synthesis could be more sensitive in moderate water stress conditions.[19] Previous study also reported higher *a** values in almond *cv.* Vairo, Marta, Guara, Lauranne and also in tomato *cv.* Sunstart growth under deficit irrigation conditions.[4,11,13] In addition, the TPC was also reported to increase with water stress in almond *cv.* Vairo but also in growth of other crops under water deficit conditions such as pistachio *cv.* Kerman, olive *cv.* Arbequina and cherry tomato cv. Kosaco, Josefina and Katalina.[3,8,12,18] The literature has reported that almond color skin is given by the polyphenol profile being unique for each cultivar,[14] which was also demonstrated for the hydroSOStainable almonds within this study. Figure 3 presents the clear correlation between SI and isorhamnetin-3-*O*-rutinoside ($R = 0.64$; $P < 0.01$), and between this phenolic compound and *a** color coordinate, demonstrating that higher water stress in the plant increases isorhamnetin-3-*O*-rutinoside and increases the values of reddish notes (*a**) in almond. Isorhamnetin is a phenolic compound that is soluble in hot water (thus removing the skin through blanching must decrease its content) less reddish-yellow than quercetin, although it is considered the principal yellow coloring matter present in the dried fruits and flowering stem of *Tithonia diversifolia* (Hemsl.), in red clover (*Trifolium pratense* L.), Indian reedmace and (*Typha elephantina*).[20] Flavonoids have been reported to be determining pigments in plants possessing color,[21] and a positive correlation between *a** values with nine individual flavonols, total kaempferols and total flavonols was previously reported in a study on the relationship between the color of rose petals (*Rosa* spp.) and polyphenol content.[22] Moreover, a positive correlation between *a** color coordinate and total anthocyanins ($R = 0.80$) was also reported for the pink-flowered group of herbaceous peony cultivars (*P. lactiflora* Pallas).[23]

The significant and positive correlation between TPC and SI, and between TPC and *a** color coordinate, together with the relationship between *L** color coordinate with two phenolic compounds, clearly demonstrated that water stress increased polyphenols in hydroSOStainable almonds, leading to anomalies in color coordinates. Higher values of *L** color coordinate were also reported in almond cv. Vairo under sustained deficit irrigation, which were the samples with the higher values of these two phenolic compounds.[11,17] This supports the present positive correlation found between *L** values and procyanidin B-type trimer and naringenin-7-*O*-glucoside, showing that water stress produces almonds with a lighter color.

Almond polyphenols are concentrated at the lipid interface and contribute to almond quality such as color, astringency, shelf life and antimicrobial activity.[14] Evidence also suggests that almond polyphenols contribute to health benefits by its consumption.[24] Many chronic and degenerative diseases such as cancer, heart, Alzheimer's, Parkinson's, as well as aging, were associated with oxidative stress.[25] The human body is able to eliminate free radicals but not entirely; thus diets rich in fruits and vegetables have been considered an excellent source of antioxidants to fight free radical accumulation.[25] Polyphenols are believed to account for a major part of the antioxidant activity in many plants,[26] and almonds have been reported to provide health benefits owing to their polyphenols and antioxidant activity.[14] The total polyphenolic intake has been reported as ∼1 g per day,[27] while the TPC of hydroSOStainable almonds has been calculated at 583 mg 100 g^{-1}.[12] Consequently, eating 43 g hydroSOStainable almonds to reduce the risk of heart disease, as claimed by the Food and Drug Administration's recommendation,[28] is equivalent to ingestion of 25% of the estimated total polyphenolic daily intake. Knowing that the average intake of almonds consumption in 2018 in Spain has been estimated at around 0.71 g per person per day according to Ministry of Agriculture, Fishery and Food in Spain, the total phenolic content intake in Spanish people is estimated at 4.13 mg TPC per person per day only from almond consumption. This represents 4% of the estimated total polyphenolic compounds, and could be raised to 25% by increasing the daily almond intake up to 43 g.

CONCLUSIONS

All these findings lead us to conclude that water stress created in plants increase the polyphenols responsible for almond's skin pigmentation and consequently produce changes in *L** and *a** color coordinates. This study reveals important results showing that isorhamnetin-3-*O*-rutinoside, isorhamnetin-3-*O*-glucoside and kaempferol-3-*O*-glucoside could be important markers of hydroSOStainable almond cv. Vairo. However, it is important to assure that this tendency occurs in a long-term experiment, as year factor might influence in the almond chemical composition. Finally, hydroSOStainable almonds might be an important source of phenols when ingesting 25% of the estimated total polyphenolic daily intake. The results play a key role in the marketability of hydroSOStainable almonds by contributing to their functionality and consumer acceptability. In addition, this work encourages the agro-food sector to invest in water use efficiency strategies, producing fruits with high functional and sensorial characteristics.

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