Saving irrigation water as a tool to increase pomegranate fruit price and enhance the bioactive compound content

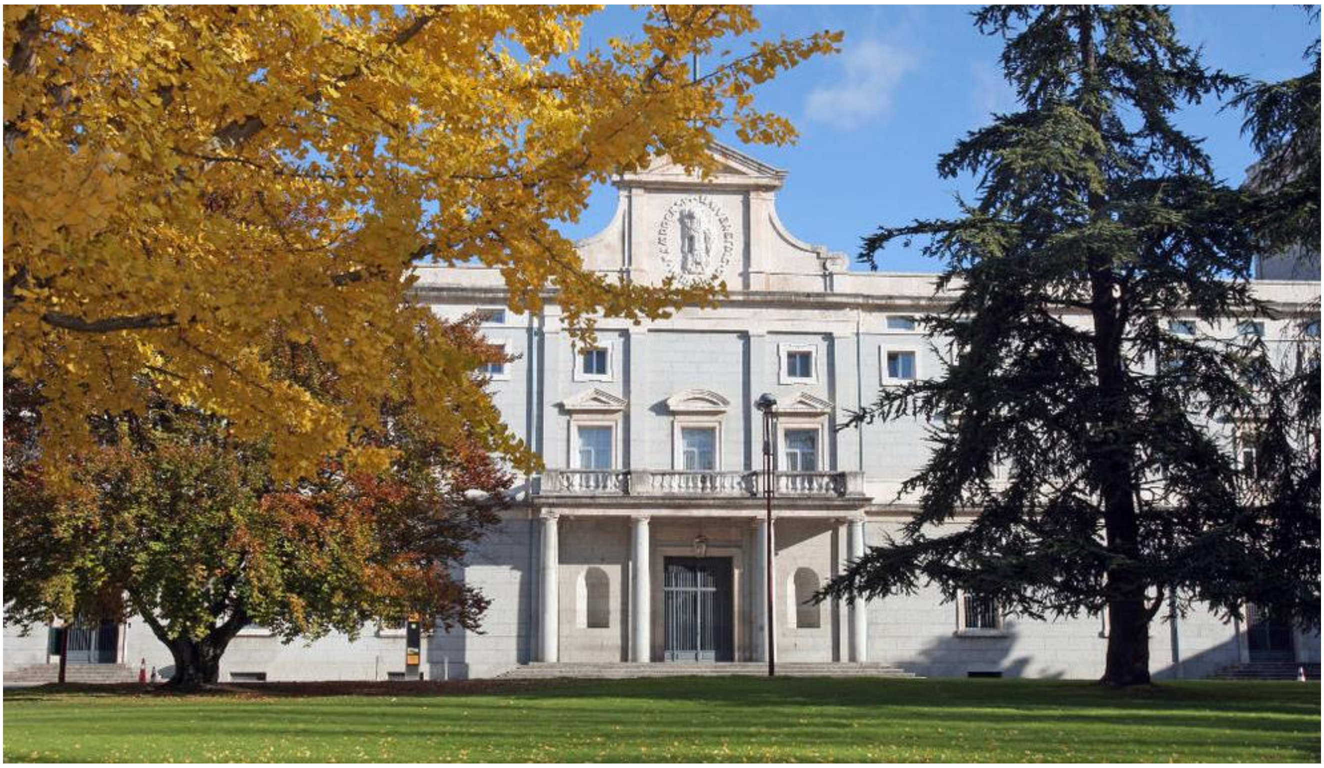
Conference Paper · October 2016

Alejandro Galindo
University of Twente

Ángel Calín-Sánchez
Universidad Miguel Hernández de Elche

Pedro Rodríguez Hernández
Corporación Colombiana de Investigación Agropecuaria (CORPOI…

Ignacio Giron
Spanish National Research Council



XIII Simposio Hispano-Portugués de Relaciones Hídricas en las Plantas

“Aprendiendo a optimizar el uso del agua en las plantas para hacer de nuestro entorno un ambiente más sostenible”

LIBRO DE RESÚMENES

(ISBN 978-84-8081-525-3)

Pamplona, 18 – 20 de octubre de 2016

Saving irrigation water as a tool to increase pomegranate fruit price and enhance the bioactive compound content

Galindo, A.[1], Calín-Sánchez, A.[2], Rodríguez, P.[3], Cruz, Z.N.[3], Girón, I.F.[4,5], Corell, M.[5,6], Martínez-Font, R.[7], Moriana, A.[5,6], Carbonell-Barrachina, A.A.[2], Torrecillas, A.[1], Hernández, F.[7]

[1] Centro de Edafología y Biología Aplicada del Segura (CEBAS-CSIC). Department of Irrigation, P.O. Box 164, E-30100 Espinardo, Murcia, Spain (atorreci@cebas.csic.es)
[2] Universidad Miguel Hernández de Elche, Department of Agrofood Technology, Food Quality and Safety Research Group, Ctra. de Beniel, km 3,2. E-03312 Orihuela, Alicante, Spain
[3] Instituto Nacional de Ciencias Agrícolas (INCA). Department of Physiology and Biochemistry, Ctra. de Tapaste, km 3.5, San José de Las Lajas, Mayabeque, Cuba
[4] Instituto de Recursos Naturales y Agrobiología (CSIC), PO Box 1052, E-41080 Sevilla, Spain
[5] Unidad Asociada al CSIC de Uso Sostenible del Suelo y el Agua en la Agricultura (US-IRNAS). Crta de Utrera Km 1, 41013, Sevilla, Spain
[6] University of Sevilla. EUITA, Dept. Ciencias Agroforestales, Carretera de Utrera km 1, E-41013 Sevilla, Spain
[7] Universidad Miguel Hernández de Elche, Department of Plant Sciences and Microbiology, Plant Production and Technology Research Group, Ctra. de Beniel, km 3,2. E-03312 Orihuela, Alicante, Spain.

ABSTRACT

The non-climateric character of pomegranate (*P. granatum*) fruit underlines the importance of determining the optimum harvest time to improve fruit quality. The effect of irrigation withholding during 6, 15, 25 and 36 d before harvest was evaluated in order to clarify whether fruit ripening is critical or non-critical from the yield, fruit characteristics and composition point of view. The results indicated that this phenological period is critical because irrigation is essential during most of this phenological period to achieve maximum yield. However, a 6 d of irrigation restriction at the end of ripening period can be used as a tool to come early harvest time, saves irrigation water, enhances the bioactive compounds (anthocyanins, phenolic compounds, punicalagin and ellagic acid) and increases the price of the fruit without affecting marketable yield and fruit size.

INTRODUCTION

All Mediterranean agrosystems must cope with water scarcity, and any policy involving greater use of the water available is unsustainable. For this, pomegranate (*Punica granatum* L.) farming must be directed towards the use of deficit irrigation strategies, maximizing crop water productivity rather than maximizing the yield per land unit, allowing significant water savings, and the profitable production of high quality fruits. Regulated deficit irrigation (RDI) is an irrigation strategy designed to save water while having a minimum impact on yield and fruit quality (Naor, 2006). This requires precise knowledge of the crop response to drought stress during the different phenological phases in order to identify phenological periods when adverse effects on productivity are minimal (non-critical periods) or maximal (critical periods). Intrigliolo et al. (2013) and Laribi et al. (2013) indicated that the period comprising flowering and pomegranate fruit set could be regarded as non-critical from the yield point of view, that irrigation water restriction during linear fruit growth period increased the concentration of many bioactive compounds in the juice, such as anthocyanins, that could be related to health and taste and that irrigation water restriction during the last part of fruit growth and ripening enhances peel red colour intensity and TSS in the juice

The aim of this research was to evaluate whether water restrictions only during the ripening stage can affect yield and whether water deficit during this period has secondary effects on fruit characteristics, which could be used to improve fruit maturity and come early harvest time.

MATERIALS AND METHODS

Drip irrigated adult own-rooted pomegranate (*P. granatum* L. cv. Mollar de Elche) plants spaced at 3 m x 5 m were irrigated above crop water requirements (control plants, T0). Also, T1, T2, T3, and T4 treatments were irrigated as T0 except for 6 (DOY 277–283, fruit late ripening), 15 (DOY 268–283, second half fruit ripening), 25 (DOY 258–283, fruit ripening) and 36 (DOY 247–283, end fruit growth and ripening) days before harvest (DOY 283), respectively, when irrigation was withheld. The total amount of water received by each treatment during the experimental period (DOY 247–283) was 128, 110, 86, 49 and 0 mm for T0, T1, T2, T3 and T4 treatments, respectively, without considering precipitation (basically the 84 mm rainfall that fell on DOY 271).

Midday leaf conductance (g_{leaf}) was measured with a porometer (Delta T AP4, Delta-T Devices, Cambridge, UK) on the abaxial surface of two leaves per tree. Midday leaf (Ψ_{leaf}) and stem (Ψ_{stem}) water potentials were measured in two leaves similar to those used for g_{leaf} using a pressure chamber (PMS 600-EXP, PMS Instruments Company, Albany, USA) (Galindo et al., 2014a). Midday fruit water potential (Ψ_{fruit}) was measured following the procedure described by McFadyen et al. (1996) and Galindo et al. (2014a). Water stress integral (SI) was calculated from the g_{leaf}, Ψ_{leaf}, Ψ_{stem} and Ψ_{fruit} data according to the expression

$$\mathrm{SI_A} = \left|\sum(\bar{\mathrm{A}} - \mathrm{H})\, n\right|$$

where A can be g_{leaf}, Ψ_{leaf}, Ψ_{stem} or Ψ_{fruit} and $\bar{\mathrm{A}}$ is the average g_{leaf}, Ψ_{leaf}, Ψ_{stem} or Ψ_{fruit} value for any interval, H is the maximum value measured during each interval and n is the number of days in the interval.

Fruit juice total soluble solids (TSS), total titrable acidity (TA), juice colour, total phenolic compounds (TPC), total anthocyanin content (TAC), total antioxidant activity (TAA), punicalagin (isomers α and β) and ellagic acid contents were determined following the procedures described by Galindo et al. (2014b) and Calín-Sánchez et al. (2013).

RESULTS AND DISCUSSION

In spite of the rainfall events, the cumulative water stress tended to increase with the number of days irrigation was withheld, the treatments T1 and T2 producing a similar and moderate water stress level and a more pronounced water stress level being observed in the treatments T3 and T4. SI_{gleaf}, $SI_{\Psi stem}$, $SI_{\Psi leaf}$ and $SI_{\Psi fruit}$ values showed some differences in describing the cumulative water deficit reached by the plants. $SI_{\Psi fruit}$ was the most reliable indicator to detect differences between the treatments at moderate water stress (T0 and T2), while SI_{gleaf} was the only indicator able to detect differences between the treatments at more pronounced water stress levels (T3 and T4) (Table 1).

The decrease in fruit yield in T2 and T3 (Table 2) confirmed the hypothesis that fruit ripening is a critical period from the yield point of view (Laribi et al., 2013; Intrigliolo et al., 2013). However, the fact that plants that were water stressed only at fruit late ripening stage (T1) showed similar marketable yield and fruit size to T0 plants clarifying some aspects of the concept of phenological critical period. In this sense, it is probable that sensitivity to water stress during a given critical phenological period is not constant and/or it is necessary to exceed a certain level of water stress to achieve adverse effects on productivity during a critical period. Whatever the case, although pomegranate trees are able to withstand severe drought conditions, irrigation was essential during most of the ripening stage to achieve optimum yield.

The first pomegranate fruits reaching the market fetch higher prices and, in this sense, 'Mollar de Elche' cultivar is often harvested when the peel has a sufficient red colouratiing. The significant increase in juice colour from T3 and T4 fruits (Table 3) is also very interesting for producers because pomegranate fruit attractiveness is primarily related to colour and taste parameters of the arils and their juice (Borochov-Neori et al., 2009). However, despite the fact that pomegranate colouration is predominantly due to anthocyanins, TAC levels in T3 and T4 fruits were similar to that observed in T0 fruits (Table 4).

The fact that (i) TAC juice levels increased only in T1 fruits, (ii) TAA levels were similar in juices from the different irrigation treatments, and (iii) redness significantly increased only in T3 and T4 fruit juices (Tables 3 and 4), confirmed the view that juice antioxidant capacity is not linearly correlated with the red colour intensity, meaning that the anthocyanins are not major contributors to the antioxidant capacity exhibited by the pomegranates and their juice (Borochov-Neori et al., 2009). Moreover, the fact that withholding water irrigation decreased TPC levels and did not affect TAA levels (Table 4) does not agree with the linear relationship between soluble phenolic levels and antioxidant capacity indicated by Borochov-Neori et al. (2009), who supported the idea that phenolic compounds are the main contributors to the antioxidant activity in pomegranate juice. In this sense, further analysis of fatty acids and organic acids must be conducted to fully understand the antioxidant capacity and bioactivity of pomegranate fruits subjected to deficit irrigation strategies (Alcaraz-Mármol et al., 2015; Calín-Sánchez et al., 2013).

It is well known that water stress influences the content of secondary metabolites in plant tissues, having also contradictory results in other crops. For example, Chaves et al. (2007) reported the substantial accumulation of anthocyanins in grape berries under water stress. In contrast, Kennedy et al. (2002) showed that osmotic stress had little or no effect on anthocyanin accumulation in grape berries. This, at first sight, confusing relation between water stress and the production of bioactive compounds could be attributed to the fact that most manuscripts are not meticulous when it comes to recording aspects of plant water stress (precise phenological period at which it takes place, water stress rate of development, duration of maximum water stress, incidence of partial recoveries and other aspects) although such information is essential for the characterisation of experimental water stress conditions. In addition, it is essential to underline that is not possible to establish a linear correlation between water stress and secondary metabolite contents (Gobbo-Neto and Lopes, 2007). For this reason, Horner (1990) proposed a quadratic model to predict the concentration of phenolic compounds as a function of plant water status. So, under a mild water stress, CO_2 assimilation could be maintained and carbon-based secondary metabolites will probably increase when carbohydrates exceed the amount required for growth. Thus, mild osmotic stress may lead to a reduction in plant growth, accompanied by an increasing concentration of non-nitrogenous secondary metabolites. When water stress increases, stomatal regulation takes place and CO_2 assimilation is reduced. In this situation, carbon will be preferentially allocated to the synthesis of primary metabolites to the detriment of the synthesis of secondary metabolites.

CONCLUSION

The results indicated that the SI calculated from g_{leaf}, Ψ_{leaf}, Ψ_{stem} and Ψ_{fruit} data vary as regards their ability to describe the cumulative water deficit reached by plants. $SI_{\Psi fruit}$ was the most feasible indicator for detecting differences between the treatments at moderate water stress levels while SI_{gleaf} was the only indicator able to detect differences between the treatments at higher water stress levels. Fruit ripening is a critical period from the yield point of view because irrigation is essential during most of this phenological period if maximum yields are to be achieved. Nevertheless, the fact that a very short irrigation restriction period (around 6 days) at the end of ripening bring the harvest time forward and so increase pomegranate fruit price, saves irrigation water and enhances the bioactive compound content (anthocyanin, phenolic compounds, punicalagin and ellagic acid) without affecting marketable yield and fruit size suggests that the sensitivity to water stress during a given critical phenological period is not constant and/or it is necessary to exceed a certain level of water stress to achieve adverse effects on productivity during a critical period. Moreover, the increase in fruits colouration as a result of water stress during fruit ripening may be considered as an interesting aspect because the appeal of pomegranate fruit is directly associated with colour. In spite of this, it is important to note that a very short irrigation restriction (around 6 days) at the end of the ripening period advances the harvest time, increases pomegranate fruit price, saves irrigation water and enhances the bioactive compound contents (anthocyanin, phenolic compounds, punicalagin and ellagic acid). Finally, the results confirmed the hypothesis that there is no a linear correlation between pomegranate water stress and secondary metabolite contents, because mild water stress may lead to a reduction in plant growth and a higher concentration of secondary carbon metabolites, whereas under a more pronounced water stress carbon are preferentially allocated to the synthesis of primary metabolites to the detriment of secondary metabolites.

***Acknowledgements*.**

This research was supported by MINECO (CICYT/FEDER AGL2013-45922-C2-1-R and AGL2013-45922-C2-2-R). This work is a result of the PR internship (19925/IV/15) funded by the Fundación Séneca - Agencia de Ciencia y Tecnología de la Región de Murcia (Seneca Foundation - Agency for Science and Technology in the Region of Murcia) under the Jiménez de la Espada Program for Mobility, Cooperation and Internationalization. AG and ZNC were funded by a FPU and an AECID grant from the Spanish Government, respectively.

REFERENCES

Borochov-Neori et al. 2009. *J Food Compos Anal* 22: 189–195
Calín-Sánchez et al. 2013. *Food Bioprocess Tech* 6: 1644-1654
Chaves et al. 2007. *Ann App Biol* 150: 237-252
Galindo et al. 2014a. *Agr Forest Meteorol* 194: 29-35
Galindo et al. 2014b. *J Sci Food Agric* 94: 2259-2265
Gobbo-Neto and Lopes 2007. *Quím Nova* 30: 374-381
Horner 1990. *Biochem Sys Ecol* 18: 211-213
Intrigliolo et al. 2013. *Irrigation Sci* 31: 959-970
Kennedy et al. 2002. *Am J of Enol Viticult* 53: 268–274
Laribi et al. 2013. *Agric Water Manag* 125: 61-70
McFadyen et al. 1996. *J Hortic Sci* 71: 469–480
Naor. 2006. *Horti Rev* 32: 111-165

Table 1. Effect of irrigation treatments on leaf conductance (SI_{gleaf}, mmol m^{-2} s^{-1} x day), stem ($SI_{\Psi stem}$, MPa x day), leaf ($SI_{\Psi leaf}$, MPa x day) and fruit ($SI_{\Psi fruit}$, MPa x day) water stress integral.

Treatment	SI_{gleaf}	$SI_{\Psi stem}$	$SI_{\Psi leaf}$	$SI_{\Psi fruit}$
T0	5434.5c	10.5b	27.6c	8.5c
T1	5911.5c	15.0b	29.1c	15.1bc
T2	6414.0c	21.5b	31.1bc	22.7b
T3	9784.0b	49.5a	49.2ab	43.7a
T4	15215.3a	62.8a	53.5a	52.9a

Table 3. Effect of irrigation treatments on pomegranate peel and juice lightness (CIE *L**), red/greenness (CIE *a**), blue/yellowness (CIE *b**), chroma (*C**) and hue angle (*H*º) values.

	Treatment	*L**	*a**	*b**	*C**	*H*º
Peel	T0	64.2a	26.5b	31.2a	41.6b	50.1a
	T1	60.8ab	30.7a	30.5a	43.9a	45.3ab
	T2	60.1b	32.0a	30.1ab	44.5a	43.7abc
	T3	57.2bc	33.5a	27.6bc	43.9a	40.1bc
	T4	55.1c	34.6a	26.8c	44.3a	38.2c
Juice	T0	32.4b	8.3b	2.3b	8.6b	14.8b
	T1	33.1ab	10.5ab	3.1ab	10.9ab	16.5ab
	T2	33.1ab	9.6ab	2.7ab	9.9ab	15.3ab
	T3	33.9a	11.7a	3.6a	12.3a	17.2ab
	T4	33.7a	11.8a	3.9a	12.5a	18.2a

Means within a column that do not have a common letter are significantly different by $LSD_{0.05}$ test.

Table 2. Effect of irrigation treatments on marketable pomegranate fruit yield (MY, kg $tree^{-1}$), average fruit weight (FW, g), fruit equatorial diameter (ED, mm), and fruit length (FL, mm).

Treatment	MY	FW	ED	FL
T0	56.8a	293a	86.9a	75.0a
T1	55.5a	258ab	80.4b	69.7b
T2	35.2b	252b	81.4b	71.4ab
T3	28.9b	249b	81.6b	69.8b
T4	17.8b	253b	78.1b	67.2b

Table 4. Effect of irrigation treatments on pomegranate juice total polyphenols content (TPC, mg GAE L^{-1}), total anthocyanin content (TAC, mg L^{-1}), total antioxidant activity (TAA, mM Trolox), α- punicalagin, β-punicalagin, and ellagic acid (mg L^{-1}).

Treatment	TPC	TAC	TAA	α-punicalagin	β-punicalagin	Ellagic acid
T0	3133a	69.5b	12.1a	168.7ab	164.5b	19.0b
T1	2681b	123.1a	13.3a	184.2a	174.2a	19.6a
T2	1945c	76.1b	13.3a	169.5ab	172.0a	19.5a
T3	1534c	75.1b	11.9a	162.2b	170.2ab	19.5a
T4	1589c	75.1b	12.0a	157.7b	168.7ab	19.3ab

Means within a column that do not have a common letter are significantly different by $LSD_{0.05}$ test.