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Ascorbic acid, carotenoid contents and antioxidant properties of Australian summer carrot with different irrigation amounts on a free-draining, sandy soil

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It is important to reduce the use of water for agricultural production in response to water scarcity and environmental concerns. The nutritive value in fruits and vegetables including carrot (*Daucus carota* L.), can be influenced by various climatic conditions, such as light intensity, temperature, and irrigation. The effect of differential irrigation treatments on the contents of ascorbic acid and carotenoid as well as antioxidant properties (antioxidant content, antiradical power, and antioxidant capacity) were studied in carrot (cv. Stefano) roots grown on a free-draining, sandy soil (Grey Karrakatta Sand) in the summer period. This soil has water holding capacities as low as 10-13% and requires irrigation up to 150% of class A pan evaporation (Epan) to optimize growth and quality. The irrigation treatments applied in this study consisted of 100% Epan replacement, 150% Epan replacement and crop factor. The soil water stress index calculation showed the soil water tension ranged from -2.4 to -7.6 kPa that was within the range between saturation and field capacity for sandy soil. The reduction of irrigation amount from 150% to 100% Epan did not differentiate the contents of ascorbic acid and total carotenoid, but it slightly decreased antioxidant properties of carrot grown in the free draining sandy soil.

Keywords: antioxidant, ascorbic acid, carotenoid, irrigation, pan evaporation

INTRODUCTION

Food nutrient quality is a significant aspect of agricultural production, especially fruit and vegetable production suited for human consumption. The consumption of high quality fruit and vegetables is able to prevent the risk of development of certain diseases and to reduce the aging process (Bahorun et al. 2004; Alasalvar et al. 2005).

Many fresh fruits, and vegetables such as carrots, are a source of natural antioxidants (Lorenz and Maynard, 1997; Ou et al. 2002). Antioxidants include vitamins that protect the human body from the effect of free radicals (Ou et al. 2002; Molyneux, 2004). Studies related to antioxidants including vitamins are becoming increasingly prominent in the literature (Zhang and Hamauzu, 2004; Alasalvar et al. 2005; Zhou and

Yu, 2006; Singh, 2007).

Sprinklers are one of the main techniques currently used for irrigation (Dechmi et al. 2003) and can be used to decrease agricultural water demand compared with flood irrigation (Quezada et al. 2011). Sprinkler irrigation systems are particularly suited to sandy soil with high infiltration rates, such as Grey Phase Karrakatta sand on the Swan Coastal Plain, Western Australia (Dechmi et al. 2003; Li and Rao, 2003). Sprinkler irrigation systems are very sensitive to wind, pressure variations and design limitations. This results in poor uniformity of water distribution (Dechmi et al. 2003), so that there will be localized water deficits and elevated rates of loss to drainage in the field (Hansen et al. 1980).

The impact of irrigation levels on carrot growth, productivity, physiological responses and water use efficiency was reported by Gibberd et al. (2000) and Ludong et al. (2013). On a free draining sandy soil irrigation treatments did not affect the growth, productivity, or physiological responses of carrot (Ludong et al., 2013). In addition, the effects of salinity (Eraslan et al. 2007) and solar radiation (Zhou and Yu, 2006) on the antioxidant activities of carrots were reported. Lee and Kader (2000) reported that ascorbic acid content in many crops can be increased with less frequent irrigation as high vitamin C content may serve as a protective strategy against drought injury. The effect of irrigation amounts on the nutrient quality related to antioxidant and vitamin composition of carrot, however, has not been much explored. This study was carried out to evaluate the effect of three different irrigation amounts (100% Epan replacement, 150% Epan replacement and carrot crop factor multiplied by 100% Epan replacement) on the content of ascorbic acid, total carotenoid, and antioxidant capacity of carrot grown in the free draining sandy soil. Hypothesis in this study was the reduction of irrigation amount would not differentiate the content of ascorbic acid, total carotenoid, and antioxidant capacity of carrot grown in the free draining sandy soil.

MATERIALS AND METHODS

Location and agronomy

An experiment was conducted at Medina Research Station of the Western Australian Department of Agriculture and Food (32.13°S and 115.38°E). The experiment was established in the warm season, i.e. November 2006. The soil type at this location is Grey Phase Karrakatta sand (Bolland, 1998). Before the experiments, the soil was hoed to 400 mm depth with a rotary hoe and basal fertilizer of double superphosphate at 175 kg ha^{-1} (680 g $plot^{-1}$ of 6.0 x 6.5 m), and Medina trace element mix ($MgSO_4$.7 H_2O 50.5 kg ha^{-1}, $MnSO_4.H_2O$ 20.0 kg ha^{-1}, $FeSO_4.7H_2O$ 18.0kgha^{-1}, $Na_2B_4O_7.10H_2O$ 18.0 kgha^{-1}, $CuSO_4.5H_2O$ 18.0 kg ha^{-1}, $ZnSO_4.H_2O$ 18.0 kgha^{-1}) at 150 kg ha^{-1} (600 g $plot^{-1}$) was applied. Beds 1.5 m wide and 0.15 m high were formed. The area was irrigated using Nelson S10 spinner sprinklers operated at 150 kPa on 1.5 m risers at 6.2 x 6.5 m spacing. Seeds of carrot cv. Stefano were sown with a precision vacuum seeder at 7 cm spacing and 1.2 cm depth into moist soil with 4 double rows per bed. Standard cultural practices were applied during the growth of the crops. In brief, nitrogen and other nutrients were applied weekly, from the first week after sowing until the 16th week with watering cans prior to irrigation. The total fertilizer application equated to 315 kg N, 255 kg K, 15 kg Mg and 10 kg Borax per ha.

Irrigation treatments and experimental design

The plants were irrigated at rates of 150% of class A pan evaporation (Epan) from sowing until before the treatments began. Irrigation treatments commenced 21 days after sowing (DAS) and the treatments consisted of three rates of sprinkler irrigation, each with four replicates, arranged in a randomized block design. There were 8 sampling times with 4 crops and 1.0 x 0.5 m spot sample each plot. The irrigation treatments were: (i) Treatment 1 (T_1) = 100% Epan replacement; (ii) Treatment 2 (T_2) = 150% Epan replacement; and (iii) Treatment 3 (T_{CF}) = Variable evaporation coefficient replacement based on carrot crop factor (CF)* multiplied by 100% Epan replacement (Ludong et al. 2013).

*The crop factor (CF) values were established by the Department of Agriculture and Food, Western Australia (DAFWA), based on previous experiments conducted by McKay et al. (nd) (unpublished report)

The daily irrigation schedule was based on the equivalent previous-day pan evaporation (Epan). The equivalent pan evaporation value was calculated every 15 minutes from weather station data (Medina weather station, Bureau of Meteorology, WA) using the modified Penman-Montieth equation (Allen et al.1998). For laboratory analysis (ascorbic acid, total carotenoid, and total antioxidant capacity), each replicated had 4 samples.

Laboratory analysis

The ascorbic acid and total carotenoid content of carrot root was determined at the post-harvest laboratory, Muresk Institute, Curtin University of Technology. Total antioxidant capacity was measured in the Wine Laboratory, located at the Centre of Wine Excellence, Curtin University of Technology, Margaret River, Western Australia. After harvesting in the field, carrot roots were washed using clean water and the samples were stored in a cool room (2 to 3°C), then the roots were "topped" and "tailed" whereby the sample was taken from the middle 1/3 of each carrot root.

The content of ascorbic acid was determined based on Jagota and Dani (1982) and AOAC (1996). Five g of sample were homogenized with 25 mL of 0.75 % metaphosphoric acid. The homogenate was centrifuged at 3000 rpm for 10 min and filtered with filter paper. The supernatant was collected and 200 µL of 3% meta-phosphoric acid and 200 µL of folin reagent (1:5) were added to 400 µL filtered supernatant. The solution was added by 1400 µL distilled water to make the total volume to 2200 µL. The content was mixed for 10 min, then the sample was read with absorbance adjusted to λ =760 nm using a 2 mL plastic disposable cuvette. The ascorbic acid sample was calculated using the standard curve with known concentration of ascorbic acid: Y = 0.0114 X – 0.0063 (R^2= 0.999).

The total carotenoid assay was conducted based on the protocol taken from Hendry and Grime (1993). In brief, 1 g of fresh mass was ground to make a tissue sample in a 20 mL volume of absolute ethanol. The ground material was placed in a centrifuge tube and spun at 10000 g for 10 min. The absorbance measurement of the whole ethanol extract was taken at λ: 480, 645, and 663 nm. Absolute ethanol was used as the blank for zeroing the machine. Total carotenoid concentration in mM = (A_{480} + 0.114 x A_{663}) – (0.638 x A_{645})): 112.5, where A_{480}, A_{645}, and A_{663} were the values for absorbance at wavelengths λ: 480, 645, and 663 nm, respectively.

The total antioxidant capacity (AOC) was determined using the diphenyl picrylhydrazyl (DPPH) assay. The sample was grated and fixed in liquid nitrogen. The sample was then ground to a fine powder using a mortar and pestle. To avoid water absorption, 0.5 g to 1 g samples of the fine powder were weighed quickly and transferred to a 59 mL centrifuge tube. Each sample was extracted with 5 mL methanol in the centrifuge tube and spun at 4400 rpm for 10 min. Half mL of supernatant was mixed with 2.5 mL of 75 µM DPPH solution in a 4 mL cuvette. The reading was taken at λ= 517 nm after 30 min (methanol was used as a reference) (Brand-Williams et al. 1995; Gil et al. 2002; Molyneux, 2004). To form a standard curve, 0.5 mL of 0, 0.04, 0.1, 0.2, 0.25 and 0.5 mM of trolox (6-hydroxy-2-carboxylic acid) solution in methanol was mixed with 2.5 mL of 75 µM DPPH solutions. The reading was taken at λ= 517 nm again, after 30 min. The 0 mM trolox was used as a "control". To determine the % DPPH scavenging, EC_{50} and $EC_{50\ sample}$, APR (antiradical power) and total AOC (trolox equivalent antioxidant capacity) concentrations were calculated by: (i) The % DPPH scavenging is defined as Q = 1 – (A0 – AC)/A0. Where A0 is the control value of absorbance (absence of any sample), AC is absorbance for the added sample concentration reading after 30 min (Molyneux, 2004); (ii) EC_{50} ("efficient concentration" value) is defined as the concentration of substrate that cause a 50% loss of DPPH activity calculated from the calibrated standard curve. The EC_{50} sample is calculated as a concentration at EC_{50}, divided by the concentration value of antioxidant in DPPH solution from the sample after 30 min [(mmol/L)/(mmol/L)] (Brand-Williams et al., 1995); (iii) APR (antiradical power) is defined as 1/ $EC_{50\ sample}$ (Brand-Williams et al., 1995); (iv) Total AOC (antioxidant capacity) is expressed as µmol trolox equivalent (TE)/g or mmol TE/kg (Brand-Williams et al. 1995).

Determination of differential soil water deficit index (DSWDI)

Differential soil water deficit index (DSWDI) was determined as the ratio of soil water potential on the average 0-45 cm depth of all treatments relative to the soil water potential of the 150% Epan treatment. The soil water deficit index for the 100% Epan and crop factor (CF) treatments was indicated as T_1 and T_{CF}, respectively. The DSWDI formula was DSWDI = $\psi_s i/\psi_s ww$
where, $\psi_s i$: soil water potential of all treatments relative and $\psi_s ww$: soil water potential on well watered treatment (T_2).

Measurement of soil moisture content and soil water potential

Soil water potential was measured at 15, 30 and 45 cm depths using low-range tensiometers (Irrometer ® Company, USA) equipped with pressure sensing transducers. Volumetric soil water content was measured using a calibrated water content reflectometer (Campbell CS625®).

The outputs from both probe types were logged by a computer every 15 minutes.

Statistical analysis

All data collected were subjected to analysis of variance (ANOVA) one-way design in randomized block using GenStat Release 8.2 statistical software. A comparison of the means of treatments each time was done using lsd's calculated at P=0.05 (Steel and Torrie, 1960; Gomez and Gomez, 1984; Payne et al. 2005).

RESULTS

Ascorbic acid and total carotenoid

The ascorbic acid and total carotenoid content in carrot roots were 4.6 mg and 12.2 mg, in 100 g fresh weight (FW), respectively. The amount of irrigation water applied to the 150% Epan treatment was significantly different from the other treatments. On the contrary, there was no significant difference in ascorbic acid and total carotenoid content among the 100% Epan replacement, 150% Epan replacement and carrot crop factor multiplied by 100% Epan replacement (Table 1).

Many previous studies indicated that some environmental factors including soil moisture might influence the contents of carotenoids (Melédez-Martinez et al. 2007; Riggi et al. 2008) and ascorbic acid (Sorensen, 1999; Lee and Kader, 2000). Reducing the irrigation level increased the carotene content of the carrot root (Rubatzky, 1999). Severe drought treatment with water potential -0.12 MPa in carrot significantly increased the concentrations of vitamin C and β–carotene based on the fresh weight of carrots 19-38 mm diameter grown on the sandy loam soil compared to weak drought with water potential -0.06 MPa (Sorensen et al. 1997) The present study showed that the differences in the amount of irrigation water applied were 33% less than the irrigation standard (150% Epan water replacement) level. The content of ascorbic acid was 4.3-4.9 mg in 100g FW and the content of carotenoid was 11.8-12.6 mg in100g FW. The irrigation amounts used in the present study, between 150% and 100% Epan water replacement, did not affect the contents of ascorbic acid and carotenoid in carrot roots.

Generally, soil fertility affected the carotene content (Rubatzky, 1999; Sorensen, 1999). Sorensen (1999) revealed that the carotene of carrot roots was positively correlated with the nutrients supplied to the medium and one of the important nutrients was nitrogen. Decreasing nitrogen supply slightly decreased the concentration of carotene of carrot roots. In conclusion, the results demonstrated that a reduction in the irrigation levels from 150% to 100% Epan water replacement did not affect the contents of ascorbic acid and carotenoid in carrot roots.

Total antioxidant capacity

The value of the trolox equivalent antioxidant capacity (TEAC) is a standardized measure of the antioxidant capacity of the sample. The antioxidant capacity of carrot roots from the three irrigation treatments ranged from 1.040 to 1.083 µmol TE (trolox equivalent) g^{-1}, respectively. Roots grown with the 100% Epan treatment had the highest antioxidant capacity (1.083 µmol TE g^{-1}), followed by the CF (1.045 µmol TE g^{-1}) and 150% Epan treatment (1.040 µmol TE g^{-1}). The total antioxidant capacity (AOC) recorded from the highest irrigation treatment was significantly different in comparison to AOC at 100% Epan. The AOC from the 100% Epan treatment was 4.2% higher than from 150% Epan treatment. However, there was no significant difference between the 100% Epan and CF treatments (Table 2).

Calculating the percentage of DPPH scavenging is the other way to determine the antioxidant activity. DPPH scavenging results ranged from 44.25 to 45.80%. The results also identified that the highest DPPH scavenging was detected in the carrot root sample extract for carrots grown with the 100% Epan treatment. The DPPH scavenging at the 100% Epan irrigation treatment was just 1.55% higher than the value at the 150% Epan treatment (Table 2).

The highest antiradical power (ARP) value was observed in the 100% Epan treatment (0.902), followed by CF (0.870) and 150% Epan treatments (0.865). Furthermore, the percentage differences among the treatments on ARP values were exactly the same as total antioxidant capacity values. The only significant difference exists between the 100% and 150% Epan treatments (Table 2).

The differential soil water deficit index (DSWDI) for the 100% Epan and CF treatments from 6th to 14th week were close to 1 in a range from 0.75 to 1.46 (Table 3).

The differential soil water deficit indices of the 100% Epan and CF treatments fluctuated between the 6th and 10th week. The lowest DSWDI in the Epan and CF treatments were 0.83 and

0.75, respectively at the 8th week. The DSWDI of 100% Epan treatment on the 16th week was 1.7-fold larger than the average DSWSI at the time before 16th week. The DSWSI of CF treatment from 10th to the 16th was relatively constant (Table 3 and Figure 1).

The difference in the volume of water applied between the 100% Epan and 150% Epan treatments was 33% and this had a small impact on the nutritive value of carrot roots. The percentage of the DPPH scavenging capacity for carrot roots grown at the 100% Epan was 1.55% greater than at the 150% Epan treatment. The percentage of total antioxidant capacity (AOC) and antiradical power (ARP) activities the for carrot roots grown at the 100% Epan was 4.2% greater than at the 150% Epan treatment. The different amount of water applied between the CF and 150% Epan treatments was 23% and this condition was did not significantly affect the antioxidant capacity. The amount of water applied at the CF treatment was only 8% higher than at the 100% Epan treatment. The antioxidant capacity in the 100% Epan treatment was 4.2% larger than crop factor (CF) irrigation treatment (Table 2). The variation in antioxidant activity values in carrot was not only influenced by geographical differences or location, weather conditions, and harvest periods, cultivars and amount of irrigation (Ou et al. 2002; Pellegrini et al. 2003; Alasalvar et al. 2005; Singh et al. 2007).

Table 1. Ascorbic acid (mg in 100 g fresh weight/FW) and caroteniods (mg in 100g fresh weight/FW) concentration in carrot roots grown under three different irrigation treatments (T_1, T_2, and T_{CF})

Treatment	Total water applied (mm)	Ascorbic Acid (mg in 100 g FW)	Carotenoids (mg in 100 g FW)
T_1	856 b	4.91	12.5
T_2	1136 a	4.57	11.8
T_{CF}	926 b	4.29	12.1
lsd (P = 0.05)	79.9		
s.e.m	23.1		

Carrot was harvested on 20 March 2007. The three irrigation treatments we T_1: 100% Epan; T_2: 150% Epan; and T_{CF}: crop factor (CF) multiplied by 100% Epan, and data are mean (n=4), different letters following data within a column indicate a significant difference between means.

Table 2. Total antioxidant capacity (AOC) (µmol TE g^{-1}), DPPH scavenging (%), and antiradical power (ARP) (dimensionless) of carrot root from three different irrigation treatments (T_1, T_2 and T_{CF}). Carrots were harvested on 20 March 2007

Treatment	Total water applied (mm)	Total AOC (µmol TE g^{-1})	DPPH scavenging (%)	ARP
T_1	856 b	1.08 a	45.80 a	0.90 a
T_2	1136 a	1.05 ab	44.45 a	0.87 ab
T_{CF}	926 b	1.04 b	44.25 b	0.86 b
lsd (P = 0.05)	79.9	0.05	1.53	0.04
s.e.m	23.1	0.01	0.44	0.04

The three irrigation treatments were T_1: 100% Epan; T_2: 150% Epan; and T_{CF}: crop factor (CF) multiplied by 100% Epan, APRs are inverse of concentration at EC_{50} divided by the concentrati value of antioxidant in DPPH solution from sample at 30 min [1/(mmol/L)/(mmol/L)] and data are mea s (n=4), different letters following data within a column indicate a significant difference between means (ns = no significant difference between treatments at P=0.05).

Table 3. Soil water potential (kPa) at 0-45 cm depths for carrot on the T_1, T_2 and crop factor (CF) (T_{CF}) irrigation treatments (Soil water potential data were 2- weekly means of

records taken at 15 minutes intervals)

Week	Soil Water Potential (kPa)		
	T_1	T_2	T_{CF}
6th	-3.59	-4.23	-3.24
8th	-3.54	-4.28	-3.22
10th	-3.98	-3.10	-3.10
12th	-4.36	-3.31	-3.32
14th	-4.84	-3.32	-3.47
16th	-5.98	-3.11	-2.95

Laboratory analysis showed that ascorbic acid and carotenoid in carrot were not sensitive to different water availability, whereas antioxidant activity was sensitive to the irrigation treatment. Although fruits and vegetables, including carrots, are rich sources of different phytonutrients, such as vitamin C, E and B-carotene, including antioxidant properties (Asalvar et al. 2005), a significant contribution of the antioxidant activity of the fruits and vegetables also comes from the phenolic compound (Gil et al. 2002). The contributions of phenolic compounds to antioxidant activity were much greater than those of vitamin C and carotenoids in the carrots in this study.

The soil matric water potential that was related to the soil water content was one of the basic soil properties required to manage irrigation effectively (James et al. 1982). The soil matric water potential influenced the water potential gradient associated with water flux into plant roots. As the soil water content increased, the water potential became less negative (Allison and Jones, 2005). The soil water deficit index of the 100% Epan and CF treatments were different from 6th to 14th week of the treatments (Figure 1) implied that there were only small soil water tension differences among all the irrigation treatments in the 0-45 cm soil depths. The soil tension ranged from –3.0 to –6.0 kPa with an average of -4.4, -3.6 and -3.2 kPa at the 100%, 150% Epan and CF irrigation treatments, respectively. These values were in the range between the saturated and field capacity for a sandy soil (0 to -10 kPa) (Hansen et al. 1980) and there was probably no soil water deficits for any of the treatments. The pan evaporation varied during the growing season and the range was 6.4-7.9 mm/day. As there was no significant impact of irrigation amount on the nutritional quality as well as on the yield (Ludong et al. 2011) of carrot, reduction of irrigation amount from 150% to 100% Epan could be applied in carrot planting.

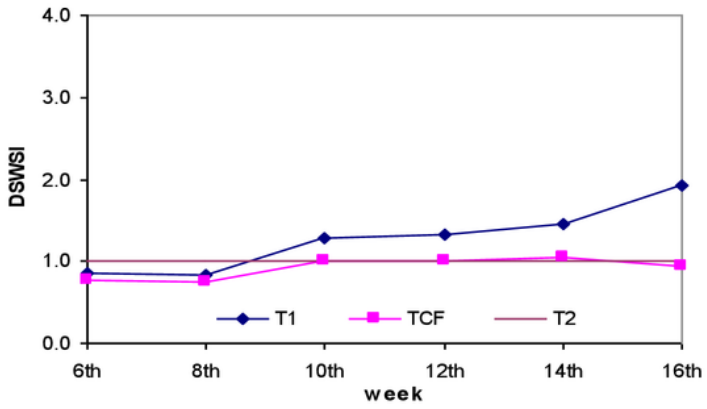

Figure 1. The average differential soil water deficit index (DSWDI) was determined as the ratio of soil water potential of the 100% Epan (T_1) and Crop factor (CF) (T_{CF}) treatments relative to the 150% Epan (T_2) treatment at 0-45 cm depth.

CONCLUSION

The reduction of irrigation amount from 150% to 100% Epan did not result in any difference in the contents of ascorbic acid and total carotenoid s,

but it slightly decreased antioxidant properties of Australian summer carrot grown in the free draining sandy soil.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

DPML designed and conducted the field experiment, carried out tissue collection, laboratory and data analysis, and also wrote the manuscript. SAN reviewed the manuscript. PO supervised field experiment. ZS and MG supervised DPML in designing and conducting the field experiment, carrying out tissue collection, and laboratory and data analysis.

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