New Approaches to Hydrosustainable Almonds Production: Agronomical, Physiological and Quality Effects

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TESIS DOCTORAL

New Approaches to Hydrosustainable Almonds Production: Agronomical, Physiological and Quality Effects

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NOTE: The entire Thesis is written in English. The summary and the conclusions are also written in Spanish to fulfil the necessary requirements to obtain the “International Doctor” degree, under the Agreement Real Decreto 99/2011.

List of works derived from this Ph.D thesis

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- García-Tejero IF, Gutiérrez-Gordillo S, Souza L, Cuadros-Tavira S, Durán-Zuazo VH (**2018**) Fostering sustainable water use in almond (*Prunus dulcis* mill.) orchards in a semiarid Mediterranean environment. Arch. Agron. Soil Sci. 65:164–181. doi:10.1080/03650340.2018.1492113

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Table of contents

Page

List of Tables

Abbreviations and Symbols
Abstract/Resumen

1. Abbreviations and Symbols

Abbreviations

AA	Antioxidant activity
$A\text{-}C_i$	Relationship between photosynthesis rate and internal concentration of C
ANCOVA	Covariance analysis
AF	Average force
AI	Atherogenic index
A_N	Photosynthesis rate
ANOVA	Analysis of variance
Ca	Ambient CO_2 concentration
C	Chroma
cv.	Cultivar
cvs.	Cultivars
CWP	Crop water productivity
DAD	Diode-array detector
DI	Deficit irrigation
DOY	Day of the year
E	Transpiration
EFSA	European Food Safety Authority
ETc	Crop Evapotranspiration
ETo	Reference Evapotranspiration
EU	European Union

F	Fracturability
FAMEs	Fatty acid methyl esters
FI	Full irrigation
GC	Ground cover
GTM	Greenwich Mean Time
g_m	Mesophyll conductance to CO_2
g_S	Stomatal conductance to CO_2
H	Hardness
HCl	Hydrochloric acid
HPLC	High performance liquid chromatography
IP	Irrigation period
IR	Irrigation requirements
IWP	Irrigation water productivity
J_{max}	Maximum rate of electron transport
K_C	Crop coefficient
KL	Kernel length
K_r	Crop reduction coefficient
KT	Kernel thickness
KW	Kernel width
LFDI	Low frequency deficit irrigation
MUFA	Monounsaturated fatty acid
n	Number of samples
NF	Number of fractures
O/L	Oleic/Linoleic ratio

PAS	Polyphenols and proanthocyanidins
PCA	Principal component analysis
PRD	Partial root drying
PUFA	Polyunsaturated fatty acid
Rad	Solar radiation
RDI	Regulated deficit irrigation
RH_{max}	Maximum relative humidity
RH_{min}	Minimum relative humidity
RH_{av}	Average relative humidity
RuBisCO	Ribulose-1,5-bisphosphate carboxylase/oxygenase
SDI	Sustained deficit irrigation
SFA	Saturated fatty acid
S	South
SW	Southwest
TI	Thrombogenic index
T_{max}	Maximum air temperature
T_{min}	Minimum air temperature
TPC	Total phenolic content
TP	Triose phosphate
TPU	Triose phosphate utilization
V_{cmax}	Maximum carboxylation rate
VPD	Vapor pressure deficit
vs.	*Versus*
WL	Whole length

WOY	Week of the year
WS	Work to shear
WT	Whole thickness
WW	Whole width
WUE	Water use efficiency

Symbols

a_w	Water activity
Ca	Calcium
ºC	Degrees Celsius
Cu	Copper
Fe	Iron
g_s^{av}	Average stomatal conductance
g_s^{max}	Maximum stomatal conductance
g_s^{min}	Minimum stomatal conductance
K	Potassium
Mg	Magnesium
Mn	Manganese
N	Hardeness
Na	Sodium
P	Phosphorous
Se	Selenium
$SI_{\Psi leaf}$	Stress integral leaf water potential
SI_{gs}	Stress integral stomatal conductance
Ψ_{leaf}	Leaf water potential
Ψ_{leaf}^{av}	Average leaf water potential
Ψ_{leaf}^{max}	Maximum leaf water potential
Ψ_{int}	Integrated leaf water potential
Y_N	Normalized yield

Y_{anual}^{av} Average yield for a single treatment and cultivar in a year

Y_{total}^{av} Average yield for a single treatment and cultivar during all the studied years

Zn Zinc

2. Abstract and Resumen

Abstract

Climate change, water resources reduction, or land degradation and abandonment are some limitations to overcome within the new model of achieving competitive and sustainable agriculture. Among the possible strategies, the implementation of drought tolerant crops with high profitability, and the application of water saving strategies such as deficit irrigation (DI) should be considered. In this regard, almond (*Prunus dulcis* Mill.) would be an excellent alternative under water scarcity and climate change scenarios. However, it is essential to reach an equilibrium between crop management to enhance its productivity and the water availability; defining its water requirements and the relationships between irrigation needs and crop development.

In this sense, the general objective of this doctoral thesis was to improve the almond irrigation management, specifically in three of the most traditional cultivars in south Spain (cvs. Guara, Marta and Lauranne) using two DI strategies; regulated deficit irrigation (RDI) and sustained deficit irrigation (SDI). In order to optimize the irrigation water management for each studied cultivar and increase the quality of almonds.

The study was carried out in two commercial almond orchards; “Montana de San José” and “Cartuja”. In the first one, the experiments were performed during the 2017 and 2018 seasons and three irrigation treatments were established: Control (FI); which was irrigated at 100% of the irrigation requirements (IR) during the entire irrigation period (IP), an overirrigated treatment (150-ETc); which was irrigated at 150% IR during all the IP, and a RDI treatment (RDI_{65}) which was irrigated covering 100% of the IR during all the IP except in the months of kernel filling where it was irrigated at 65% of the IR. In the second orchard, the experiments were carried out in 2019 and 2020 and three irrigation treatments were established: Control (FI) that was irrigated at 100% IR during all the IP, and two SDI treatments (SDI_{75} and SDI_{65}) which received 75 and 65% of IR during the IP. The IP in both experiments was from March to October. The response to irrigation strategies was monitored at the agronomic level [almond yield, kernel weight, irrigation water productivity (IWP),

among other factors], physiological level [leaf water potential (Ψ_{leaf}), stomatal conductance (g_s) and photosynthetic capacity (measured only at the Cartuja orchard in each phenological stage)] and the quality of almond [physical-chemical, morphological and sensory parameters].

At the agronomic level, it was found that the cultivars response to the irrigation strategies was very different. Indeed, cv. Guara produced in terms of kernel yield ($kg \cdot ha^{-1}$) about 30% higher than the cvs. Marta and Lauranne with RDI strategy. However, under SDI strategy, the cvs. Marta and Lauranne obtained the highest yields (~2,200 $kg \cdot ha^{-1}$), while cv. Guara reached ~2,000 $kg \cdot ha^{-1}$, although these differences were not statistically significant. Nevertheless, the yields in the most restrictive treatments (in both DI strategies) were very similar to the control trees, which indicates that the yield losses were minimal compared to the savings in water and energy. Regarding the tested irrigation treatments, it is worth highlight the response obtained by the three cultivars to the overirrigated treatment in which only cv. Marta yielded 46% more than the FI treatment. In relation to the IWP, both DI strategies improved the use of water, showing similar values ~0.40-0.50 $kg \cdot m^{-3}$ in the deficit treatments.

Relating to the physiological response, the water stress produced by the imposed deficit irrigation treatments was not enough to affect the g_s, though, the Ψ_{leaf} was affected; evidencing the almond capability to cope with drought. In terms of cultivars, the greatest water stress level was observed in cv. Guara under the SDI strategy, which could justify its lower yield in this irrigation strategy. In reference to the photosynthetic capacity, the highest photosynthetic rate (A_N) was shown in the vegetative stage and kernel-filling (14-19 $\mu mol \cdot m^{-2}\ s^{-1}$) while it decreased during postharvest (10-12 $\mu mol \cdot m^{-2}\ s^{-1}$). On the other hand, the use of triose phosphate (TPU) was higher in the vegetative period (12.47 $\mu mol \cdot m^{-2}\ s^{-1}$) and then progressively decreased in kernel-filling and postharvest stages (5.74 $\mu mol \cdot m^{-2}\ s^{-1}$). This showed that although the A_N was still high during the kernel-filling stage, the TPU decreased. This fact could be related to a possible limitation by the maximum rate of electronic transport (J_{max}) during the kernel-filling stage causing a down-regulation in the photosynthetic capacity of the almond tree and explaining why this period could considered the least sensitive to drought conditions.

Finally, taking into consideration the quality parameters of the almonds, the physical-chemical parameters that were most affected by the application of DI were sugars (sucrose and glucose), fatty acids [oleic/linoleic ratio, monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA)] and organic (oxalic and citric acids)]. Concerning sugars, they were decreased by the overirrigated treatment (40-50 g·kg^{-1} for the irrigated treatment and 50-60 g·kg^{-1} in the deficit treatment), which indicates that an excess of water is not beneficial to accumulate sugars in the almond nuts. With respect organic and fatty acids, they showed a very noticeable cultivar effect, being cv. Lauranne registered the highest concentration, cv. Marta the highest content of MUFA, and cv. Guara the best morphological characteristics when applying DI. The data obtained at the physicochemical level was transferred to the sensory level in the panel of tasters. In this sense, the dilution of sugars in the overirrigated treatment was perceived at a sensory level, since the less sweet almonds on the palate were of the overirrigated treatment. In terms of crispiness, this variable depended more on the cultivars than on the irrigation treatments because cv. Marta in RDI$_{65}$ had the lower crispiness but cv. Guara in RDI$_{65}$ had the highest one.

According to the findings in this doctoral thesis, it can be concluded that DI strategies are a good tool to improve irrigation management in almond, being the RDI strategy which showed the best results at the level of final yield. The most suitable period to apply water restrictions in this crop, through the monitoring of the main photosynthetic limitations at phenological stages, it can be concluded that kernel-filling stage would be the best stage to apply restrictions without important impact on final yield. At the cultivars level, it is necessary to recalculate the irrigation doses based not only on the irrigation strategy, but also on the cultivar. Not all cultivars need the same amount of water as is the case with cv. Marta in which the water demand is higher than cv. Guara or Lauranne.

In relation to the quality of almond nuts, it has been shown that the water stress improved its quality at a physical-chemical and sensory level, which promotes the marketability of almonds by providing an added value in the market. Thus, under the current climate change scenario and water, scarcity, especially in arid and semi-arid areas of south Spain, it is crucial to implement water saving strategies able to produce hydrosustainable products as the case of almonds.

Taking into account the framework of this doctoral thesis, it is evident that the almond crop is a profitable and viable option in the long term when subjected to DI strategies.

El cambio climático, la reducción de los recursos hídricos o la degradación y el abandono de la tierra son algunas de las limitaciones que hay que superar en la búsqueda de una agricultura competitiva y sostenible. Dentro de las posibles estrategias, debe considerarse la implementación de cultivos tolerantes a la sequía con alta rentabilidad y la implementación de estrategias de ahorro de agua como las estrategias de riego deficitario (RD). Dentro de las posibilidades de cultivo, el almendro (*Prunus dulcis* Mill.) sería una excelente alternativa ante un escenario de escasez de agua y cambio climático. Sin embargo, es esencial alcanzar un equilibrio entre la gestión de cultivos y la disponibilidad de agua, definiendo sus necesidades hídricas y las relaciones entre las necesidades de riego y las prácticas agronómicas.

En este sentido el objetivo general de esta tesis doctoral fue mejorar el manejo del riego del cultivo del almendro, concretamente en tres de las variedades más implementadas en el sector almendrero (cvs. Guara, Marta y Lauranne) usando dos estrategias de RD diferentes; riego deficitario controlado (RDC) y riego deficitario sostenido (RDS). Con el objetivo de optimizar el manejo del agua de riego para cada variedad estudiada y aumentar la calidad de la almendra obtenida.

El estudio se llevó a cabo en dos fincas comerciales de almendro; "Montana de San José" y "Cartuja". En la primera finca los experimentos se llevaron a cabo durante las campañas 2017 y 2018 y se estableció tres tratamientos de riego: Control (FI); el cual se rego al 100% de las necesidades de riego (NR) durante todo el periodo de riego (PR), un tratamiento sobre regado (150-ETc); el cual se regó al 150% NR durante todo el periodo de riego y un tratamiento de RDC (RDC_{65}) el cual se regó cubriendo el 100% de las NR excepto en los meses de llenado de grano donde se regó al 65% de las NR. En la segunda, los experimentos se llevaron a cabo en las campañas 2019 y 2020 y se establecieron tres tratamientos de riego: un tratamiento control (FI) el cual se regó al 100% NR durante todo el PR, y dos tratamientos en RDS, (RDS_{75} y RDS_{65}) los cuales recibieron 75 y 65% de las NR durante todo el PR. El PR en ambas fincas experimentales fue de marzo a octubre. Se monitorizó la respuesta a ambas estrategias de riego a nivel agronómico [producción final

obtenida almendra grano, peso de grano, productividad del uso del agua, entre otros factores], a nivel fisiológico [potencial hídrico de hoja (Ψ_{hoja}), conductancia estomática (g_s) y capacidad fotosintética (medida solo en finca Cartuja y en cada uno de los periodos fenológicos del cultivo)] y de calidad de la almendra [parámetros físico-químicos, morfológicos y sensoriales de la almendra].

A nivel agronómico, se obtuvo que la respuesta varietal a las estrategias de riego fue muy diferente. En este sentido, cv. Guara obtuvo en términos de producción final de almendra grano ($kg \cdot ha^{-1}$) un 30% más que las cvs. Marta y Lauranne bajo la estrategia RDC. Sin embargo, bajo la estrategia SDI, cvs. Marta y Lauranne obtuvieron las mayores producciones almendra grano (~2.200 $kg \cdot ha^{-1}$), mientras que cv. Guara alcanzó ~2.000 $kg \cdot ha^{-1}$, aunque estas diferencias no fueron significativas. Sin embargo, las producciones almendra grano en los tratamientos hídricos más restrictivos (en ambas estrategias de riego) fueron muy similares a las del tratamiento FI, lo cual indicaría que las pérdidas de producción son mínimas con los ahorres de energía y agua. Respecto a los tratamientos de riego ensayados, hay que resaltar la respuesta obtenida por las tres variedades al tratamiento sobre regado en el cual solo la cv. Marta produjo un 46% más que el tratamiento FI. En relación a IWP, ambas estrategias mejoraron el uso del agua, mostrando valores similares ~0.40-0.50 $kg \cdot m^{-3}$ en los tratamientos deficitarios.

En referencia a la respuesta fisiológica, el estrés alcanzado por los tratamientos de riego impuestos fue suficiente para afectar a g_s, sin embargo, el Ψ_{hoja} si se vio afectado; evidenciando la capacidad del almendro para hacer frente a la sequía. En relación a las variedades el mayor estrés hídrico lo presentó cv. Guara bajo la estrategia RDS lo cual puede justificar su menor producción en esta estrategia de riego. En referencia a la capacidad fotosintética, se obtuvo que la mayor tasa fotosintética (A_N) se alcanzó en el periodo vegetativo y llenado de grano (14-19 $\mu mol \cdot m^{-2} \cdot s^{-1}$) mientras que disminuía en postcosecha (10-12 $\mu mol \cdot m^{-2} \cdot s^{-1}$). Por otra parte, el uso de las triosas fosfato (TPU) fue mayor en el periodo vegetativo (12.47 $\mu mol \cdot m^{-2} \cdot s^{-1}$) y luego disminuía progresivamente en el llenado de grano y postcosecha (5.74 $\mu mol \cdot m^{-2} \cdot s^{-1}$). Esto demostraba que a pesar de que la A_N seguía siendo elevada en el periodo de llenado de grano el TPU se veía disminuido. Esta disminución podría estar relacionado con una posible limitación por parte de la tasa máxima de transporte electrónico (J_{max}) en el periodo de llenado de grano que provocó una *down-regulation* en la capacidad fotosintética del almendro y que podría explicar por qué este periodo es menos sensible a condiciones de estrés hídrico.

Finalmente, teniendo en cuenta los parámetros de calidad de la almendra, los parámetros físico-químicos que fueron más afectados por la aplicación de RD fueron los azúcares (sucrosa y glucosa), ácidos grasos [ratio oleico/linoleico, ácidos grasos monoinsaturados (MUFA), ácidos grasos polinsaturados (PUFA)] y ácidos orgánicos (ácidos oxálico y cítrico). En lo referente a los azúcares, se vieron disminuidos por el tratamiento sobre regado (40-50 $g \cdot kg^{-1}$ en el tratamiento sobre regado y 50-60 $g \cdot kg^{-1}$ en el tratamiento deficitario), lo cual indica que un exceso de agua no es beneficioso para acumular azúcares en el almendro. Respecto a los ácidos orgánicos y grasos, mostraron una respuesta varietal muy marcada siendo cv. Lauranne la que mayor concentración de azúcares mostraba, cv. Marta el mayor contenido de MUFA y cv. Guara la que mejor características morfológicas presentaba a la hora de aplicar RD. Los resultados obtenidos a nivel físico-químico fueron los detectados a nivel sensorial en el panel de catadores. En este sentido, la dilución de los azúcares en el tratamiento sobre regado fue percibida también a nivel sensorial, ya que las almendras de este tratamiento en el paladar fueron menos dulces. En cuanto a la crujibilidad, dependió más de las variedades que de los tratamientos de riego debido a que el cv. Marta en RDI_{65} tuvo la crujibilidad más baja, pero cv. Guara en RDI_{65} obtuvo la más alta.

De acuerdo a los resultados obtenidos en la presente tesis doctoral, se puede concluir que las estrategias de RD son una buena herramienta para mejorar la gestión del riego en el cultivo del almendro, siendo la estrategia RDC la que mejores resultados muestra a nivel de producciones finales. En referencia a cuando aplicar las restricciones hídricas en este cultivo, a través de la monitorización de las principales limitaciones fotosintéticas en los estados fenológicos, se puede concluir que el periodo de llenado de grano puede ser el mejor momento de aplicarlas sin penalizar la producción final. A nivel varietal, es necesario recalcular las dosis de riego basadas no solo en la estrategia de riego seleccionada, sino también en la propia variedad. No todas las variedades necesitan la misma cantidad de agua, como es el caso de cv. Marta en el cual la demanda es mayor que en la cvs. Guara o Lauranne.

En relación a la calidad de la almendra, se ha visto que el estrés hídrico mejora la calidad de la almendra a nivel físico-químico y sensorial, lo cual mejora la comerciabilidad de la almendra aportándole un valor añadido en el mercado. Por lo tanto, bajo el escenario actual de cambio climático y escasez de agua, especialmente en las zonas áridas y semiáridas del sur de España, es fundamental implementar estrategias de ahorro de agua capaces de producir productos hidrosostenibles como es el caso de las almendras.

Teniendo en cuenta, el marco de desarrollo de esta tesis doctoral, se evidencia que la implantación del cultivo del almendro en esta zona es rentable y viable a largo plazo cuando se somete a estrategias RD.

Chapter 1

Introduction and Objectives

Part of the introduction has been published as a book chapter "Linking Sustainability and Competitiveness of Almond Plantations under Water Scarcity and Changing Climate", in Resources Use Efficiency in Agriculture. Springer, Singapore 695-728.

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1. Introduction

1.1 Agriculture under climate change scenarios.

There is a consensus about the weak management of water resources in the Mediterranean areas of southern Europe, and the need of reaching an equilibrium between rural development, food security, and environmental protection[1,2]. Climate change will significantly affect this equilibrium, with even higher challenges concerning the sustainable management of natural resources, these being between the main constraints to be solved[3–7].

Different works have been recently developed to assess the effects of climate change and its impact on the agricultural systems[8–12]. On overall, these studies have remarked the unsustainability of the current management systems of water at farm level, especially in regions of southern Europe[13,14], with particular emphasis in south Spain[15,16]. Rising temperatures will cause changes in hydrological cycles affecting precipitation and evaporation[17]. The increase in rainfall observed in the mid and high latitudes contrasts with reductions in the northern sub-tropics and with the fact that the area affected by scarcity has been increasing since 1970[17]. According to this, Mediterranean countries of southern Europe will be significantly affected in the future by climate change scenarios, with significant increases in the average air temperature (>2–4 ^{o}C), more heat waves events, or decrease in precipitations (~30%), which will increase the risks of drought and biodiversity losses and decreases in crop yields[18]. Moreover, climate change will promote not only substantial changes about the natural resources management, but also in the crop phenological development; these changes being associated with a shortening in the crops cycles, an earlier flowering, and a higher heat and water stress[19–21]. In this regard, there are three key factors that ultimately will cause significant changes in crops development: higher temperatures, water resources depletion for crop development (-15 to -25%)[22], and the increase of atmospheric carbon dioxide (CO_2)[23,24], which could reach values close to 700 ppm[10,25].

In relation to how much climate change affect to phenological stages, the works developed are relatively scarce and almost all of them are related to the temporal variations of the different phenological stages as it has been recently reported by

Gabaldón-Leal et al.[19] and Lorite et al.[21] in olives. In this context, according to De Ollas et al.[26], phenological changes on fruit trees derived from climate change will probably determine not only the yield but also the fruit quality and marketability. Thus, it is expected that higher temperatures during the flowering and fruit-setting period could promote a massive flower dropping, with significant reductions in the yield, as it has been suggested by other authors such as Albrigo and Saúco[27] and Iglesias et al.[28].

In relation to the increase of CO_2, currently, there is no clear consensus about the interactions between the increasing atmospheric temperature and CO_2 concentration, and the expected water scarcity scenarios[29]. Authors such as Medlyn[30], Flexas et al.[25] have suggested that the increase of CO_2 content could be accompanied by a reduction in the crop transpiration (E) levels, and hence higher intrinsic water-use efficiency (WUEi). On the contrary, some authors have observed in plants grown under high CO_2 content during long-term periods, some modifications in the parenchyma of mesophyll and the chloroplasts, a decrease in the photosynthetic rate (A_N), alterations in the ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) activity and during the photorespiration[31,32].

The European Environment Agency (EEA) has recently described the main impact derived from climate change, among them, significant alterations in the average temperature, a higher frequency of extreme events, and the rainfall irregularity[33,34]. These constraints will affect not only to the crops final yield and its components, but also put the detrimental effects in the remaining processes such as the storage, transport conditions, and/or product transformation. Moreover, these effects are not appearing in the same way along the European Union (EU), the Mediterranean countries being the most affected, especially the southern regions. As a consequence, a progressive deterioration of rural areas, and a descend in terms of productivity of agroecosystems, and, ultimately, the land abandonment is expected[35]. In this sense, the agricultural sector will require a rapid adaptation with the aim of ensuring a sustainable production throughout crop management practices at the farm level[36].

As a response, the EU have included as the primary objective for the new Common Agricultural Policy 2021–2027, the promotion of practices to ensure the adaptation and mitigation to the climate change; throughout investments, incentives, and improving the final returns[37]. According to Iglesias and Garrote[38], under these environmental conditions, the use of adapted crops to arid and semi-arid environments or the use of tolerant cultivars to drought must be seriously considered. Also, the use of different techniques related to precision agriculture and the improvement of the irrigation water productivity (IWP) is within the whole of the required actions. In this sense, at the farm level, the implementation of these strategies will also encourage for the sustainability, profitability, and viability of Mediterranean agroecosystems. These actions are even more necessary in those regions where the agricultural intensification have promoted land degradation, such as in many rural areas as south Spain[39,40].

1.2 Almond production in a global, national and regional scale.

Almond (*Prunus dulcis* Mill.) does not represent a newness crop in the south of Europe, this being widely cultivated in many Mediterranean countries such as Italy, Greece, Syria, Tunisia, Argelia, and Morocco, although, up today, Spain is the most representative country in terms of surface worldwide (Fig. 1.1A). However, these data contrast with those related to the crop productivity (in terms of the surface), the USA and Australia being the most relevant producers (Fig. 1.1B,C), providing 80% of the global market[41].

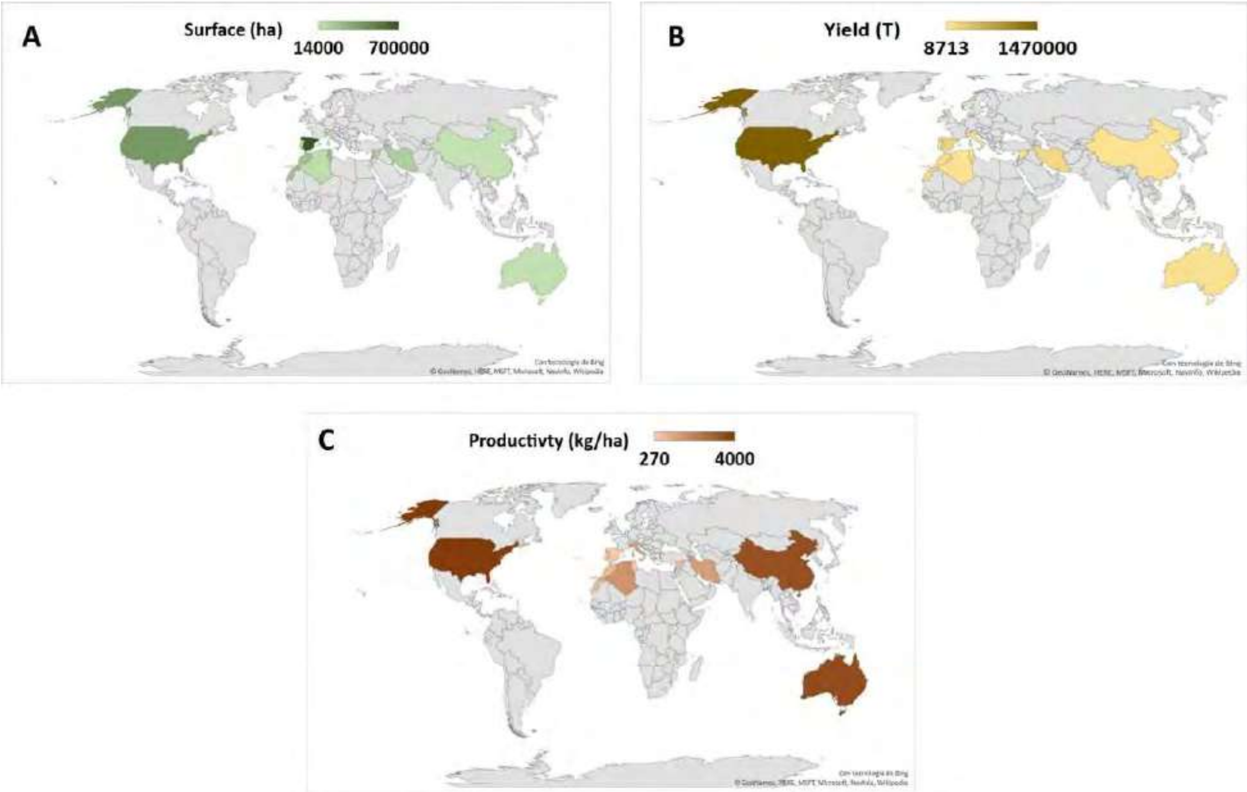

Figure 1.1 Almond surface (A), yield (B), and productivity (C) worldwide. Data resources: FAOSTAT.

Moreover, the USA, with a surface close to 400,000 ha, is able to reach an annual production of almonds close to 1.5 million tonnes, with an average productivity between 3,500 and 4,000 kg·ha^{-1}. By contrast, Spain, with 700,000 ha, produces ~200,000 tonnes and the average productivity[40] would be close to 300 kg·ha^{-1}. These values are related to the water availability because almond has been traditionally cultivated under rainfed conditions in marginal areas of south Spain[42].

The almond represents the third crop in terms of area in Spain, contributing to 84% of European production and 5% of world production[41]. In Andalusia (S Spain), there are just over 191,361 ha, of which almost 88% are in dry conditions; and the rest, are mostly plantations, relatively new, and deficient in terms of water demand versus availability[43]. By provinces, Almeria and Granada cover a good part of the cultivated area (53,621 ha and 97,543 ha, respectively); although, in recent years, there has been a very significant increase in the area dedicated to almond cultivation in provinces such as Córdoba or Seville (35% and 27%, respectively) most of the new plantations in irrigated areas in the Guadalquivir River basin. Thus, of the 30,000 new hectares dedicated to almond plantation between 2013 and 2018; 25,000 ha were put into operation in irrigated areas; largely due to the increase in prices registered in recent years and the stability achieved by them[43]. Despite this, in general terms the productivity of this crop in Andalusia (as in Spain) is low, since the production of the 191,361 ha available is 161,546 tn.

Recently, a significant increase in the surface devoted to this crop has been observed, especially in irrigated areas traditionally occupied by other species[44]. This fact has been primarily associated with the relevant increases in the almond prices during 2014–2016, and after this, price stability around to 6 euros per kg[45].

As a response, this crop has been progressively introduced under irrigated conditions, to be developed under those traditional strategies of management designed in those countries where the maximum productivity is reached. Under these circumstances, it is worth to consider the possibilities and capability of these viable management strategies under the current conditions registered in Mediterranean countries such as Spain.

In the case of almond, despite being a drought tolerant crop, water availability is the most limiting factor to reach maximum yield values in terms of number and size of fruit[46]. It has been shown that optimum water requirements for the almond crop would range between 9,000 and 13,500 $m^3 \cdot ha^{-1}$, depending on location, rootstock, variety, canopy size, and tree spacing[46,47]. Thus, considering the water requirements of the almond, its acceptance as an alternative crop would be exclusively justified within an equilibrium between the crop management and the water availability, focusing the efforts in search of equilibrium among agricultural activity, competitiveness, and environmental protection[48]. Thus, exclusively from the acceptance limitations of production systems it will be possible to maximize the final yield, the fruit quality, and redesigning the irrigated agriculture for environmental constrains under climate change context.

1.3 *Phenological stages of almond: The importance of the crop phenology in irrigation management*.

The sharp phenology of almonds allows differentiating the main effects of water stress, depending not only on the intensity, but mainly its phenological development (Fig. 1.2), characterized by different stages dormant, bloom (Stage I), fruit growth, and vegetative development (Stage II), kernel-filling with dry-matter accumulation and pre-harvest (Stage III), and post-harvest, when reserves accumulation and buds differentiation occurs before leaf-fall[49].

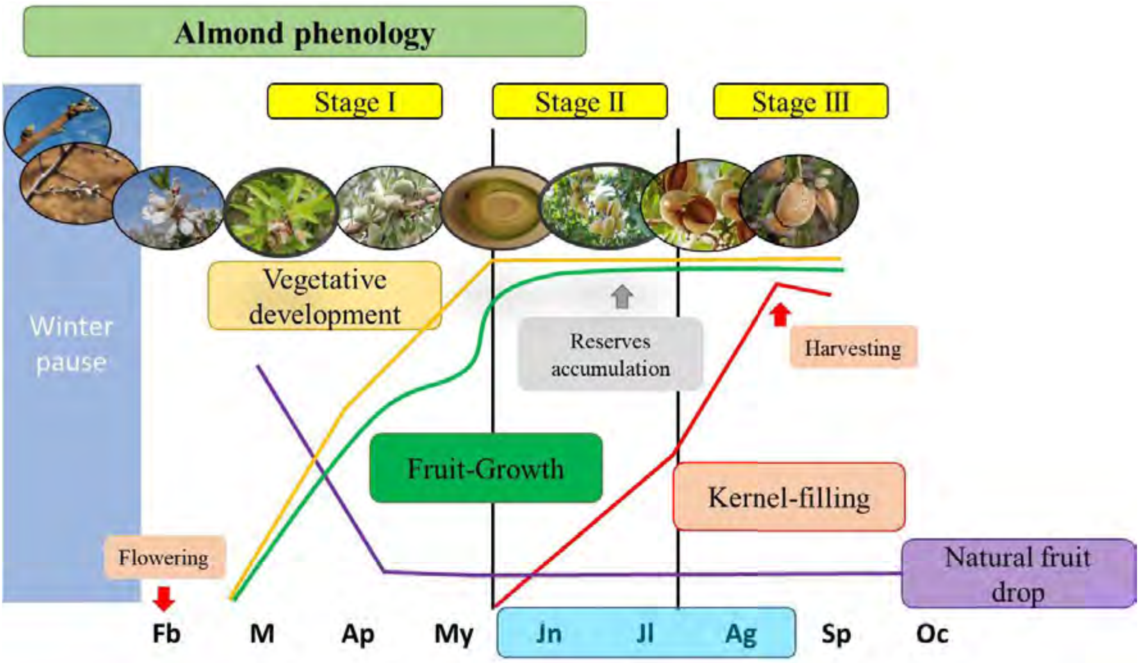

Figure 1.2 Almond tree phenology throughout the nut production process.

In Mediterranean countries, almond flowering and its vegetative development occur in the first months of the year. The harvesting occurring between the end of July and September, depending on the cultivar and the registered climatic conditions[50]. Flowering and canopy growing take place almost simultaneously, once the crop has accumulated the necessary cold hours (number of hours below to 7.2 °C). According to Tabuenca[51], this requirement is highly cultivar-dependent, and it can range between 150 and 220 h for cultivars such as Desmayo Largueta, Marcona, or Nonpareil; between 220 and 350 h for varieties such as Ferraduel, Primorskii, Texas Drake, or Guara; or even up to 350 h for Cristomorto, Ferragnès or Yaltinski, and start to increase the temperatures at middle-end of winter. These processes are going to be directly affected by the stored reserves in the previous season during the end of Stage II and Stage III, just before the leaf-fall process. In this agreement, during pre-harvest and after this, the carbohydrates accumulation occurs, and ultimately, it will directly affect the yield potential in the following season[52], not only in terms of flowering potential but determining the fruit-setting and growing in the next season[53].

Considering this sharp differentiation in the almond phenological development, different authors have pointed out relevant results for strategies in which the water stress imposed at different phenological stages through deficit irrigation strategies. Girona et al.[54,55] and Micke[56] concluded that water stress imposed during Stage I could promote fruits abortion, small fruits, and poor canopy development, which ultimately will affect the photosynthetic capacity. However, considering the climatic conditions registered in Mediterranean countries during this period, with a low evapotranspiration demand and a scarce canopy development during the first months, it would be very difficult to reach severe water stress situations. Something similar occurs when a water stress is imposed during Stage III. Although after harvesting, the crop water demand progressively comes down, if drought occurs, significant adverse effects on yield can be registered for the following season. In this agreement, authors such as Micke[56], Goldhamer and Viveros[57], and Romero et al.[58] reported adverse effects on bud differentiation and carbohydrates accumulation, these facts being reflected during the fruit-setting and vegetative development in the coming year.

Considering all these facts, after flowering, the presence of carbohydrates reserves is necessary to ensure proper shoots development together with the initial fruit growth, coinciding with a fast cell division process. However, the bloom is determined by the crop status during the previous year, until as vegetative development is moving forward, the crop produces photoassimilates, this being determinant for the following stages of fruit growth[50].

Therefore, fruit size would be affected by the available resources during the first stage. Flowering and fruit set are directly influenced by the reserve's accumulation during the previous season[53] and the fruit growth is more dependent on the water and nutrients provided to the crop during the current season.

Moreover, several authors reported the exceptional capability of the almond crops to offer an excellent response to water stress when it is imposed during the kernel-filling stage (phenological stage II). Goldhamer et al.[59] or Romero et al.[58] concluded that the optimum response of almond to water withholding occurs during this stage. Lately, García-Tejero et al.[60] did not observe any significant difference either in terms of kernel weight and final yield when water stress was applied during the kernel-filling stage. On the contrary, Girona et al.[55] observed yield losses when water withholding was applied during kernel-filling stage, mainly because of depletion in the dry mass accumulation.

Thus, there is no scientific consensus on when to apply water stress in almond to save water without severely penalizing yield.

1.4 Almond cultivars differentiation to increase the marketability and quality of almond nuts.

The propagation of the almond tree is carried out through the union of a part that provides the root, known as rootstock, and another part that provides the aerial part (known as cultivar)[61]. The part of the cultivar is the one that is commercialized and it is the one that must be well known to ensure a good yield. There are many varieties of almond trees, which can be classified into two main groups; hard-shell and soft-shell cultivars; the first ones being mainly developed in European countries; and the second ones in USA. The main cultivars obtained in USA are; Nonpaeril, Carmel, Monterey, Sonora and Butte among others[61]. The main cultivars by country in the Mediterranean area are; Ferraduel, Ferragnès, Lauranne, Ferrastar and Ferralise among others in France[61], Supernova, Tuono, Genco and Cristomorto among others in Italy and Marcona, Desmayo Largueta, Guara, Marta, Antoñeta, Vairo, Soleta, Penta, Glorieta, among others in Spain.

The almond market in Spain has a simple characterization based on the name of the cultivar (Table 1.1), three main groups being defined in order to prize establishment and commercialization: Marcona, Largueta and "Comuna".

Table 1.1 Spanish almond market organization.

Spanish almond market	
Name of the group	Characteristics
"Marcona"	Almonds of the Marcona cultivar. They are characterized by having a sweet flavor, round and white grain. It is considered the best cultivar in the market.
"Largueta"	Almonds of the cultivar Desmayo. It is characterized by having an elongated grain. They are used in the industrial pastry and their market is in Italy and Germany.
"Comuna"	It is the most important group in reference to its commercialization (50% of Spanish exports). A large number of varieties form part of this group (cvs. Guara, Marta, Lauranne, Antoñeta, Ferraduel, Ferragnès, etc). Its shell is hard.

Regarding the price of almonds, in order to establish it for each of the seasons, it is necessary to know in advance both current stocks and harvest forecasts in the main production areas. From this perspective, in the USA the farmers are in charge for establishing the almond price for each year (Agricultural Statistics Service in California with funding from Almond Board of California), which for every season carries out an appraisal to predict the harvest. Two appraisals are carried out, one at the beginning of May with estimated results, the other with real results at the end of June/July[61]. In the case of Spain, these appraisals are achieved by the Confederation of Agricultural Cooperatives of Spain on a sample of 104,000 ha.

In this line, as the market moves by the law of supply and demand and as almonds are a product that can be stored if prices are not favourable, the harvest is usually stored waiting for the change in the market trend. The weekly progress of the prices, published in the different markets, allows knowing the prices every Monday. In Spain, the best known are Slice of Reus, Albacete, and Murcia (Fig.1.3); responsible for collecting the indicative and non-binding values of the different

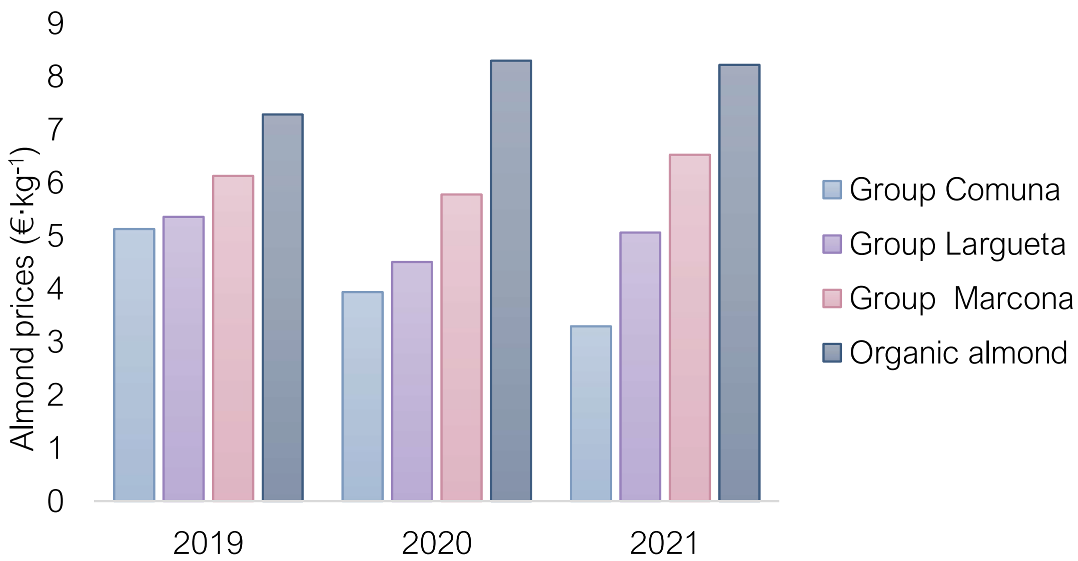

Figure 1.3 Almond prices in slice of Murcia. Data resources: historical data of Slice of Murcia (MercaMurcia).

cultivars at the national level.

The Spanish almond market has focused on the cultivar Marcona, this being (within conventional management) the one that has had the highest price in the market (Fig. 1.3). However, several authors[62,63] have shown that the group Comuna have a similar almond quality to the Marcona cultivar.

The amount of irrigation water supplied to the almond is a factor that will directly influence on the nut's quality. According to the morphological parameters, fruit weight, size, colour, and texture are the most relevant parameters that could be modified when a water stress is imposed, being possible to find different responses in terms of the cultivar. In this regard, it is worth mentioning the most relevant results obtained by Lipan et al.[64] in cv. Vairo (group Comuna) irrigated under two different water stress strategies in which it has been concluded that water stress do not affect almond fruit quality; being possible to increase the final quality of nuts. Recently, Lipan et al. [65] in the same cultivar (cv. Vairo) evidenced the use of moderate water stress significantly increased the total phenolic content in skin, polyphenols and proanthocyanidins (PAs), and the antioxidant activity.

Very few research groups have carried out consumer opinion surveys to verify the influence of water stress strategies on the final harvested product[66,67]. However, Noguera-Artiaga et al.[68] proposed an identifying brand for the products obtained under DI strategies and crop management (named as hydroSOS); as strategy to stablish a quality certification to recognize those products that have been obtained under sustainable and environmental friendly strategies; particularity, under water saving practices. Under this brand would be included those products obtained under DI strategies, many of them being benefited from an increase in secondary metabolites and quality composition increasing their functionality. In study by Noguera-Artiaga et al.[68] was found that consumers were willing to pay more for hydrosustainable products under a well-identified label.

1.5 Deficit irrigation strategies to achieve hydrosustainable and competitive almond yields under water scarcity scenarios.

According to Allen et al.[69], almond water requirements are defined to cover the evapotranspiration losses under optimum conditions; that is, a disease-free crop, without nutritional deficiencies and proper soil characteristics. Total almond water requirements have been intensely studied under very different conditions[70–73]. The actual annual water requirements in mature almond trees in California[46] would be close to 13,000 $m^3 \cdot ha^{-1}$ or for the case of south Spain[47,72], close to 8,000 $m^3 \cdot ha^{-1}$ about 50% higher than those estimated three decades ago in orchards with yields that were practically half of current yields (~3,500 – 4,000 $kg \cdot ha^{-1}$)[42,46,57]. Within a context of water scarcity scenarios, it is noteworthy to consider those strategies focused on reaching an equilibrium between the crop management and water requirements.

Almond has been traditionally considered as a proper alternative under drought scenarios, and for this reason, its development has been traditionally associated with rainfed conditions in many areas of south Spain[74,75]. Within the advantages of this crop would be its sharp phenology, which promotes different results depending on the phenological period in which the water stress is imposed. In this line, deficit irrigation (DI) is defined as the application of water below full crop-water requirements without compromising the final yield[76]. Different key factors should be considered when a DI strategy is imposed, such as the irrigation strategy, crop phenological development, and threshold values of physiological indicators. On overall, water stress strategies imposed in almond can be defined in four different ways (Fig. 1.4):

- Sustained deficit irrigation (SDI)[77], consists of applying a sustained water reduction throughout the whole growth cycle. This strategy is applied to achieve an equilibrium between canopy and fruit development.
- Regulated deficit irrigation (RDI)[78], characterized by applying a smaller amount of water in the period in which the crop is less sensitive to this withholding of water. This strategy is focused on the sharp differentiation in the phenological development.
- Low-frequency deficit irrigation (LFDI)[79], consists of applying irrigation-restriction cycles which are derived by means of physiological threshold values previously defined. Thus, its application has required proper crop water monitoring.
- Partial root drying (PRD)[80], part of the root system is exposed to drying soil while the remaining part is irrigated normally. This strategy aimed to the chemical signals produced under water stress conditions, which are responsible for the control of leaf stomata.

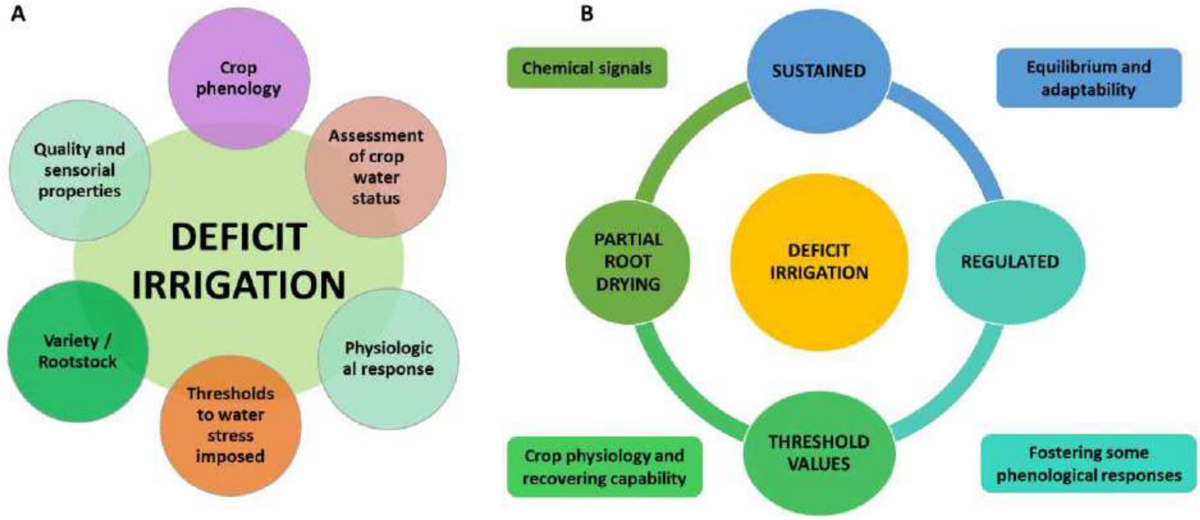

Figure 1.4 Key factors under deficit irrigation (A) and different types of strategies with the main characteristic (B).

Many authors have reported the advantages and opportunities of DI in the almond crop, this being able of obtaining competitive yields under moderate to severe water stress situations (Table 1.2).

Table 1.2 Deficit irrigation studies concerning almond yield and its components.

DI strategies defined	Main conclusions	References
RDI during the kernel-filling stage.	RDI did not promote significant reductions in kernel yield without effect on its size. Improvements on water use efficiency (WUE) with 30% less irrigation water respect to control trees were reached.	Romero et al.[58]
RDI during the kernel-filling stage.	During the first two seasons, kernel dry matter accumulation did not decrease with RDI. Yield and kernel growth were reduced during the third and fourth seasons. Yield reductions for RDI were significant and water savings close to 60%.	Girona et al.[81]

Three PRD and RDI during the kernel-filling stage.	Kernel yield showed a linear decrease with decreasing water applied that means that a 1% water reduction lead implies 0.43% in yield.	Egea et al.[82]
RDI during kernel-filling stage receiving, moderate and severe sustained deficit irrigation (SDI_m SDI_s).	The water stress imposed had not intensified the negative impact of deficit irrigation on final yield. Irrigation water productivity (IWP) increased with water stress. RDI and SDI_m showed similar responses. Therefore, the SDI_s appears to be the best option under severe water scarcity conditions.	Egea et al.[83]
The use of HYDRUS-2D model for drip-irrigated almond orchard, evaluating the daily fluctuations in water under: full pulsed (FIp) with replacing of 100% ET_C, sustained deficit pulsed (SDIp) irrigated to replace 65% ET_C, and full continuous (FIc) irrigation with replacing of 100% ET_C.	Water uptake efficiency under SDIp (68%) was higher respect to full water application of FIp and FIc (54-55%). The IWP increased (37%), the yield was reduced by 8%, and 35% of irrigation water was saved with SDIp compared to FIp. Thus, SDIp appears to be a promising strategy, and irrigating almonds above the SDIp level may enhance unproductive water usage in the form of accelerated drainage.	Phogat et al.[84]

Six irrigation treatments: no irrigation, SDI irrigated at 25% ET_C, RDI irrigated at 50% ET_C with an exception during the kernel-filling stage irrigated at 15% ET_C, SDI irrigated at 50% ET_C, RDI irrigated at 100% ET_C with the exception during the kernel-filling stage irrigated at 20% ET_C, and a control.	Significant differences in nut yield and WUE among irrigation treatments were found. The optimum yield response was from control treatment throughout the study period. Additionally, there were no significant differences in almond production and WUE between RDI and SDI strategies.	Mañas et al.[85]
Five irrigation treatments: control at 100% ET_C, three RDI levels applied for specific periods during the growing season, or SDI throughout the growing season, and a high irrigation level at 120% ET_C.	Irrigation at 85% ET_C had no impact on kernel weight and yield, but 70% ET_C or 55 % ET_C decreased kernel yield regardless of strategy, except for SDI 70%. During the last season, trees with SDI produced higher kernel yield than those subjected under RDI.	Monks et al.[79]
Four irrigation treatments: control, moderate SDI, moderate RDI irrigated as control, but only at 40% of control during the kernel-filling stage, and severe RDI irrigated as control trees and only 15% of control during the kernel-filling stage.	The maximum average yield of was obtained from control trees. Although values varied, water productivity averaged 0.23 $kg \cdot m^{-3}$ and did not differ among treatments.	López-López et al.[86]

Three irrigation regimes were defined: control, RDI_{50} irrigated at 50% ET_C during the kernel-filling stage, and LFDI during the kernel-filling stage.	Significant improvements for WUE were found, and no differences in nut yield between control and LFDI, leading to important water savings can be achieved without compromising the almond productivity.	García-Tejero et al.[77]

Even though many DI strategies for almond trees have been developed as shown in Table 1.2, up today, there are no precise conclusions in terms of yield when comparing SDI and RDI during kernel filling. Within this full of experiments, it is worth to remark some relevant results provided in the last few years under Mediterranean conditions. Goldhamer et al.[59] concluded that under moderate water stress conditions, and with similar irrigation amounts, SDI offered lower yield reductions compared to RDI, and even more, SDI allows to obtain similar productions to those registered under full irrigated conditions as reported by Girona et al.[55]. By contrast, Egea et al.[83] or Alcón et al.[87] did not found differences between SDI and RDI strategies in terms of fruit yield, through RDI trended to lower values than SDI.

Significant findings were revealed by García-Tejero et al.[77] for mature almond trees in a long-term experiment. These authors applied three irrigation treatments: a FI treatment; and RDI during the kernel-filling stage (50% of ET_C, RDI_{50}), and LFDI treatment (consecutive irrigation-restriction cycles during the same period of RDI_{50}). According to these findings, LFDI was able to obtain similar productions from those reported in FI during the studied years. Moreover, this treatment was liable for improving the yields registered in RDI_{50}, where this strategy offered significantly worse results under FI.

Relating to PRD strategies, Egea et al.[88,89] concluded that a PRD strategy that at the end of the irrigation period had received 50% ET_C, was able to obtain similar productions to those with an RDI strategy that on overall, had been received 20% more water than the previous one. More relevant results were obtained in a PRD strategy in which water withholdings close to 70% were imposed. This treatment offered similar yields to those obtained in the RDI_{70} previously discussed, without significant effects in terms of water potential and gas exchange parameters. These absences of differences suggest that PRD strategy did not show a relevant chemical signal from abscisic acid synthesis able to reduce the stomatal conductance (g_s) rates and maintain the leaf water potential values.

Although there are many authors who have worked evaluating the effects of the different available DI strategies, there are very few works that focus their efforts on comparing the productive response of different cultivars or their physiological response to water stress. Even those works in which two DI strategies are evaluated do so in a single cultivar or those that compare cultivars do so in a single DI strategy.

Therefore, in this doctoral thesis, the response of different cultivars to the application of different irrigation strategies will be studied, in order to apply irrigation more efficiently.

1.6 Physiological and productive water stress indices.

When a DI is imposed, it is necessary to monitor the water status of the crop to ensure that we are not causing damage in the plant[90]. There are many techniques to monitor the crop water status, but what all of them must meet the following conditions: have a physiological basis, robustness, representativeness, easy to use, low prices, continuous monitoring, and irrigation control[91]. In this line, the most used variables would be: leaf/stem water potential ($\Psi_{leaf/stem}$)[92,93], stomatal conductance (g_s)[90], and leaf turgor [94], trunk diameter[95], sap flow [96], canopy themperature[97,98], among others.

Agronomical response (total yield and its related components) is directly determined, although not only, by photosynthetic rate (A_N) because it conditions biomass accumulation. However, we studied the main changes occurring in almond trees when water stress is applied; especially in those physiological variables susceptible of being measured to ease the taking-decisions and irrigation scheduling; specially under water scarcity scenarios.

In this agreement, according to Hsiao[99] when plants are subjected to water stress, the first evidence are reflected in g_s reductions, this being a defensive response to reduce the water losses throughout stomata. Subsequently, this reduction would limit carbon assimilation which could be accompanied by other biochemical limitations at RuBisCO level and electron transport chain[89,100]. This apparent relationship between water stress and A_N reduction does not occur in the same way for the different plants species[100]. In this context, after different research experiences, it has been demonstrated that the resistance of almond to water stress is relatively high, comparing to other woody Mediterranean crops as olives[101]. In this line, Romero et al.[58] observed that g_s reductions close to 50% from its maximum rate would be accompanied by A_N depletion of only around 30%. Applying these reduction levels of g_s and A_N to the results obtained by García-Tejero et al.[77], it could be assumed that, a decrease of around 50% in Ψ_{leaf}, would promote depletion of carbon assimilation rate close to 15–20%, evidencing the high almond capability to keep maximum g_s values (~0.3 $mol \cdot m^{-2} \cdot s^{-1}$) even when Ψ_{leaf} values are close to -2.5 MPa. According to these findings, almond would be able to keep optimum rates of g_s, A_N, and hence increasing the WUEi[102]. By contrast, this down-regulation of g_s can be accompanied by a leaf senescence when drought conditions are very severe and supported during a long-term period[103]. By considering these physiological processes, it is determinant to define the most appropriate parameter to assess the crop physiological status, especially when DI strategies are being imposed to avoid significant effects on vegetative development, yield, and fruit quality.

1.7 Justification of the Doctoral Thesis

As agricultural land use expansion has increased the pressure on the available water in the Mediterranean area, important efforts must be made to develop efficient water management systems in almond cultivation and subsequent irrigation scheduling under the climate change scenario. One of the main reasons for the low productivity of the almond tree compared to other more productive areas, such as California, is the marginal location that this crop has in Spain and in Andalusia. That is why it is necessary to carry out an appropriate management of this crop under DI strategies. In this line, there are many works focused on evaluating yields in terms of kernel yield but with some contrasting results. Moreover, the studies focused on comparing the yield and quality of almond cultivars are limited. In addition, many few works have valued the nuts obtained under this type of DI strategies capable of increasing the functional properties of the nut. Therefore, the enhancement of nut quality through adaptive and water-saving strategies and the use of specific cultivars, could be represented an unquestionable advance for the Spanish almond sector under changing climate and limited water resources scenarios, allowing adaptation to new consumer demands, and guaranteeing quality throughout the food chain. And fundamentally, allowing the farmer to obtain a product with a high added value, which helps to compensate for the possible losses in yield derived from the implementation of this type of strategy.

All of the above mentioned, establishes the framework of this doctoral thesis; improving the available information regarding to almond cultivation under water scarcity conditions, taking into account the differential cultivar response, comparing physiological, productive and quality terms of the almond obtained from different DI strategies.

2. Objectives

The overall aim of this Doctoral Thesis was to improve the management of water in almond cultivation (cvs. Guara, Marta, and Lauranne) using different deficit irrigation strategies, considering varietal differentiation in a scenario of limited water resources. All of this with the purpose to reduce the irrigation water, increase the quality of the yield, identify optimal deficit irrigation strategies, and water stress achieved for each of the monitored cultivars.

To reach the main purpose, the following specific objectives were established:

- **Objective 1**. To identify the agronomic and physiological response of different almond cultivars (Guara, Marta, and Lauranne) under regulated deficit irrigation (RDI) and sustained deficit irrigation (SDI) strategies.

- **Objective 2**. To analyze the photosynthetic capacity of almond throughout phenological stages (vegetative, kernel-filling, and postharvest) in response to water stress in three almond cultivars (Guara, Marta, and Lauranne) by determining the main photosynthetic limitations in each phenological stages.

- **Objective 3**. To elucidate the effects of RDI and SDI strategies on the quality of almonds in terms of physical-chemical and sensory characteristics produced under water scarcity scenarios.

Chapter 2

Materials and Methods

1. Location, experimental design and irrigation treatments.

The experiments conducted in this doctoral thesis were carried out from 2017, to 2020 in two commercial almond orchards "Montana de San José" (37° 29′ 3.19″ N; 5° 59′ 55.1″ O) and "Cartuja" (37°30′27.4″ N, 55°50′48.7″ W) both located in La Rinconada (Seville, Spain) (Fig. 2.1). In Montana de San José, the experiments were carried out in 2017 and 2018, while in Cartuja in 2019 and 2020. In both commercial orchards the almonds were grafted onto GN15 rootstock, and the studied cultivars were Guara, Marta, and Lauranne. For the case of "Montana de San José" trees were planted in 2007 whereas for the case of "Cartuja" trees were planted in 2013. In both cases with trees spaced at 8×6 m, and drip irrigated using two pipe lines with emitters of 2.3 $L \cdot h^{-1}$, and 16 emitters per tree.

Figure 2.1 Satellite view of the experimental plots. (A). Montana de San José orchard. (B). Cartuja orchard.

In both orchards, the experimental design was of randomized blocks, with four replications per irrigation treatment and cultivar. Each replication had 12 trees (3 rows and 4 trees per row), the two central trees for each replication being used for physiological measurements and yield monitoring. Thus, eight trees per treatment of irrigation strategy were monitored (n = 8).

The climatic classification of the study area is attenuated meso-Mediterranean, with a hot-summer Mediterranean climate (csa) in the Köppen climate classification[103] an annual ET_0 rate of 1,400 mm and accumulated rainfall of 540 mm, mainly distributed from October to April and with an annual average temperature of 17-18 ^{o}C (historical data for the last 20 years; own development from the Andalusian Weather Information Network, depending on the Andalusian Institute of Training and Agricultural Research, IFAPA in Spanish[104]).

The soil in both orchards was classified as a silty loam typical Fluvisol (USDA, 2010) (Fig. 2.2), with more than 2.5 m deep, and organic matter content<1.5%. Roots were located predominately in the first 50 cm of soil (> 90%), corresponding to the intended wetting depth, although these exceed more than one meter in depth. Soil-water content values at field capacity (–0.033 MPa) and permanent wilting point (–1.5 MPa) are 0.42 and 0.17 $m^3 \cdot m^{-3}$ respectively, with an allowable soil-water depletion level of 0.35$m^3 \cdot m^{-3}$.

Figure 2.2 Soil sampling of the orchards and laboratory analysis.

Regarding irrigation strategies, for the case of Montana de San José (Fig. 2.3) three irrigation treatments were performed: i) a full irrigated treatment (FI), which was irrigated in order to cover the 100% of irrigation requirements (IR) during the irrigation period, ii) an overirrigated treatment (150-ET_C), which was irrigated at 150% of IR during the entire irrigation period; iii) and a regulated deficit irrigation (RDI_{65}) covering the full IR during the whole irrigation period, except during the kernel-filling stage (from the beginning of this to harvesting), when it was irrigated at 65% IR.

Figure 2.3 Montana de San José orchard. (A) cv. Guara. (B) cv. Marta. (C) cv. Lauranne. (D). View of the tree in the experimental plot.

Furthermore, in Cartuja a sustained deficit irrigation (SDI) strategy was applied (Fig. 2.4). Three irrigation treatments were designed; (i) a full irrigated treatment (FI), which received 100% IR during the irrigation period, (ii) a sustained deficit irrigation (SDI_{75}) treatment with 75% IR, and (iii) a sustained deficit irrigation (SDI_{65}) treatment with 65% IR.

Figure 2.4 Cartuja orchard. (A) cv. Guara. (B) cv. Marta. (C) cv. Lauranne. (D). View of the tree in the experimental plot.

Irrigation doses were calculated, in both experimental orchards, according to the methodology proposed by Allen et al.[69] (Eq. 2.1 and 2.2), obtaining the values of reference evapotranspiration (ET_0) by using a weather station installed in the same experimental orchard (Davis Advance Pro2, Davis Instruments, Valencia, Spain). The irrigation was applied from the middle of March to the end of October. The local crop coefficients (K_c) used during the experimental period (Table 2.1) ranged from 0.4 to 1.2, according to the results obtained by García-Tejero et al.[72].

$$ET_C = K_C \cdot K_r \cdot ET_0 \qquad Eq. 2.1$$

$$IR\ (mm) = ET_C - Rainfall \qquad Eq. 2.2$$

where ETc is the crop evapotranspiration; K_C is the single-crop coefficient; K_r is the crop reduction coefficient, which depends on the percentage of shaded area cast by the tree canopy; ET_0 is the reference evapotranspiration; and IR is the irrigation requirements. In the case of K_r, since it depended on the canopy volume, and taking into account the different trees ages in both orchards, a different K_r were used for each orchard.

Table 2.1 Local crop reduction (K_r) and crop coefficient (K_c) values used in the experiment.

Coefficients	March	April	May	Jun	July	August	September	October
K_c	0.4	0.6	0.9	1.1	1.2	1.1	0.8	0.7
K_{r1}	0.4	0.7	0.8	0.9	0.9	0.9	0.8	0.7
K_{r2}	0.4	0.6	0.7	0.7	0.7	0.7	0.7	0.4

K_c, Single-crop coefficient; K_{r1}, Crop reduction coefficient in Montana de San Jose orchard; K_{r2}, Crop reduction coefficient in Cartuja orchard.

2. Plant measurements

Crop physiological response to water stress, was monitored by means of measurements of leaf water potential (Ψ_{leaf}) and the stomatal conductance to water vapor (g_s). These readings were taken between 12:00 and 13:30 GTM, and weekly (**Chapters 3 and 4**). These measurements were done during the maximum evapotranspirative demand period, coinciding with the kernel-filling and preharvest stages (Figure 1.2), in both experimental orchards.

The Ψ_{leaf} was measured, in both experimental orchards, using a pressure chamber (Soil Moisture Equipment Corp., Sta. Barbara, CA, USA), monitoring 8 trees per irrigation treatment (two leaves per tree), located in the north side of the tree and being totally mature, fresh, and shaded[105], at 1.5 m of height, approximately (Fig. 2.5).

Figure 2.5 (A). Scholander camera. (B). Measurement with the Scholander camera in the field.

Additionally, in these same trees, g_s was measured, using a porometer SC-1 (Decagon Devices, INC, WA, USA), these measurements being done in two leaves per monitored tree, completely exposed to the sun and at 1.5 m of height, approximately and preferably with south-eastern facing (Fig. 2.6).

Figure 2.6 (A). Porometer SC-1. (B). Field measurement with the porometer SC-1.

With the aim of comparing the water stress supported by the crop at the end of the season, a normalization of the data was made by calculating the Stress Integral (SI) in terms of Ψ_{leaf} and g_s, following the methodology proposed by Myers et al.[106] (Eq. 2.3 and 2.4).

$$SI_{\Psi leaf} = \sum \left| \left(\Psi_{leaf}^{av} - \left(\Psi_{leaf}^{max} \right) \right) \cdot n \right| \qquad Eq.2.3$$

$$SI_{g_s} = \sum \left(g_s^{av} - g_s^{min} \right) \cdot n \qquad Eq.2.4$$

where $SI_{\Psi leaf}$ is the stress integral in terms of leaf-water potential values, Ψ_{leaf}^{av} is the average leaf water potential for any interval; Ψ_{leaf}^{max} is the maximum value of leaf-water potential registered during the experimental period; SI_{gs} is the stress integral in terms of stomatal conductance values, g_s^{av} is the average stomatal conductance for any interval; g_s^{min} is the minimum value of stomatal conductance during the experimental period; and n is the days numbers within each interval.

According to these indexes, higher water stress gathered by the crop would be related to higher values of $SI_{\Psi leaf}$ and lesser values of SI_{gs}.

At the end of each season, crop agronomical response was assessed in terms of nut and kernel weight (**Chapters 3 and 4**), harvesting being done using specific machinery for almond.

In Montana de San Jose, a mechanic vibrator to throw the almond on the ground (Fig.2.7A) (previously covered with a plastic mesh) (Fig.2.7B). Collected almonds were processed with a mechanic peeling to remove the hull (Fig. 2.7C). Finally, once cleaned, almonds were left to air dry and weighing once reached am humidity content around 6%. Finally, almonds were processed with shelling machine, obtaining the kernel yield for each irrigation treatment and cultivar. In relation to the harvesting dates these were different for each cultivar and season. In this sense, in 2017 harvesting was developed at 207, 220, and 234 day of the year (DOY) for Guara, Marta, and Lauranne respectively; whereas in 2018, harvesting was carried out at 226, 233, and 243 DOY for Guara, Marta and Lauranne; respectively.

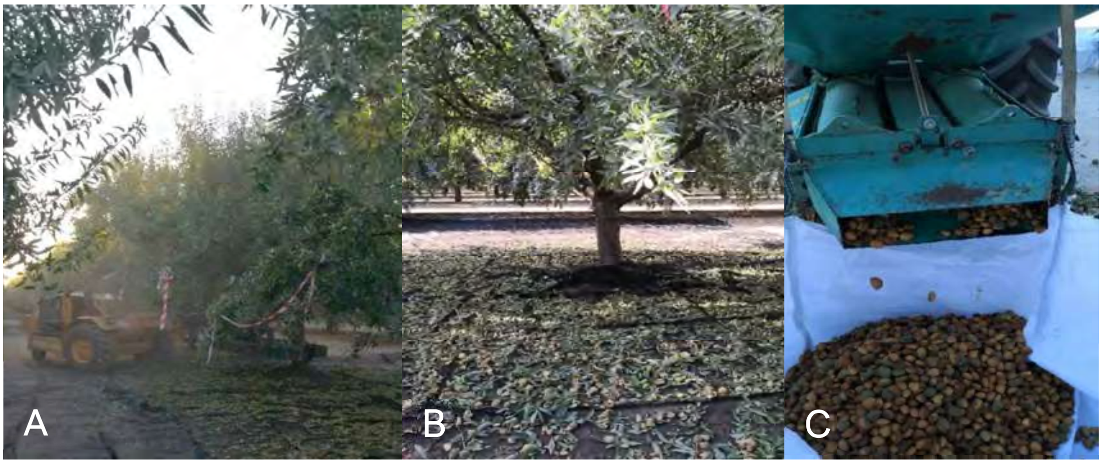

Figure 2.7 (A). Mechanic vibrator. (B). Almond covered with a plastic mesh. (C). Almonds processed with a mechanic peeling to remove the hull.

In Cartuja orchard the harvest was performed by using a mechanical vibrator with a mechanical peeling to remove the hull (Fig.2.8A and B). Once cleaned, almonds

Figure 2.8 (A). Mechanical vibrator with a mechanical peeling to remove the hull. (B). Almonds coming out of the mechanical peeling. (C). Almonds left to air dry in the laboratory.

were left to air dry and weighed once they reached a humidity content of around 6% (Fig. 2.8C). Finally, almonds were processed with shelling machine, obtaining the kernel yield for each irrigation treatment and cultivar. In relation to the harvesting dates these were different for each cultivar and season. In 2018, the almond harvest labors were done at 232 DOY for cv. Guara and 239 DOY for cvs. Marta and Lauranne; meanwhile in 2019, these were done at 219, 221, and 235 DOY for Guara, Marta, and Lauranne, respectively.

Finally, the size and number of almonds per monitored tree were quantified in both experimental orchards (**Chapters 3 and 4**). The first one was obtained by weighing 100 almonds per monitored tree (n = 8); obtaining the kernel unit weight and ratio between kernel and nut (kernel + shell). After this, the second component (number of almonds per tree) was estimated by dividing “the kernel yield per monitored tree” by “the kernel unit weight”. Lastly, was estimated the irrigation water productivity (IWP; kg·m^{-3}), as the ratio between kernel yield and the irrigation applied.

In addition, in Cartuja orchard the photosynthetic capacity of almond throughout the different phenological stages (vegetative, kernel-filling, and postharvest) in response to water stress was analyzed (**Chapter 5**). The maximum rate of ribulose biphosphate (RuBP) carboxylation (V_{cmax}), maximum rate of electron transport (J_{max}), mesophyll conductance to CO_2 (g_m) and triose phosphate utilization (TPU) were determined from A-Ci (net CO_2 assimilation rate-calculated internal CO_2 concentration) curves, i.e., the response of photosynthesis to varying concentrations of CO_2. These curves were done in three replications per irrigation treatment and cultivar in each phenological stage (n = 27), i.e., developing the curves in one of the central trees of the experimental plots.

Figure 2.9 (A). Three LI-6400 portable photosynthesis system (LI-COR, Lincoln, NE, USA). (B). Field measurement with LI-6400.

The A-Ci curves were measured between 9:00 and 13:00 GMT during the experimental period (May 30, Vegetative; June 24, Kernel-filling; September 26, Postharvest), using three LI-6400 portable photosynthesis system (LI-COR, Lincoln, NE, USA) (Fig. 2.9) at ambient temperature, saturating photosynthetic photon flux density (1,500 μmol·m^{-2}·s^{-1}) and ambient CO_2 concentration (C_a) between 50 and 1,500 μmol·mol^{-1}. The response of A_N to varying Ci was measured by lowering C_a stepwise from 400 to 50 μmol·mol^{-1}, returning to 400 μmol·mol^{-1} and then increasing Ca stepwise from 400 to 1,500 μmol·mol^{-1}. Each A-Ci curve comprised 14 measurements, each made after at least two minutes at each C_a.

V_{cmax}, J_{max}, g_m and TPU was estimated by the curve-fitting method proposed by Ethier and Livingston[107] and adapted from Díaz-Espejo et al.[108]. Diffusion leaks were corrected following the procedure by J. Flexas et al.[109]. Rubisco Kinetic parameters were taken from Bernacchi et al.[110].

In addition, at the Cartuja orchard, fruit growth was monitored. To monitor fruit growth, 12 fruits per treatment and cultivar were collected weekly from the central trees from March to July. Once the fruits were excised, they were placed in a closed bag with soaked paper to create an atmosphere saturated with H_2O and prevent moisture loss. This bag was placed in a second bag inside a field cooler with ice containers. This sampling method also allowed us to reduce the variability between them due to different collection times.

Figure 2.10 Weighing and measuring the size of the fruits in the laboratory.

In the laboratory, they were weighed on the same day they were collected to obtain the fresh weight and their longitudinal and equatorial diameters were measured with a caliper (Mitutoyo Digital ABS Caliper 0-450mm, Aurora, IL). Later they were put in an oven (Dry-Big, J.P. SELECTA®) during 72h at 65^{o}C. After this time they were weighed to obtain the dry weight (Fig. 2.10). In addition, the nuts harvested were opened to identify exactly when the kernel-filling stage began.

3. Physicochemical and sensorial analysis

In collaboration with the Food Quality and Safety group "Sensofood Solutions" of the Department of Food Quality and Technology of the Miguel Hernández University (Spain), different physicochemical and sensorial analyses were carried out on almond samples obtained in the different irrigation treatments studied in this doctoral thesis.

The physicochemical analysis was done in both orchards (**Chapters 6 and 7**) and the sensorial analysis only in Montana de San Jose orchard (**Chapter 6**).

In terms of physicochemical analysis in Montana de San Jose orchard were analyzed: kernel ratio, weight, and size, instrumental color, instrumental texture analysis, dry weight and water activity, minerals, organic acid and sugars, and fatty acids.

By the contrast, in Cartuja orchard additional parameters were determined: antioxidant activity and total phenolic content (TPC), organic acids, sugars, and fatty acids. In order to examine the influence of irrigation at the nutraceutical level of the almonds.

3.1 Physicochemical analysis

3.1.1 Kernel Ratio, Weight, and Size

The ratio between the mass of in-shell almonds and kernels was calculated from 1 kg of fruits per cultivar and irrigation treatment. Moreover, 25 almonds per cultivar and irrigation treatment, randomly selected, were analysed by measuring the weight of both in-shell and kernel almonds using a precision scale (model AG204 scale; Mettler Toledo, Barcelona, Spain) and the size (length, width, thickness) with a digital caliper (model 500-197-20, 150 mm; Mitutoyo Corp., Aurora, IL) (Fig.2.11).

Figure 2.11 Measurements of the size of almonds in the laboratory.

3.1.2 Instrumental color

Color determinations were carried out using a colorimeter (model CR-300, Minolta, Osaka, Japan), which uses an illuminant D65 and a 10° observer (Fig. 2.12). Color measurements were done three times per kernel at 25 ± 1 °C, measuring a total of 25 kernels per cultivar and irrigation treatment. Results are presented as CIEL*a*b* coordinates, which define a color in a three-dimensional space: (i) L* shows lightness (0–100 values), (ii) a* represents the green-red coordinate (negative values represent green, while positive values red), and (iii) b* represents the blue-yellow coordinate (negative values represent blue and positive values yellow).

Figure 2.12 Colorimeter.

3.1.3 Instrumental texture analysis

The texture of 25 almonds per cultivar and irrigation treatment was determined with a texture analyser (Stable Micro Systems, model TA-XT2i, Godalming, U.K.) with a 30 kg load cell and a probe (Volodkevich Bite Jaw HDP/VB): the trigger was set at 15 g, and the test speed was 1 $mm \cdot s^{-1}$ over a specified distance of 3 mm. The measured parameters were fracturability (mm), hardness (N), work done to shear (N s), average force (N), and the number of fractures (peak count).

3.1.4 Dry weight and water activity

The dry weight of almonds was determined using 2 g of ground almonds dried to a constant weight in an oven at 60 °C. Additionally, the water activity determination of ground almonds was done with a water activity (aw, ratio between the vapor pressure of the food and the vapor pressure of distilled water under identical conditions) meter (Novasina aw-Sprint TH500; Pfaffikon, Zurich, Switzerland). Three replications per cultivar and irrigation treatment were done.

3.1.5 Minerals

Mineral content was determined by digesting 0.5 g of the sample, with four samples per cultivar and irrigation treatment. Ground almonds were put in a muffle furnace (Hobersal, Barcelona, Spain), model 12 PR/300 series 8B, set at 650 °C for 6 h (Fig. 2.13). Additionally, 1 mL of HCl (6 N) was added to the obtained ash and transferred to a 25 mL volumetric flask. Dilutions of 1:25 and 1:10 were prepared using ultrahigh-purity deionized water and stored at 4 °C until analysis. Determination of macro (Ca, Mg, K, and Na) and micro (Fe, Cu, Mn, and Zn) elements in previously mineralized samples was studied.

Figure 2.13 Muffle furnace.

3.1.6 Organic acid and sugars

Organic acids and sugars were identified and quantified with high-performance liquid chromatography (HPLC) (Fig.2.14), as previously described by Lipan et al.[63], and 1 g of ground almond was homogenized with 5 mL of 50 mM phosphate buffer (pH = 7.8) with a homogenizer (Ultra Turrax T18 Basic) for 2 min at 11 300 rpm; all this time, the tube was maintained in an ice bath and then centrifuged (Sigma

3–18 K; Sigma Laborzentrifugen, Osterode and Harz, Germany) for 20 min at 15 000 rpm and 4 °C and filtered (0.45 µm Millipore membrane filter). The supernatant (10 µL) was injected into a Hewlett Packard (Wilmington DE) series 1100 (HPLC) using 0.1% orthophosphoric acid elution buffer. Sugars were determined using a Supelcogel TM C-610H column (30 cm × 7.8 mm) with a precolumn (Supelguard 5 cm × 4.6 mm; Supelco, Bellefonte, PA) and detected with a refractive index detector (RID). Organic acids were separated as sugars, and absorbance was measured at 210 nm with a diode-array detector (DAD). Calibration curves were run in triplicate with different standards of organic acids and sugars provided by Sigma (Poole, U.K.). Analyses were run in triplicate, and results were expressed as $g \cdot kg^{-1}$ dry weight.

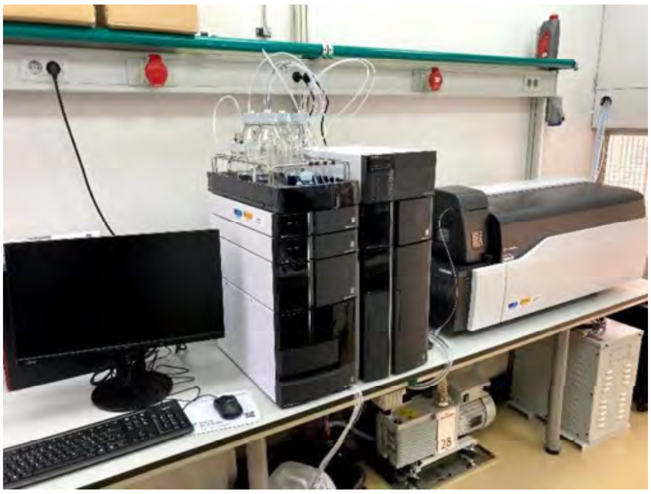

Figure 2.14 High performance liquid chromatography (HPLC) equipment.

3.1.7 <u>Fatty acids</u>

Fatty acid methyl esters (FAMEs) were prepared as previously described by Lipan et al.[64] and analysed according to Tuberoso et al.[111] FAMEs were separated in a Shimadzu GC17 A gas chromatograph with a flame ionization detector and a DB-23 capillary column (30 m length, 0.25 mm internal diameter, 0.25 µm film thickness, J&W Scientific, Agilent Technologies) (Fig.2.15). Helium was used as a carrier gas at flow rates of 1.1 and 35 $mL \cdot min^{-1}$ at the makeup point, with an injector temperature of 240 °C and the detector temperature of 260 °C. The injection volume was 0.8 µL (split ratio 1:34). The temperature program was as follows: the initial temperature 100 °C held for 1 min, temperature gradient of 3 °C min^{-1} until 220 °C, followed by a gradient of 5 °C min^{-1} until 245 °C and keeping 245 °C for 1 min. The identification of methylated fatty acid (FAME) peaks was made by comparing the retention times of the FAME Supelco MIX-37 standards. Analysis was carried out in triplicate, and the results were expressed as g·kg^{-1} concentration, using methyl nonadecanoate as the internal standard.

Figure 2.15 Shimadzu GC17 A gas chromatograph.

3.1.8 <u>Antioxidant activity and total phenolic content (TPC)</u>

Extraction was done in 0.5 g of sample sonicated with 10 mL of extractant for 15 min and stored for 24 h at 4 °C. Then the mixture was sonicated again under the same conditions and subsequently centrifuged at 10,000 rpm for 10 min. Two methods, DPPH• (2,2-diphenyl-1-picrylhydrazyl) and ABTS•+ (2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)), were used to measure the antioxidant activity of the obtained extracts. More details about the methodology can be found in Lipan et al.[111].

3.2 Sensorial analysis

The main goal of the sensory tests (only done in Montana de San Jose orchard, (**Chapter 6**) was to establish those samples with the highest and lowest intensity of three key descriptors (almond-ID, sweetness, and crispiness) (Fig. 2.16). To avoid overcomplication of the descriptive sensory analysis, the group "Sensofood Solutions" of the Miguel Hernández University (Spain) conducted a ranking test. These types of tests are helpful in checking whether significant differences in the intensity of evaluated parameters exist, although the intensity of the differences among the samples cannot be estimated. A total of 24 panelists were asked to objectively rank the intensity of three sensory descriptors of almonds: aromatics reminiscent of almond (almond-ID), sweetness, and crunchiness. First, the panelists evaluated the almonds grouped by cultivar. Then, the panel evaluated the almonds grouped by irrigation treatment. Water and crackers were used in between samples to clean the palate. Three evaluations per sample were done.

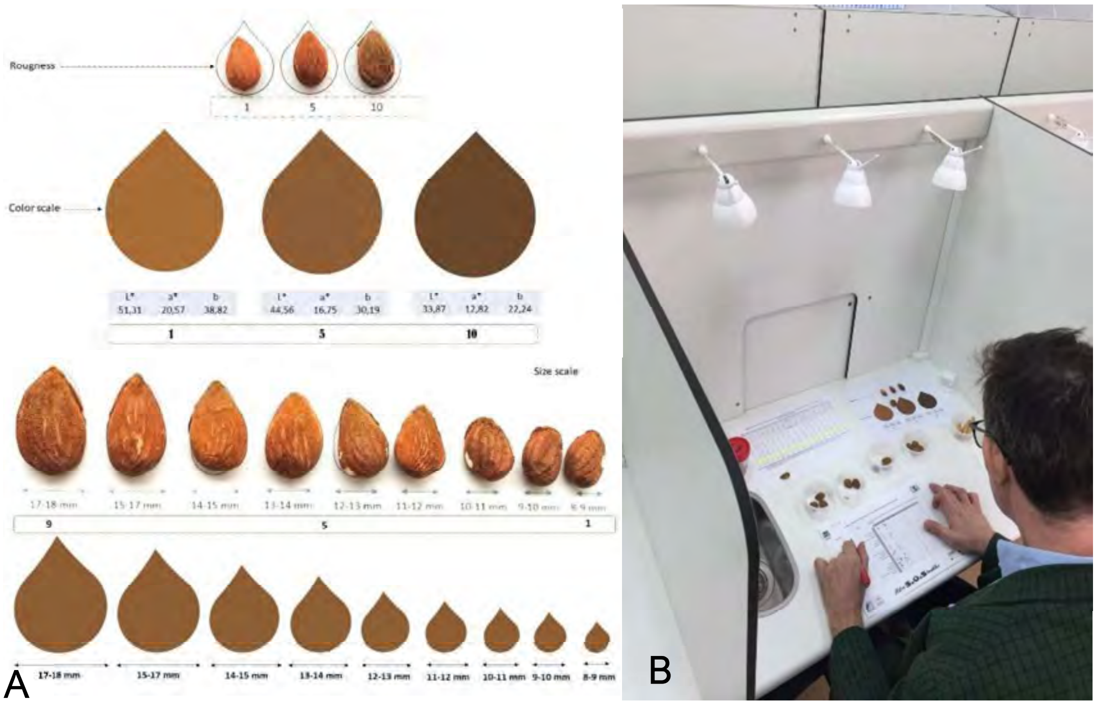

Figure 2.16 (A). Example of scales reference used by trained panel to evaluate the almond appearance. (B). Panellist in the trained panel.

4. Statistical analysis[1]

4.1 Experimental data derived from the Montana de San José Orchard

To analyze the physiological and agronomical response of almonds to different irrigation regimes (**Objectives 1; Chapters 3**) different statistical analysis were performed for the information derived from both experimental orchards.

The dataset derived from the Montana de San Jose orchard were subjected to a statistical analysis developed by using the Sigma Plot statistical software (version 12.5, Systat Software, Inc., San Jose, CA, USA). An exploratory descriptive analysis of data was done for each measurement day (Ψ_{leaf} and g_s), applying a Levene's test to check the variance homogeneity of the variables studied. Within each cultivar, significant differences between irrigation treatments ($p \leq 0.05$) in these physiological variables were identified by applying a two-way analysis of variance (ANOVA), and a Tukey's test to study the means separation between treatments.

The relationships between $SI_{\Psi leaf}$ and SI_{gs} for each cultivar were examined, using a linear-correlation analysis. The slope and intercept obtained from the regressions were compared using a covariance analysis (ANCOVA) to determine the variation in terms of the cultivar.

At the end of each season, the kernel yield was analyzed together with the kernel unit weight and the ratio between kernel weight *vs*. almond weight (kernel+shell) for each treatment within each cultivar, applying a Levene's test to check the variance homogeneity and a two-way ANOVA with a tukey's test to study the means separation between treatments. Finally, the IWP was calculated, in order to decide the best irrigation strategy in terms of irrigation water applied and almond yield.

Taking into account that all of the yield data were obtained in different seasons, a dataset normalization for each irrigation treatment and cultivar was done, taken

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[1] Additional information about Statistical procedures can be separately found in the published articles in each Chapters

into consideration the methodology proposed by Sterk and Stein[112]. This methodology tries to minimize the variability associated to the particular conditions in each season, grouping the values and allowing applying an overall analysis with the entire of data, according to this equation (Eq. 2.5):

$$Y_N = \left(\frac{Yield_i}{Yield_{annual}^{av}}\right) \cdot Yield_{total}^{av} \qquad Eq. 2.5$$

where Y_N is the normalized yield for each treatment and cultivar $Yield_i$ is the yield obtained for each replication; $Yield_{annual}^{av}$ is the average yield for a single treatment and cultivar in a year; and $Yield_{total}^{av}$ is the average yield for a single treatment and cultivar during all the studied years.

Additionally, to analyze the effect of different irrigation regimes on the quality of the final product, (**Objective 3, Chapter 6**) statistical analyses were done using two-way ANOVA, and data were submitted to Tukey's multiple range test to compare means. Statistically significant differences were considered when $p < 0.05$ and were studied using XLSTAT Premium 2016 (Addinsoft, New York). Friedman's test was carried out with the same program in which data were subjected to nonparametric tests, comparison of K samples. Finally, a principal component analysis (PCA) was made with the aim of identifying those variables or underlying factors that better explain the correlation or covariance matrix of several variables. Therefore, linear combinations of the factors or components may explain a large part of the variability

4.2 Experimental data derived from de Cartuja Orchard

The dataset derived from the Cartuja orchard were subjected to a statistical analysis in order to analyze the almond physiological and agronomical response to different SDI treatments (**Objectives 1; Chapter 4**) a statistical analysis was developed by using the Sigma Plot statistical software (version 12.5, Systat Software, Inc., San Jose, CA, USA) and the SPSS software (SPSS Inc., 15.0 Statistical packages: Chicago, IL, USA). Year-to-year, an exploratory descriptive analysis of the whole of physiological measurements (Ψ_{leaf} and g_s) for each treatment and cultivar was done, applying a Levene's test to check the variance homogeneity of the variables studied. After this, for each cultivar, an ANOVA for repeated measures was developed (three treatments and 2 freedom degrees), applying a Bonferroni test to compare pairs of treatments when significant differences in the ANOVA were detected. Moreover, and with the aim of identifying those days in which differences between irrigation treatments were detected, a one-way ANOVA and a Tukey's test were done for each measurement day.

Additionally, considering the results provided by SI_{gs} and $SI_{\Psi leaf}$; for each irrigation treatment and cultivar, a two-way ANOVA was developed, followed by a Tukey's multiple range test. Moreover, the linear regressions between the average values of $SI_{\Psi leaf}$ and SI_{gs} registered for each treatment in both years were obtained ($n = 6$), applying an ANCOVA to evaluate the differences in the interception points and slopes of these regressions. These analyses allowed the determination of whether these relationships are cultivar dependent and if this dependence is accompanied by similar yield responses.

Finally, and year-to-year, the kernel yield and its components were analyzed (kernel unit weight, the ratio between kernel weight vs. almond weight (kernel + shell), and fruits number per tree); by applying a Levene's test to check the variance homogeneity and ANOVA with a Tukey's test, considering as factors, the irrigation treatment, the cultivar, and their interactions.

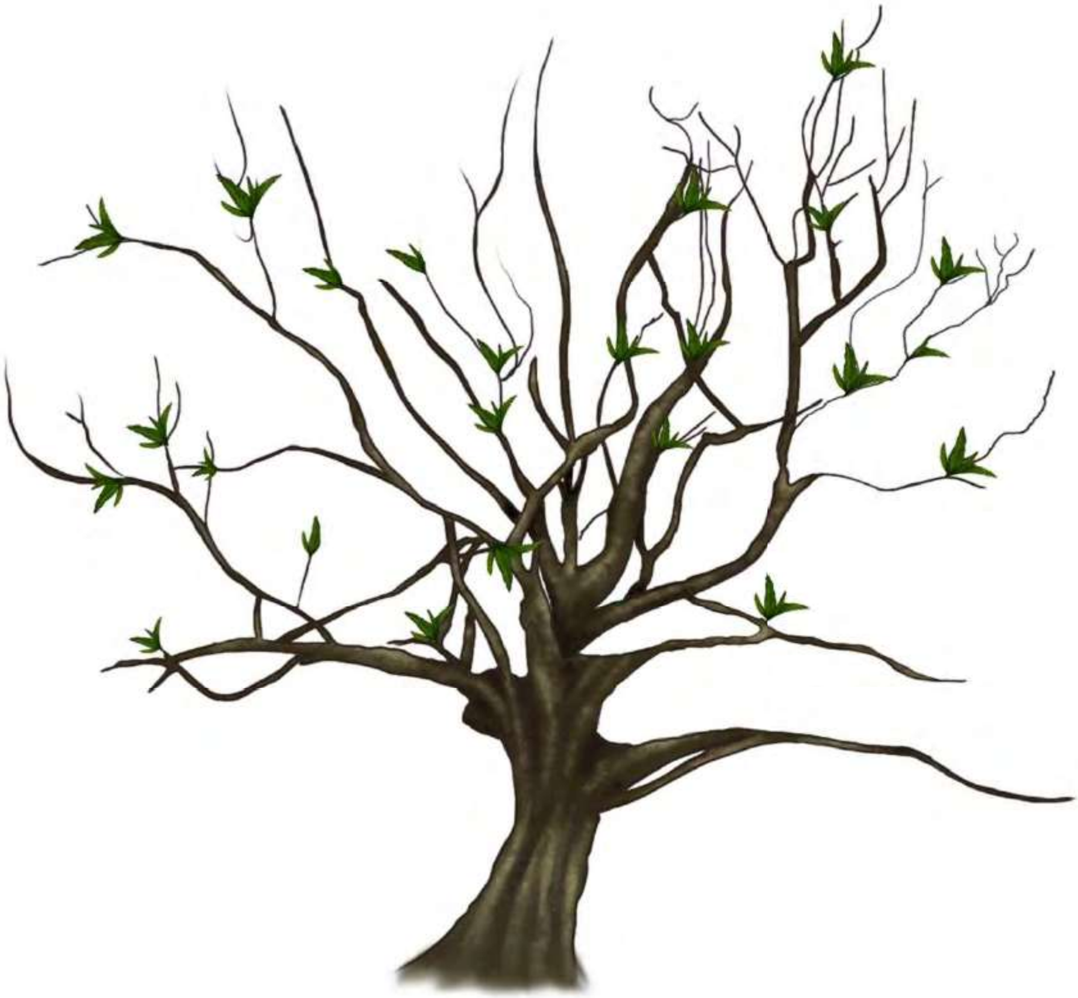
In terms of photosynthetic capacity in response to different SDI treatment and cultivars (**Objective 2; Chapter 5**) in the Cartuja orchard, (A_N, V_{cmax}, J_{max}, and g_m) a three-way ANOVA (phenological stage x irrigation treatment x cultivar) was done with Tukey's post-hoc comparisons at $p < 0.001$. Once observed that there were no significant differences in any of the variables studied (except in Ψ_{leaf}) produced by the irrigation treatments, a two-way ANOVA (phenological stage x cultivar) analysis was performed with Tukey's post-hoc comparisons at $p < 0.05$.

The photosynthetic limitations in each stage and cultivar a contribution analysis was done taking as a reference in each one the vegetative phenological stage because in this period the highest A_N was observed[113.] In addition, a two-way ANOVA was done with a Tukey's post-hoc comparisons at $p < 0.05$ to identify the main differences between the limitation in each phenological stage and cultivar. Statistical analysis was developed using the R software (R Core team version 4.0.3 and R studio version 1.3.1093) using R packages “nlme”[114] and “multcompR” [115].

Finally the influence of the SDI on the nutraceutical compounds of almonds in Cartuja orchard (**Objective 3; Chapter 7**), XLSTAT Premium 2016 (Addinsoft, New York, NY, USA) was used to detect statistically significant differences, with a significance level of $p < 0.05$. Eight replications per irrigation treatment and cultivar were considered. Two-way ANOVA was done, applying Tukey’s multiple range test for means comparison, taking into consideration the variance homogeneity previously analyzed by Levene’s test.

Chapter 3

Response of three almond cultivars subjected to different irrigation regimes in Guadalquivir River basin



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1.Introduction

Almond (*Prunus dulcis* Mill.) represents the third crop in terms of surface in Spain and contributes to the 84% of the European production of almonds. However, this performance represents only 5% worldwide, USA being the largest international producer, with 80% of the world market[41].

Almond production in Spain is generally low. This is because this crop has been traditionally associated to marginal areas, i.e., cultivated under very limited water conditions; leading to those low yields (~150 $kg \cdot ha^{-1}$)[42]. These data contrast with those obtained in other countries, such as USA or Australia, where this crop is cultivated under intensive practices and without irrigation restrictions obtaining higher yields[44], in many cases up to 3,000 $kg \cdot ha^{-1}$.

Irrigation is considered the main limiting factor for almond trees, determining the yield in terms of fruit numbers and kernel unit weight; and the nut quality. Goldhamer et al.[50] estimated that almond water requirements for the climatic conditions of California would range between 9,000 and 13,500 $m^3 \cdot ha^{-1}$, depending on the plant density, rootstock, crop management or pruning system; among other factors.

Recently, López-López et al.[85] defined the optimum irrigation doses for almond (cv. Guara) under the climatic conditions of Guadalquivir River basin (S Spain); these being close to 8,000 $m^3 \cdot ha^{-1}$, and similar to other irrigation doses applied under non limiting conditions for this same cultivar by García-Tejero et al.[60]. These data contrast with those reported by Goldhamer and Fereres[46], who concluded that in California, almond crop reached the maximum yield (close to 4,000 $kg \cdot ha^{-1}$) when the irrigation doses applied were around 1,250 mm. Notwithstanding, these results were obtained under other climatic conditions (San Joaquin River basin, California) and for a soft-shell cultivar (cv. Nonpareil).

Therefore, according to the maximum irrigation rates required by this crop, its introduction as alternative to other traditional crops in arid and semiarid zones would be justified only if its productivity is improved by means of deficit irrigation (DI) strategies. In this sense, almond is considered a drought-tolerant species and its response under water scarcity conditions has been defined by several authors to improve its development under drought conditions, minimizing the losses of production and fruit quality[58,59,80,82]. In this context, Egea et al.[87] reported the effects of various irrigation strategies and water stress intensities on the growth and quality of almond nuts. These authors demonstrated that DI strategies does not affect the overall fruit growth pattern, but a negative impact on the final kernel dry weight for the most severely stressed treatment was found. In the same vein, García-Tejero et al.[60] concluded that the kernel-filling stage would be a suitable phenological stage to apply water withholdings close to 50% ET_C for a long-term period, minimizing the yield losses.

On the other hand, the cultivar is also important, because not all have the same behavior when a DI strategy is applied. Up to day, there are few works related to the study of the differential response of almond cultivars when these are subjected to different irrigation doses. Among the known experiments, Gomes-Laranjo et al.[116] studied the physiological response of five almond cultivars (cvs. Lauranne, Masbovera, Ferragnès, Francoli and Glorieta) under non-watered and a watered situation. These authors concluded that cv. Ferragnès was the most sensitive to drought conditions. Something similar was reported by Oliveira et al.[117], who compared the leaf anatomy and water relations in different cultivars of almond (cvs. Ferragnès, Glorieta, Bonita, Casanova, Parada and Pegarinhos), concluding that cv. Ferragnès was the most sensitive to water stress due to its thin cuticle, and the high stomatal density.

Taking into account the previous experiments, we hypothesize that on one hand, a differential responsiveness in terms of yield can be observed depending on the cultivar when different irrigation regimes are applied in almond; and on other hand, a sustainable DI strategy could be defined, obtaining maximum yields for total irrigation doses close to 6,000 $m^3 \cdot ha^{-1}$.Thus, the aim of the present study was to assess the nut yield and physiological response of three most popular almond cultivars (cvs. Guara, Marta and Lauranne) when a moderate DI strategy is imposed; and corroborate the tree response when these three cultivars are subjected to different irrigation regimes in a semiarid Mediterranean environment (SW Spain).

2.Results

2.1. Climatic conditions and water supply

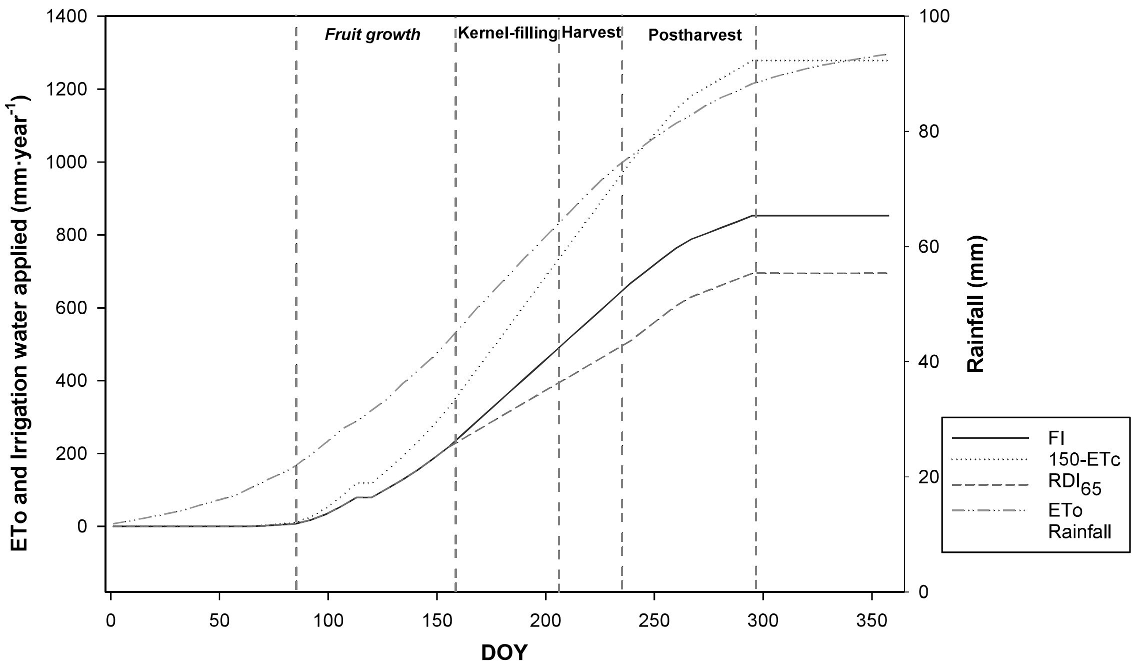
During the studied seasons, the climatic conditions were very similar to those historically registered in the experimental area. During 2017 (Table 3.1), total rainfall and reference evapotranspiration was 210 and 1337 mm, respectively.

Table 3.1 Monthly average values of weather parameters registered during irrigation period in 2017.

	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct
T_{max}	21.2	25.7	28.8	36.2	37.2	37.6	33.6	30.5
T_{min}	7.3	11.1	13.4	17.8	17.8	18.7	14.9	14.3
T_{av}	13.6	17.7	20.8	26.9	27.0	27.7	23.7	21.2
RH_{max}	96.7	90.3	92.5	81.4	82.6	80.3	83.1	87.4
RH_{min}	41.5	33.1	31.6	20.8	19.9	18.9	21.9	29.5
RH_{av}	75.0	63.5	62.7	48.2	50.2	47.4	50.6	60.0
Rad	16.2	22.0	24.9	28.2	27.5	24.1	20.4	15.2
Rain	88.8	80.6	18.44	0	0	0	0	22
ET_0	85.3	128.2	155.9	193.1	193.5	171.8	124.7	92.1

T_{max}, maximum air temperature (ºC); T_{min}, minimum air temperature (ºC), T_{av}, average air temperature (ºC); RH_{max}, maximum relative humidity (%); RH_{min} minimum relative humidity (%), RH_{av}, average relative humidity (%); Rad, solar radiation ($W{\cdot}m^{-2}$); Rain, rainfall (mm); ET_0, reference evapotranspiration (mm).

According to these conditions, FI, 150-ET_C and RDI_{65} received 852, 1,279 and 554 mm, respectively (Fig. 3.1A), considering that RDI_{65} from the beginning to 13th June (DOY 164, when water stress was applied), and after harvesting, it was irrigated with similar irrigation amounts than FI (100% ET_C).



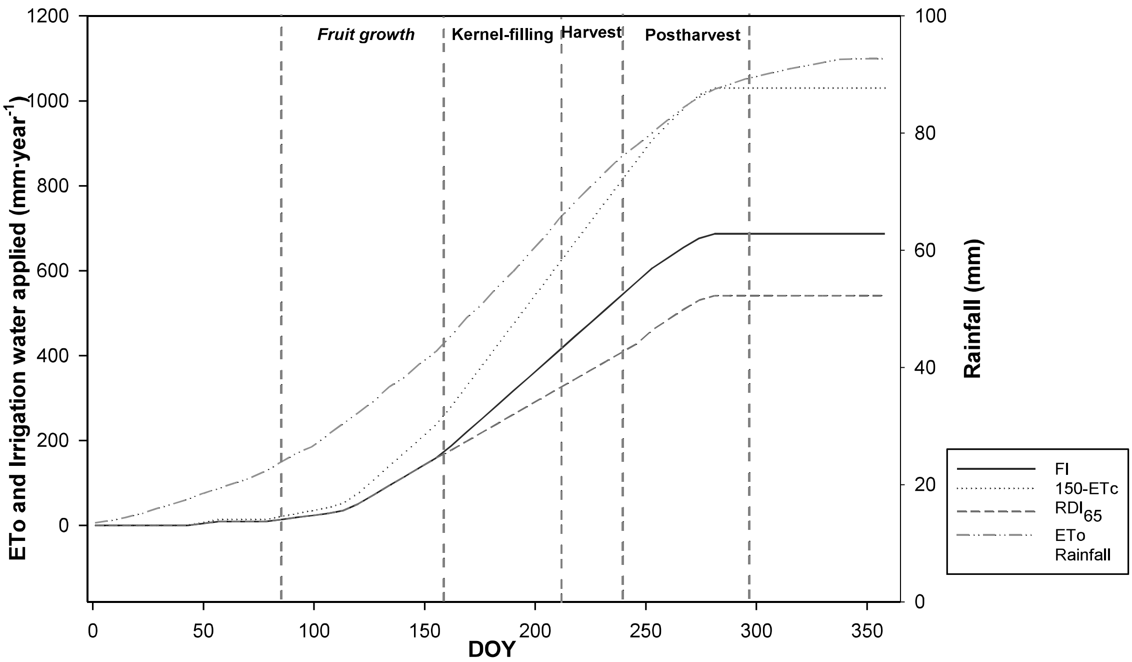


Figure 3.1. Rainfall, Cumulative water supplies by irrigation treatment, and potential evapotranspiration (ET_o) registered in 2017 (A) and 2018 (B). All values are in mm. DOY = Day of the year. Phenological stages are represented with vertical bars.

During 2018 (Table 3.2), rainfall and reference evapotranspiration were 514 mm and 1,102 mm, respectively. According to this, the accumulative amounts of irrigation water applied in FI, 150-ET_C and RDI_{65} were 687, 1,030 and 541 mm, respectively (Fig. 3.1B). Regarding to RDI_{65}, from the beginning of the irrigation period to 12th June (DOY 163, when water stress was imposed), and after harvesting, it was irrigated with similar irrigation amounts than FI.

Table 3.2 Monthly average values of weather parameters registered during irrigation period in 2018.

	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct
T_{max}	18.1	22.0	25.6	30.5	33.7	37.7	33.6	26.3
T_{min}	7.3	9.5	12.1	15.2	15.9	18.7	18.3	13.1
T_{av}	12.5	15.2	18.2	22.5	24.4	27.6	24.9	19.0
RH_{max}	98.7	97.3	96.5	93.6	96.1	87.9	89.7	95.3
RH_{min}	52.5	44.1	37.3	33.3	27.5	20.7	30.7	41.0
RH_{av}	81.1	75.6	71.3	62.4	60.9	51.9	61.9	71.8
Rad	12.5	17.3	21.9	24.9	27.0	23.5	19.6	13.9
Rain	187.8	97.2	103.0	5.4	0.0	0.6	21.4	98.4
ET_0	64.7	96.6	125.5	150.8	172.1	168.8	125.4	198.1

T_{max}, maximum air temperature (ºC); T_{min}, minimum air temperature (ºC), T_{av}, average air temperature (ºC); RH_{max}, maximum relative humidity (%); RH_{min} minimum relative humidity (%), RH_{av}, average relative humidity (%); Rad, solar radiation ($W \cdot m^{-2}$); Rain, rainfall (mm); ET_0, reference evapotranspiration (mm).

2.2. Physiological response to irrigation

During the first studied season (2017) similar values of Ψ_{leaf} were observed among the different irrigation treatments in the periods of low evaporative demand which correspond to fruit growth and postharvest stages, these being significant different during the kernel-filling stage (Fig. 3.2).

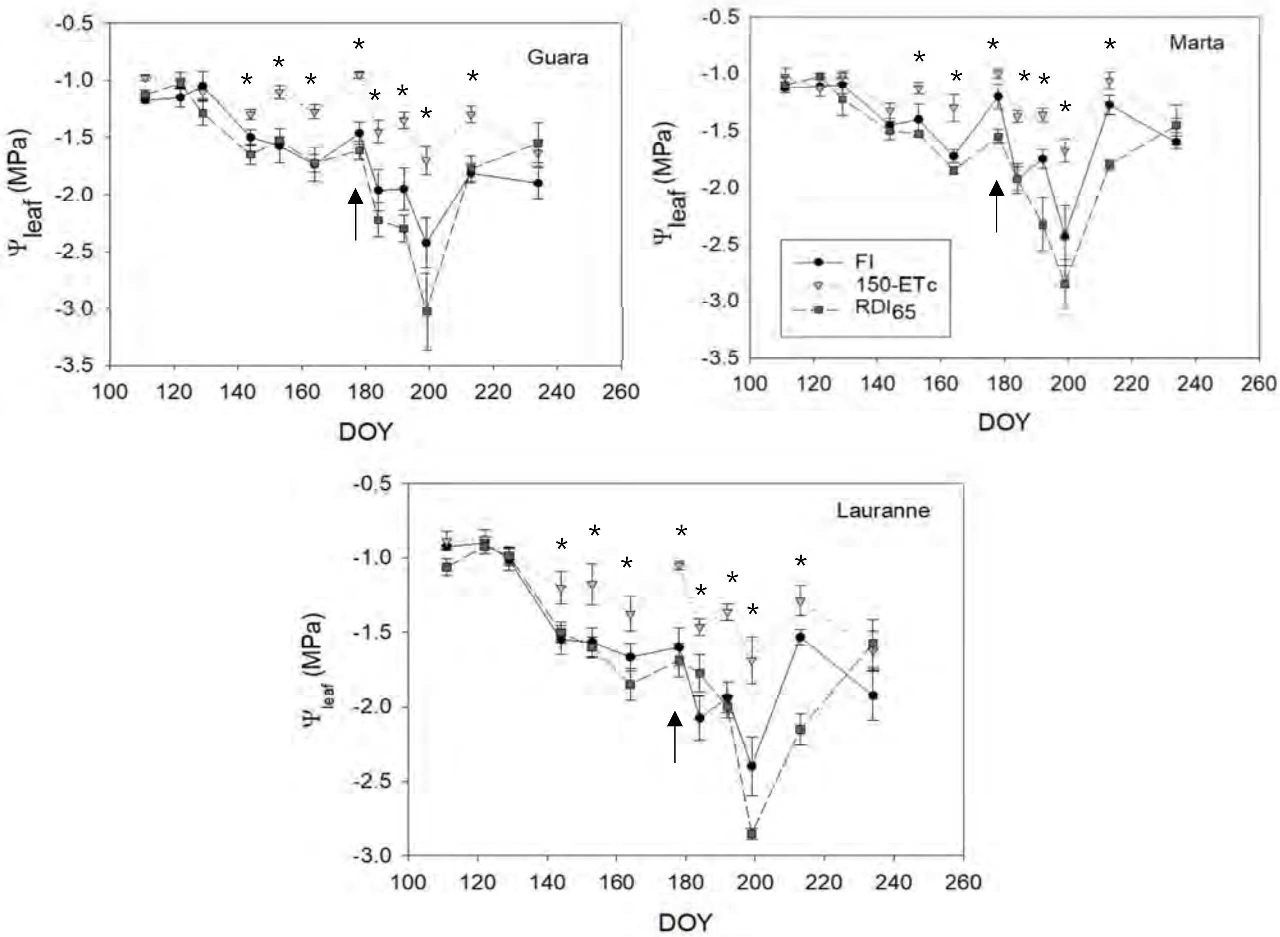


Figure 3.2 Seasonal evolution of leaf water potential in shaded leaves (Ψ_{leaf}) during the experimental period of 2017. FI: Full Irrigated treatment, 150-ET_C: overirrigated treatment, RDI_{65}: regulated deficit irrigation treatment. Black arrow pointed up the start of the stress period. Asterisks show the moments in which significant differences between treatments were observed ($p < 0.05$).

In this regard the values of Ψ_{leaf} in 150-ET_C treatment were significantly higher than those obtained in the remaining three studied treatments. In relation to RDI_{65}, the Ψ_{leaf} values were similar to FI during the fruit growth period, and significantly lower when water withholding was applied during the kernel-filling stage (from 172 DOY to harvesting). Finally, after harvesting, Ψ_{leaf} recovered till reach similar levels than those registered in 150-ET_C (Fig. 3.2).

A similar trend was observed in g_s for each cultivar and irrigation regime in 2017 (Fig. 3.3). As it was observed for Ψ_{leaf}, the most noticeable differences among irrigation treatments were observed during the kernel-filling period; specially between 150-ET$_C$ etc and the remining treatments, these differences being more pronounced at 192, 199, 200 and 234 DOYs. Moreover, it is noticeable that cv. Guara reached the highest values of g_s, in contrast with cv. Marta, which reached the lowest, especially during maximum evapotranspirative demand period.

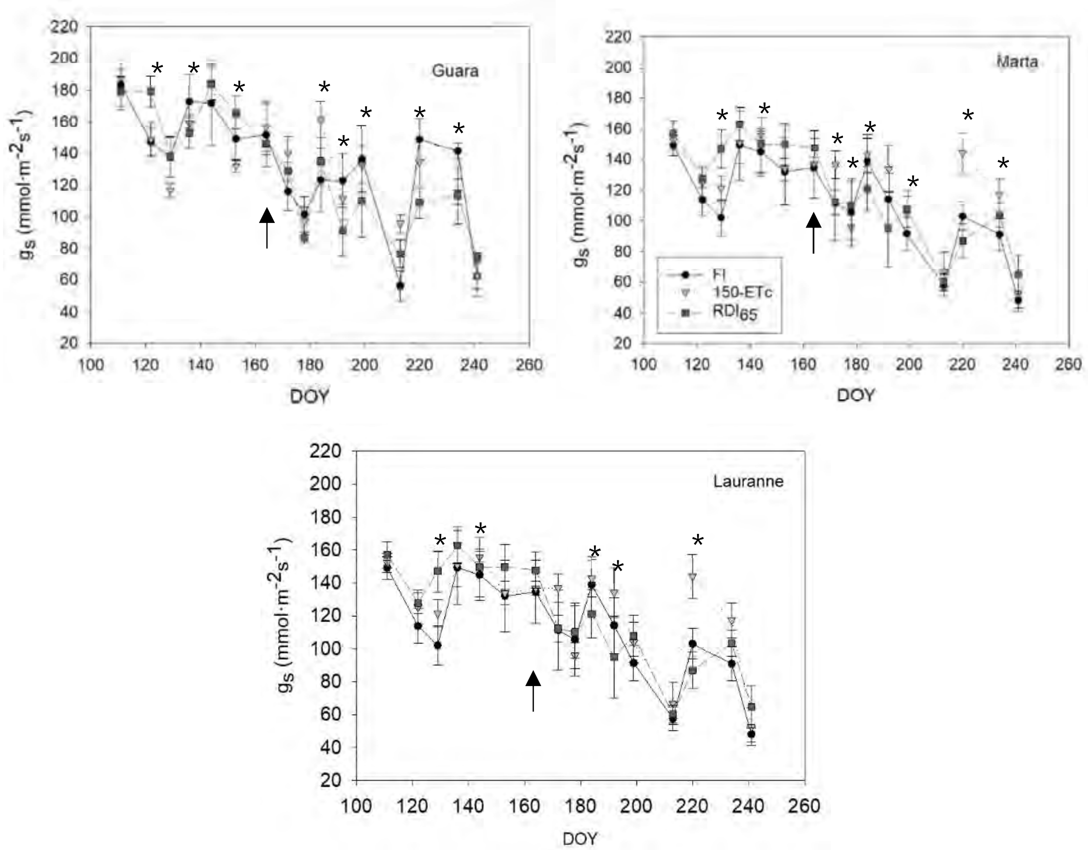


Figure 3.3 Seasonal evolution of stomatal conductance (g_s) during the experimental period of 2017. FI: Full Irrigated, 150-ET$_C$: an overirrigated, treatment, RDI$_{65}$: regulated deficit irrigation treatment. Black arrow pointed up the start of the stress period. Asterisks show the moments in which significant differences between treatments were observed ($p < 0.05$).

During the second experimental year, as it was in 2017, the differences in Ψ_{leaf} between irrigation treatments were negligible during the fruit growth period, and more significant during the kernel-filling stage, especially between 150-ET$_C$ and the remaining irrigation regimes (Fig. 3.4).

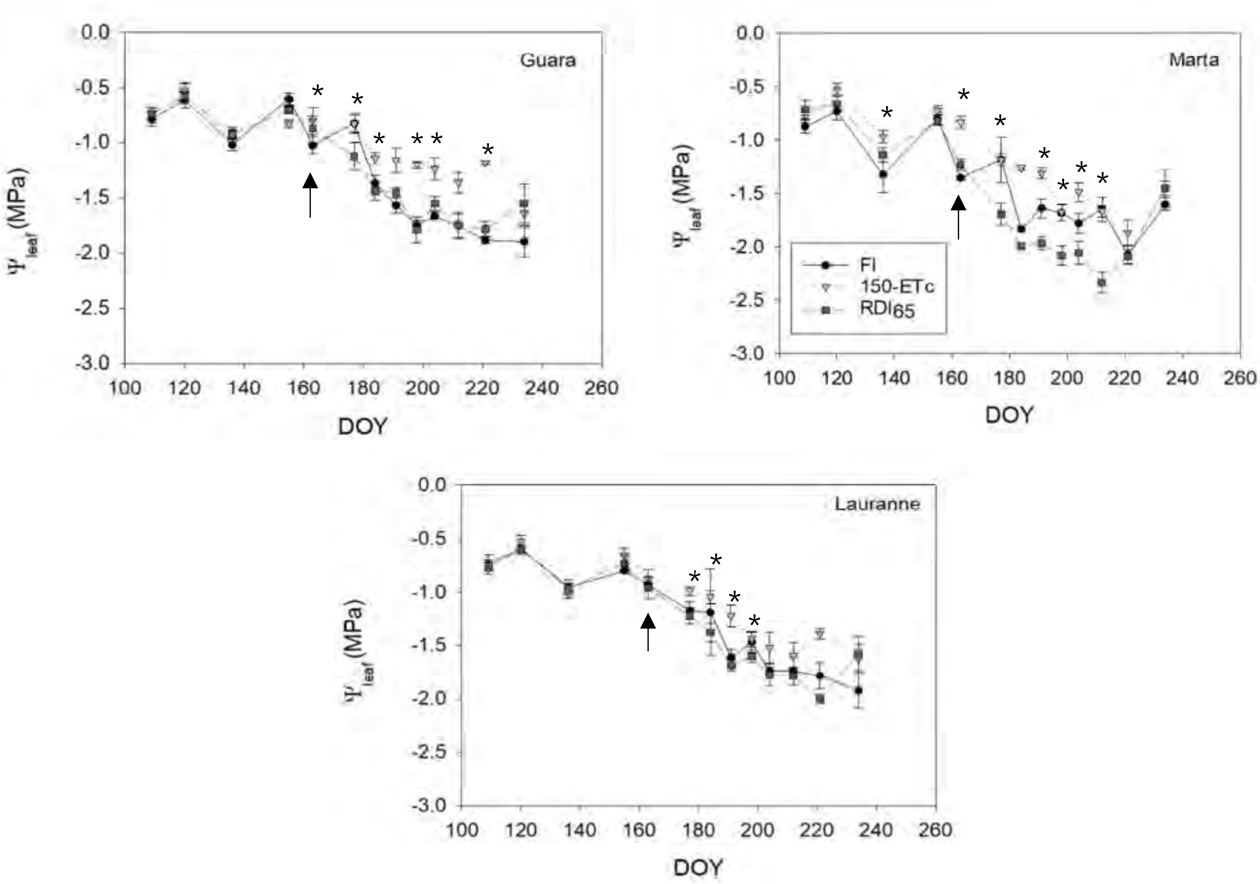


Figure 3.4 Seasonal evolution of leaf water potential in shaded leaves (Ψ_{leaf}) during the experimental period of 2018. FI: Full Irrigated, 150-ET_C: an overirrigated treatment, RDI_{65}: regulated deficit irrigation treatment. Black arrow pointed up the start of the stress period. Asterisks show the moments in which significant differences between treatments were observed ($p < 0.05$).

In relation to RDI_{65}, the Ψ_{leaf} values were similar to FI during the fruit growth period and sensitively lower when the stress started in 163 DOY. Regarding to g_s values during 2018 (Fig. 3.5), the most remarkable were the significant differences in cv. Marta between 150-ET_C and RDI_{65}, in contrast with cultivars Guara and Lauranne, where these differences were not as patent as in the previous cultivar.

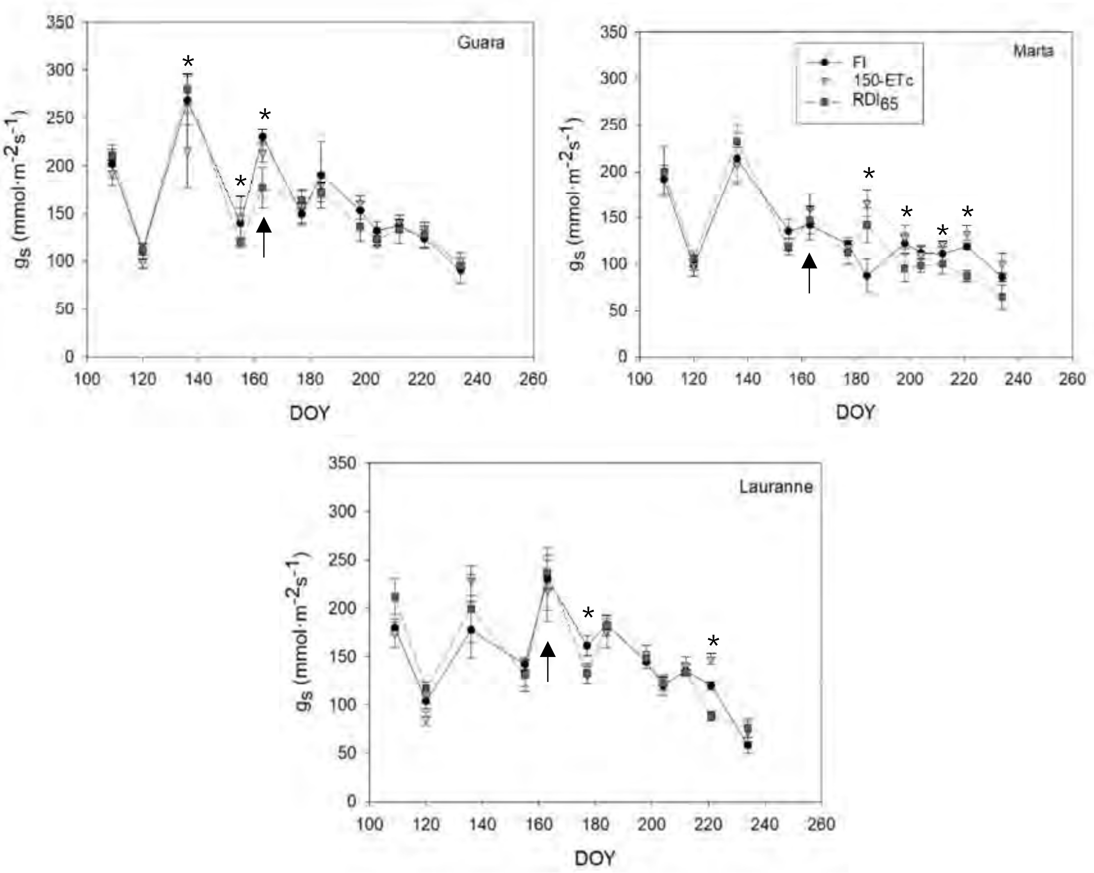


Figure 3.5 Seasonal evolution of stomatal conductance (g_s) during the experimental period of 2018. FI: Full Irrigated, 150-ET$_C$ etc: an overirrigated, treatment, RDI$_{65}$: regulated deficit irrigation treatment. Black arrow pointed up the start of the stress period. Asterisks show the moments in which significant differences between treatments were observed (p < 0.05).

More interesting were the obtained values for the stress integral in terms of Ψ_{leaf} during the kernel-filling period (Table 3.3). In this sense; during 2017 as Guara as Lauranne offered similar values for FI and RDI$_{65}$ within each cultivar, and these being significant higher (p < 0.05) than those registered in 150-ET$_C$. However, for Marta, these differences were slightly different, not appearing significant differences between FI and 150-ET$_C$. During 2018, this pattern was very similar to those registered in the previous season. In this regard, the registered values in 150-ET$_C$ for cv. Marta were 9 and 20% lower than those registered in FI and RDI$_{65}$; whereas for cvs. Lauranne and Guara, these differences were equal to those registered in the previous season.

Table 3.3 Stress integral in terms of leaf-water potential ($SI_{\Psi leaf}$) and stomatal conductance (SI_{gs}) values during the kernel filling period in the studied seasons.

	2017					
	$SI_{\Psi leaf}$ (MPa·day)			SI_{gs} (mmol·m^{-2}· s^{-1}·day)		
Cultivar	F_I	150-ET_C	RDI_{65}	F_I	150-ET_C	RDI_{65}
Marta	164ab	144b	180a	5,073a	3,711b	5,100a
Lauranne	177a	152b	182a	5,277a	3,229b	4,670a
Guara	181a	154b	193a	3,444a	3,439a	4,150a
	2018					
	$SI_{\Psi leaf}$ (MPa·day)			SI_{gs} (mmol·m^{-2}· s^{-1}·day)		
Cultivar	F_I	150-ET_C	RDI_{65}	F_I	150-ET_C	RDI_{65}
Marta	140ab	127b	158a	7,086a	6,093b	7,337a
Lauranne	130a	120b	135a	4,923a	4,942a	5,487a
Guara	130a	111b	131a	4,657a	4,786a	5,297a

FI, Full irrigated treatment; 150-ET_C, an overirrigated treatment; RDI_{65}, regulated deficit irrigation treatment. $SI_{\Psi leaf}$, stress integral leaf water potential; SI_{gs}, stress integral stomatal conductance. Different letters show significant differences by Tukey test (p < 0.05) between irrigation treatments within each cultivar and season.

Regarding to the values of SI_{gs} during the kernel-filling period in both studied seasons, cv. Guara showed similar levels of accumulated water stress in terms of the stomatal conductance values. There were observed increases in FI and RDI_{65} in comparison to 150-ET_C during 2017, and these were negligible for FI and close to 17% for RDI_{65}. Moreover, during 2018, these reductions did not appear for FI, and were close to 10% for RDI_{65}. The opposite situation was observed for cv. Marta; in this line, during 2017, these increases were 27% in FI and RDI_{65} comparing to 150-ET_C; whereas during 2018, these were 14 and 17% for FI and RDI_{65}, respectively.

Considering these differences among cultivars in terms of the accumulated water stress, the relationships between the $SI_{\Psi leaf}$ and SI_{gs} were estimated for each measurement interval (Fig. 3.6).

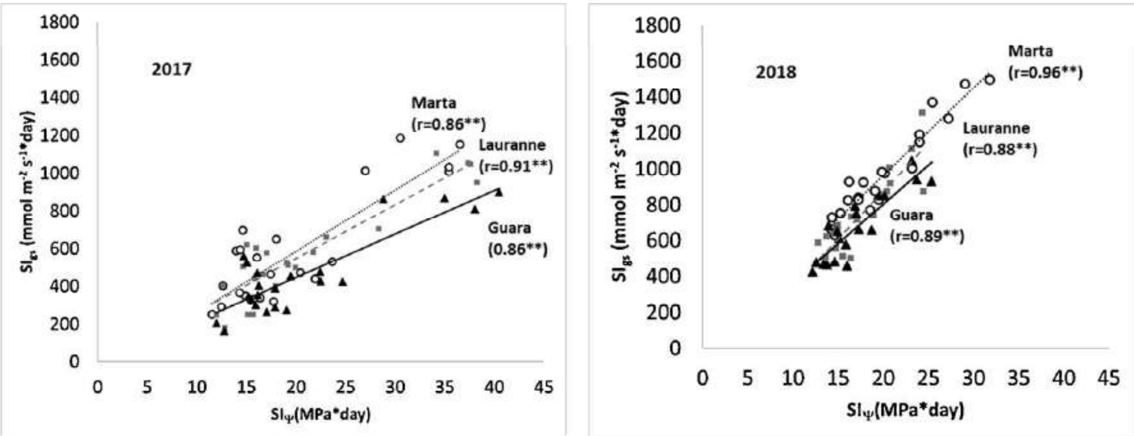


Figure 3.6 Relationships between the values of the stress integral in terms of water potential (MPa) and stomatal conductance (mmol·m^{-2}·s^{-1}) registered for each measurement interval (between 7–10 approximately) during 2017 and 2018. *r* shows the Pearson's coefficient at $p < 0.01$.

According to these relationships, it can be observed that cv. Marta registered higher SI_{gs} values for similar $SI_{\Psi leaf}$ in comparison to the remaining studied cultivars. This was similar in both seasons, showing the same patterns for cvs. Lauranne and Guara. Moreover, for 2017, significant differences were observed as in the slope as in the interception points between cultivars, but, during 2018, the obtained relationships for Guara and Lauranne were similar, and different with Marta.

2.3 Yield response and irrigation water productivity (IWP)

Figs. 3.7 and 3.8 show the yield for each cultivar and irrigation treatment in 2017 and 2018, the same pattern being observed during the two-year monitoring period. For cvs. Guara and Lauranne, no differences were obtained, while for cv. Marta a clear pattern was fixed with significant improvements for 150-ET_C.

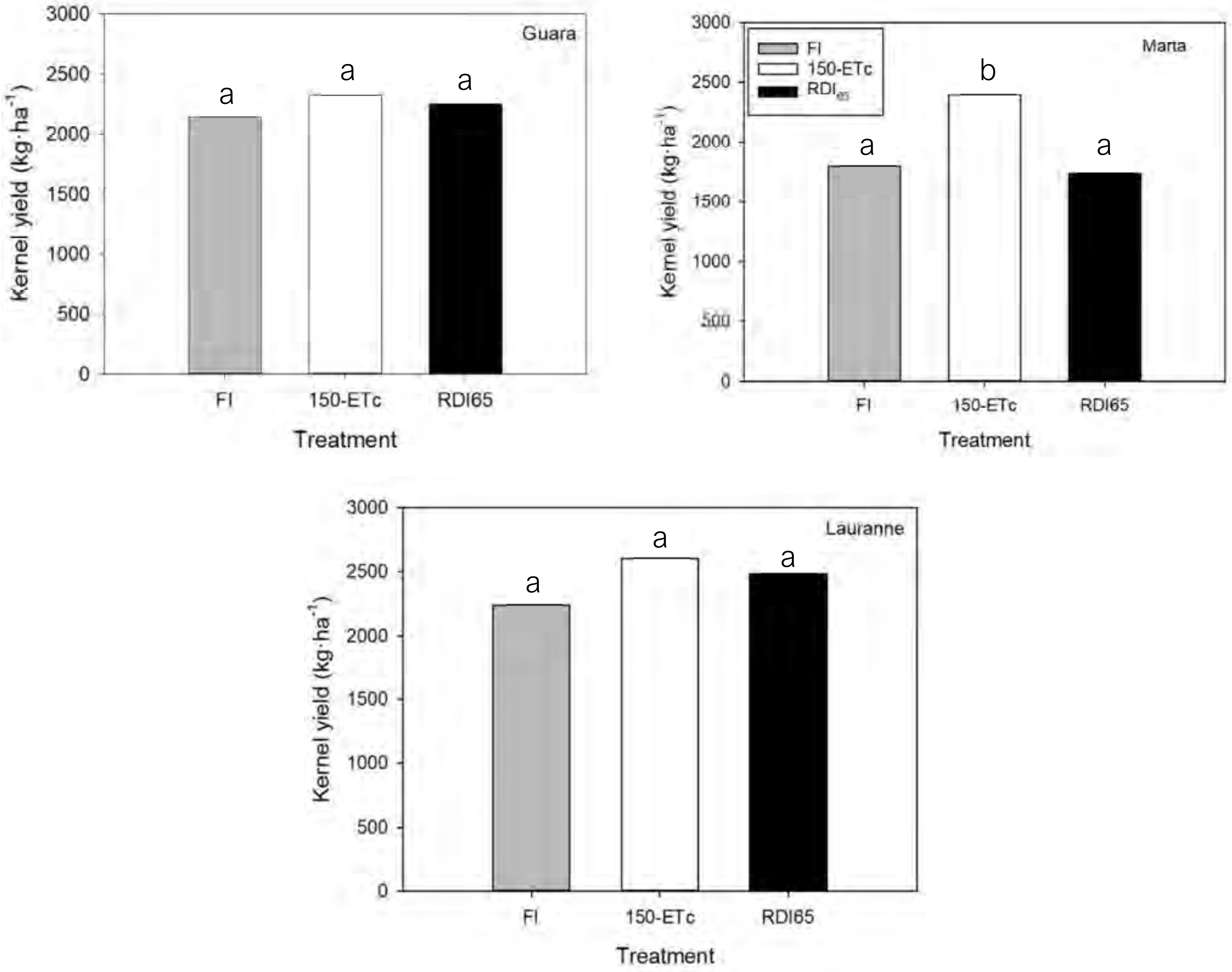


Figure 3.7 Kernel yield in 2017. **Fig. 3.7A**: cv. Guara, **Fig. 3.7B**: cv. Marta, **Fig. 3.7C**: cv. Lauranne. FI: Full Irrigated, 150-ET_C an overirrigated, RDI_{65}: regulated deficit irrigation. Different letters are significant different by Tukey test (p < 0.05).

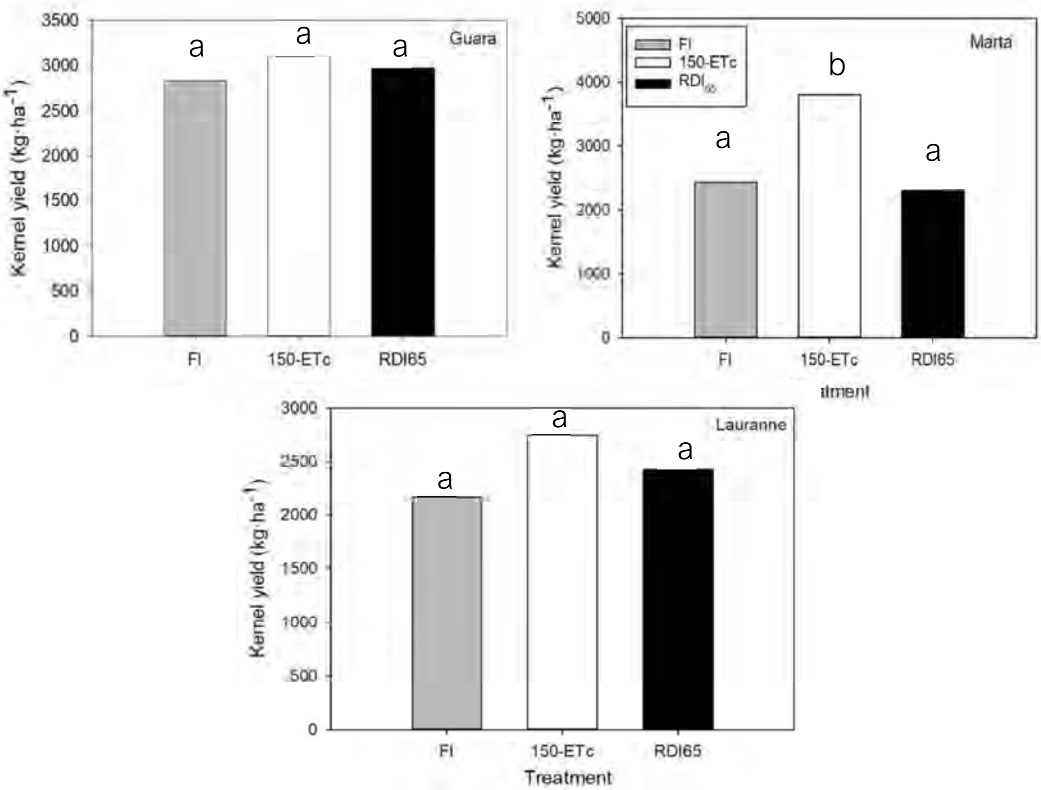


Figure 3.8 Kernel yield in 2018. **Fig. 3.8A**: cv. Guara, **Fig. 3.8B**: cv. Marta, **Fig. 3.8C**: cv. Lauranne. FI: Full Irrigated, 150-ET_C: an overirrigated, RDI_{65}: regulated deficit irrigation. Different letters are significant different by Tukey test (p < 0.05).

In addition, the kernel unit weight was calculated in both seasons, these results being summarized in Table 3.4. There were no significant differences between treatments within each cultivar in 2017, except in the cv. Marta that showed higher values in the 150-ET_C etc treatment.

Table 3.4 Kernel unit weight (g) for the defined irrigation treatments and almond cultivars during the study period.

	2017			2018		
	Treatments					
Cultivar	FI	150-ETc	RDI_{65}	FI	150-ETc	RDI_{65}
Guara	1.07a	1.14a	1.19a	1.36b	1.49a	1.43ab
Marta	1.19b	1.37a	1.10b	1.17ab	1.83a	1.11b
Lauranne	0.92a	1.06a	0.90a	1.04a	1.32a	1.16a

F_I, Full irrigated treatment; 150-ETc, an overirrigated treatment; RDI_{65}, regulated deficit irrigation treatment. Different letters show significant differences by Tukey test (p<0.05) between irrigation treatments within each cultivar and season.

Something similar was observed in 2018 for the case of Marta, although during this second year, some differences between irrigation treatments were observed in the case of Guara.

Finally, and taking into account the normalization of the final yield according to the Sterk and Stein[112] methodology, no significant differences among irrigation regimes were registered for cvs. Guara and Lauranne, whereas for Marta, 150-ET_C etc treatment reached significant higher values than FI and RDI_{65}, as it was previously discussed within each experimental season.

Regarding to the irrigation water productivity (IWP, kernel yield per unit of irrigation water applied), the obtained data for the two seasons are shown in Table 3.5. In both years and for all the cultivars, the treatment with better values of IWP were reached in RDI_{65}. This treatment with 35% less water than the FI treatment was able to produce about 10–14% more of kernel yield in terms of unit of irrigation regime applied.

Table 3.5 Irrigation water productivity (IWP, $kg \cdot m^{-3}$) for each irrigation treatment during the study period.

	2017			2018		
	Treatments					
Cultivar	FI	150-ETc	RDI_{65}	FI	150-ETc	RDI_{65}
Guara	0.25b	0.18c	0.32a	0.41b	0.30c	0.55a
Marta	0.21a	0.19b	0.25a	0.36b	0.37b	0.43a
Lauranne	0.26b	0.20c	0.36a	0.32b	0.27c	0.45a

F_I, Full irrigated treatment; 150-ETc, an overirrigated treatment; RDI_{65}, regulated deficit irrigation treatment. Different letters show significant differences by Tukey test (p<0.05) between irrigation treatments within each cultivar and season.

3. Discussion

This experiment was developed in order to define the response of three almond cultivars to different irrigation regimes, confronting both the physiological and agronomical responses. Although the climatic conditions were something different, the results showed a similar pattern in both seasons, these being enough robust to extract relevant responses to the issues considered at the beginning of this experiment. In this regard, two main issues were raised: the importance of the cultivar when a DI strategy is going to be imposed and corroborate the almond response when is subjected to the three irrigation regimes is clearly differentiated.

In relation to the first issue, and according to the obtained results, two differential patterns were observed in the studied cultivars. On one side, Guara and Lauranne showed similar responses when an overirrigation and a regulated deficit irrigation treatment was applied, with similar values of Ψ_{leaf} during entire study period (Figs. 3.2 and 3.4). This similarity between these cultivars was confirmed by means of the SI values (Table 3.3), and the relationships between them (Fig. 3.6). Unlike this latter, higher values of Ψ_{leaf} were registered for case of cv. Marta, together with lower values of g_s in FI and especially in RDI_{65} in comparison to the observed in the remaining cultivars; indicating that cv. Marta would have more pronounced stomatal sensitiveness, comparing to cvs. Guara and Lauranne.

On the other hand, these physiological responses were directly related with the observed results in terms of final yield. In this sense, not all the studied cultivars showed similar responses to the overirrigated treatment (comparing to the obtained yields in the remaining treatments). That is, cv. Marta evidenced a higher sensibility to water stress, showed significant improvements on yield when this received irrigation amounts above to the theoretical requirements (150-ET_C vs. FI).

In spite of the absence of similar works comparing the differential responses of almond cultivars to water stress; Gomes-Laranjo et al.[116] evidenced that Lauranne was a cultivar with a higher capability of maintaining similar photosynthesis rates (directly related with the stomatal conductance) under moderate to severe drought conditions than those obtained under full irrigation conditions; in comparison to other almond cultivars such as cvs. Ferragnès, Masbovera, Glorieta and Francoli. In our case, the highest values of g_s were registered in Guara and Lauranne being able to keep higher transpiration rates than cv. Marta for similar values of Ψ_{leaf} as it can be observed in Figs. 3.2– 3.5. This relation makes strong the hypothesis that cv. Marta would show a higher sensitiveness in terms stomatal control under a water stress, adopting a conservative approach to water use under drought scenarios.

These findings endorse the obtained in our experiment and corroborate that to establish a proper irrigation strategy, we must know the physiological behaviour for a given cultivar.

Relating to the second issue considered in this work, the obtained results sustain the viability of the RDI_{65} treatment, which was able to reach similar yield values to those under full irrigated conditions in the three studied cultivars, and even similar to the yields registered under overirrigation conditions with cvs. Guara and Lauranne. In relation to these findings, SI_{gs} were similar in the three irrigation treatments in both cultivars (Table 3.3), which would largely explain the absence of effects in the yields. Similar responses to these obtained for cvs. Guara and Lauranne have been reported by other authors. Girona et al.[80], in a four-year experiment with the cultivar Ferragnès, concluded that any improvement was detected for an irrigation treatment in which crop was irrigated at 130% of ET_C. This capability of reaching similar yield values under a moderate water stress situation encouraged significant improvements in the IWP for RDI_{65} treatment (Table 3.5), these values being similar to those reported by Egea et al.[81] for stressed treatments reporting values from 0.25 to 0.45 $kg \cdot m^{-3}$. Thus, according to our findings, significant yields could be reached under a moderate-water stress situation in the Guadalquivir River basin, with water savings around $2000 m^3 \cdot ha^{-1}$.

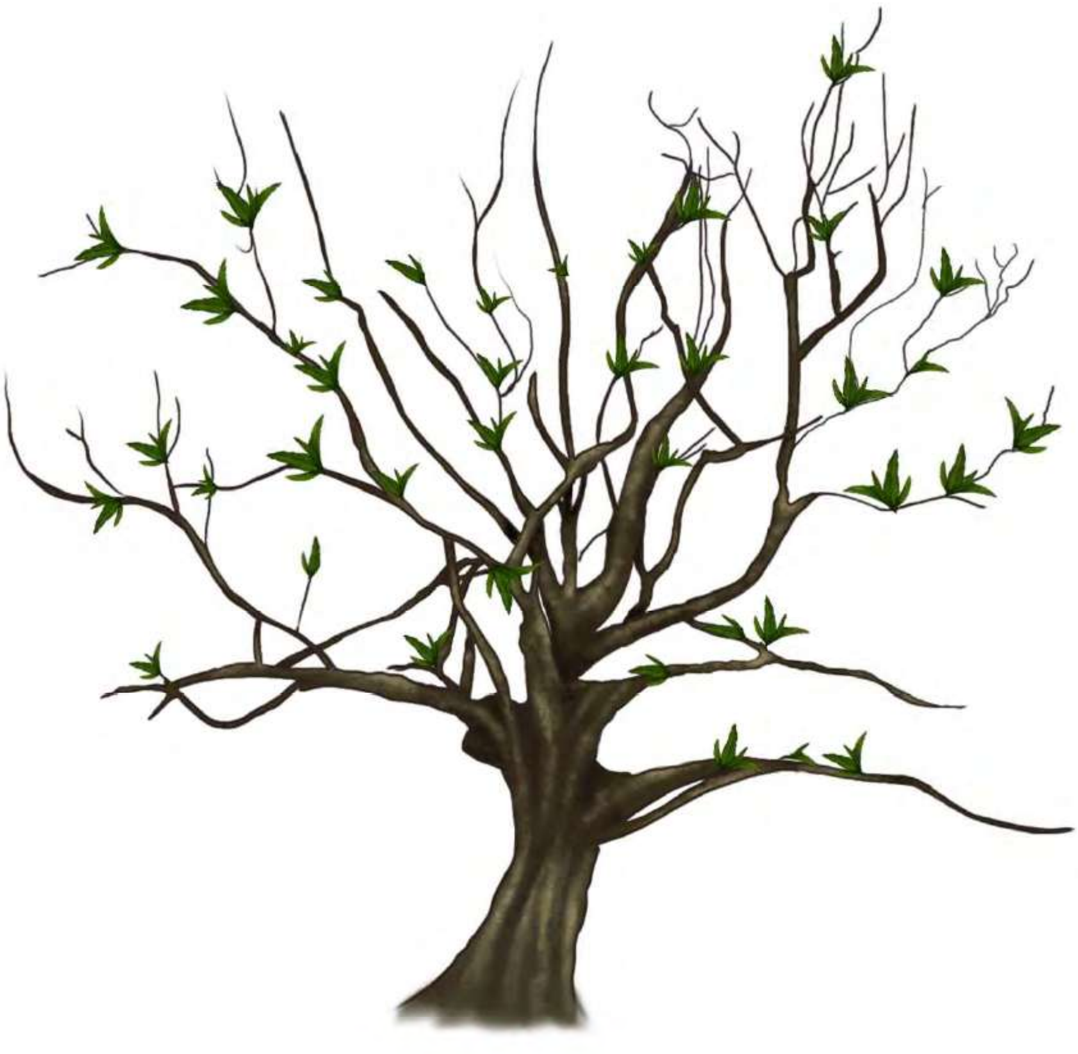
4. Conclusions

Almond is a drought tolerant crop, and many works have been developed in order to perform the best DI strategy. However, up to day, no differences between cultivars have been considered. In this way, we have determined that Guara and Lauranne can reach similar yields with water savings close to 3,000$m^3 \cdot ha^{-1}$, whereas Marta would be more sensitive to drought conditions as it has been corroborated by using the stress integral defined in terms of water potential and stomatal conductance.

In this regard, according to our findings, the cultivar was a determinant factor to take into consideration when a DI is going to be apply. Moreover, according to the final yield harvested in the studied cultivars, we can conclude that, on overall, similar production values to full irrigated conditions can be reached with annual irrigation doses around 6,000 $m^3 \cdot ha^{-1}$.

Chapter 4

Cultivar Dependent Impact on Yield and Its Components of Young Almond Trees under Sustained Deficit Irrigation in Semi-Arid Environments



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1. Introduction

Today, deficit irrigation (DI) strategies cannot be considered as a novelty, and their effects and consequences have been widely studied in order to improve water resources management under water shortage conditions. With them, irrigation can be reduced until certain levels, with the main aim of maximizing the water savings keeping the yields within an acceptable range, close to those obtained under non-water restrictions[76]. Several authors have reported positive results when these strategies have been applied in different woody crops, many times with promising results of water savings, low yield reductions[118], and even improvements on fruit quality[119-121]. Although almond is a well-known drought tolerant crop (Torrecillas et al.[74], water availability is the main restricting factor in semi-arid environments, determining the nut yield and its components (kernel unit weight and fruit numbers per tree)[122]. Moreover, yield reductions are not exclusively an effected by the water stress but also the DI practice imposed; among them; regulated deficit irrigation (RDI), sustained deficit irrigation (SDI), partial root zone (PRD), or low frequency deficit irrigation (LFDI)[42]. RDI and SDI are the most studied strategies; the first one, characterized by applying a smaller amount of water in that period in which the crop is less sensitive to this withholding of water[77], whereas SDI consists of applying a sustained water reduction throughout the growth cycle[78]. Very promising results have been obtained when LFDI is applied during the kernel-filling period, without yield losses even under long term experiences[60]. This DI strategy consists of applying irrigation-restriction cycles which are derived by means of physiological threshold values previously defined. Thus, its application has the disadvantage of requiring proper crop water monitoring, which many times results in being very difficult for farmers and technicians. Finally, in PRD strategies, part of the root system is exposed to drying soil while the remaining part is irrigated normally[79], and in the case of almond, its application has not improved the obtained results in comparison to RDI or SDI treatments that had received similar irrigation amounts[87].

Currently, the RDI is the most-studied strategy for the case of almond and many authors have described its response under different water stress levels[58,59,77,80], but there are few works about the response of almond trees to SDI. In this sense, Egea et al.[82] compared the response of almond (cv. Marta) productivity to different irrigation strategies, reporting yield reductions of 12% and water savings of 37% with SDI strategy. Phogat et al.[83] evaluated the response of soil plant system in cvs. Nonpareil, Carmel and Ne Plus Ultra under SDI, obtaining that water uptake efficiency was substantially higher under SDI, compared to normal application conditions. Other authors as Alcon et al.[86] have evaluated the financial viability of applying SDI or RDI, concluding that SDI was the strategy that allowed greater savings in financial terms.

Not only the DI strategy is determinant, but also the almond cultivar, because the response to water stress could be different among them[123,124]. In this sense, Gómez-Laranjo et al.[116] studied the differences in terms of physiological response of five almond cultivars (cvs. Lauranne, Masbovera, Ferragnès, Francoli, and Glorieta) under full-irrigated and rainfed situations. This author concluded that cv. Lauranne and Francoli were the less sensitive to irrigation withholdings. Moreover, Oliveira et al.[117] studied leaf anatomy changes and water relations in five traditional cultivars (cvs. Bonita, Casanova, Parada, Pegarinhos, and Verdeal) and two commercials (cvs. Ferragnès and Glorieta), highlighting cv. Ferragnès as most sensitive cultivar to drought conditions. This fact was associated with low values for cuticle and for the ratio between palisade and spongy parenchyma; as well as higher values of vulnerability index, conducting vessels area, or xylem area. Yadollahi et al.[125] apprised that six almond genotypes had different reactions to water stress, displaying the ability to tolerate moderate and severe water stress conditions. In addition, Barzegar et al.[126] studied the responses of six almond cultivars (cvs. Azar, Marcona, Mission, Nonpareil, Sahand, and Supernova) to water stress, reporting as less sensitive cvs. Supernova and Azar in contrast to cvs. Marcona and Sahand. More recently, Gutiérrez-Gordillo et al.[127] analyzed the yield of three almond cultivars (cvs. Guara, Marta, and Lauranne) under RDI and over irrigated conditions, revealing that, although no significant reductions were observed between full irrigation and RDI; cv. Marta offered significant improvements in terms of yield and physiological response when this cultivar received irrigation doses around 150% of crop evapotranspiration, this response not being observed in the remaining cultivars. In summary, plants' reactions to water stress are complex, implicating adaptive changes and/or detrimental consequences, and the different responses are regulated by the innate plant features as well as the duration and intensity of the imposed stress. In this line, we hypothesize that under water stress almond cultivars are able to exhibit different tolerance levels, and the physiological reactions could be divergent in response to SDI strategies, which ultimately effects on yield and its components. Thus, the aim of this study was to assess the nut yield and physiological response of three commercial almond cultivars namely Guara, Marta, and Lauranne subjected to sustained deficit irrigation strategies, elucidating the tolerant cultivar under these strategies in a semi-arid Mediterranean environment.

2. Results and Discussion

2.1 Physiological Response to Water Stress

According the climatic conditions the irrigations doses received for FI, SDI_{75} and SDI_{65}, during 2018 were 4,974, 3,713 and 3,342 $m^3 \cdot ha^{-1}$; and during 2019 were 7,700, 5,744 and 5,159 $m^3 \cdot ha^{-1}$, respectively.

In the course of the first experimental season (2018) the ANOVA for repeated measures did not show significant differences between irrigation treatments within each cultivar. That is, on overall as Ψ_{leaf} as g_s evidenced similar results for the studied treatments; although afterwards, the analysis done independently for each monitoring day reflected significant differences between treatments. These differences between treatments were especially noticeable for the case Ψ_{leaf} (Figure 4.1), in comparison to the higher similarities reported by g_s measurements (Figure 4.2). When confronting the Ψ_{leaf} values registered in each cultivar, these ranged from -0.9 to -2.3 MPa for cvs. Guara and Lauranne, meanwhile for cv. Marta these ranged between -0.8 and -2.0 MPa.

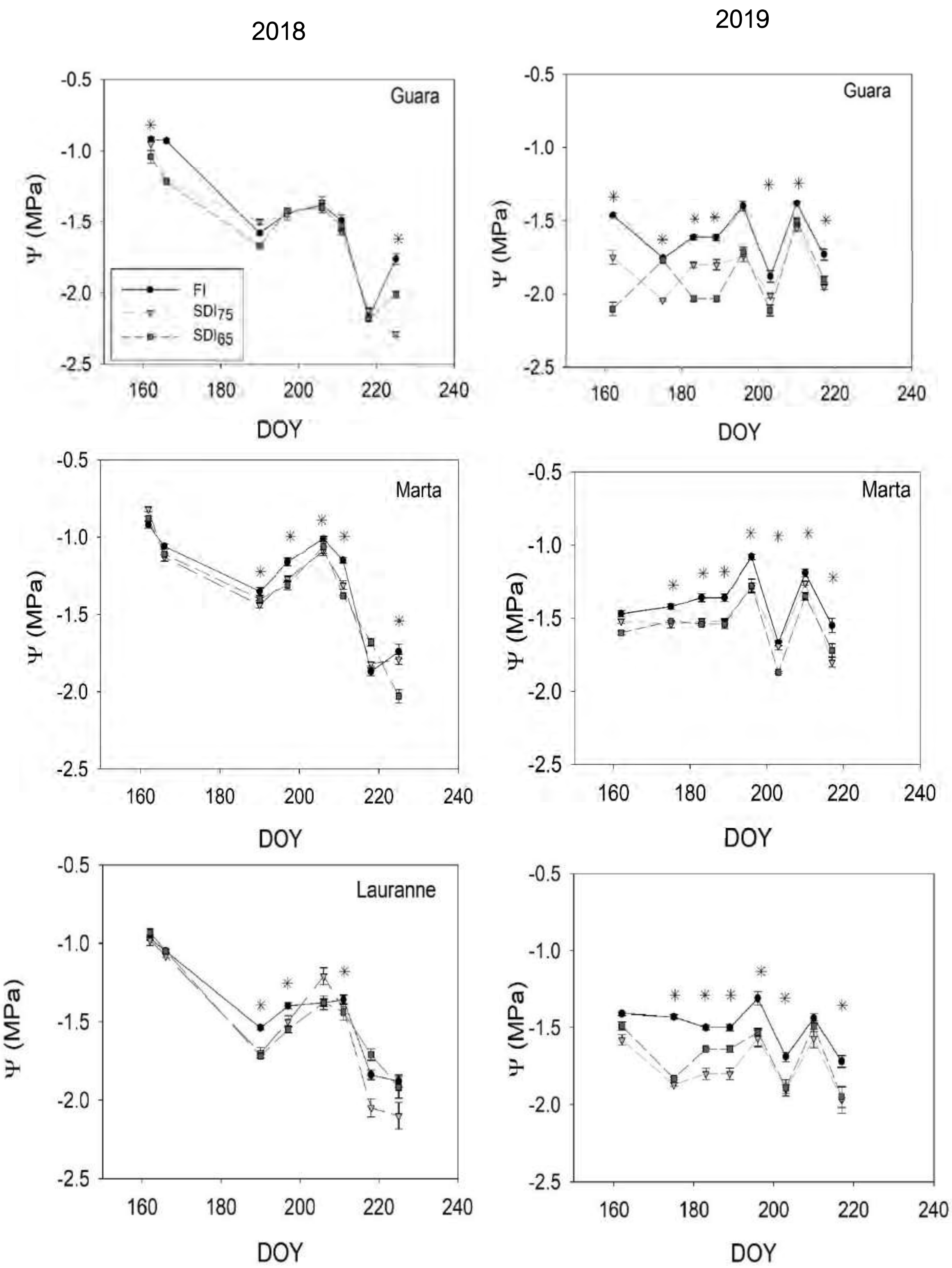

Figure 4.1 Seasonal dynamics of leaf water potential (Ψ_{leaf}) during 2018 and 2019. FI, Full irrigated treatment; SDI_{75}, sustained deficit irrigation at 75% during the irrigation period (IR); SDI_{65}, sustained deficit irrigation at 65% IR, DOY; Day of the year. Vertical bars are standard deviation. Asterisks show the intervals with significant differences between FI and SDI treatments (p < 0.05).

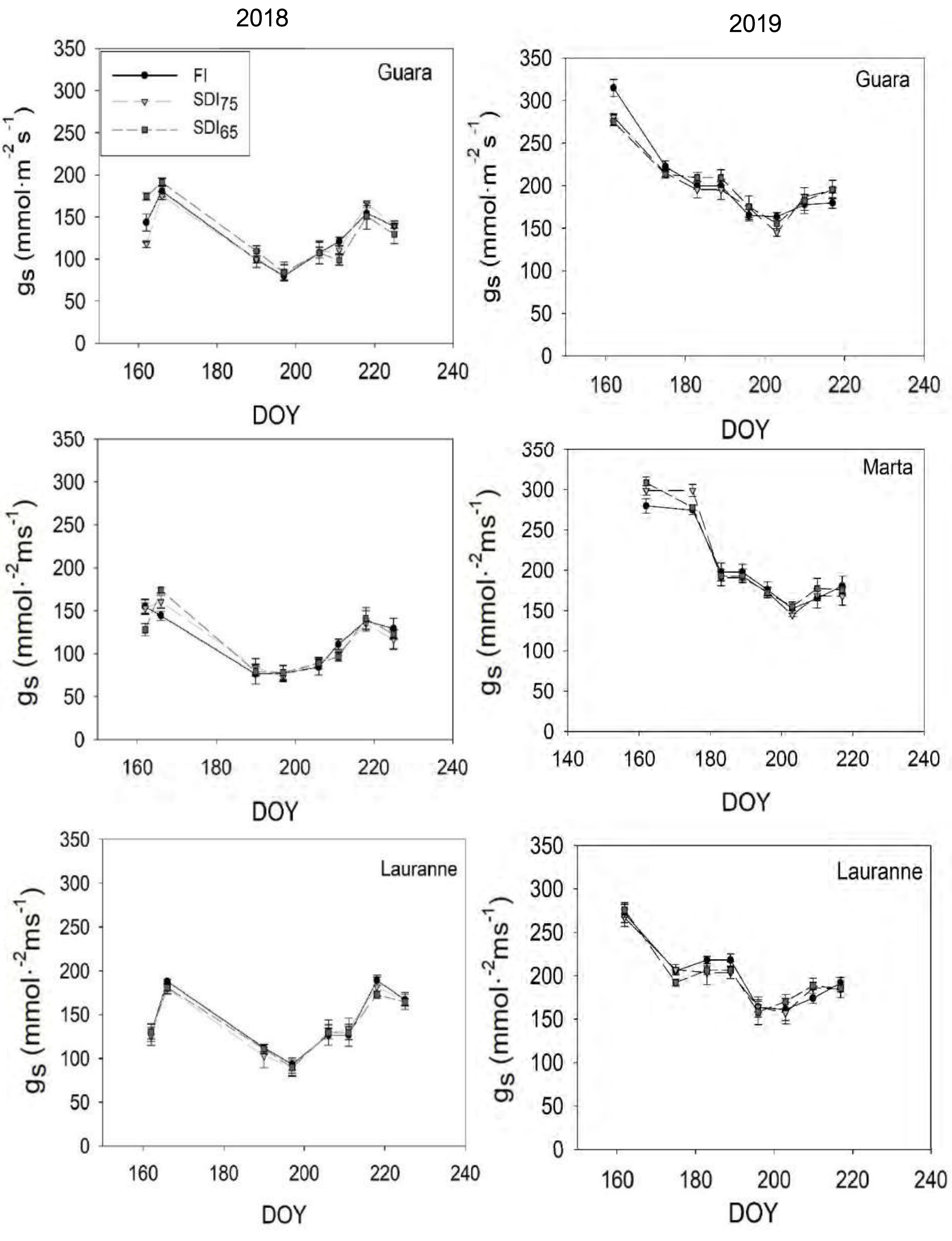

Figure 4.2 Seasonal dynamics of stomatal conductance (g_s) during 2018 and 2019. FI, Full irrigated treatment; SDI_{75}, sustained deficit irrigation at 75% during the irrigation period (IR); SDI_{65}, sustained deficit irrigation at 65% IR, DOY; Day of the year. Vertical bars are standard deviation. Asterisks show the intervals with significant differences between FI and SDI treatments ($p < 0.05$).

In relation to g_s, the obtained values between treatments and cultivars were very similar, which was very evident by the absence of significant differences. Comparing the g_s values for each cultivar, cv. Guara showed values between 80 and 175 $mmol{\cdot}m^{-2}{\cdot}s^{-1}$, cv. Marta between 75 and 180 $mmol{\cdot}m^{-2}{\cdot}s^{-1}$, and cv. Lauranne registered g_s rates between 90 and 175 $mmol{\cdot}m^{-2}{\cdot}s^{-1}$.

As happened in 2018, during 2019 the differences among treatments were higher in Ψ_{leaf} than in g_s. (Figure 4.1). Again, the ANOVA for repeated measures did not reflect significant differences between irrigation treatments. For cv. Guara, Ψ_{leaf} values ranged from -1.4 to -1.9 MPa in FI, whereas in SDI treatments these values oscillated from -1.7 to -2.5 MPa. Somewhat lower were the values for cv. Lauranne with Ψ_{leaf} between -1.1 and -1.7 MPa in FI, and for both SDI_{75} and SDI_{65} between -1.5 and -2.0 MPa. Also, some differences for cv. Marta were observed with Ψ_{leaf} with values between -1.1 and -1.6 MPa in FI; and for both SDI treatments between -1.3 and -1.9 MPa. In relation to g_s in 2019, no significant differences among treatments were observed for any studied cultivar (Figure 4.2), very similar to that reported in the previous season. In particular, for cv. Guara, the g_s rates were between 163 and 314 $mmol{\cdot}m^{-2}{\cdot}s^{-1}$whereas cv. Lauranne displayed similar rates between 161 and 275 $mmol{\cdot}m^{-2}{\cdot}s^{-1}$. Finally, cv. Marta registered g_s values that ranged from 150 to 310 $mmol{\cdot}m^{-2}{\cdot}s^{-1}$ these being similar in all studied irrigation treatments.

In summary and considering the observed values during the two studied years, cv. Marta showed a higher capability to register higher values for Ψ_{leaf} with respect to the other remaining studied cultivars, especially under SDI conditions.

More perceptible findings were achieved in relation to the water stress integral (Table 4.1).

Table 4.1 Effect of deficit irrigation strategy and cultivar on water stress integrals for $SI_{\Psi leaf}$ and SI_{gs}.

	First season (2018)		Second season (2019)	
ANOVA	$SI_{\Psi leaf}$ (MPa·day)	SI_{gs} (mmol m^{-2} s^{-1}·day)	$SI_{\Psi leaf}$ (MPa·day)	SI_{gs} (mmol m^{-2} s^{-1}·day)
Irrigation	ns	ns	ns	*
Cultivar	*	*	*	**
Irrigation × cultivar	ns	*	*	*
	Tukey multiple range test			
	Irrigation			
FI	198.7	3,069	188.7	3,758a
SDI_{75}	199.4	3,071	197.9	3,426ab
SDI_{65}	197.9	3,152	199.6	3,136b
	Cultivar			
Marta	189.6b	2,301b	187.8b	2,651b
Lauranne	201.6a	3,685a	194.3ab	4,127a
Guara	204.7a	3,306a	204.1a	3,532a
	Irrigation × cultivar			
cv. Marta				
FI	190.9a	2,174a	178.8d	3,005b
SDI_{75}	189.8a	2,306a	187.6c	2,874b
SDI_{65}	188.2a	2,422a	197.2ab	2,075c
cv. Lauranne				
FI	201.9b	3,784b	193.9bc	4,151a
SDI_{75}	202.9b	3,633b	197.7ab	4,023a
SDI_{65}	200.2b	3,639b	191.3c	4,207a
cv. Guara				
FI	203.3b	3,249b	193.5bc	4,089a
SDI_{75}	205.5b	3,276b	208.5a	3,381b
SDI_{65}	205.4b	3,395b	210.2a	3,127b

FI, Full irrigated treatment; SDI_{75}, sustained deficit irrigation at 75% during the irrigation period (IR); SDI_{65}, sustained deficit irrigation at 65% IR. ns, not significant; * and **, significant at $p < 0.05$ and $p < 0.01$, respectively. Values followed by the same letter within the same column and factor are not significantly different ($p > 0.05$) by Tukey's test.

During the first experimental season (2018) significant effects in relation to cultivar (p < 0.05) were found in $SI_{\Psi leaf}$ and SI_{gs}. In this sense, cvs. Guara and Lauranne showed similar values of $SI_{\Psi leaf}$ and SI_{gs}, and significant (p < 0.05) higher than those calculated in cv. Marta. Particularly noticeable were the values of SI_{gs} for cv. Marta, which were, globally, 38% and 30% lower than those registered in cvs. Lauranne and Guara, respectively. Respect to the irrigation doses effects, no significant differences were observed for $SI_{\Psi leaf}$ and SI_{gs}, this being in agreement with the absence of differences reported by the ANOVA for repeated measured previously discussed.

During 2019, the highest differences were observed again among cultivars, as for $SI_{\Psi leaf}$ as for SI_{gs}. Thus, the lowest and highest values of $SI_{\Psi leaf}$ were reached by cvs. Marta and Guara, respectively, very similar to the response observed the previous season. Moreover, the highest differences in 2019 were determined in SI_{gs} among cultivars, with values for cvs. Guara and Lauranne higher that those obtained for cv. Marta (p < 0.01). That is, irrigation doses did not promote differences in $SI_{\Psi leaf}$ (as the previous season), different to the observed in SI_{gs} with values for SDI_{65} 20% higher than those detected in FI.

Taking into account the interaction between both considered factors (irrigation x cultivar), these were detected only the second studied year, as for $SI_{\Psi leaf}$ as for SI_{gs}, the cultivar being the main factor of this interaction. More interesting were the relationships between the $SI_{\Psi leaf}$ and SI_{gs}, which were estimated for each cultivar (Figure 4.3). Whereas cvs. Guara and Lauranne showed similar relationships, and the ANCOVA did not reveal differences for the intercepts and slopes between both cultivars; significant differences were registered with the interception point obtained in cv. Marta, although the slopes were very similar.

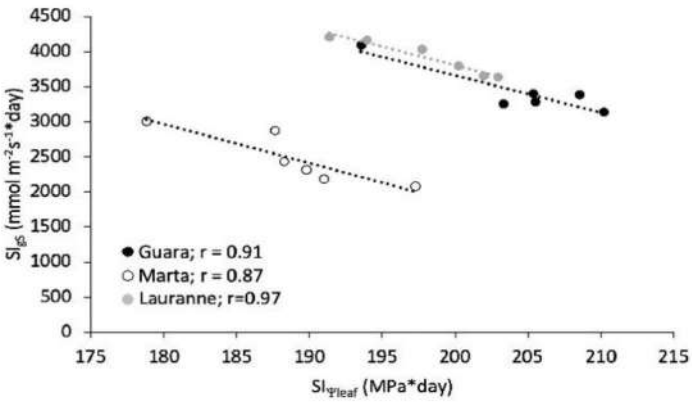

Figure 4.3 Linear relationships between the stress integral of leaf water potential ($SI_{\Psi leaf}$) and stomatal conductance (SI_{gs}) for cvs. Guara, Marta, and Lauranne. Each point is defined by the average values for each irrigation treatment, season, and cultivar.

According to these results, when physiological response was analyzed by using $SI_{\Psi leaf}$ and SI_{gs}, these differences were more evident, this being mostly relevant in comparing the type of cultivars. Considering previous results reported by Gutiérrez-Gordillo et al.[127], cv. Marta was more sensitive to water stress in physiological terms, evidencing a stronger stomatal control under RDI strategy. On the contrary, cv. Guara triggered physiological mechanisms that were able to maximize the gas-exchange rates by increasing g_s and decreasing Ψ_{leaf} values. By contrasting these findings with those outlined by García-Tejero et al.[128], declines of leaf encourage to lessen the carbon assimilation rate, disclosing the almond capability in maintaining high g_s values even when leaf is close to -2.5 MPa[100]. Moreover, before reaching important exhaustion in g_s rates, reductions in leaf are not accompanied by relevant depletions in g_s[128]. This might happen because almond is competent at holding optimum g_s and carbon assimilation rates, improving the water use efficiency, at least under moderate water stress situations. Therefore, almond reaction to water stress would demand different regulation mechanisms to counteract the adverse impact of water stress as was corroborated with the results of the present study. In addition, according to Fernández et al.[129] and Fu et al.[130], the almond has a lower capability to regulate the stoma under mild water stress situations. Moreover, these findings corroborate with those revealed by García-Tejero et al.[60] who defined two threshold values for $SI_{\Psi leaf}$; the first about -1.4 MPa without reductions for g_s, and a second of -2.0 MPa, when significant depletions in g_s are observed. Also, other authors reported that under moderate water stress conditions the almond tree decreased the leaf rates much more than g_s, and only when a certain threshold value is reached, significant depletions on photosynthesis rates are detected[131-133].

2.2 Nut Yield and Irrigation Water Productivity (IWP)

Yield and its related components showed results that agreed with the physiological responses observed in the three studied cultivars (Table 4.2). During the first experimental season, significant differences on kernel yield were observed in cv. Guara, with yield reductions around 13% in SDI_{65} in comparison to FI treatment.

Table 4.2 Impact of irrigation strategies on yield components (total yield, kg·ha^{-1}), the ratio between kernel and nut (kernel+shell), kernel unit weight (g), and fruit number per tree (n), during the study period.

Cultivar	First season (2018)			Second season (2019)		
	FI	SDI_{75}	SDI_{65}	FI	SDI_{75}	SDI_{65}
	Kernel yield (kg·ha^{-1})					
Guara	1,928.3a	1,659.2b	1,704.1b	2,254.4a	2,081.1ab	1,871.4b
Marta	1,933.1a	1,676.8b	1,775.3b	2,218.2a	2,208.5a	2,243.2a
Lauranne	2,349.2a	2,343.1a	2,241a	2,325.6a	2,104.6a	2,195.6a
	Ratio (kernel·nut^{-1})					
Guara	0.41a	0.41a	0.41a	0.34a	0.39b	0.37b
Marta	0.32a	0.31a	0.33a	0.34a	0.34a	0.34a
Lauranne	0.36a	0.36a	0.36a	0.33a	0.33a	0.32a
	Kernel unit weight (g)					
Guara	1.40a	1.40a	1.41a	0.99b	1.00b	1.22a
Marta	1.31a	1.37b	1.33a	1.21a	1.18a	1.18a
Lauranne	1.12a	1.13a	1.13a	1.03a	1.05a	1.08a
	Fruits number tree^{-1}					
Guara	6,611a	5,688b	5,801b	10,930a	9,989ab	7,363b
Marta	7,083a	5,875b	6,407ab	8,799a	8,984a	9,125a
Lauranne	10,068a	9,952a	9,519a	10,828a	9,621b	9,758b

FI, Full irrigated treatment; SDI_{75}, Sustained deficit irrigation at 75% during the irrigation period (IR); SDI_{65}, Sustained deficit irrigation at 65% IR. Vertical bars are standard deviation. Different letters are significant different by Tukey test (p < 0.05).

These differences were exclusively reflected in fruits number per tree, with a fruit number depletion similar to that reported on total yield. Something similar happened with cv. Marta, with yield reductions around 11% in SDI_{65} comparing to FI treatment, as consequence of fruits number reductions of 13.5% on average. However, these depletions were partially corrected by increasing the kernel unit weight, especially in SDI_{75}. Moreover, no differences on kernel yield were observed for cv. Lauranne, with similar results among treatments in all the yield components.

During the second year, cv. Guara showed again significant differences between SDI_{65} and FI conditions, although in this case SDI_{75} offered similar productions to those detected under FI; with yield reductions around 8% and 17% on SDI_{75} and SDI_{65}, respectively. These depletions were associated with fruit number reductions roughly 9% on SDI_{75} and 32% on SDI_{65}. It is noticeable that these reductions in the fruits number were partially corrected because of a significant increasing of kernel unit weight on SDI_{65} (23% higher than the obtained value of FI). Something different were the obtained values for cv. Marta during the second year, which did not evidence relevant yield losses by effect of SDI strategies. Finally, as it was previously discussed for the first studied season, cv. Lauranne registered the best response to SDI strategies, without relevant differences in the studied yield components.

Analysing these results regarding to the irrigation water productivity (IWP, $kg \cdot m^{-3}$) (Table 4.3), in the first studied season and within each treatment, cvs. Guara and Marta evidenced similar results, these being 20%, 40%, and 31% higher in cv. Lauranne for FI, SDI_{75}, and SDI_{65}; respectively.

Table 4.3 Irrigation water productivity (IWP) in each cultivar, treatment and studied season.

Cultivar	First season (2018)			Second season (2019)		
	(kg· m^{-3})					
	FI	SDI_{75}	SDI_{65}	FI	SDI_{75}	SDI_{65}
Guara	0.39c	0.45b	0.51a	0.29b	0.36a	0.36a
Marta	0.39c	0.45b	0.53a	0.29b	0.34b	0.44a
Lauranne	0.47b	0.63a	0.67a	0.30b	0.37ab	0.43a

FI. Full irrigated treatment; SDI_{75}. Sustained deficit irrigation at 75% during the irrigation period (IR); SDI_{65}. Sustained deficit irrigation at 65% IR. Different letters are significant different by Tukey test ($p < 0.05$).

Regarding to the effects of water stress, all treatments fixed significant improvements in comparison to the results detected under FI. Thus, comparing the IWP obtained in SDI_{65} with that registered under FI, the average values for both studied seasons offered improvements on IWP of 31%. 36%, and 43% for Guara, Marta, and Lauranne; respectively. These results were confirmed during the second season. All cultivars offered similar values of IWP for FI and SDI_{75}, meanwhile, for the case of SDI_{65} relevant improvements were detected in cvs. Marta and Lauranne in comparison to cv. Guara. Regarding to the effects of water stress, all cultivars registered significant increasing trend under SDI strategies, cvs. Lauranne and Marta offering the best response versus cv. Guara when sustained water withholdings of 35% were imposed. These values would be comparable to those reported by Egea et al.[81] for cv. Marta (0.25–0.40 kg·m^{-3}) or Phogat et al.[83] who highlighted that water productivity increased substantially respect to full irrigated trees when SDI strategies were applied.

Taking into account the whole of data, cv. Guara was the most sensitive cultivar to water stress under sustained deficit irrigation strategies. This fact is especially noticeable, taking into account that this cultivar evidenced a very positive response when water withholding was applied under RDI strategies during the kernel-filling period[85.127.128]. This contrary response would evidence that the final response to DI strategies would be determined by the added effect of three factors: The cultivar, the water stress and the irrigation strategy. However, taking into consideration the findings in the present work when this water stress was applied during the whole irrigation period, it was detected a significant fruit dropping (during the fruit setting period) (Table 4.2) and ultimately this determined the final yield with significant reductions linked to less fruit numbers per tree. These results agree with those reported by other authors. In this sense, for a SDI study (2.500 $m^3 \cdot ha^{-1}$). Alegre et al.[134] in Catalonia (North-Eastern Spain) reported productions for seven-year-old almond plantations of cvs. Guara and Lauranne of 1.65 and 2.02 $t \cdot ha^{-1}$, respectively. The absolute difference with respect to our findings could mainly be ascribed to the amount of irrigation water applied in water stressed treatments (4.250 and 4.730 $m^3 \cdot ha^{-1}$) and the climatic conditions of South Western Spain. Likewise, these results would confirm the better response of cv. Lauranne in comparison to cv. Guara under SDI strategies. This is in line with Miarnau et al.[135] who outlined that kernel yield of new almond plantations could be ranged between 1.50 and 2.0 $t \cdot ha^{-1}$ with water allocations of 2.000–3.000 and 6.000 $m^3 \cdot ha^{-1}$, respectively. In general, the yield potential of almond is highly related to the irrigation amount provided as it was revealed by Miarnau et al.[136]. These authors pointed out that under a SDI strategy with water applications around 2.000 $m^3 \cdot ha^{-1}$, the nut yield for cvs. Guara and Marta amounted to 1.20 and 1.85 $t \cdot ha^{-1}$. whereas under FI conditions (~7.500 $m^3 \cdot ha^{-1}$) these values were 2.80 and 3.55 $t \cdot ha^{-1}$, respectively. Consequently, this fact would suggest that cv. Marta would be able to activate a physiological prevention mechanism to mitigate the water stress, yielding more than cv. Guara, as was observed in the present work. Moreover, similar agronomical and physiological responses to water stress of cvs. Guara and Lauranne were highlighted by Girona et al.[80].

According to effects of SDI in the fruit unit weight, Alegre et al.[134] reported similar values than those found in the present experiment with kernel unit weights of 1.5 and 1.2 g for cv. Guara and Lauranne. In addition, according to Miarnau et al.[137] kernel unit weight FI conditions for cvs. Guara, Lauranne, and Marta would be around 1.50, 1.20, and 1.50 g, respectively implying that water stress provoked weight reduction as it was found in the present study. Likewise, it is worth mentioning the improvements in terms of fruit unit weight observed in cv. Marta under SDI_{75} during the first experimental season; and something similar in cv. Guara under SDI_{65}.

during the second year of this experiment. These results would reinforce the possibility of improving the fruit size when SDI is imposed, this being an added value in relation to fruit marketability and consumer acceptance[62].

3. Conclusions

Combining the type of almond cultivars with water stress through deficit irrigation will be vital to reach an equilibrium between water allocations and sustainable nut yields under climate change scenarios. In the framework of the present experiment, the almond response to SDI strategies was cultivar-dependent, and hence, this fact should be considered before designing a proper DI strategy.

The findings allow for a conclusion on the importance of the cultivar when a DI strategy is being applied because different physiological behaviours will promote different responses in terms of yield and its components. In this way, the cv. Marta exhibited the most conservative behaviour to water stress in physiological terms, which allowed it to obtain very similar productions than those registered under FI conditions. Furthermore. cv. Lauranne, despite showing a physiological behaviour similar to cv. Guara. it was able to reach the best yield values when a moderate-to-severe SDI was applied. Also, according to our findings. cv. Guara registered the lesser promising results, with significant yield reductions (~14%) when water restrictions around 35% of irrigation requirements were applied; these being particularly promoted by depletions in the fruit number per tree. That is. SDI_{65} would be suitable strategies to cvs. Lauranne and Marta, whereas for the case of cv. Guara we should select a more moderate SDI strategy (as SDI_{75}) or re-consider the application of other more appropriate treatments such as RDI during the kernel-filling period.

Finally, taking into consideration the absence of differences in cvs. Lauranne and Marta, and the differing results observed in cv. Guara; long-term experiment could be advisable; in order to get a deeper knowledge respect to cumulative effects of more severe water stress strategies imposed during several consecutive seasons.

Chapter 5

The effect of nut growth limitation on Triose Phosphate Utilization and down-regulation of photosynthesis in almond

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1. Introduction

Among all the abiotic and biotic stresses that plants suffer, water stress is accepted as one of the most important restricting factors for crop growing. Drought alone is expected to limit the productivity of more than half of the cultivated land in the next 50 years [138]. Irrigation can be applied to alleviate this situation, but in areas where water resources are scarce, deficit irrigation (DI) strategy is necessary for adaptation and saving water. Specifically, regulated deficit irrigation (RDI) and sustained deficit irrigation (SDI) are the main strategies that have been applied for different crops with promising but variable results[78,139].

Indeed, some fruit tree species such as almond (*Prunus dulcis* Mill.), respond positively to water stress[58,59,80,82], and hence, crop yield may not be severely affected by significant water reductions[81]. According to several works on almond, the phenological stage when water stress is applied plays a key role in the final yield. However, there is no consensus on when to impose water stress. Some studies found that the final yield was not significantly affected when water stress was applied during the kernel-filling stage[58,87,105,140]. By contrast, others authors reported significant differences in the yield produced by water stress applied during this stage[59,80,82]. Moreover, water stress during the postharvest stage could impact negatively the bloom density and fruit set of the following year, compromising yield, and thus Goldhamer and Viveros[57] suggested avoiding severe water stress in this stage. Therefore, it is critical for an optimal application of a DI strategy to elucidate how the physiological traits that ultimately determine the yield response to water stress would vary with the phenological almond stage.

According to Nortes et al.[141], the almond cycle is characterized by five stages. Stage I in the flowering period (from February to March), where the carbon (C) sinks are mainly invested in flower buds. Stage II-III or vegetative stage (from April to June) is when leaves develop and fruit growth occur, in this stage is vital the synthesis of carbohydrates for shoots and fruit growth. Stage IV or kernel-filling stage (from June to August) with dry matter accumulation, in this period, the available C is used in the fattening of the fruit and accumulation of reserves. Finally, Stage V or postharvest (from September to November) is when almond trees accumulate reserves for the next season.

Yield is partly determined by fruit growth which in turn, is limited on turgor pressure and the accumulation of carbohydrates[142]. In almond fruits most of the biomass accumulation occurs in the vegetative stage when the tree needs to build all its new foliage and nuts. During the kernel-filling stage, carbohydrates are still demanded by nuts, but it is expected that in a lower intensity than in the previous stage. Turgor and carbon availability may act as limiting factors in different stages of fruit development, as was demonstrated in olive trees [94]. The photosynthetic capacity of a plant is a major determinant of C fixation, together with stomatal conductance (g_s) and mesophyll conductance (g_m). The photosynthetic capacity can be defined as the maximum carboxylation rate (V_{cmax}), maximum rate of electron transport (J_{max}), and triose phosphate utilization (TPU). Because TPU is usually only observed at high concentration of CO_2, much higher than ambient CO_2, it has been rarely studied [143] and even less in almond where only V_{cmax} and J_{max} have been evaluated [88]. However, it could be of great importance for fruit growth, and thus, yield, since it indicates the demand of photoassimilates by the sink organs of the plant. Due to its connection to the export of sugars from the leaves to other sink organs, it is a good candidate for being such an indicator. Importantly, the TPU can lead to down-regulation of photosynthetic capacity [144,145].

The contradictory findings with respect to the almond's sensitiveness to water stress and the scarce information on its photosynthetic capacity influence on yield have motivated the present study. Improving our knowledge with respect to this particular physiological behaviour will allow us to understand better the stages when water deficit must be relieved, thereby, the fruit yield is not penalized severely, assisting in defining the most proper DI practices.

Thus, the main objective of this work was to analyze the photosynthetic capacity of almond throughout different phenological stages (vegetative, kernel-filling, and postharvest) in response to water stress for three almond cultivars (Guara, Marta, and Lauranne). We specifically focus the experiment: (1) to describe and compare the variation of the major determinants of C fixation (Vcmax, Jmax, gm, and TPU) for each almond cultivar at the three considered phenological stages and (2) to analyze the main limitations (totals, biochemical, and diffusional) that AN has in each phenological stage and its relation to yield.

2. Results

2.1 Plant water status and yield

Significant differences were found among irrigation treatments in Ψ_{leaf} readings (Figure 5.1), being both SDI treatments statistically different from FI, throughout the whole almond phenological cycle with exception of the vegetative phenological stage. As vapor-pressure deficit increased (data not shown), the Ψleaf became more negative in all the cultivars. That is, cv. Guara ranged from -0.8 to -1.9 MPa, cv. Marta between -0.5 and -1.5 MPa, and cv. Lauranne between -0.7 and -1.7 MPa for FI treatment. In the case of both SDI treatments, these ranged between -0.9 and -2.2 MPa, -0.7 and -1.9 MPa, and-1.0 and -2.1 MPa for cvs. Guara, Marta, and Lauranne, respectively. During the kernel-filling stage, cv. Guara denoted more significant differences between SDI treatments and FI than the other two cultivars

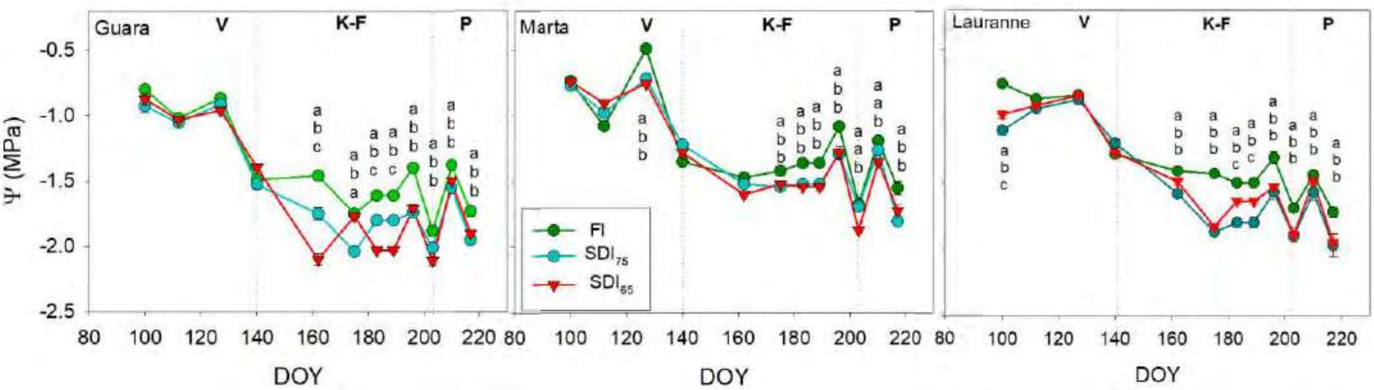

Figure 5.1 Leaf water potential (Ψ_{leaf}) dynamics for each cultivar in relation to irrigation treatment. FI, Full irrigated treatment; SDI_{75}, sustained deficit irrigation at 75% irrigation requirement (IR); SDI_{65}, sustained deficit irrigation at 65% IR; DOY, Day of the year. Vertical bars are standard deviation. Lowercase letters show statistical differences among treatments (p <0.05). **V**: Vegetative, **K-F**: Kernel-filling, **P**: Postharvest.

Despite the differences in Ψ_{leaf} found, there were not significant differences in almond yield among irrigation treatments and cultivars (Figure 5.2).

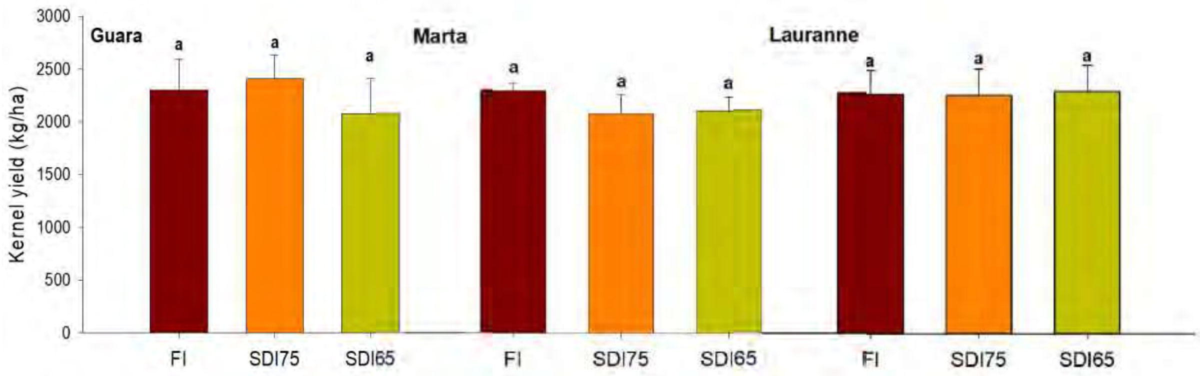

Figure 5.2 Kernel yield in relation to irrigation treatment in each almond cultivar. FI, Full irrigated treatment; SDI_{75}, sustained deficit irrigation at 75% irrigation requirement (IR); SDI_{65}, sustained deficit irrigation at 65% IR. Different letters are significant different by Tukey test (p < 0.05). Cultivars: Guara, Marta and Lauranne.

2.2 Determinants of C fixation in response to water stress and phenological stage

There were no differences in A_N among cultivars in any of the treatments (Figure 5.3). However, in all the treatments the phenological stages showed a very marked trend in which the vegetative and kernel-filling stages reached similar values of A_N (~14-19 µmol·m^{-2}·s^{-1}) without significant differences between them (except for FI in the cv. Lauranne), while the postharvest stage presented significantly lower values than the other two stages.

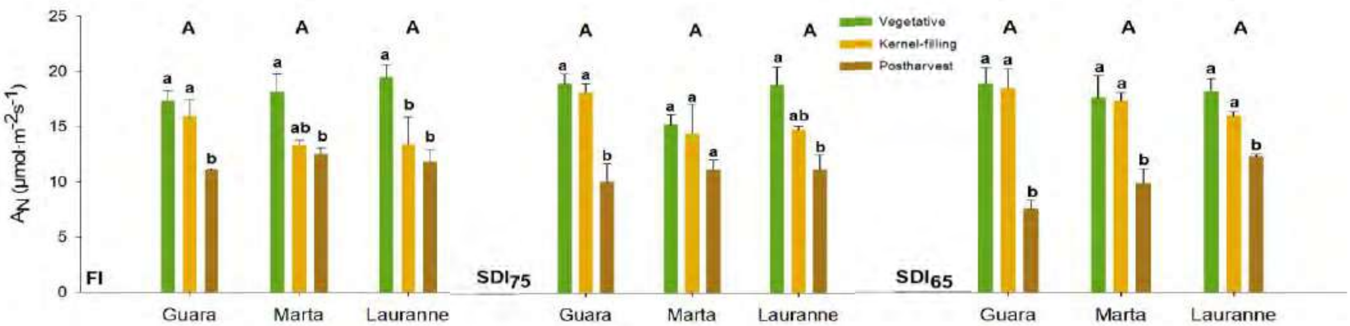

Figure 5.3 Net photosynthesis rate (A_N) for cvs. Guara, Marta, and Lauranne. FI, Full irrigated treatment; SDI_{75}, sustained deficit irrigation at 75% irrigation requirement (IR); SDI_{65}, sustained deficit irrigation at 65% IR. Vertical bars are standard deviation. Lowercase letters show statistical differences among phenological stages within each cultivar (p <0.001). Capital letters show statistical differences among cultivars (p < 0.001).

The leaf photosynthetic capacity, represented by V_{cmax} and J_{max}, showed as well a marked seasonality in all cultivars and treatments (Figure 5.4). The highest values occurred in vegetative stage (~ 193-268 μmol·m^{-2}·s^{-1} for V_{cmax} and 160-210 μmol·m^{-2}·s^{-1} for J_{max}) and the lowest, as occurred in A_N, in postharvest (~ 57-97 μmol·m^{-2}·s^{-1} for V_{cmax} and 73-113 μmol·m^{-2}·s^{-1} for J_{max}), presenting the kernel-filling stage an intermediate behavior. There were no significant differences among cultivars in V_{cmax}, while for J_{max} in the SDI_{75} treatment there were differences between cultivars, being *cv*. Lauranne the one with the highest values (~ 209 μmol·m^{-2}·s^{-1}).

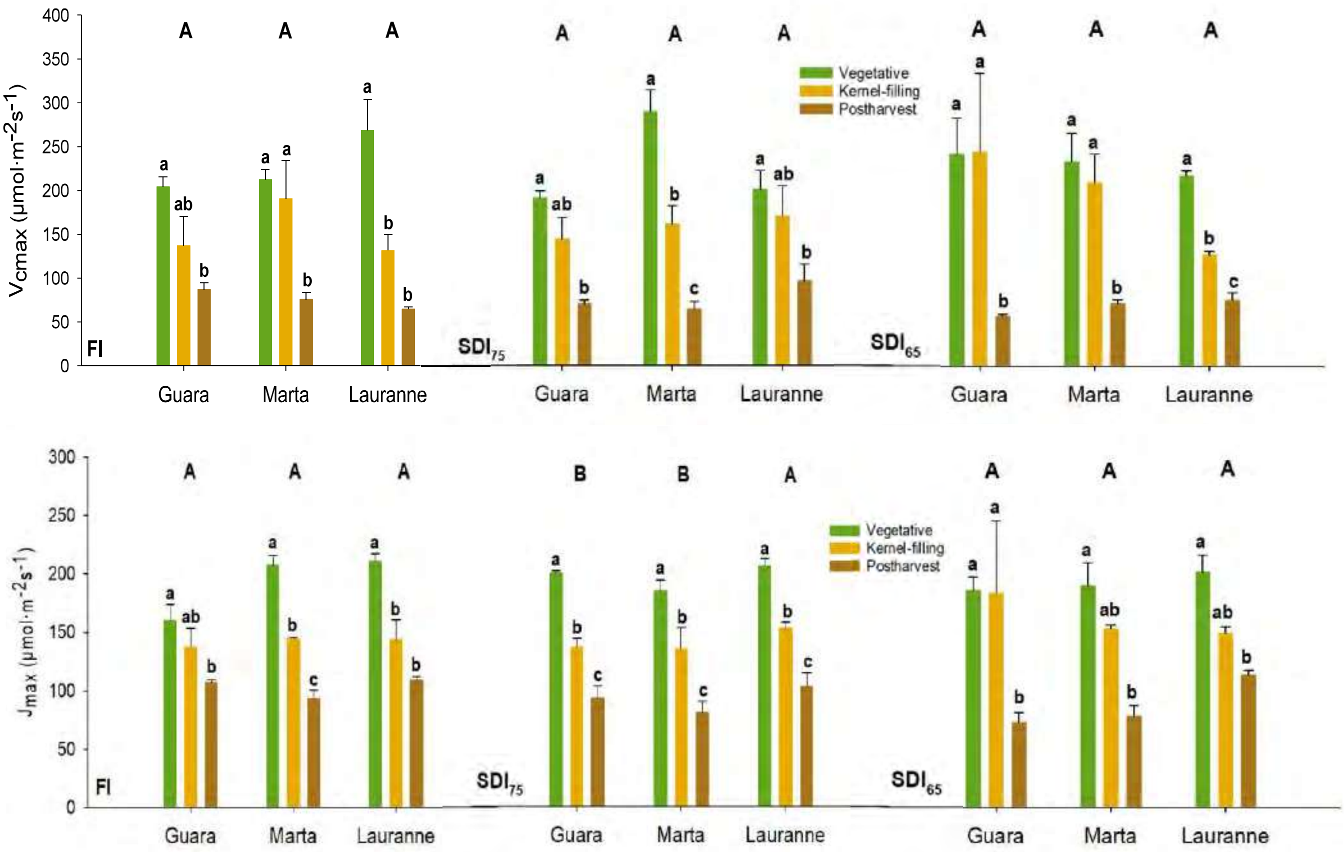

Figure 5.4 Major determinants of C fixation: Maximum carboxylation rate (V_{cmax}) and Maximum rate of electronic transport (J_{max}) for cvs. Guara, Marta and Lauranne. FI, Full irrigated treatment; SDI_{75}, sustained deficit irrigation at 75% irrigation requirement (IR); SDI_{65}, sustained deficit irrigation at 65% IR. Vertical bars are standard deviation. Lowercase letters show statistical differences among phenological stages within each cultivar (p <0.001). Capital letters show statistical differences among cultivars (p < 0.001).

The use of triose phosphate (TPU) showed no differences among cultivars in any of the treatments (Figure 5.5), except in SDI_{75} where *cv*. Lauranne (as the trend found in J_{max}) presented the highest value (9.48 μmol·m^{-2}·s^{-1} on average), while the cv. Marta presented the lowest TPU rate (7.44 μmol·m^{-2}·s^{-1} on average).

As previously for V_{cmax} and J_{max}, the vegetative stage presented the highest values of TPU (12.47 μmol·m^{-2}·s^{-1}), and postharvest the lowest (5.74 μmol·m^{-2}·s^{-1}). In this case, the kernel-filling stage presented values similar to postharvest, being significantly different in some cases (*cv*. Guara and Marta in SDI_{65}).

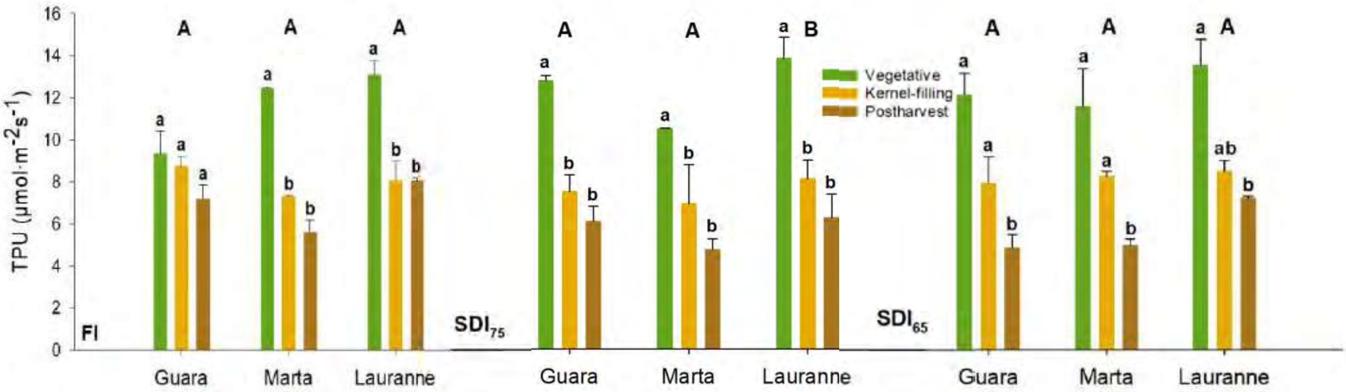

Figure 5.5 Triose phosphate utilization (TPU) for cvs. Guara, Marta and Lauranne. FI, Full irrigated treatment; SDI_{75}, sustained deficit irrigation at 75% irrigation requirement (IR); SDI_{65}, sustained deficit irrigation at 65% IR. Vertical bars are standard deviation. Lowercase letters show statistical differences among phenological stages within each cultivar (p <0.001). Capital letters show statistical differences among cultivars ($p < 0.001$).

Moreover, TPU correlated significantly with fruit growth rate (Figure 6). The greatest TPU coincided with the vegetative stage in which the fruit growth is higher. During the kernel-filling stage when fruit growth was reduced, the use of triose phosphate decreased.

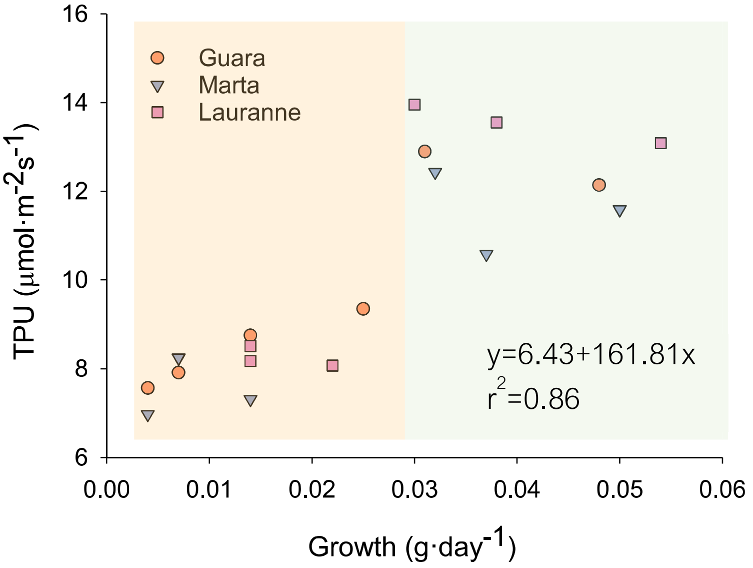

Figure 5.6 Triose phosphate utilization (TPU) relationship with fruit growth in vegetative and kernel-filling stages. The yellow and green backgrounds correspond to the kernel-filling, and vegetative phenological stages, respectively.

The reduction in TPU was also significantly related to the reduction in J_{max}, and this latter one to V_{cmax} (Figure 5.7). Although both showed a significant correlation, it was stronger in the case of TPU vs J_{max} (R^2=0.89, p< 0.001) than for the J_{max}-V_{cmax} relationship (R^2=0.77, p< 0.001), likely indicating a putative mechanism sharing the downregulation of both.

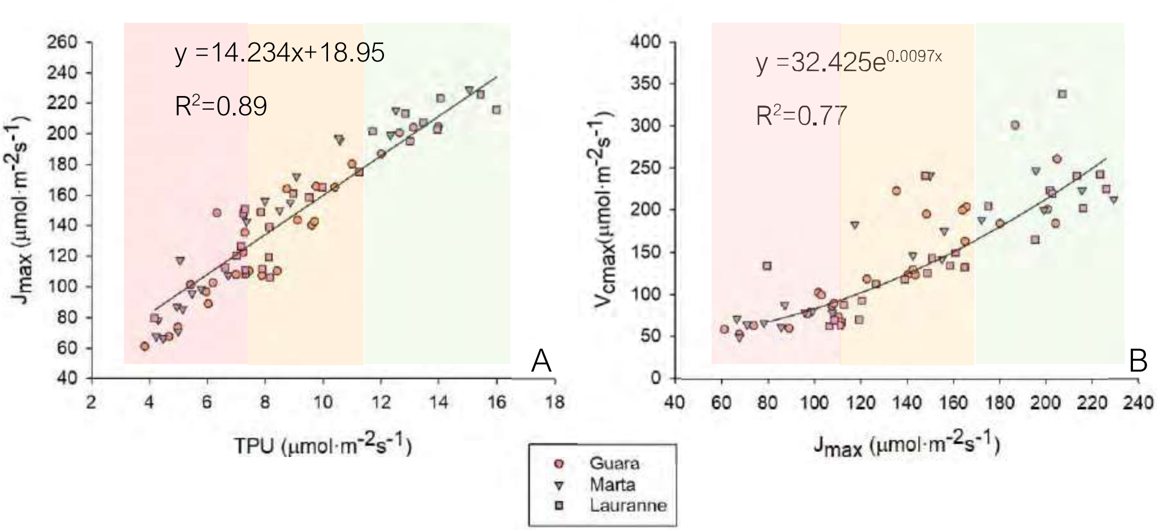

Figure 5.7 Relationships between J_{max} and TPU (A) and V_{cmax} and J_{max} (B). The red, yellow, and green backgrounds correspond to the postharvest, kernel-filling, and vegetative phenological stages, respectively.

Figure 5.8 shows the response of g_s and g_m, the main diffusional limitations for A_N. Stomatal conductance showed significant differences between the cultivars being cv. Lauranne the one with the highest values in all treatments (0.77, 0.55, and 0.61 $mol \cdot m^{-2} \cdot s^{-1}$ in FI, SDI_{75}, and SDI_{65} treatment respectively). As regards the phenological stages in each of the treatments, there were no notable significant differences, except in the cv. Lauranne where g_s increased notably in the postharvest stage.

Mesophyll conductance showed significant differences among cultivars in the SDI_{65} treatment where *cv*. Lauranne had the highest values. In the rest of the treatments there were no significant differences among the cultivars. Concerning the phenological stages, there were differences between them depending on the cultivar. In the case of FI treatment, *cvs*. Guara and Marta obtained the highest g_m values in kernel-filling, while *cv*. Lauranne in vegetative stage. On the other hand, *cv*. Guara obtained lower values in postharvest as well as in *cv*. Marta. Lastly, in the SDI_{65} treatment, *cvs*. Guara and Marta obtained the lower value in postharvest being different of the other two treatments. In the case of cv. Lauranne the trend was the same that in the other treatments, being higher in postharvest.

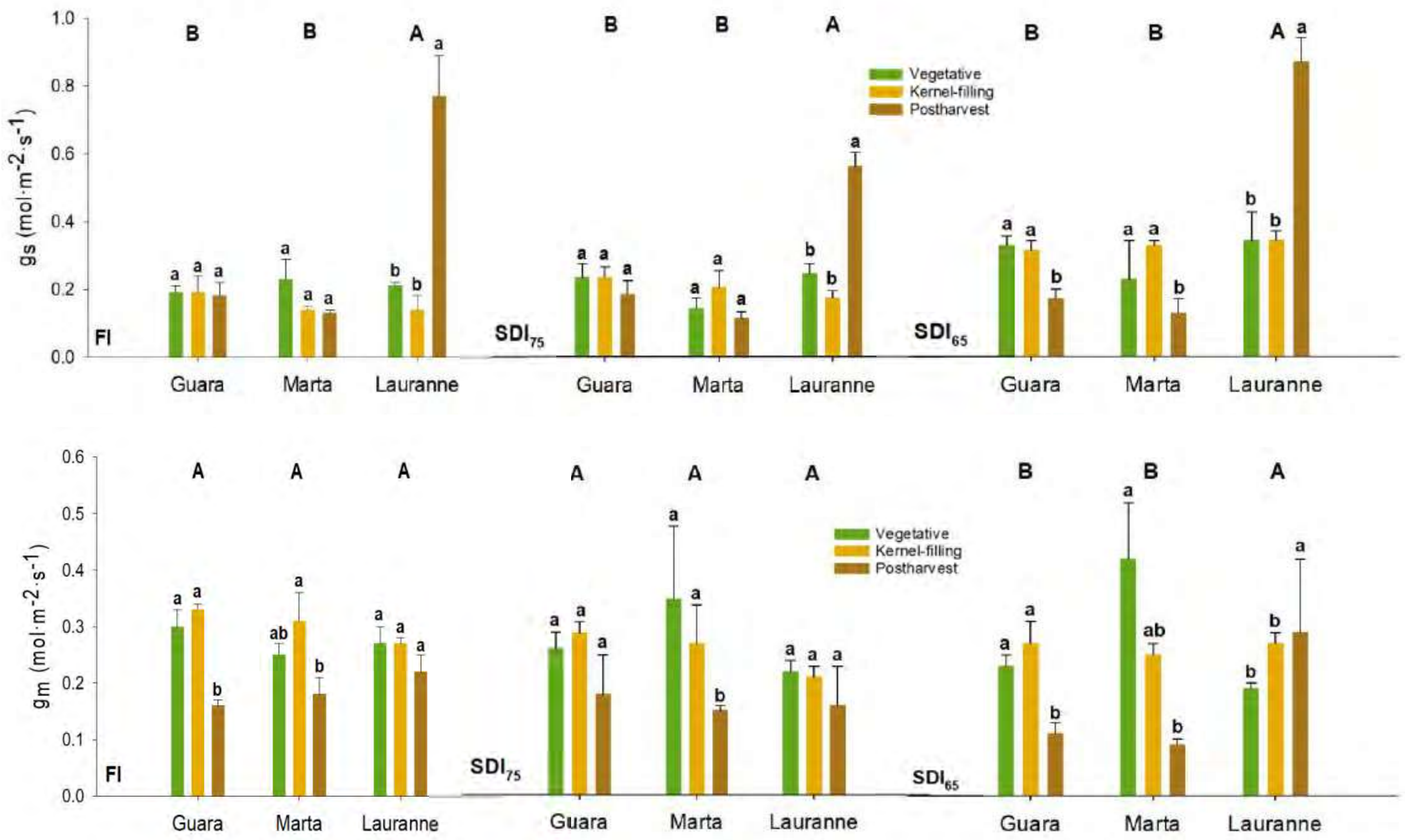

Figure 5.8 Stomatal conductance (g_s) and mesophyll conductance (g_m) for cvs. Guara, Marta and Lauranne. FI, Full irrigated treatment; SDI_{75}, sustained deficit irrigation at 75% irrigation requirement (IR); SDI_{65}, sustained deficit irrigation at 65% IR. Vertical bars are standard deviation. Lowercase letters show statistical differences among phenological stages within each cultivar (p <0.001). Capital letters show statistical differences among cultivars (p < 0.001).

2.3 Principal limitations of A_N in each phenological stage

In terms of total limitation to A_N, no differences were observed among the cultivars or treatments, showing a similar pattern: the limitation was greater for postharvest than for kernel-filling stage (Figure 5.9) in relation to the vegetative period. These differences were significant in cvs. Guara and Marta but non-significant for cv. Lauranne.

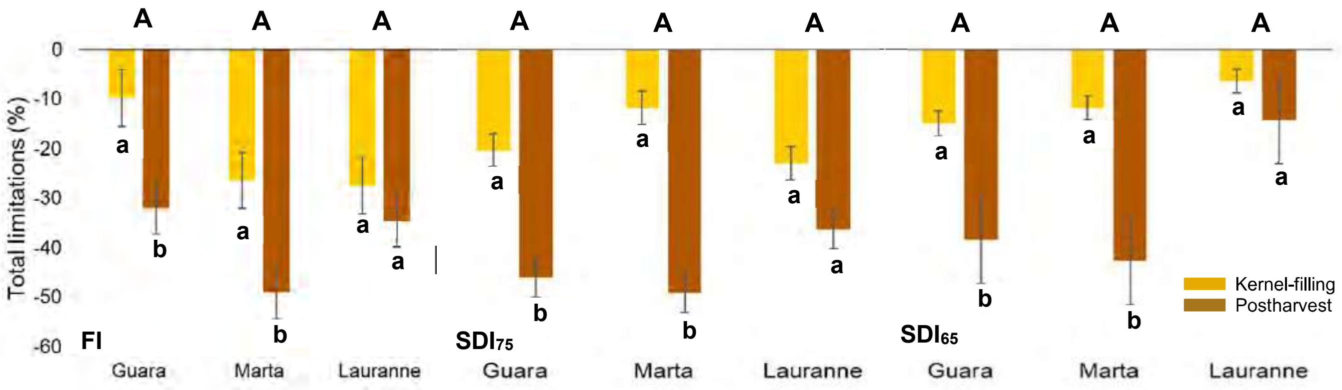

Figure 5.9. Total limitations (%) of photosynthesis compared to vegetative stage for cvs. Guara, Marta, and Lauranne. FI, Full irrigated treatment; SDI_{75}, sustained deficit irrigation at 75% irrigation requirement (IR); SDI_{65}, sustained deficit irrigation at 65% IR. Lowercase letters show statistical differences among phenological stages within each cultivar (p <0.001). Capital letters show statistical differences among cultivars (p < 0.001).

The biochemical limitations were more relevant than the diffusional ones (Table 5.1). Moreover, although cvs. Guara and Marta showed important diffusional limitations, cv. Lauranne only presented biochemical limitations. The major limitation came from J_{max}, which responded to the irrigation treatments in cv. Guara. Its effect was greater with the increasing irrigation deficit. Regarding diffusional limitations, the greatest limitation of photosynthesis was g_s in both phenological stages, except for the cv. Lauranne, which did not present limitation by g_s in the postharvest stage.

Table 5.1 Biochemical and diffusional limitations (%) in kernel-filling and postharvest stages compared to vegetative stage.

	Kernel-filling Stage							Postharvest Stage						
	Biochemical limitations (%)				Diffusional limitations (%)			Biochemical limitations (%)				Diffusional limitations (%)		
	J_{max}	V_{max}	TPU	**TOTAL**	g_m	g_s	**TOTAL**	J_{max}	V_{max}	TPU	**TOTAL**	g_m	g_s	**TOTAL**
cv. Guara														
FI	-10	0	0	**-10**	1	-1	**0**	-22	0	0	**-22**	-8	-2	**-10**
SDI_{75}	-22	0	0	**-22**	2	0	**2**	-37	0	0	**-37**	-5	-4	**-9**
SDI_{65}	-25	0	0	**-25**	4	-1	**3**	-39	0	0	**-39**	-9	-9	**-19**
cv. Marta														
FI	-18	0	0	**-18**	3	-12	**-9**	-35	0	0	**-35**	-5	-9	**-14**
SDI_{75}	-20	0	0	**-20**	3	5	**8**	-35	0	0	**-35**	-6	-8	**-14**
SDI_{65}	-15	0	0	**-15**	-4	1	**-2**	-35	0	0	**-35**	-15	-14	**-29**
cv. Lauranne														
FI	-18	0	0	**-18**	0	-9	**-10**	-42	-5	0	**-47**	-3	16	**13**
SDI_{75}	-14	0	0	**-14**	-2	-8	**-9**	-40	0	0	**-40**	-6	10	**4**
SDI_{65}	-17	0	0	-17	8	0	8	-40	0	0	-40	8	11	19

FI, Full irrigated treatment; SDI_{75}, sustained-deficit irrigation at 75% irrigation requirement (IR); SDI_{65}, sustained-deficit irrigation at 65% IR. J_{max}, maximum rate of electron transport; V_{cmax}, maximum carboxylation rate; TPU, triose phosphate utilization; g_s, stomatal conductance; g_m, mesophyll conductance.

3. Discussion

The most remarkable result of our study is that we have identified different demands of photoassimilates by the plant depending on the phenological stage. This seasonal change is reflected physiologically in the TPU, and can be estimated from An-Ci curves with the parameter TPU. The general reduction in TPU in the kernel-filling and postharvest stages in relation to the vegetative stage, likely reflects the decrease in the sink strength of the plant. This explains well the further reduction in the plant's photosynthetic capacity (J_{max} and V_{cmax}) and the consequential increase of the relevance of biochemical limitations in relation to diffusional ones. These results open new avenues to manage irrigation and identify new physiological indicators to design DI strategies rationally.

Our results showed that there were no differences among irrigation treatments in terms of g_s, photosynthetic-related variables or the yield, despite the differences in Ψ_{leaf} (Figure 5.1), which were greatly diminished in all cultivars and treatments over time. These results agreed with those obtained by Fathi et al.[146], who verified in a two-year experiment with almond cultivars (*cvs*. Ferragnès and Sahand), that under moderate stress, the trees significantly reduced their Ψ_{leaf} and even that most of the almond genotypes had an osmotic regulation mechanism to maintain turgor and photosynthetic capacity during the first phases of applied stress. In this line, Barzegar et al.[126], with six almond cultivars (Azar, Marcona, Mission, Nonpariel, Sahand, and Supernovoa) reported that Ψ_{leaf} decreased rapidly due to the osmotic adjustment in order to avoid a reduction of its photosynthetic capacity in the early stages of stress. In addition, other authors [147-149] demonstrated that leaves of drought tolerant species such as almond or olive can reach lower values of $\Psi_{leaf,}$ before losing turgor. Thus, it seems that under moderate water stress levels, such as the ones applied here, the almond tree tends to decrease the Ψ_{leaf} rather than decrease the photosynthetic capacity, as the measurement in A_N in our study shows.

Although the photosynthetic capacity was not affected significantly by the irrigation treatment, A_N, $g_{m,}$ V_{cmax}, J_{max} and TPU were significantly different among phenological stages (Figures 5.3, 5.4, 5.5, and 5.8), being in all cases the highest values found in the vegetative stage. The highest photosynthetic capacity during the vegetative stage and the progressive descend during kernel-filling and postharvest stages can be explained by the characteristics of the phenological development of almond crop. In the vegetative stage the shoots and fruits are developed[141]. Moreover, in this stage a fast cell division process occurs in this species[150], which has been recently related with a greater importance of photosynthesis than turgor for growth in olive fruits[151]. In terms of fruit growth almond showed a double sigmoid pattern with a kernel-filling stage influenced by the length of the reproductive cycle[152]. In this sense, the fruit growth is much higher in the vegetative stage, where the cell division process occurs, than in the kernel-filling stage. For this reason, the use of photoassimilates is much greater in vegetative, consuming more TPU than in kernel-filling, as shown in Figure 5.6.

The A_N limitation analysis explained further that the major limitation of kernel-filling and postharvest stages compared to vegetative stage was mainly produced by the biochemical limitations, specifically J_{max} (Table 5.1). Accordingly, Egea et al.[88] in a four-year experiment with the *cv*. Marta, found that the highest rates of A_N and J_{max} were observed during the vegetative stage, rapidly decreasing until the postharvest stage and the main photosynthetic limitation due to the seasonal effects were the biochemical ones. Other authors [153-155] confirmed the importance of biochemical limitations in woody species.

This J_{max} limitation in the kernel-filling and postharvest stage compared to the vegetative stage can be a consequence of the regulation occurring due to the decrease of TPU in the last two stages. A decrease in TPU means that the use of triose phosphates, the direct product of photosynthesis, is reduced. This is likely because almond sustains high A_N which is greatly used in the vegetative stage but not to the same extent in the two other stages. As described before, during the kernel-filling stage, there is no significant vegetative growth and nuts have achieved their full size, which reduces the sink strength and therefore the demand of photoassimilates. In the postharvest stage, the harvest has already been carried out, and the tree is beginning to prepare for the winter rest phase, with hardly any growing organs demanding any photoassimilates. Thus, during the kernel-filling stage and postharvest, part of the triose-phosphates produced may not be exported via phloem to other organs, which leads to their accumulation in the leaves. This accumulation of triose-phosphates would produce a photosynthesis inhibition or a down-regulation of the photosynthetic capacity [144,145], reducing J_{max}, V_{cmax} and finally, g_m to maintain the balance. When high photosynthetic rate and TPU accumulation occurs, the phosphorus that ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) needs to regenerate from TPUs is not available and therefore, the Calvin cycle stops [156](Raines 2003).

We can not rule out the possibility that the photosynthetic limitation happened in the kernel-filling stage produced by a higher demand of nitrogen during the fruit fattening process than in the other stages (30% of the estimated total nitrogen is recommended to be applied during kernel-filling[61]). Nitrogen is essential for growth and development of the crop. It supports photosynthesis and strong productive growth leading to high almond yields [157]. Moreover, Walcroft et al.[158] verified that decreases in nitrogen content negatively affect J_{max}, which suggests that almond leaf may preferentially lose nitrogen from the light harvesting complex or electron transport chain[159]. However, the maintenance of A_N across water treatments, the similar application of the amount nitrogen applied despite the SDI, and especially the appearance of TPU limitation, makes this possibility unlikely. If electron transport rate, estimated in this study as J_{max}, would have been the triggering factor downregulating the photosynthetic capacity, TPU limitation would have been more unlikely to have taken place. Another potential explanation might be the decrease in Ψ_{leaf} due to the water treatments, which could have limited the export of sugar via phloem[160]. But this explanation is also unlikely as there are no differences in TPU between FI and both SDI treatments, although it is true that FI treatment also showed a decrease in Ψ_{leaf} coinciding with the progressive increment of vapor pressure deficit. The mechanistic reason for the decrease in TPU during kernel filling and post-harvest merits further verification in the future to identify.

On the view of the results, we hypothetise that the irrigation dose could be reduced in these two stages, kernel-filling and post-harvest, without penalizing severely the fruit growth and thus, yield. The foundation for this hypothesis rest upon the fact that if such an amount of photoassimilates is not needed because the plant is doing more photosynthesis than it really needs, in that case g_s could be reduced by means of the irrigation reduction with the consequent save of water in transpiration. However, our results are not enough to validate this approach, since this imposition of moderate water stress by reduction of the irrigation dose need to consider that plant growth in general, and fruit growth specifically, are limited by photosynthesis reduction but also, by turgor loss[142,151,161]. Thus, the DI strategy applied should consider that the turgor threshold below which growth would not occur[162], should not be reached. The water stress level applied must also consider that at postharvest stage water withholdings had a reduction in bloom density and fruit set in the following seasons[57]. Our research group is currently working on testing these hypotheses.

Scheduling irrigation based on physiological data as the ones obtained in the present study, contribute to the traditional practice in which it is stated that the almond tree in the kernel-filling stage is less sensitive to water restrictions than in the other phenological stages[58,87,105,140]. Our result could help to answer the long-debated question on when it is better to apply deficit irrigation in almond. It is obvious that the measurement of TPU can not be implemented in regular practices in the field due to the complexity of its measurement and following analysis. But the consequence of the reduction of TPU in the electron transport rate can be easily monitored with chlorophyll fluorescence techniques[163], now with low-cost devices[164], estimated remotely with terrestrial sensors[165], and even estimated from satellites with several vegetation indices[166]. This opens the possibility to real and practical use of the conclusions of this work.

4. Conclusions

To our knowledge this is the first attempt to explain what happens with the major determinants of C fixation (A_N, V_{cmax}, J_{max}, g_m and TPU) in each phenological stage of almond and also this study breakdowns the different photosynthetic limitations based on the phenological stages of the crop. Our main conclusion is that the major limitation in photosynthetic capacity was found in the kernel-filling and postharvest stage compared to vegetative stage, and this suggest that this fact was mainly produced by the biochemical limitation of J_{max}, due to the down regulation induced by the triose-phosphates TP accumulation. Thus, we also conclude that TPU could be a good indicator to apply DI to almond. In this line, according to our results the kernel-filling stage is the best moment to apply this water withholding, being this stage the less sensitive stage in terms of photosynthetic limitation. The physiological findings from this study could help to advance the irrigation deficit scheduling of almond by providing relevant evidence for the importance of a high photosynthetic capacity in the vegetative stage and also the existence of TPU limitation in almonds.

Chapter 6

Enhancing Nut Quality Parameters and Sensory Profiles in Three Almond Cultivars by Different Irrigation Regimes

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1. Introduction

Water scarcity is the most limiting factor in arid and semiarid areas of the Mediterranean basin such as south Spain because of the rainfall shortage and variability and the high annual evapotranspiration rates[38]. This situation will be even more extreme in the coming years, considering the past forecasts of climate change, which predict significant reductions in the annual rainfalls (close to 30%) and an increase in the average temperatures between 2 and 3 °C[21,33]. The implementation of sustainable strategies aiming at improving water management in irrigated areas will be crucial to maintain the long-term development and competitiveness of the agricultural activity. Among them, the introduction of profitable alternative drought tolerant crops in irrigated areas and the use of deficit irrigation (DI) strategies (not as an alternative but as a requirement) should be considered[3].

Almond (*Prunus dulcis* Mill.) is considered a drought-tolerant crop. Many results have been reported in relation to its physiological and agronomical responses when it is subjected to DI strategies, to achieve a proper tree development under water stress conditions, minimizing the yield losses and adjusting the irrigation inputs to water resource availability[60,88,167]. In Spain, almond represents (after olive and vineyards) the third woody crop in terms of the surface, producing more than 80% of the European production[168]. However, the almond yields in Spain are very low because it has been traditionally associated with marginal areas with very limiting conditions, with average kernel yield of about 150 $kg \cdot ha^{-1}$. This value contrasts with those obtained in other countries, such as the United States or Australia, where this crop is cultivated under intensive agronomic practices and without irrigation restrictions, leading to enormously higher productions, in many cases up to 3,000 $kg \cdot ha^{-1}$ of the kernel yield[46].

Despite the positive response of almond trees to moderate water stress conditions, irrigation is considered the main limiting factor for this crop[42]. Recently, López-López et al.[85] reported that the optimum irrigation doses under the climatic conditions of the Guadalquivir River basin would be close to 8,000 $m^3 \cdot ha^{-1}$. Similar results were reported by Garciá-Tejero et al.[60] in a long-term experiment performed for mature almond trees cv. Guara. These findings differ from those published for mature almond trees (cv. Nonpareil) in California, concluding that almond crop reached the maximum yield (~4,000 $kg \cdot ha^{-1}$) under irrigation close to 12,500 $m^3 \cdot ha^{-1}$[46]. Taking into account these maximum irrigation requirements and the actual water allocations for almond in south Spain (~3,500 $m^3 \cdot ha^{-1}$), its introduction as an alternative crop would only be justified if irrigation productivity can be improved under water scarcity scenarios by means of DI strategies. Lately, new research lines focused on food production under hydrosustainable strategies (hydroSOS products) have been successfully developed[68,169,170]. These studies highlight the positive effects of sustainable practices of water management on different crops, with significant improvements in the amount of bioactive compounds, fruit quality, sensory profiles, and consumer acceptance in different products such as pistachios[171] and olive oils[172]. Recently, Lipan et al.[64] reported novel results in almond (cv. Vairo), concluding that DI strategies did not reduce the main nut quality parameters and even led to improvements in fat (especially unsaturated fatty acid) and potassium contents when a moderate DI strategy was used. Following these promising results, it can be hypothesized that the reported improvements in the almond nut quality (cv. Vairo) might also be observed in other more representative commercial cultivars. Thus, the aim of this work was to assess the impact of different irrigation strategies in terms of almond morphological, physicochemical, functional, and sensory parameters on three commercial almond cultivars (Guara, Marta, and Lauranne) under Mediterranean semiarid conditions.

2. Result and discussion

2.1 Crop Physiological Response to Irrigation Water Applied.

Table 6.1 shows the average of climatic conditions together with the irrigation doses applied in each treatment during the experimental period at the different phenological stages. Total rainfall and reference evapotranspiration registered were 513.5 and 1,102 mm, respectively. During this season, average daily temperatures ranged between 10.2 and 38.5 °C, whereas the relative humidity between 15.0 and 96.0%. According to the crop-water requirements, during the irrigation period (IP), the treatments FI and 150% ET_C received 687 and 1,030 mm, respectively. On the other hand, the treatment RDI_{65} received 542 mm during the IP, considering that, from the beginning to June 12th (DOY 163, when water stress was initially imposed), and during post-harvesting stage (232-302 DOY) this treatment received similar irrigation amounts to those of FI.

Table 6.1 Crop water requirements and irrigation dose applied in each studied treatment.

DOY	Stage I (1-47)	Stage II-III (48-162)	Stage IV (163-231)	Stage V (232-302)	Total (1-302)
			(mm)		
ETo	68.6	375.0	383.7	273.7	1,102
Rainfall	145.2	299.0	0.0	69.3	513.5
ET_C	0.0	235.0	420.0	212.0	867.0
RDI_{65}	0.0	110.0	266.6	166.0	542.6
FI	0.0	110.0	410.2	166.0	686.2
150%ET_C	0.0	165.5	615.0	249.0	1,029.5

Stage I, flowering stage; Stage II-III, vegetative development and fruit-growth stage; Stage IV, kernel-filling stage; Stage V, post-harvesting; DOY, day of the year; ET_0, reference evapotranspiration; ET_C, crop evapotranspiration; F_I, full-irrigated treatment at 100 ET_C; 150-ET_C, over-irrigated treatment at 150% ET_C; RDI_{65}, regulated deficit irrigation treatment, which received 65% of water applied in F_I during the stage IV.

The different irrigation doses applied in each treatment and cultivar promoted a significant response in terms of integrated leaf water potential (ΨInt) during the stage IV (kernel filling stage), when the water restriction in RDI_{65} was imposed (from 24 to

31 week of the year) (Fig. 6.1). According to Figure 6.1, Guara and Lauranne showed similar values of ΨInt for FI and RDI$_{65}$, but they were significantly higher ($p<0.05$) than those registered in 150% ET$_C$. However, for Marta, these differences were slightly different, not appearing significant differences between FI and 150% ET$_C$ at the end of monitoring period. Taking into account the ΨInt for the whole monitoring period, the registered values in 150% ET$_C$ for cv. Marta (127 MPa day) were 9 and 20% lower than those registered in FI (140 MPa day) and RDI$_{65}$ (158 MPa day). By the contrary, for cv. Lauranne 150% ET$_C$ reached ΨInt=120 MPa day, this being 8 and 12% lower than those detected in FI (130 MPa day) and RDI$_{65}$ (135 MPa day). Finally, cv Guara reached very similar values of ΨInt in FI and RDI$_{65}$ (130 and 131 MPa day, respectively), and 15% lower in 150% ET$_C$.

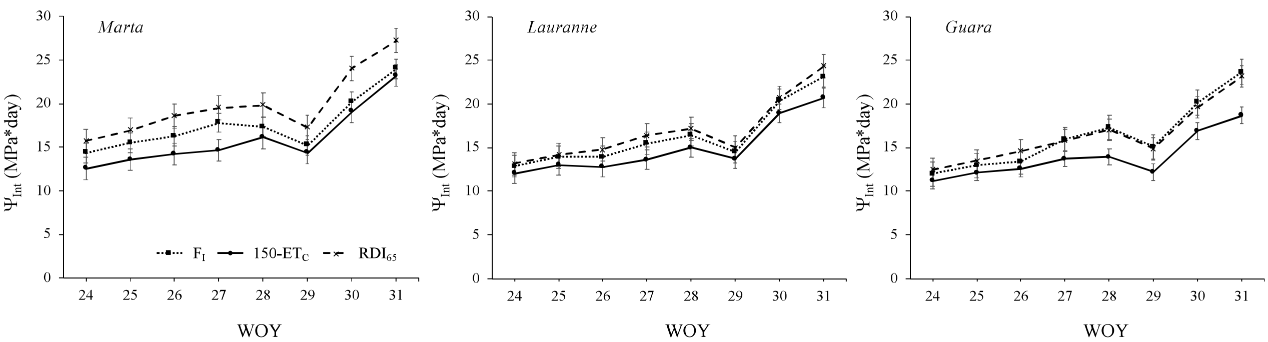


Figure 6.1 Integrated leaf water potential (Ψ_{Int}) on a weekly basis for the different irrigation treatments and studied cultivars. WOY, week of the year; FI, full irrigated treatment at 100 ET$_C$; 150-ET$_C$, overirrigated treatment at 150% ET$_C$; RDI$_{65}$, regulated deficit irrigation treatment, which received 65% of the water applied in FI during the stage IV.

In general, Lauranne and Guara evidenced similar patterns under FI and RDI$_{65}$ strategies. In this regard, these cultivars showed similar values of ΨInt in both treatments which agreed with results previously[60,74]. By the contrary, cv. Marta evidenced a higher sensibility to water stress and showed significant descends on ΨInt when this cultivar was irrigated at 150% ET$_C$ above to the theoretical requirements (150% ET$_C$ vs. FI).

Although there are not many similar experiments comparing the differential responses of almond cultivars to water stress in physiological terms, Gomes-Laranjo et al.[116] reported a higher ability to maintain similar photosynthesis rates (directly related to the stomatal conductance) under moderate-to-severe drought conditions than those obtained under non stressed trees (FI) in cv. Lauranne as compared to other cultivars, including Ferragnès, Masbovera, Glorieta, and Francoli.

2.2 Morphological and physical parameters

Table 6.2 summarizes the main results related to the effects of irrigation dose and cultivar on the morphological and physical parameters. In general, the irrigation dose promoted significant differences in almond weight, kernel thickness, color and kernel cutting force parameters. Regarding to the cultivar and the interaction irrigation dose x cultivar, significant differences were also observed for all the morphological parameters except number of fractures. According to irrigation treatments, almonds under 150% ET_C registered the highest values of weight and for the a* and b*coordinates but the lowest values for color L* and almonds hardness. This means that softer almonds with lighter color, less red and more yellow notes were produced under 150%ET_C). A softer texture was expected for almonds from 150% ET_C treatment (70.4 N) and gradually harder ones for FI (72.8 N) and RDI_{65} (74.2 N) due to the higher amount of applied water during the growing season. The cultivar Guara produced almonds with the highest weight, size and hardness (together with Lauranne ~75 N). On the other hand, the lowest hardness value was observed for nuts of the cv. Marta (68 N). Texture parameters (Table 6.2) are of utmost importance for consumer acceptability. In this line, both the irrigation treatment and the cultivar promoted significant effects. On overall, RDI_{65} reported significant improvements in terms of fracturability and hardness, whereas, in terms of the cultivar, Guara and Lauranne reported the most promising results (highest hardness and work to shear). Color coordinates showed a lighter skin for cv. Guara and darker for cv. Marta, this last being characterized by the highest values of a* (reddish) but lowest of b* (bluish). The RDI_{65} was the treatment leading to the darkest and most reddish almonds which is in agreement with other results recently published[64].

Table 6.2 Morphology, instrumental color and instrumental texture of raw almonds as affected by irrigation dose and cultivar.

	Weight (g)			Size (mm)						Kernel Color Coordinates					Kernel Cutting Force				
	Whole	Kernel	Shell	WL	KL	WW	KW	WT	KT	L*	a*	b*	C	Hue	F(mm)	H(N)	WS(Ns)	AF(N)	NF
	ANOVA†																		
Irrigation	***	***	**	NS	NS	NS	NS	NS	***	***	**	***	***	***	***	**	*	*	NS
Cultivar	***	***	**	***	***	***	***	***	***	***	**	***	***	***	***	**	*	*	NS
Irrigation x Cultivar	***	***	**	***	***	***	***	***	***	***	**	***	***	***	***	**	*	*	NS
	Tukey Multiple Range Test‡																		
	Irrigation																		
RDI_{65}	3.43b	1.25b	2.18b	32.5	24.6	23.6	14.3	16.2	7.84b	48.0b	18.4a	34.7b	39.33b	61.8b	2.09a	74.2a	84.4a	40.1a	9.85
FI	3.59b	1.28b	2.31ab	32.4	24.8	23.5	14.4	16.2	8.15a	47.9b	18.1ab	34.0b	38.5b	61.8b	2.09a	72.8b	83.9a	39.5ab	9.68
150-ETc	3.86a	1.37a	2.48a	32.6	24.8	23.8	14.7	16.7	8.36a	50.6a	18.0b	37.0a	41.2a	63.8a	1.98b	70.4c	75.6b	37.6b	10.3
	Cultivar																		
Marta	3.61b	1.24b	2.37b	31.9b	25.0a	21.3c	13.2c	15.9b	8.29a	46.3c	18.7a	33.8b	38.7b	60.8c	2.12a	68.0b	80.1b	36.9b	9.28
Guara	3.89a	1.49a	2.41b	33.5a	25.4a	26.3a	16.0a	17.4a	8.14ab	50.9a	18.0b	37.5a	41.7a	64.1a	2.00b	75.8a	81.7a	40.6a	10.0
Lauranne	3.38b	1.18b	2.20a	32.1b	23.9b	23.3b	14.2b	15.8b	7.92b	49.3b	17.7b	34.3b	38.7b	62.4b	2.04b	73.5a	82.1a	39.7ab	10.5
	Irrigation x Cultivar																		
Marta																			
RDI_{65}	3.36b	1.16c	2.20b	32.2b	24.8abc	21.5c	13.0d	15.9c	7.84bc	44.0d	18.9a	32.3c	37.5c	59.5d	2.39a	71.9ab	95.4a	39.3a	8.04
FI	3.72ab	1.26bc	2.46ab	32.2b	25.3ab	21.2c	13.4bcd	15.7c	8.31abc	45.7cd	18.9a	33.1c	38.1c	60.0d	2.01b	66.3b	74.1c	35.9c	9.64
150-ETc	3.76ab	1.31bc	2.45ab	31.3b	24.7abc	21.2c	13.3cd	15.9c	8.71a	49.1bc	18.3ab	36.1abc	40.5abc	62.9bc	1.95b	65.7b	70.8c	35.4c	10.2
Guara																			
RDI_{65}	3.69b	1.42ab	2.27ab	33.0ab	25.0abc	25.9a	15.7a	17.0b	7.98bc	51.4ab	18.6av	35.6av	42.9ab	64.2ab	1.89b	77.9a	80.8b	42.2a	10.1
FI	6.69b	1.43ab	2.26ab	3.2ab	25.0abc	26.0a	15.7a	17.2b	8.11abc	48.5bc	17.4b	34.1c	38.4c	62.7bc	2.14ab	86.9b	40.2a	9.04	9.04
150-ETc	4.61a	1.61a	2.69a	34.3a	26.0a	26.8a	16.5a	18.1a	8.33ab	52.9a	18.0ab	39.8a	43.8a	65.5a	1.96b	75.8ab	77.3c	39.4b	10.9
Lauranne																			
RDI_{65}	3.25b	1.18c	2.08b	32.5ab	24.0bc	23.3v	14.3b	15.8c	7.72c	48.7bc	17.7ab	33.2c	37.7c	61.5cd	1.98b	72.6ab	76.9c	38.7b	11.4
FI	3.37b	1.16c	2.22ab	31.6b	23.9bc	23.4b	14.1bc	15.7c	8.03bc	49.5ab	17.9ab	34.7bc	39.1bc	62.6bc	2.11ab	78.3a	90.7a	42.5a	10.4
150-ETc	3.51b	1.20c	2.31ab	32.3b	23.7c	23.3b	14.2b	15.9c	8.02bc	49.7ab	17.6ab	35.1bc	39.4bc	62.9bc	2.03b	69.6ab	78.8bc	38.0b	9.76

† NS = not significant at $p > 0.05$; *, **, and *** significant at $p < 0.05$, 0.01, and 0.001, respectively. ‡ Values (mean of 25 replication) followed by the same letter, within the same column and factor, were not significantly different (p>0.05), according to Tukey's least significant difference test. WL=Whole Length; KL=Kernel Length; WW=Whole Width; KW= Kernel Width; WT=Whole Thickness; KT=Kernel Thickness; L*, a*, b*= Color coordinates; C= Chroma; F=Fracturability; H=Hardness; WS=Work to Shear; AF=Average Force; NF=Number of Fractures.

The interaction irrigation dose x cultivar showed no relevant differences for the main morphological parameters in cvs. Marta and Lauranne. However, significant improvements were observed for the almonds under 150% ET_C in cv. Guara with a comparable pattern to those of other varieties in the remaining physical parameters. These findings agreed with those reported by other authors in almonds and other nuts such as pistachios, in which many quality parameters were not significantly affected by moderate deficit irrigation doses[42,87,173].

Kernel ratio and dry weight were affected by the irrigation dose and the cultivar (Table 6.3). In this sense, RDI_{65} showed the best results in kernel ratio (358 $g \cdot kg^{-1}$), followed by FI (354 $g \cdot kg^{-1}$) and 150% ET_C (352 $g \cdot kg^{-1}$). Regarding cultivars, significant differences were also observed with Guara having the highest value of the kernel ratio (363 $g \cdot kg^{-1}$), followed by Lauranne (359 $g \cdot kg^{-1}$) and Marta (342 $g \cdot kg^{-1}$). Within each cultivar, the best kernel ratio was observed in RDI_{65} for Lauranne and Guara, not being detected significant effects in these varieties for the case of dry weight. These improvements could be associated with reductions in the fruit number in the stressed trees, which could be associated with a more effective kernel-filling process when the fruit number per tree is sub-optimal[42].

Finally, the water activity (a_w) was not significantly affected neither by irrigation nor by cultivar (Table 6.3). However, all found values could be considered within the optimum range for almonds stored in cool and dry conditions (values in the range 0.3 to 0.6), as it has been reported by Gama et al.[174] and Huang[175].

Table 6.3 Effect of irrigation dose and cultivar on kernel ratio, dry weight and water activity.

	Kernel Ratio	Dry weight	Water activity
	($g\ kg^{-1}$)		(a_w)
	ANOVA†		
Irrigation	***	*	NS
Cultivar	***	*	NS
Irrigation × Cultivar	***	*	NS
	Tukey Multiple Range Test‡		
	Irrigation		
RDI_{65}	358a	967b	0.57
F_I	354b	966b	0.57
150% ET_C	352c	969a	0.57
	Cultivar		
Marta	342c	971a	0.57
Guara	363a	965b	0.57
Lauranne	359b	966b	0.57
	Irrigation × Cultivar		
Marta			
RDI_{65}	333g	969ab	0.57
F_I	351d	970ab	0.57
150% ET_C	342f	973a	0.57
Guara			
RDI_{65}	371a	965ab	0.57
F_I	352d	965ab	0.57
150% ET_C	367b	966ab	0.58
Lauranne			
RDI_{65}	369ab	966ab	0.57
F_I	360c	964ab	0.57
150%ET_C	348e	967ab	0.57

† NS = not significant at $p > 0.05$; *, **, and *** significant at $p < 0.05$, 0.01, and 0.001, respectively.

‡ Values (mean of 3 replication) followed by the same letter, within the same column and factor, were not significantly different ($p > 0.05$), according to Tukey's least significant difference test.

2.3 Contents of ash, minerals, organic acids, and sugars

Significant effects of the irrigation and cultivar factors on the contents of Ca, Mg, Cu, and Mn were detected (Table 6.4). In this sense, it is of importance to mention that RDI_{65} almonds presented the highest values of some of these minerals (Ca Mg, and Cu), which would suppose an advantage of DI strategies in nutritional terms. Moreover, these increases were mainly detected in cv. Lauranne under RDI_{65} and were not found in the remaining cultivars. However, it is noticeable that, RDI_{65} did not promote lower mineral contents of any of the cultivars under study.

According to Yada et al.[176], Ca, Cu, Fe, Zn, K, P, Se, and Na are the most relevant nutrients in almond fruits. Similar results to the obtained in this work were reported for the case of cv. Vairo almonds that were subjected to moderate RDI during the kernel-filling period[64]. For instance, they reported a higher K content in nuts obtained under moderate RDI and a mean value of all treatments of 7.2 $g\cdot kg^{-1}$.On the other hand, Ca (2.13 $g\cdot kg^{-1}$) and Mn (0.29 $g\cdot kg^{-1}$) were reduced in almonds when severe RDI was applied[64]. By the contrast, in the present study, K element was not influenced by none of the irrigation treatments applied, while Ca and Mn contents were similar to those of the control treatment (FI) and Cu content was slightly increased by RDI_{65}.

Regarding the K content, lower levels were found in cvs. Marta, Guara and Lauranne than those reported for cv. Vairo. However, these almond cultivars are source of K (mean values 508 mg 100 g^{-1}) as this value is above the minimum threshold defined in the Annex to Directive 90/496/ECC (300 mg 100 g^{-1}). The Ca levels detected in the present study were 2-3 times higher than those reported by Lipan et al.[64] in cv. Vairo, and the highest Ca content was found for the combination of RDI_{65} or cv. Lauranne. Similar results were also reported by Carbonell-Barrachina et al.[171], who found higher contents of Ca and Zn of pistachio nuts produced under water stress conditions. On the other hand, Alimohammadi et al.[177] did not find significant effects of DI in different phenological stages for almond mineral contents. Other studies in different crops, such as grapes, olive, or apple, have also concluded that DI can be applied without relevant impact on the mineral nutrition in fruits[178].

Table 6.4 Ash and minerals content of raw almonds as affected by irrigation dose and cultivar.

	Ash content	Ca	Mg	K	Fe	Cu	Mn	Zn
	($g \cdot kg^{-1}$)				($mg \cdot kg^{-1}$)			
	ANOVA†							
Irrigation	NS	*	**	NS	NS	*	*	NS
Cultivar	NS	*	**	NS	NS	*	*	NS
Irrigation x Cultivar	NS	*	**	NS	NS	*	*	NS
Tukey Multiple Range Test‡								
Irrigation								
RDI$_{65}$	32.8	6.99a	1.97a	5.86	28.7	6.13a	15.9b	31.2
FI	34.3	6.34a	1.99a	6.08	26.4	5.93b	15.5b	28.7
150-ETc	32.8	5.91b	1.93b	5.41	27.3	6.60a	16.8a	28.2
Cultivar								
Marta	32.6	5.69b	1.80b	5.72	25.2	5.49b	13.2b	26.5
Guara	33.6	5.15b	1.93b	5.80	30.4	6.84a	17.4a	29.2
Lauranne	33.7	8.39a	2.16a	5.82	26.8	6.34ab	17.5a	32.3
Irrigation x Cultivar								
Marta								
RDI$_{65}$	34.3	6.13b	1.82bc	6.03	27.5	5.87ab	14.1ab	28.9
FI	32.8	6.03b	1.87abc	6.07	26.1	5.43b	13.3ab	27.3
150-ETc	30.6	4.91c	1.70c	5.08	21.8	5.18b	12.2b	23.3
Guara								
RDI$_{65}$	33.3	6.36b	1.96abc	5.58	31.5	6.19ab	16.7ab	32.4
FI	34.9	4.46c	1.93abc	6.29	25.6	6.18ab	15.6ab	25.5
150-ETc	32.7	4.64c	1.88abc	5.52	34.1	8.14a	20.0a	29.7
Lauranne								
RDI$_{65}$	30.7	8.46a	2.12ab	5.97	27.0	6.33ab	16.9ab	32.2
FI	35.4	8.52a	2.17ab	5.88	27.4	6.18ab	17.5ab	33.3
150-ETc	35.2	8.19a	2.19a	5.62	26.1	6.50ab	18.1ab	31.5

† NS = not significant at $p > 0.05$; *, **, and *** significant at $p < 0.05$, 0.01, and 0.001, respectively.

‡ Values (mean of 3 replication) followed by the same letter, within the same column and factor, were not significantly different ($p > 0.05$), according to Tukey's least significant difference test.

Table 6.5 summarizes the effects of irrigation and cultivar on the organic acids and sugars profiles. Organic acids and sugars were significantly higher in RDI_{65} than in 150% ET_C nuts. In terms of cultivar, Guara (10.6 $g·kg^{-1}$) and Lauranne (10.3 $g·kg^{-1}$) were the cultivars having the highest total contents of organic acids, while Marta had the lowest one (9.43 $g·kg^{-1}$). Meanwhile, Lauranne (56.3 $g·kg^{-1}$) showed the highest total sugar content, followed by Marta (51.7 $g·kg^{-1}$) and Guara (49.2 $g·kg^{-1}$). As observed, organic acids and sugars are highly cultivar-dependent. For instance, Marta is high in oxalic acid, Guara in citric acid and Lauranne in citric, tartaric and fumaric acids. Similar findings were observed for sugar content, with Marta and Guara being lower in sucrose and higher in glucose and the opposite was observed for Lauranne. Regarding the interaction irrigation dose x cultivar, it might be highlighted that all cultivars were positively influenced by deficit of irrigation water (RDI_{65}) and negatively affected by excess of water (150% ET_C) in both organic acids and sugars contents. Similar results were also reported by Lipan et al.[64] with a clear relationship between water stress and total organic acids content, although other authors concluded that water stress did not promote relevant effects in these compounds[87,179]. Other authors working with grapes and tomatoes under water deficit condition also reported an increase in sugars and this sugar accumulation was attributed either to a post effect of inhibiting lateral shoot growth, which induces a reallocation of carbohydrates to the fruit, or as a direct impact of the root abscisic acid signal on fruit maturation[180]. Moreover, the increase in sugars also might be due to the osmotic adjustment, activated by the accumulation of solutes (sugars) in the cytoplasm under water stress conditions[181].

Table 6.5 Organic acids and sugars content (g·kg^{-1}) in raw almonds as affected by irrigation dose and cultivar. cultivar.

	Organic acids						Sugars					
	Oxalic	Citric	Tartaric	Malic	Fumaric	Total	Maltoheptaose	Maltotriose	Sucrose	Glucose	Fructose	Total
	ANOVA†											
Irrigation	***	***	***	***	***	***	***	***	***	***	***	***
Cultivar	***	***	***	***	***	***	***	***	***	***	***	***
Irrigation x Cultivar	***	***	***	***	***	***	***	***	***	***	***	***
	Tukey Multiple Range Test‡											
	Irrigation											
RDI65	2.16a	2.77a	1.79a	4.43a	0.27a	11.4a	3.64a	3.36a	33.2a	12.5a	3.52a	56.3a
FI	2.09b	2.58b	1.57b	3.18b	0.23b	9.65b	3.64a	2.86b	29.1b	12.2ab	3.25b	51.1b
150-ETc	2.02c	2.43c	1.50b	3.06b	0.24b	9.20b	3.49b	2.62c	28.6b	11.9b	3.28b	49.9b
	Cultivar											
Marta	2.18a	2.41b	1.20c	3.38b	0.26b	9.43b	3.73a	4.02a	26.1b	14.6a	3.32b	51.7b
Guara	2.03c	2.69a	1.74b	3.93a	0.18c	10.6a	3.55b	3.13b	23.7b	15.8a	3.08c	49.2c
Lauranne	2.06b	2.68a	1.92a	3.37b	0.30a	10.3a	3.49b	1.70c	41.2a	6.27b	3.65a	56.3a
	Irrigation x Cultivar											
Marta												
RDI65	2.33a	2.81ab	1.65b	4.63a	0.29a	11.7a	3.83ab	4.99a	29.0cd	15.4a	3.53ab	56.7ab
FI	2.13b	2.29de	1.09c	3.05bc	0.24b	8.82de	3.85a	3.68b	25.8de	14.1a	3.42abc	50.8cd
150-ETc	2.08c	2.12e	0.87c	2.46c	0.23b	7.76e	3.53c	3.39bc	23.4de	14.4a	3.00cd	47.8de
Guara												
RDI65	2.07c	2.73ab	1.82ab	4.32a	0.19c	11.1ab	3.64abc	3.36bc	25.9de	17.3a	3.34bcd	53.5abc
FI	2.04cd	2.88a	1.84ab	3.66ab	0.16c	10.6abc	3.53c	3.18cd	25.5de	16.0a	2.89d	51.1bcd
150-ETc	1.98e	2.45cd	1.56b	3.79ab	0.18c	10.0bcd	3.47c	2.83d	19.6e	14.4a	3.00cd	42.9e
Lauranne												
RDI65	2.08c	2.76ab	1.91ab	4.35a	0.32a	11.4ab	3.46c	1.72e	44.8a	4.96b	3.68ab	58.6a
FI	2.09c	2.55bcd	1.78ab	2.83bc	0.29a	9.54cd	3.55bc	1.72e	36.1bc	6.52b	3.45abc	51.6bcd
150-ETc	2.01de	2.72abc	2.08a	2.93bc	0.30a	9.89bcd	3.46c	1.65e	42.8ab	7.32b	3.83a	59.0a

† NS = not significant at p>0.05; * and ** significant at p< 0.05 and 0.01, respectively. ‡ Values (mean of 8 replications) followed by the same letter, within the same column and factor, were not significantly different (p>0.05), according to Tukey's multiple range test.

2.4 Fatty acids profile

The almond nuts have a high nutritive value mainly due to their balanced lipid content which represents a good caloric source without increasing the cholesterol level in humans. The chemical composition of the almond lipid fraction consists of monounsaturated (MUFAs) fatty acids, mainly oleic acid (C18:1n9), polyunsaturated fatty acids (PUFAs) mainly linoleic acid (C18:2n6), while the saturated fatty acids (SFAs), especially palmitic (C16:0) and stearic (C18:0) acids, are found in very low concentration[64,182]. All these findings were confirmed by the present work in which 17 fatty acids were identified and quantified (Table 6.6), with oleic acid, being the predominant compound, followed by linoleic, palmitic, stearic and palmitoleic acids.

Regarding the effect of the irrigation treatments, statistically significant differences were found for myristic, palmitoleic, cis-heptadecenoic, oleic, linoleic, α-linolenic, arachidic, eicosenoic and erucic acid (Table 6.6). Palmitic and arachidic acids (SFAs) were higher in FI than RDI_{65} and 150% ET_C. On the contrary, RDI_{65} evidenced an increase in some MUFAs and PUFAs such as palmitoleic, cis-heptadecenoic, linoleic in comparison to the rest of treatments.

Moreover, the same fatty acids were also significantly affected by the cultivar (Table 6.6) Marta genotype was found to have more myristic, cis-heptadecenoic, oleic, α-linolenic, arachidic, eicosenoic, and erucic fatty acid contents. Guara registered the highest content of arachidic acid together with Marta, while cv. Lauranne recorded the highest content of palmitoleic, linoleic, and was statistically similar to Marta genotype with regard to the cis-heptadecenoic and eicoseoic acids.

The interaction irrigation dose x cultivar showed that cv. Marta at FI and Lauranne at 150% ET_C were the combinations leading to the highest content of myristic acid. Lauranne RDI_{65} had the highest contents of palmitoleic, cis-heptadecenoic, and linoleic acid. On the other hand, cv. Marta at 150% ET_C was found to have the highest content of oleic acid, while Marta and Lauranne at RDI_{65} showed the highest content of linoleic acid. These facts suggest that oleic acid was decreased by the RDI_{65} while linoleic acid increased under this irrigation strategy. These results agree with those of other authors in almonds and other woody crops[64,171,179].

Table 6.6 Fatty acids content (g·kg^{-1}) in raw almonds as affected by irrigation dose and cultivar.

	C14:0	C15:0	C16:0	C16:1	C17:0	C17:1cis	C18:0	C18:1n9	C18:2n6	C18:3n3	C20:0	C20:1n9	C20:3n6	C21:0	C22:0	C22:1	C23:0
								(g·kg^{-1})									
								ANOVA†									
Irrigation	**	NS	NS	***	NS	**	NS	***	***	*	*	*	NS	NS	NS		NS
Cultivar	**	NS	NS	***	NS	**	NS	***	***	*	*	*	NS	NS	NS		NS
Irrigation x Cultivar	**	NS	NS	***	NS	**	NS	***	***	*	*	*	NS	NS	NS		NS
								Tukey Multiple Range Test‡									
								Irrigation									
RDI65	0.09b	0.06	22.1	1.67a	0.21	0.37a	6.67	219b	65.9a	0.28b	0.54b	0.06b	0.27	0.16	0.19	0.07b	0.15
FI	0.10a	0.06	22.0	1.58b	0.23	0.36b	7.14	227a	60.9b	0.30a	0.63a	0.07a	0.28	0.17	0.220	0.08ab	0.13
150-ETc	0.09b	0.06	21.66	1.54b	0.23	0.36b	5.07	227a	58.8b	0.29ab	0.54b	0.06b	0.29	0.16	0.19	0.09a	0.19
								Cultivar									
Marta	0.10a	0.05	21.7	1.53b	0.25	0.37a	6.39	241a	62.8b	0.36a	0.60a	0.08a	0.26	0.16	0.18	0.12a	0.18
Guara	0.09b	0.07	21.7	1.36c	0.24	0.32b	6.69	218b	56.4c	0.26b	0.65a	0.06b	0.27	0.16	0.20	0.07b	0.15
Lauranne	0.09b	0.06	22.3	1.91a	0.20	0.40a	5.80	213b	66.5a	0.24b	0.47b	0.06b	0.30	0.17	0.20	0.04c	0.14
								Irrigation x Cultivar									
Marta																	
RDI65	0.10abc	0.05	22.1	1.57bcd	0.21	0.34abc	7.13	228abc	71.7a	0.34ab	0.55ab	0.08ab	0.29	0.16	0.19	0.13a	0.14
FI	0.11a	0.05	22.1	1.53bcd	0.21	0.40abc	7.44	245ab	60.3bc	0.39a	0.75a	0.09a	0.24	0.18	0.19	0.12a	0.14
150-ETc	0.08c	0.04	21.1	1.49cd	0.34	0.36abc	4.60	251a	56.6c	0.36a	0.52ab	0.06av	0.26	0.14	0.17	0.13a	0.26
Guara																	
RDI65	0.09abc	0.06	20.6	1.47d	0.21	0.35abc	6.75	206c	55.9c	0.23c	0.59ab	0.06ab	0.22	0.15	0.21	0.05c	0.16
FI	0.09abc	0.07	22.3	1.33d	0.24	0.29c	8.31	225abc	58.3bc	0.27bc	0.64ab	0.05b	0.28	0.15	0.18	0.10ab	0.13
150-ETc	0.09abc	0.06	22.2	1.28d	0.18	0.32bc	5.00	223abc	55.0c	0.28bc	0.71a	0.07ab	0.31	0.18	0.20	0.07bc	0.15
Lauranne																	
RDI65	0.08bc	0.06	23.6	1.98a	0.20	0.42a	6.14	222bc	70.2a	0.26c	0.50ab	0.06ab	0.29	0.18	0.17	0.03c	0.15
FI	0.09abc	0.05	21.8	1.89ab	0.22	0.38abc	5.665	209c	64.3ab	0.24c	0.51ab	0.06ab	0.31	0.16	0.23	0.03c	0.11
150-ETc	0.11a	0.07	21.5	1.85abc	0.19	0.39ab	5.62	207c	64.9ab	0.23c	0.39b	0.05b	0.31	0.17	0.20	0.06c	0.16

† NS = not significant at p> 0.05; *, **, and *** significant at p< 0.05, 0.01, and 0.001, respectively. ‡ Values (mean of 3 replication) followed by the same letter, within the same column and factor, were not significantly different (p>0.05), according to Tukey's least significant difference test. Myristic=C14:0, Pentadecylic=C15:0, Palmitic=C16:0, Palmitoleic=C16:1, Margaric=C17:0, cis-Heptadecenoic=C17:1, Stearic=C18:0, Oleic=C18:1n9, Linoleic=C18:2n6, α-Linolenic=C18:3n3, Arachidic=C:20:0, Eicosenoic=C20:1n9, Eicosatrienoic=C20:3n6, Heneicosylic=C21:0, Behenic=C22:0, Erucic=C22:1, Tricosylic=C23:0.

It was observed that the oleic:linoleic ratio decreased in RDI_{65} samples which might produce almonds more sensitive in terms of oil stability (Table 6.7), because low linoleic (PUFAs) content is related to high oil stability[182]. However, referring to the health properties, linoleic acid, which plays an important role in the death of cardiac cells, among others functions, is an essential PUFA (omega 6) for the human body[183]. This fatty acid cannot be synthesized by the human body and it is necessary to maintain the metabolic integrity. The reference intake value for linoleic acid according to European Food Safety Authority (EFSA) is 10 g per day linoleic acid, which means that consuming 50 g of RDI_{65} almonds will cover approximately 33% of the linoleic acid daily intake recommended by EFSA.

In this regard, MUFAs decreased and PUFAs increased in RDI_{65} almonds. On the other hand, nuts of the cv. Marta had the highest contents of MUFAs, while cv. Lauranne the highest contents of PUFAs. The increase in PUFAs in DI products was also observed by other author in studies on almonds, pistachios and olives[64,121,171,172].

Regarding the atherogenic index (AI) which provides information about whether a diet could promote coronary diseases, the obtained results did not evidence significant differences among irrigation treatments; however, the factor “cultivar” affected these indexes, with Marta nuts having the lowest values. Moreover, the thrombogenic index (TI) offers information about clots development in the blood vessels and almonds irrigated at 150% ET_C and from cv. Marta were found to have the lowest content of TI, although the differences were not too high but were statistically significant.

2.5 Principal component analysis

In order to estimate which of the studied parameters were more influenced by the water stress supported by the crop, a principal component analysis (PCA) was prepared (Figure 6.2), taking the ΨInt variable as reference in order to localize those groups of variables more influenced by the water stress imposed.

In order to estimate which of the studied parameters were more influenced by the water stress supported by the crop, a PCA was prepared (Figure 6.2), taking the

ΨInt variable as reference in order to localize those groups of variables more influenced by the water stress imposed.

According to the obtained results, the two main components (F1 and F2) explained 32.4 and 31.9% of the total variance (Figure 6.2). As observed, samples for each cultivar were grouped separately, and for the case of irrigation treatments, samples were clustered together for Lauranne, and slightly separated for Guara, Marta being the cultivar in which a higher separation treatment was observed. In this sense, Lauranne samples were mainly described by the contents of Ca, Mg, some organic acids such as Citric and Malic, the total content of sugars and the sucrose content or the ratio between PUFA:MUFA or PUFA SFA. In the case of Guara, samples were mainly described by morphological parameters such as almond weight, length, kernel width color parameters or kernel ratio. Finally, samples of Marta were mainly characterized by the dry weight and the fatty acids, specially total fatty acids, MUFA, the ratio (MUFA+PUFA)/SFA, and the ΨInt. Moreover, negative correlations between ΨInt and all the morphological parameters was observed, being especially noticeable for the cases of almond weight (R=-0.62), kernel weight (R=-0.60), almond width (R=-0.59) and kernel width (R=-0.65). In this way, it is remarkable the effect of water stress in relation to the almond size, although these effects were not significant, as it has been previously discussed (Table 6.2). According to the color parameters, significant negative relationships were observed between ΨInt and the color parameters L* (R=-0.73*), b* (R=-0.80**), C (R=-0.78*) and Hue (R=-0.83**), reflecting that the water stress would be related to significant reduction in the luminosity, chroma, and the yellow color; and a slight tendency to the red color (although this relationship was not significant).

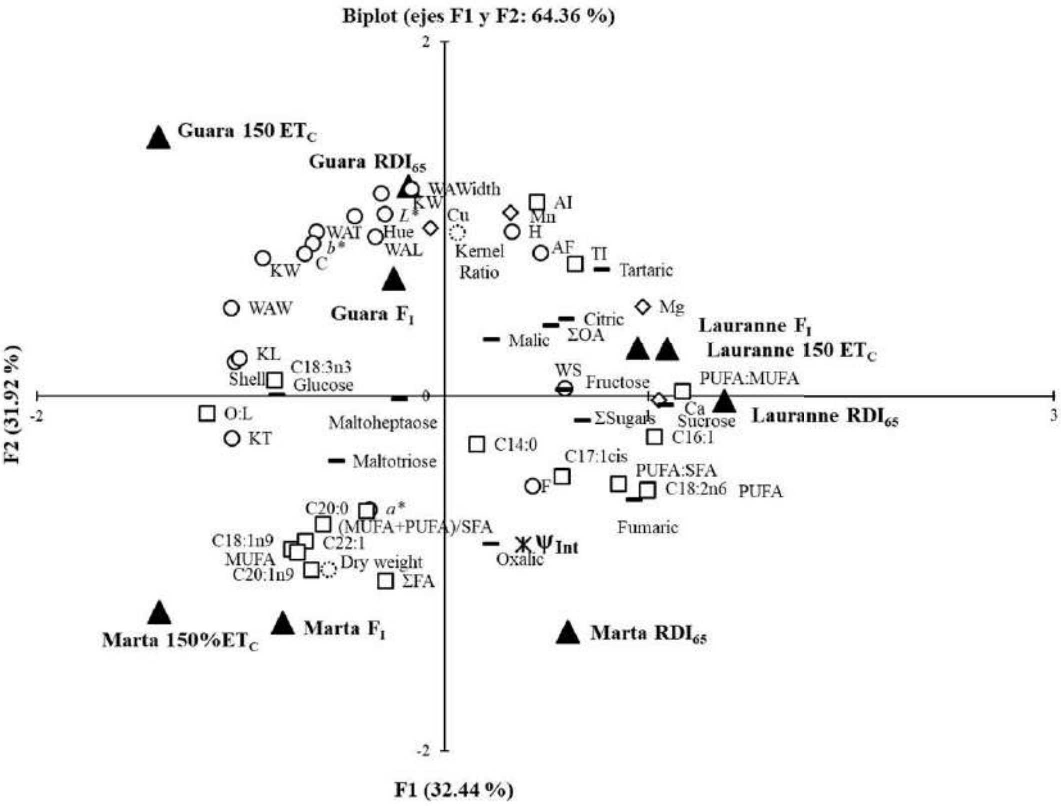


Figure 6.2 Principal Components Analysis (PCA) scores biplot showing the relationship among stress integral, morphological and physicochemical parameters. Legend: ▲samples; x stress integral; ○morphological parameters; ○ kernel ratio and dry weight; ◊ minerals; − organic acids; □ fatty acids; WAW=Whole almond weight; KW= Kernel weight; WAL=Whole almond length; KL=Kernel length; WAWidth=Whole almond width; KW= Kernel width; L*, a*, b*=color coordinates; C=Chrome; F=Fracturability; H=Hardness; WS=Work to Shear; AF=Average Force; ΣOA=Total organic acids; C14:0=Myristic, C16:1=Palmitoleic, C17:1cis=cis-Heptadecenoic, C18:1n9=Oleic, C18:2n6=Linoleic, C18:3n3=α-Linolenic, C:20:0=Arachidic, C20:1n9=Eicosenoic, C22:1=Erucic; O/L=Oleic/linoleic ratio; MUFAs=Monounsaturated fatty acids; PUFAs=Polyunsaturated fatty acids; AI=Atherogenic index, TI = Thrombogenic index.

In relation to the content of organic acids, the most relevant relationships between ΨInt and organic acids contents were obtained for oxalic acid (R=0.96**) malic, fumaric, and the total organic acids content (R no significant). On overall, high water stress levels were accompanied with increases in the amounts of oxalic, citric, malic and fumaric, while the content of tartaric acid was not affected (Figure 6.2).

Attending to the sugars content, although the linear correlations between ΨInt and the studied sugars were not significant ($p>0.05$), the pattern observed showed a trend to increases with the water stress imposed

Finally, regarding to the effects of water stress on fatty acid profile, it is especially noticeable the positive relationship between ΨInt and total PUFAs, and the ratios PUFA/SFA and PUFA/MUFA, as result of the relationships obtained between ΨInt and the total content of different unsaturated fatty acids.

Similar recent works have concluded that, in general the water stress imposed in almonds does not affect the chemical composition of nuts, although slight improvements in some parameters can be found. In this way, Lipan et al.[62,64,149] concluded that water stress did not affect the most relevant quality parameters in almonds cv. Vairo, with moderate deficit irrigation promoting redder color and higher contents of fat, potassium and unsaturated fatty acids. Changes in chemical composition under deficit irrigation conditions were also reported in different crops and food products such as pistachio[171], grapes[184,185], olives[186], and olives oil[187]. These authors reported an increase in important compounds such as fatty acids, organic acids, sugars, volatiles or phenolic compounds under water deficit conditions. For instance, an increase in mono- and polyunsaturated fatty acids and a decrease in saturated fatty acids was reported in pistachio[171], olive oil[187] and olives[186], and for this last was concluded that the higher the water stress the higher the content in linolenic acid and MUFAs. These results agree with those obtained in the present study, with water stress leading to improved PUFA/SFA ratio, and healthier values of the atherogenic and thrombogenic indexes[188,189].

2.6 Sensory analysis

Although Figure 6.3 shows different position in the ranking tests for each sample under analysis, according to a specific almond cultivar, there were only significant effects of the irrigation treatment on the intensity of the almond-ID attribute for the Lauranne cultivar, with the sample under RDI_{65} strategy having the highest intensity. The sweetness of the FI and RDI_{65} almonds of the cultivars Guara and Lauranne was significantly higher than that of the 150% ET_C nuts. This finding means that an excess of irrigation water led to a significant reduction of the sweetness of almonds cvs. Guara and Lauranne. The crispiness intensity was significantly affected by the irrigation treatment on Marta and Guara almonds. However, the trends of the irrigation treatments were not easy to interpret, because Marta RDI_{65} had the lowest crispiness but Guara RDI_{65} had the highest one; implying that the sensory profile of almonds depends more on the cultivar than on the irrigation treatments. On the other hand, the results of the ranking tests of samples grouped by irrigation treatment (e.g., RDI_{65} samples of the cvs. Marta, Guara and Lauranne) gave no statistically significant differences and thus, they will not be further discussed.

On overall, an taking into account the relationships between the water stress supported by the crop and the parameters considered, almond crop subjected to over irrigation would not improve the nut quality; however, the almond quality can be slightly improved when a moderate RDI strategy is imposed. These results open the possibility of showcasing those hydroSOS products that have been obtained within a framework of water scarcity and natural resources sustainability. Moreover, the results evidence the absence of improvements when a crop is over-irrigated not only in terms of final yield but also from a quality point of view. This fact was previously discussed by Gutiérrez-Gordillo at al.[167] when they studied the main effects in terms of yield registered in these cultivars when these were subjected to the irrigation strategies described in this work. In this agreement, no significant differences were detected between the three irrigation strategies for the case of Guara and Lauranne, with kernel yields around to 2,800 and 2,500 $kg \cdot ha^{-1}$, respectively. Something similar was reported for the case of Marta, without differences between FI and RDI_{65} and kernel yield values around 2,200 $kg \cdot ha^{-1}$, although in the case of this cultivar significant improvements were obtained when an excess of water was imposed (150% ET_C). Moreover, no differences were observed for the case of nut-splitting and humidity content between irrigation strategies described in this experience. Similar results were also reported for almond trees cv. Vairo grown under moderated deficit irrigation and also in olives and pistachios[62,170-172].

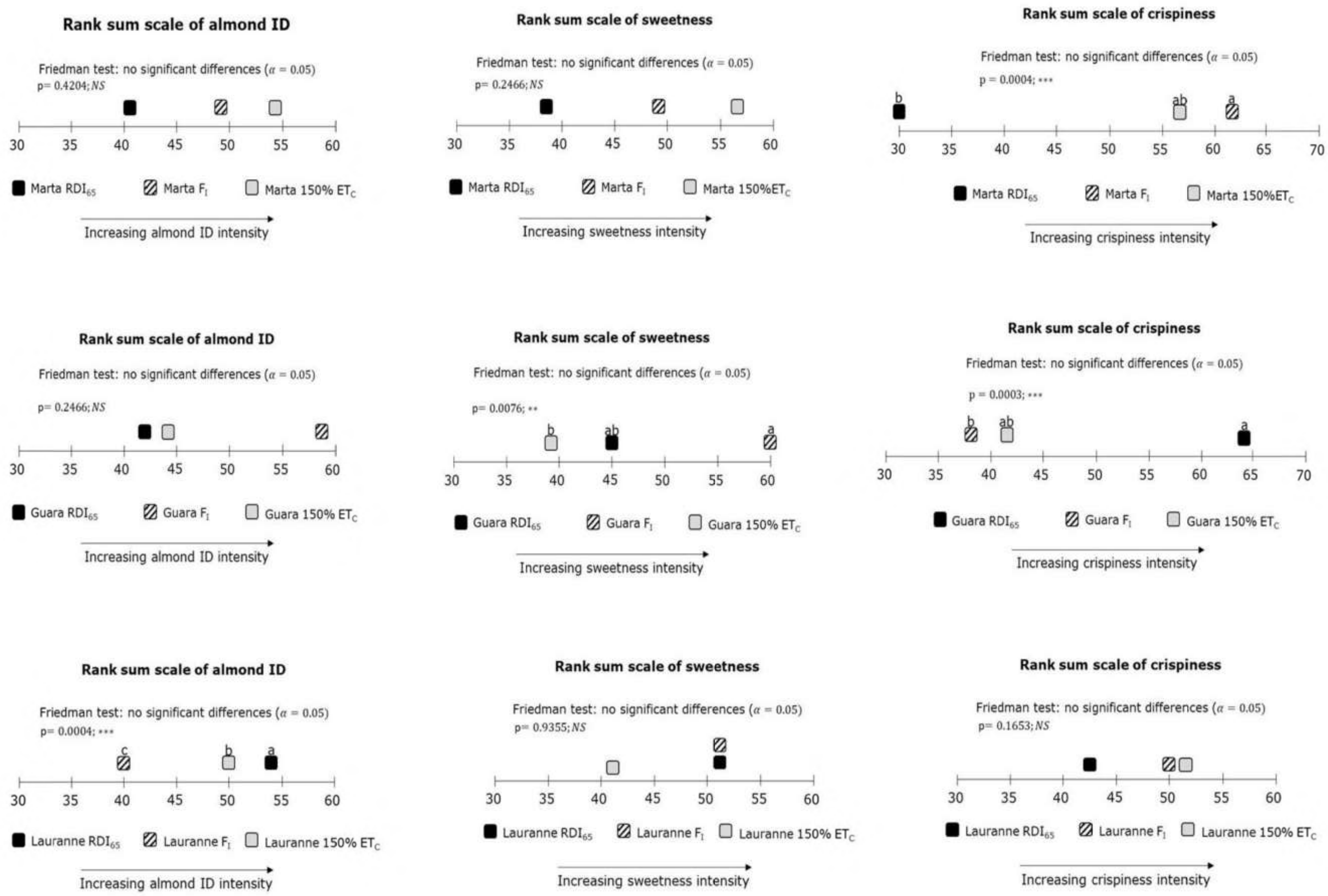


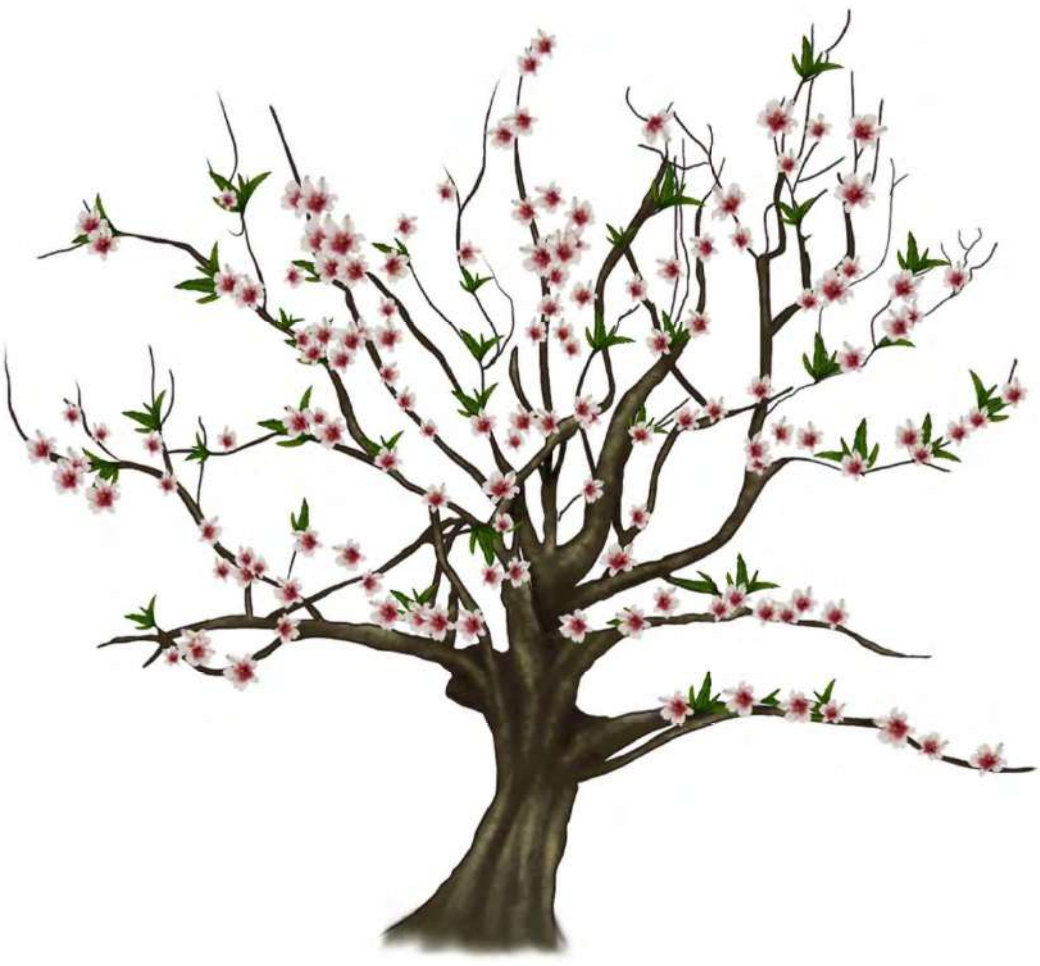
Figure 6.3 Rank sum scale of panellists scores of the 3 almond cultivars as affected by irrigation strategies. NS= not significant at $p < 0.05$; and *, **, and *** significant at $p < 0.05$, 0.01 and 0.001, respectively.

3. Conclusions

A moderate DI during the kernel filling period would be a suitable strategy, without jeopardizing the yield and/or the almond nut quality, allowing to save irrigation amounts close to ~1,400 $m^3 \cdot ha^{-1}$ compared to FI treatment. Finally, regarding sensory analysis, a reduced volume of irrigation water could slightly increase the intensity of key positive sensory attributes in Guara and Lauranne almonds, although results were clearly cultivar-dependent.

Chapter 7

Deficit Irrigation as a Suitable Strategy to Enhance the Nutritional Composition of HydroSOS Almonds



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1. Introduction

In the last 10 years, due to climate change, the planet has warmed at a global average of around 1.41 °C[190]. In addition, together with the increased temperature, irregular rainfall and extreme events are the main effects caused by climate change, as described by the European Environment Agency[33]. These phenomena not only affect the amount of water available for agriculture but will also cause changes in the growing cycles of plants, affecting the final production. For example, for each degree of increase in global temperature, a 4–6% decrease in crop yields is expected[191]. Thus, considering water-scarcity and climate-change scenarios, the introduction of sustainable irrigation strategies to boost the proper water management for irrigated crops is crucial[2].

In this sense, many Mediterranean fruit crops are well adapted to drought, able to respond positively when water withholding is applied during different phenological stages, such as almond[141], olive[129], and citrus[192], among others. More concretely, almond (*Prunus Dulcis* (Mill.) D.A. Webb) is considered as a drought-tolerant species[74], with high positive responses under deficit irrigation (DI) strategies. Many authors have described the almond response to DI, defining its behavior in terms of final yield[127,193], physiology[116], or nut quality[87].

Moreover, almond has high added value because of its nutritional and functional properties, with a composition of macro- and micronutrients that are beneficial to human health[194], with higher amounts of vegetable protein and fat-soluble bioactives. They are also dense in a variety of other nutrients and provide dietary fiber, vitamins, minerals, and many other phytochemicals such as phenolic acids, flavonoids, lignin, hydrolysable tannins, carotenoids, alkaloids, and phytates, among others[195]. In this line, many diseases such as type 2 diabetes, high blood pressure, and neurodegenerative and cardiovascular diseases can be prevented with a healthy diet and one serving of nuts per day[196].

In the last four years, the hydroSOStainable concept has gained great importance in agriculture. This concept arises from society's dedication to the consumption of products that are sustainable for the environment and also have health benefits[64]. The definition of hydroSOStainable products as fruits and vegetables cultivated under regulated deficit irrigation (RDI) strategies was first made by Noguera-Artiaga et al.[68]. HydroSOS products are those obtained from plants subjected to DI strategies and are characterized by high amounts of bioactive and functional compounds, among other properties. Taking this concept into consideration, the hydroSOStainable index was created to help farmers develop sustainable practices, ensuring recognition for their products[197]. Since then, hydroSOStainable strategies have been used in many crops, such as olives[172], pistachios[171], and almonds[63]. Although DI strategies slightly affect the final yield (compared with fully irrigated conditions), they improve the quality of the final product, with greater consumer acceptance, and produce environmentally friendly products. According to the scientific literature, previous studies on the effect of DI on almond quality were only focused on cv. Vairo. However, it is well known that each almond cultivar behaves differently in water-deficit conditions. In this line, Lipan et al.[198] evaluated the effect of RDI and overirrigation doses on the nut quality of three almond cultivars, Guara, Marta, and Lauranne. Later, Garcia-Tejero et al.[199] studied the effect of sustained-deficit irrigation (SDI) on the same cultivars, focusing their attention on quality parameters related to the almonds' marketability and how this type of deficit irrigation strategy gave added value to the final product.

Considering these aspects, the aim of this work was to evaluate the irrigation and cultivar effects on the main chemical components of almonds (antioxidant activity, sugars, organic acids, and fatty acids), defining the most suitable irrigation dose that would ensure improvement of the almond quality in relation to its functional composition.

2. Results and Discussion

2.1 Climatic conditions, physiological response, and final yield

The climatic conditions during the irrigation period were characterized by mild average temperatures (19.8–26.9 °C) and low rainfall. The cumulative ET_0 and rainfall were 840 and 85 mm, respectively. Maximum evapotranspiration rates occurred in June (203 mm), July (239 mm), and August (170 mm), whereas rainfall was concentrated during April (71.2 mm) and October (10 mm).

Thus, according to the climatic conditions, the total amount of irrigation received for FI, SDI_{75}, and SDI_{65} at the end of the season was 7,700, 5,744, and 5,159 $m^3{\cdot}ha^{-1}$, respectively.

In relation to the crop water status, significant differences in Ψ_{leaf} were found among cultivars and irrigation treatments (Table 7.1), taking into consideration not only all the data but also the daily measurements. In this regard, according to three-way ANOVA for repeated measures, significant differences ($p < 0.01$) were found for timing evolution, cultivars, and irrigation treatments. Focusing on the cultivars, cv. Marta registered the highest average Ψ_{leaf} value (−1.55 MPa), which was significantly different from cv. Guara (−1.80 MPa) and cv. Lauranne (−1.71 MPa), without differences between them. Regarding the irrigation dose, FI registered an average value of −1.55 MPa, which was significantly higher than those of SDI_{75} (−1.76 MPa) and SDI_{65} (−1.71 MPa), and the SDIs were similar.

The interactions between irrigation and cultivars showed the highest Ψ_{leaf} value for cv. Marta under FI (−1.45 MPa) and the lowest for cv. Guara under SDI_{75} (−1.91 MPa) and SDI_{65} (−1.90 MPa). A very similar trend was observed for each measuring day, which reflects that cv. Marta had a different water status than the other cultivars, whereas SDI treatments were similar.

Table 7.1 Evolution of crop water status in terms of leaf water potential (Ψ_{leaf}).

DOY	162–217	162	175	183	189	196	203	210	217
Timing	**	–	–	–	–	–	–	–	–
Irrigation	**	**	**	*	*	*	*	*	*
Cultivar	**	NS	**	**	**	**	**	**	*
Timing × Irrigation	*	–	–	–	–	–	–	–	–
Timing × Cultivar	**	–	–	–	–	–	–	–	–
Irrigation × Cultivar	*	*	*	*	**	**	*	**	**
Tukey's Multiple Range Test ‡									
Irrigation									
FI	−1.55a	−1.42a	−1.49a	−1.58a	−1.46a	−1.24a	−1.76a	−1.32a	−1.65a
SDI$_{75}$	−1.76b	−1.60ab	−1.81b	−1.85b	−1.74b	−1.54b	−1.85a	−1.47b	−1.90b
SDI$_{65}$	−1.71b	−1.72b	−1.73b	−1.71ab	−1.69b	−1.48b	−1.88a	−1.45b	−1.82ab
Cultivar									
Marta	−1.53a	−1.52a	−1.49a	−1.47a	−1.47a	−1.21a	−1.72a	−1.26a	−1.67a
Guara	−1.80b	−1.69a	−1.85b	−1.80b	−1.77b	−1.59b	−1.93b	−1.47b	−1.80ab
Lauranne	−1.71ab	−1.53a	−1.71b	−1.89b	−1.66b	−1.48b	−1.85ab	−1.51b	−1.89b
Irrigation × Cultivar									
Marta									
FI	−1.45a	−1.47a	−1.42a	−1.36a	−1.36a	−1.09a	−1.67a	−1.19a	−1.55a
SDI$_{75}$	−1.57ab	−1.52a	−1.54a	−1.52a	−1.52ab	−1.29ab	−1.69ab	−1.26ab	−1.80ab
SDI$_{65}$	−1.60ab	−1.60ab	−1.52a	−1.54a	−1.54ab	−1.28ab	−1.87ab	−1.35abc	−1.72ab
Guara									
FI	−1.65abc	−1.46a	−1.75b	−1.61ab	−1.61ab	−1.42abc	−1.88ab	−1.38abc	−1.73ab
SDI$_{75}$	−1.91c	−1.75ab	−2.00b	−1.80ab	−1.80bc	−1.74c	−2.01ab	−1.55c	−1.95b
SDI$_{65}$	−1.90c	−2.11b	−1.77b	−2.03ab	−2.03c	−1.71c	−2.22b	−1.50c	−1.90b
Lauranne									
FI	−1.58ab	−1.41a	−1.43a	−1.50ab	−1.50ab	−1.31ab	−1.69ab	−1.44bc	−1.72ab
SDI$_{75}$	−1.78bc	−1.58a	−1.87b	−1.80ab	−1.80bc	−1.57bc	−1.90ab	−1.57c	−1.97b
SDI$_{65}$	−1.71abc	−1.49a	−1.83b	−1.64ab	−1.64ab	−1.53bc	−1.89ab	−1.49bc	−1.95b

Note: ns, not significant at $p < 0.05$; *, ** significant at $p < 0.05$ and 0.01, respectively. ‡ Values followed by the same letter within the same column and factor are not significantly different ($p < 0.05$) according to Tukey's multiple range test. DOY, day of year; FI, fully irrigated control treatment; SDI$_{75}$, sustained- deficit irrigation at 75% of irrigation requirement (IR); SDI$_{65}$, sustained-deficit irrigation at 65% of IR.

While the almond quality parameters are determined by water stress imposed during the kernel-filling period, final yield responded to the level of water stress produced over the entire period of crop development (including the first stages of vegetative and fruit growth, or even the postharvest conditions from the previous season)[150]. In our case, SDIs were applied, hence, water stress was imposed during the whole season (even during the postharvest period of the previous year, 2018). In response to this strategy, cv. Guara registered the highest yield reduction in SDI$_{65}$ (17% lower than FI), while the yield in SDI$_{75}$ was significantly similar to the yield in FI (Table 7.2).

The yield reductions obtained with cv. Guara were in agreement with the Ψ_{leaf} values previously registered by this cultivar (Table 7.1). In contrast, the yield of cvs. Marta and Lauranne was not significantly affected by SDI treatments (Table 7.2); these cultivars showed the best results in terms of Ψ_{leaf} (Table 7.1), as has been previously discussed.

Table 7.2 Kernel yield for different cultivars and irrigation treatments.

Cultivars	FI	SDI_{75}	SDI_{65}
		(kg·ha^{-1})	
Marta	2218a	2209a	2243a
Guara	2254a	2081ab	1871b
Lauranne	2326a	2105a	2196a

Note: FI, fully irrigated control treatment; SDI_{75}, sustained-deficit irrigation at 75% of irrigation requirement (IR); SDI_{65}, sustained-deficit irrigation at 65% of IR. Values followed by the same letter within the same row and factor are not significantly different (p < 0.05) by Tukey's test.

This differential response between cultivars is not new, as is it has been proved by other authors. Gomes-Laranjo et al.[116] reported different physiological responses among cultivars when they were subjected to different DI strategies. The authors concluded that cv. Lauranne was less sensitive to water restrictions than Ferragnès. More recently, authors such as Miarnau et al.[135] highlighted that under the SDI strategy, with a total amount of water around 200 mm, the nut yield for cvs. Guara and Marta amounted to 1,200 and 1,900 kg·ha^{-1}, whereas under FI conditions (750 mm) the values were 2,800 and 3,600 kg·ha^{-1}, respectively. All these results suggest that cv. Marta may mitigate the water stress by means of an internal mechanism, yielding more than cv. Guara, as was observed in the present work.

2.2 Antioxidant Activity and Total Phenolic Content

The results of antioxidant activity (AA) and total phenolic content (TPC) are shown in Table 7.3. Highly significant effects ($p < 0.001$) were found in response to the applied irrigation treatments and cultivars.

AA was studied through two methodologies: ABTS•+ and DPPH•. The ABTS•+ results showed significant differences among cultivars but not irrigation treatments, with cv. Marta showing the highest values. Moreover, it was notable that the highest values of ABTS•+ were for cv. Guara under SDI_{65}, followed by cv. Marta under FI and SDI_{65} strategies. These results evidence the importance of the cultivar in the antioxidant activity of almonds, which can be increased by using DI strategies, as has been observed for cv. Guara under SDI_{65}. The DPPH• assay corroborated the results obtained using ABTS•+, showing that cv. Marta recorded the highest AA. In terms of irrigation strategies, it was observed that AA increased with the highest level of stress (SDI_{65}). Regarding the interaction irrigation × cultivar for DPPH•, cv. Marta under SDI_{65} registered the highest values, followed by cv. Marta SDI_{75} and FI. Overall, the obtained results highlight how cultivar and irrigation can positively affect almond's antioxidant activity.

For TPC, the highest values were also reached with SDI treatments and, in terms of cultivar, Lauranne had the highest value. Regarding the interaction irrigation × cultivar, the highest values were shown for cv. Guara SDI_{65}, followed by cvs. Lauranne SDI_{75} and Marta SDI_{65}; this trend was consistent with the water-stress values found in this experiment. These findings agreed with Lipan et al.[64], who found a positive correlation between TPC and imposed water stress. Antioxidant activity and TPC are parameters of great importance for health properties. Ros et al.[200] and Lopez- Uriarte et al.[201] reviewed a total of 21 clinical studies that evaluated almond's antioxidant activity, reporting numerous cardiovascular health benefits such as decreased blood pressure and visceral adiposity. Polyphenols contribute to almond color and astringency and increase its shelf life[202]. The highest polyphenol concentration is found in almond skin, and many times this is eliminated through the operations of processing[65]. Moreover, almond skin also has antimicrobial properties, which are promoted by synergistic interactions between phenolic acids and flavonoids and involved in fighting against diseases caused by Salmonella enterica, Listeria monocytogenes, and Escherichia coli[203]. The main contributions of almond polyphenols to health are in reducing inflammation and type 2 diabetes[204]; they also have antiproliferative and antitumoral effects, including proanthocyanins, the major polyphenol found in almonds.

The relationship between cultivar and antioxidant activity in almond has been investigated[202], and it was concluded that each cultivar has its own characteristic antioxidants. Considering our results, cvs. Marta and Guara had greater amounts of antioxidants than cv. Lauranne, as shown by ABTS•+ and DPPH•. The values obtained by ABTS•+and DPPH• may be a result of the activity of tocopherols rather than polyphenols, since cv. Lauranne had the highest TPC index. It is well known that food antioxidant activity can be given by polyphenols but also by other compounds such as tocopherols, which can increase under water-stress conditions, as previously reported by Zhu et al.[173]. and Lipan et al.[65], who demonstrated the importance of DI strategies to increase the antioxidant activity and TPC in raw almonds.

Table 7.3 Antioxidant activity (ABTS•+ and DPPH• indices) and total phenolic content (TPC) for different cultivars and irrigation treatments.

	ABTS•+	DPPH (mmol Trolox·kg^{-1})	TPC (g GAE·kg^{-1})
ANOVA†			
Irrigation	ns	***	***
Cultivar	***	***	***
Irrigation × Cultivar	***	***	***
Tukey Multiple Range Test‡			
Irrigation			
FI	10.8	40.2b	2.97b
SDI$_{75}$	10.0	39.8b	3.81a
SDI$_{65}$	10.9	42.0a	3.80a
Cultivar			
Marta	11.2a	45.0a	3.40b
Guara	10.5ab	38.8b	3.50b
Lauranne	9.99b	38.2b	3.68a
Irrigation × Cultivar			
Marta			
FI	12.8ab	41.7bc	2.79de
SDI$_{75}$	9.77d	45.3ab	3.44cd
SDI$_{65}$	11.1abc	48.0a	3.98bc
Guara			
FI	8.85cd	37.3ef	2.29e
SDI$_{75}$	9.53cd	39.2cde	3.14cde
SDI$_{65}$	13.1a	40.0cde	5.06a
Lauranne			
FI	10.8bc	41.6bcd	3.82c
SDI$_{75}$	10.8bc	35.0f	4.86ab
SDI$_{65}$	8.40d	37.9def	2.37e

Note: † ns, not significant at $p < 0.05$; *** significant at $p < 0.001$. ‡ Values (mean of eight replications) followed by the same letter within the same column and factor are not significantly different ($p < 0.05$) according to Tukey's multiple range test. FI, fully irrigated control treatment; SDI$_{75}$, sustained-deficit irrigation at 75% of irrigation requirement (IR); SDI$_{65}$, sustained-deficit irrigation at 65% of IR.

2.3 Organic Acid and Sugar Content

In relation to organic acid results, strongly significant effects were observed between irrigation treatments and cultivars. That is, cv. Guara showed the highest amounts of all studied organic acids, followed by cvs. Lauranne and Marta (Table 7.4). Thus, as described for antioxidant activity and TPC, the cultivar factor was of great importance. In relation to the imposed irrigation strategy, it is noticeable that SDI$_{75}$

treatment obtained the highest values in all organic acids and, consequently, in total organic acid amount.

Regarding the interaction irrigation × cultivar, it can be highlighted that all cultivars were positively influenced by SDI_{75}. The interaction of cv. Guara and SDI_{75} treatment registered the highest amount of organic acids except fumaric acid; cv. Lauranne × SDI_{75} had the highest value of fumaric acid. Similar results were reported by Lipan et al.[64] for cv. Vairo, which showed a clear relationship between water stress and total organic acid content, although other authors concluded that water stress did not have such effects on these compounds[87,179]. Comparing these results with those obtained for Ψ_{leaf}, it is noticeable that the best results in relation to organic acid content were found in the cultivar that registered the highest water-stress values, which is in line with the results of Lipan et al.[64], who concluded there was a direct relationship between imposed water stress and organic acid content for these cultivars (Guara, Marta, and Lauranne).

Table 7.4 Organic acids in raw almonds as affected by irrigation dose and cultivar.

	Organic acids					
	Oxalic	Citric	Tartaric	Malic	Fumaric	Total
	ANOVA Test					
Irrigation	ns	***	***	***	***	***
Cultivar	**	***	***	***	***	***
Irrigation x Cultivar	***	***	***	***	***	***
	Tukey's Multiple Range Test					
	Irrigation					
FI	2.00	2.98b	2.05b	1.72b	0.21b	8.97b
SDI_{75}	2.04	3.14a	2.20a	2.00a	0.30a	9.68a
SDI_{65}	2.02	2.98b	2.08b	1.61b	0.28a	8.97b
	Cultivar					
Marta	2.04a	2.43c	1.23c	1.35c	0.24b	7.29c
Guara	2.14a	3.63a	2.98a	2.11a	0.27a	11.1a
Lauranne	1.88b	3.05b	2.11b	1.87b	0.28a	9.19b
	Irrigation x Cultivar					
Marta						
FI	2.00abc	2.40e	1.26d	1.66bc	0.20d	7.52de
SDI_{75}	1.99abc	2.36e	1.12d	1.21c	0.23cd	6.91e
SDI_{65}	2.13abc	2.53de	1.32d	1.18c	0.28abc	7.45de
Guara						
FI	2.17ab	3.58ab	3.07a	1.68bc	0.19d	10.7b
SDI_{75}	2.20a	3.97a	3.27a	2.77a	0.32ab	12.5a
SDI_{65}	2.04abc	3.33bc	2.61b	1.89b	0.29abc	10.1bc
Lauranne						
FI	1.83c	2.97cd	1.83b	1.83b	0.23b	8.69cd
SDI_{75}	1.93abc	3.09c	2.21b	2.21b	0.34b	9.59bc
SDI_{65}	1.89bc	3.08c	2.30bc	2.30bc	0.28bc	9.30bc

Note: † ns, not significant at $p < 0.05$; **, *** significant at $p < 0.01$ and 0.001, respectively. ‡ Values (mean of eight replications) followed by the same letter within the same column and factor are not significantly different ($p < 0.05$) according to Tukey's multiple range test. FI, fully irrigated control treatment; SDI_{75}, sustained-deficit irrigation at 75% of irrigation requirement (IR); SDI_{65}, sustained deficit irrigation at 65% of IR.

Sugar content was also highly influenced by the cultivar and the irrigation strategy (Table 7.5). All studied sugars showed a clear response to the irrigation treatments and cultivars, except for maltoheptaose. In terms of irrigation treatment, SDI_{75} and SDI_{65} showed the highest total sugar content of 62.9 and 62.2 g·kg^{-1}, respectively. In terms of cultivar, Guara (65.5 g·kg^{-1}) and Lauranne (63.7 g·kg^{-1}) had the highest amount of sugars. Total sugars, and specifically sucrose, tend to be higher as water stress increases, which is in line with other studies[205] that observed that total sugars and sucrose increased with water stress. Relating to the interaction of irrigation ×

cultivar, it can be highlighted that all cultivars were positively influenced by DI, especially by SDI_{75}, as occurs with organic acids. For maltrotriose and sucrose, the best combination was observed in cv. Guara under SDI_{75}; for glucose, the best combination irrigation × cultivar was in cv. Marta SDI_{65}; and for fructose, cv. Lauranne under the SDI_{65} strategy reached the highest value. According to Sánchez-Bel et al.[179], sucrose is the principal sugar in almond cultivars, due to its preferential production and accumulation in the almond during ripening, and probably its synthesis and accumulation would be influenced by water stress. In this line, there is a strong effect between water stress and the sugar composition of nuts. During the water-stress period, almond leaves begin to lose turgor due to dehydration and, to avoid this, the tree closes the stoma by decreasing the transpiration process. Therefore, to restore the osmotic balance, the tree concentrates sugars to recover the turgor that was lost by dehydration. This is in line with Prgomet et al.[206], who found in a two-year experiment that non-irrigated trees accumulated more leaf water soluble sugars than control trees to maintain cell turgor, similar to what occurred in almonds, as previously discussed[63,64,198].

Table 7.5 Sugar content in raw almonds as affected by irrigation dose and cultivar.

	Sugars					
	Maltoheptaose	Maltotriose	Sucrose	Glucose	Fructose	Total
	ANOVA Test					
Irrigation	ns	***	***	***	***	***
Cultivar	ns	***	***	***	***	***
Irrigation x Cultivar	ns	***	***	***	***	***
	Tukey's Multiple Range Test					
	Irrigation					
FI	3.34	3.20ab	33.5b	9.78b	2.80b	52.6b
SDI_{75}	3.45	3.56a	41.2a	10.9a	3.81a	62.9a
SDI_{65}	3.35	3.08b	40.5a	11.2a	4.06a	62.2a
	Cultivar					
Marta	3.38ab	3.39b	26.5b	12.5a	2.85c	48.5b
Guara	3.56a	4.45a	44.3a	9.66b	3.55b	65.5a
Lauranne	3.20b	2.00c	44.4a	9.79b	4.27a	63.7a
	Irrigation x Cultivar					
Marta						
FI	3.40	3.27c	25.2e	10.0bcde	2.52c	44.4f
SDI_{75}	3.50	3.38bc	26.6e	11.4b	2.78c	47.6ef
SDI_{65}	3.24	3.51bc	27.6e	15.9a	3.24bc	53.6d
Guara						
FI	3.31	3.57bc	33.4d	9.46cde	2.35c	52.1de
SDI_{75}	3.76	5.60a	52.0a	10.5bcd	4.21ab	76.1a
SDI_{65}	3.60	4.19b	47.5b	9.05de	4.09ab	68.4b
Lauranne						
FI	3.30	2.74c	41.8c	9.87bcde	3.52bc	61.3c
SDI_{75}	3.10	1.69d	44.9b	10.9bc	4.44ab	65.1bc
SDI_{65}	3.22	1.56d	46.4b	8.62e	4.86a	64.7bc

Note: † ns, not significant at $p < 0.05$; *** significant at $p < 0.001$. ‡ Values (mean of 8 replications) followed by the same letter within the same column and factor are not significantly different ($p < 0.05$) according to Tukey's multiple range test. FI, fully irrigated control treatment; SDI_{75}, sustained-deficit irrigation at 75% of irrigation requirement (IR); SDI_{65}, sustained-deficit irrigation at 65% of IR.

2.4 Fatty Acids

Almond's fatty acid profile has a broad spectrum, which makes this product a food that is a good energy source and does not increase cholesterol levels[207]. A total of 25 fatty acids were identified in this experimental work, classified as saturated fatty acids (SFA) (Table 7.6) and unsaturated fatty acids (Table 7.7), which are subdivided into monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). The almond lipid fraction identified was mainly composed of oleic acid (C18:1n9), linoleic acid (C18:2n6), palmitic acid (C16:0), and stearic acid (C18:0).

These were significantly affected by water stress, which increased their concentration in both SDI treatments compared to the FI treatment. In particular, oleic and linoleic acids increased 6.0% and 10%, respectively, in SDI treatments.

Regarding the cultivar, Guara had the highest amount of oleic acid, while cv. Lauranne had the highest amount of linoleic acid (6.2% and 3.8% more than Marta and Guara, respectively). The interaction irrigation × cultivar showed that cv. Guara in SDI_{75} and cv. Lauranne in SDI_{65} led to the highest content of palmitic acid; whereas cv. Marta in the three treatments and cv. Guara in SDI_{65} and cv. Lauranne in both SDI treatments showed the highest amounts of oleic acid.

The best results in terms of the oleic/linoleic (O/L) ratio were found for SDI_{75} (this index improves as its value decreases), while in terms of the cultivar, the lowest values were obtained for cvs. Guara and Lauranne. Linoleic acid is related to the stability of the oil. Thus, the lower the amount, the greater the stability[182]. This fatty acid is essential for humans and cannot be synthesized by itself. Thus, although stability is reduced, the increase in linoleic acid by deficit irrigation treatment gives the almond added value since this acid plays a fundamental role in the death of cardiac cells[205]. Additionally, as water stress increases, total phenolic content and antioxidant activity increase, which could help in protecting against lipid oxidation.

Table 7.6 Saturated fatty acid (SFA) profile in raw almonds affected by cultivar and irrigation dose.

	C14:0 (Myristic)	C15:0 (Pentadecylic)	IsoC16:0	C16:0 (Palmitic)	C17:0 (Margaric)	C18:0 (Stearic)	C20:0 (Arachidic)	C21:0 (Heneicosylic)	C22:0 (Behenic)	C23:0 (Tricosylic)
ANOVA Test †										
Irrigation	***	*	*	***	***	***	***	*	***	ns
Cultivar	***	*	ns	***	***	***	***	*	***	***
Irrigation × Cultivar	***	*	*	***	***	***	***	*	***	***
Tukey Multiple Range Test ‡										
Irrigation										
FI	0.11b	0.05b	0.04b	21.5b	0.30b	9.92b	0.56ab	0.09a	0.14a	0.19
SDI_{75}	0.13a	0.06a	0.05a	23.4a	0.36a	11.0a	0.63a	0.09a	0.14a	0.19
SDI_{65}	0.11b	0.05b	0.04b	23.0a	0.28b	10.7ab	0.53b	0.08b	0.13b	0.19
Cultivars										
Guara	0.12a	0.05b	0.04	21.8b	0.37a	9.92b	0.57b	0.09a	0.13b	0.25a
Marta	0.11b	0.06a	0.04	22.5ab	0.25b	12.9a	0.67a	0.08b	0.16a	0.18b
Lauranne	0.12a	0.06a	0.04	23.6a	0.33a	8.70c	0.47c	0.09a	0.12b	0.15c
Irrigation × Cultivar										
Marta										
FI	0.12ab	0.05ab	0.04b	21.6bc	0.34c	9.30de	0.59b	0.09ab	0.12bc	0.25a
SDI_{75}	0.12ab	0.05ab	0.04b	21.8bc	0.50a	9.90de	0.53b	0.08ab	0.13abc	0.24a
SDI_{65}	0.12ab	0.05ab	0.04b	21.9bc	0.25b	10.6cd	0.59b	0.10ab	0.14abc	0.26a
Guara										
FI	0.10c	0.05b	0.04b	20.8c	0.25b	11.8bc	0.63ab	0.08ab	0.17a	0.17b
SDI_{75}	0.12ab	0.07a	0.05a	22.6bc	0.25b	13.8a	0.81a	0.09ab	0.16ab	0.18b
SDI_{65}	0.11bc	0.06ab	0.04b	24.1ab	0.26b	13.2ab	0.57b	0.08ab	0.14abc	0.18b
Lauranne										
FI	0.11bc	0.05ab	0.04b	22.0bc	0.32b	8.70e	0.44b	0.11a	0.12bc	0.15b
SDI_{75}	0.14a	0.07a	0.05a	25.8a	0.34b	9.14de	0.54b	0.11a	0.13abc	0.17b
SDI_{65}	0.11b	0.05ab	0.04b	22.9bc	0.33b	8.26e	0.43b	0.07b	0.10c	0.14b

Note: † ns, not significant at $p < 0.05$; * and *** significant at $p < 0.05$ and 0.001, respectively. ‡ Values (mean of three replications) followed by the same letter within the same column and factor are not significantly different ($p < 0.05$), according to Tukey's least significant difference test. FI, fully irrigated control treatment; SDI_{75}, sustained-deficit irrigation at 75% of irrigation requirement (IR); SDI_{65}, sustained-deficit irrigation at 65% of IR.

Table 7.7 Monounsaturated and polyunsaturated fatty acid (MUFA and PUFA) profile in raw almonds affected by cultivar and irrigation dose (g·kg^{-1} dry weight).

	MUFA												PUFA		
	C15:1 (Pentadecenoic)	C16:1c7	C16:1c9 (Palmitoleic)	C16:1c10	C17:1 (cis-Heptadecenoic)	C18:1t9 (Elaidic)	C18:1c9 (Oleic)	C18:1n7 (cis-Vaccenic)	C20:1c9 (Eicosenoic)	C20:1c11 (Eicosenoic)	C20:1c15 (Eicosenoic)	C24:1 (Nervonic)	C18:2t8c13 (Linoleaidic)	C18:2n6c9,12 (Linoleic)	C18:3n3 (α-Linolenic)
ANOVA Test†															
Irrigation	ns	***	***	***	ns	ns	*	***	ns	**	ns	***	***	*	***
Cultivar	ns	***	***	***	ns	ns	*	***	**	**	ns	***	***	*	***
Irrigation x Cultivar	ns	***	***	***	ns	ns	*	***	**	**	ns	***	***	*	***
Tukey Multiple Range Test‡															
Irrigation															
FI	0.03	0.11b	2.10c	0.08a	0.57	0.17	139b	15.7a	0.09	0.42b	0.02	0.08a	0.12a	50.9b	0.18ab
SDI75	0.03	0.10c	2.22b	0.07b	0.54	0.17	141ab	15.4ab	0.09	0.41b	0.02	0.07b	0.10b	56.5a	0.20a
SDI65	0.03	0.15a	2.33a	0.07b	0.55	0.14	147a	14.7b	0.09	0.49a	0.02	0.07b	0.08c	53.0ab	0.17b
Cultivars															
Guara	0.03	0.14a	2.12b	0.06b	0.57	0.18	149a	16.0a	0.09aq	0.47a	0.02	0.06b	0.09b	51.9c	0.17c
Marta	0.03	0.09c	1.78c	0.05c	0.52	0.16	137b	13.9b	0.10a	0.42b	0.02	0.08a	0.09b	53.2b	0.20a
Lauranne	0.03	0.12b	2.75a	0.11a	0.57	0.15	142ab	15.9a	0.07b	0.42b	0.03	0.07b	0.13a	55.3a	0.19b
Irrigation x Cultivar															
Marta															
FI	0.03	0.14b	2.15bc	0.09abc	0.64	0.19	145ab	16.1abc	0.09ab	0.46b	0.02	0.09a	0.09cde	51.6ab	0.20abc
SDI75	0.03	0.10bc	2.03bc	0.04d	0.49	0.17	146ab	15.2abcd	0.08ab	0.45b	0.02	0.05c	0.08de	52.3ab	0.16bc
SDI65	0.03	0.18a	2.17bc	0.05cd	0.58	0.17	156a	16.6ab	0.10ab	0.50a	0.02	0.06bc	0.08de	51.7ab	0.17abc
Guara															
FI	0.04	0.09cd	1.63c	0.04d	0.49	0.18	136b	14.6abcd	0.11a	0.40b	0.02	0.08ab	0.13abc	48.5b	0.16bc
SDI75	0.03	0.06d	1.57c	0.04d	0.52	0.14	133b	13.8cd	0.11a	0.39c	0.02	0.08ab	0.08de	57.2ab	0.23a

Note: † ns, not significant at $p < 0.05$; *, **, and *** significant at $p < 0.05$, 0.01, and 0.001, respectively. ‡ Values (mean of three replication) followed by the same letter, within the same column and factor, were not significantly different ($p < 0.05$), according to Tukey's least significant difference test. I, irrigation; C, cultivar; FI, fully irrigated control treatment; SDI_{75}, sustained-deficit irrigation at 75% of irrigation requirement (IR); SDI_{65}, sustained-deficit irrigation at 65% of IR.

Regarding the total monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) content (Table 7.8), these values were higher in both SDI treatments; cv. Marta had a higher amount of MUFA and cv. Lauranne of PUFA. These results agree with those in the study of Lipan et al.[198], which found the same relation of these cultivars under different irrigation strategies. In contrast, in other cultivars, such as cv. Vairo, decreased MUFAs and increased PUFAs with increasing water stress after three years of experimentation was highlighted by Lipan et al.[205]. In other nuts, such as pistachio, these compounds were not affected by water stress[208]. However, significant differences were found with values higher than 50% of MUFA and 30% of PUFA depending upon the pistachio cultivar used. In olive cv. Arbequina, Garcia et al.[209] reported an increase in the MUFA/PUFA ratio in the RDI treatment compared to the control treatment due to the desaturation of oleic acid with high stress and consequent linoleic formation. Bitok and Sabaté[210] reported that products rich in MUFA and PUFA could contribute to the prevention of coronary heart and cardiovascular diseases as well as diabetes and obesity. To prevent these diseases, the US Food and Drug Administration recommends 42.5 g of almond[211]. This provides greater functionality for almonds cultivated under water-stress conditions. The PUFA:SFA ratio provides information about whether a diet is atherogenic or could promote coronary heart disease. In this case, this ratio had a high relation with irrigation, cultivar, and irrigation × cultivar, and was higher in SDI_{75} with cv. Lauranne.

Table 7.8 Types of fatty acids, ratios and health indices in different almond cultivars under sustained deficit irrigation (g·kg^{-1} dry weight).

	O:L	SFA	MUFA	PUFA	PUFA:SFA	PUFA:MUFA	(MUFA+PUFA)/SFA	Total fatty acids
ANOVA Test†								
Irrigation	***	***	**	*	***	***	***	*
Cultivar	***	***	**	*	***	***	***	*
Irrigation × Cultivar	***	***	**	*	***	***	***	*
Tukey Multiple Range Test‡								
Irrigation								
FI	2.73a	32.9b	158b	51.2b	1.56a	0.32b	6.38a	242b
SDI_{75}	2.51b	36.0a	160ab	56.8a	1.57a	0.35a	6.05b	253a
SDI_{65}	2.79a	35.0a	166a	53.2ab	1.52b	0.32b	6.30a	254a
Cultivar								
Marta	2.88a	33.3b	169a	52.1b	1.56b	0.31b	6.63a	254a
Guara	2.59b	36.9a	154b	53.5b	1.45c	0.35a	5.64b	245b
Lauranne	2.57b	33.7b	162ab	55.6a	1.65a	0.34a	6.47a	251a
Irrigation × Cultivar								
Marta								
FI	2.82ab	32.5c	164ab	51.9ab	1.59ab	0.31cd	6.65ab	249b
SDI_{75}	2.80ab	33.4bc	165ab	52.5ab	1.57abc	0.32d	6.51ab	251ab
SDI_{65}	3.02a	34.0bc	177a	51.9ab	1.53abc	0.29d	6.72a	262a
Guara								
FI	2.81ab	34.0bc	154b	48.8b	1.44bc	0.32cd	5.97d	237c
SDI_{75}	2.32d	38.1a	150b	57.6ab	1.51abc	0.38a	5.44e	245b
SDI_{65}	2.63bc	38.7a	159ab	54.2ab	1.40c	0.34bc	5.50de	251ab
Lauranne								
FI	2.57bcd	32.0c	156b	53.0ab	1.65a	0.34bc	6.51ab	241b
SDI_{75}	2.41cd	36.5ab	166ab	60.3a	1.65a	0.36ab	6.21bc	263a
SDI_{65}	2.71b	32.4c	163ab	53.6ab	1.65a	0.33c	6.68ab	249b

† NS = not significant at *p*> 0.05; *, **, and *** significant at *p*< 0.05, 0.01, and 0.001, respectively. ‡ Values (mean of 3 replication) followed by the same letter, within the same column and factor, were not significantly different (p>0.05), according to Tukey's least significant difference test. Oleic:linoleic ratio=O:L, Saturated Fatty Acids=SFAs, Monounsaturated Fatty Acids=MUFAs, Polyunsaturated Fatty Acids=PUFAs, Atherogenic Index=AI, Thrombogenic Index=TI.

2.5 Relationships between crop water status at different stages and healthy composition of almond

With the aim of finding the potential relationships between Ψ_{leaf} and quality parameters and estimate which of them were more affected by imposed water stress, principal component analysis (PCA) was done (Figure 7.1).

According to the obtained results, the two main components (F1 and F2) explained 41.9% and 21.4% of total variance. As observed, samples of each irrigation treatment were grouped mainly separately, except for SDI_{75} for cvs. Lauranne and Marta grouped together in FI treatments, and totally opposite to SDI_{65} treatments. In this regard, SDI_{65} samples were mainly surrounded by sucrose, glucose, fructose, SFA, and total fatty acids. In addition, cv. Guara under SDI_{75} was characterized by total organic acids (especially malic, citric, and tartaric), maltoheptaose, maltotriose, and the PUFA/MUFA ratio. Finally, FI samples were characterized by Ψ_{leaf} and the (MUFA + PUFA/SFA) ratio.

Significant relationships were observed between Ψ_{leaf} values and the quality parameters that had previously shown significant differences between irrigation treatments. No significant correlations were obtained between Ψ_{leaf} and antioxidant activity (in terms of ABTS•+ and DPPH• index with r =−0.10 and 0.08, respectively). However, a significant correlation was observed for TPC (r= −0.59). On the contrary, Lipan et al.[205] found a significant relationship between accumulated water stress and the ABTS•+ index (r = 0.79), which was not detected in the present study, probably because this experiment was developed with different cultivars.

More interesting were the Pearson's coefficients among Ψ_{leaf}, organic acids, and sugar content. Within the organic acids, fumaric showed the best correlations (r = −0.96 **), whereas for the sugar profile, the best relationships were found for sucrose (r = −0.85 **), fructose (r = −0.92 **), and total sugars (r = −0.86 **). Similar results were reported by Lipan et al.[212], who noted improvements in terms of sucrose (r = −0.42 **), fructose (r = −0.36 *), and total sugar content (r = −0.39 **). Thus, according to these findings, high water-stress levels (lower values of Ψ_{leaf}) would be accompanied by higher sugar and fumaric acid content.

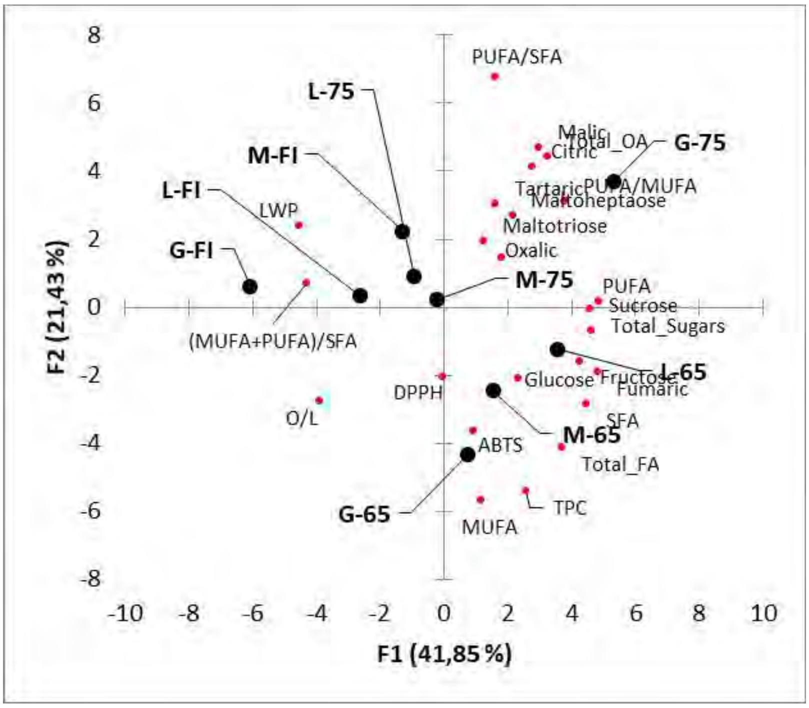

Figure 7.1 Principal component analysis (PCA) score biplot showing the relationship among leaf water potential (LWP) and quality parameters. ● Samples; O/L, oleic/linoleic ratio; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; Total_FA, total fatty acids; TPC, total phenolic content.

3. Conclusions

In view of the obtained results, almond cultivars which have been subjected to SDI strategies improved the fruit quality parameters, thus these being suitable strategies to enhance healthy composition of almonds. Specifically, SDI_{65} treatment improved the content of fatty acids and TPC, while SDI_{75} improved sugars and organic acids. These results also demonstrate that these parameters were strongly affected by the almond cultivar. In addition, organic acids, sugars and fatty acids were the most affected parameters, being evidenced strong correlations with the water stress imposed. With these results, a new line of research is opened in which it could be possible to evaluate how the quality parameters evolve during the kernel-filling period, even defining different threshold values of Ψ_{leaf} to increase specific components, and with different objectives depending on the cultivar. Furthermore, the SDI strategies not only would improve the almond quality with relevant reductions on water consumption, but also print an added value to the final product, within the concept of hydroSOS products. Additionally, it would be convenient to continue this type of work to check this effect in long-term experiments and introducing new cultivars.

Chapter 8

General discussion

1. Relationship between agronomic, physiological and quality variables of almonds cultivars in response to different irrigation strategies.

In this doctoral thesis the impact of different DI strategies, irrigation doses as well as the cultivar response have been studied in terms of final yield, nut quality and plant physiology. The efforts have been focused on two main important issues: (i) the effect of the water stress through DI strategies and, (ii) the effect of the cultivar (Fig. 8.1).

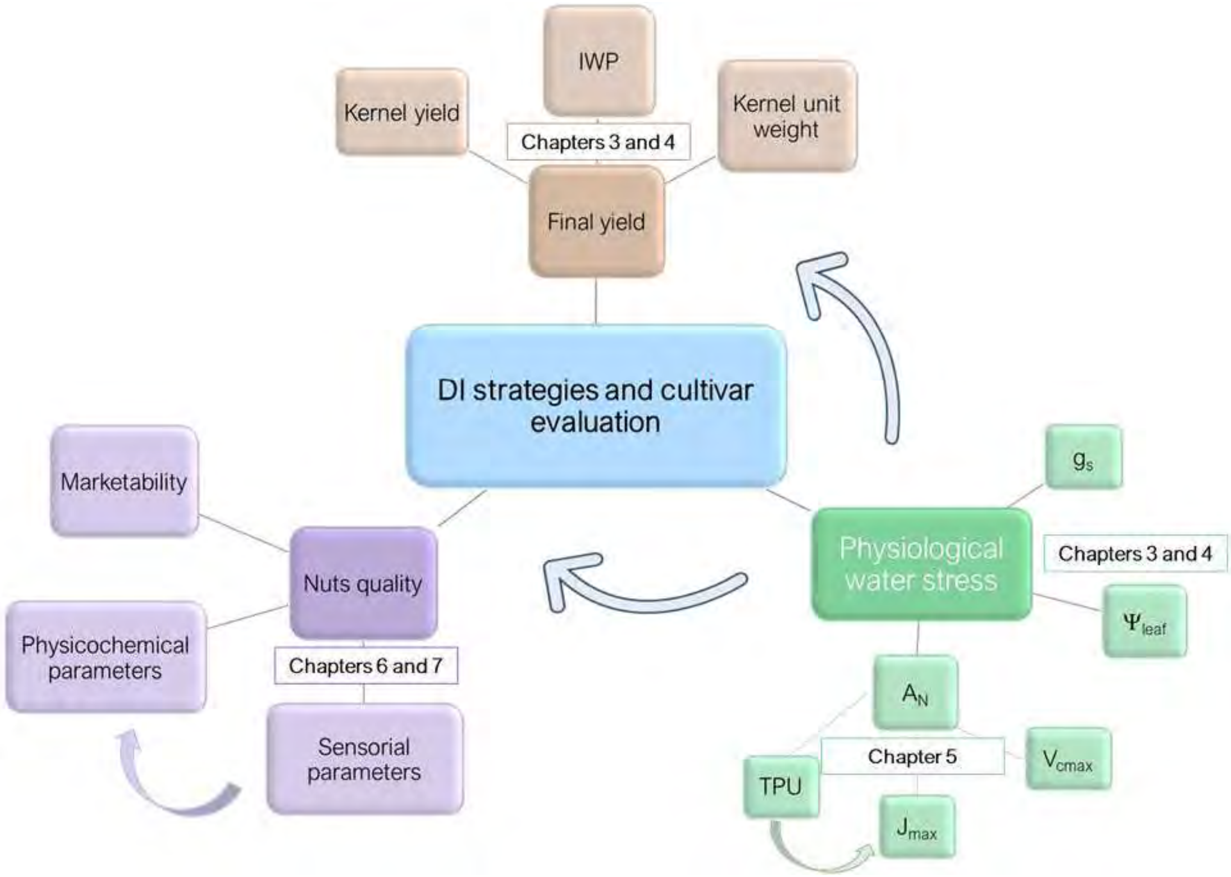

Figure 8.1 Graphical interconnections of the research and relationships among studied parameters in the doctoral thesis. Arrows indicate the relationships found between the different parameters in this doctoral thesis.

According to the results obtained and represented in Figure 8.1, it is necessary to highlight that the water stress applied directly affected the final yield (including all its variables) and the nut quality. Hence, the importance documented by Fernandez[214] of monitoring the crop response to water withholdings has been demonstrated, since the final yield and quality of the product obtained will depend on the stress levels reached by the crop and the duration of these conditions.

2. **How is the final yield affected by the irrigation doses imposed in different almond cultivars?**

Respect to the agronomic response (**Chapters 3 and 4)**, the greatest effect of the DI strategies and cultivars studied was obtained on the kernel yield, IWP and kernel unit weight.

In relation to the kernel yield, it is not easy to determine which DI strategy is more efficient because it was observed that the response of each almond cultivar was different. In this line, in **Chapter 3**, a positive yield response has been evidenced in RDI_{65} for cv. Guara in comparison with the other cultivars which indicates that in this cultivar a better response is obtained under RDI strategies in accordance with the studies carried out by López-López et al.[85]. In contrast, in **Chapter 4** for the same cultivar, a negative response (in comparison with the remaining cultivars and the yield obtained in RDI_{65}) was evidenced in SDI_{65}, although this point was compensated with the higher kernel unit weight obtained for this cultivar, compared to cvs. Marta and Lauranne, which would supply the yield losses at the market level. Finally, cvs. Marta and Lauranne adapted in a similar way to both irrigation strategies (**Chapters 3 and 4**), although in productive terms, lesser yield reductions were observed in RDI_{65} in both cultivars than in SDI_{65}. Since all cultivars had the same rootstock and therefore the same root efficiency in response to water availability, it is hypothesized that the difference between cultivars in kernel yield could be explained by studying the physiological response of each cultivar at leaf level which will be developed throughout the discussion section.

As already mentioned, there are few works[83,116,117,125,126] in which different irrigation strategies and cultivars have been compared. Egea et al.[82] in a six-year trial but only with one cultivar of almond tree (cv. Marta) was able to compare the response of RDI and SDI, obtaining that from an agronomic point of view, the water stress imposed had not intensified the negative impact of deficit irrigation on final yield. This finding corroborates the results obtained for cv. Marta in this doctoral thesis. Furthermore, an innovative aspect of this doctoral thesis is the fact that the irrigation strategies for almond should be adapted to each cultivar, as the studied ones here showed a different response in terms of kernel yield.

Another parameter that is important at the agronomic level is the kernel unit weight (one of the compounds that determine the final yield, together with the total fruit number). In this line, as the kernel unit weight is greater, the yield could be greater and therefore the incomes from the total yield would be higher. In this sense, RDI_{65} and SDI_{65} obtained similar values of kernel unit weight in all the cultivars (**Chapter 3**, Table 3.4 and **Chapter 4**, Table 4.2) and also 7% higher than the controls. These results would reinforce the possibility of improving the fruit size when DI strategy is imposed, this being an added value in relation to fruit marketability and consumer acceptance[62].

In terms of IWP, because of the low effects of DI on yield, deficit treatments (RDI_{65} and SDI_{65}) showed higher IWP values (~0.40-0.50 $kg \cdot m^{-3}$) than those registered under FI. In relation to the SDI_{65} treatment, the most relevant results were observed in cvs. Marta and Lauranne (0.49 and 0.55 $kg \cdot m^{-3}$, respectively) in comparison with cv. Guara (0.44 $kg \cdot m^{-3}$). This point was a response to the yield reductions in this cultivar mainly produced by a depletion of the total fruit number (**Chapter 4**, Table 4.2); although this negative effect was partially compensated with the kernel unit weight (almonds of SDI_{65} were 23% higher than FI). Other authors such as Phogat et al.[83], have emphasized that IWP increased substantially under DI strategies compared to FI tress; and others such as Egea et al.[81] obtained similar IWP values in RDI (0.30-0.40 $kg \cdot m^{-3}$) to those shown in this doctoral thesis.

Finally, it is worth highlighting the interesting results obtained in the overirrigated treatment (150-ETc; **Chapter 3)**. This treatment was designed considering the findings in a study by Goldhamer and Fereres (2017)[46]. These authors concluded that, for mature almond trees, well developed, and cultivated under the climatic conditions of California (similar to the current conditions in the experimental area of this thesis), the maximum yield values (~ 4000 $kg \cdot ha^{-1}$) would be reached under irrigation doses between 12,500 and13,000 $m^3 \cdot ha^{-1}$. Thus, in spite of being these irrigation doses beyond the crop water requirements estimated in the experimental area, this treatment was defined with the aim of testing if the final yield could be improved when almond trees were subjected to the irrigation amounts close to those irrigation doses reported by these authors. Through the analysis in kernel yield ($kg \cdot ha^{-1}$) of this treatment (**Chapter 3**, Figure 3.7 and 3.8) it was showed that not all cultivars significantly increased yield as the water supplied was higher. In this sense, cvs. Guara and Lauranne did not show a significant increase in yield in 150-ETc compared to the FI. However, cv. Marta, increased its yield by 46% compared to the FI treatment. These results would point out the importance of cultivar (and its physiological mechanisms) to manage the available water when irrigation doses and treatments are imposed.

3. **What physiological mechanisms explain the almond response to water stress and the yield values in different cultivars?**

In order to determine the physiological response of this crop to different irrigation doses and strategies, the most relevant parameters and variables that regulates tree water use and kernel growth were studied. The efforts were focused in seeking differences among the studied cultivars and the relationships of these responses with the obtained yield values.

When the relationship of SI was estimated in terms of Ψ_{leaf} and g_s (**Chapter 3,** Figure 3.6), in RDI_{65} treatment, cv. Marta showed greater water stress tolerance in terms of g_s for a particular Ψ_{leaf} than the other two cultivars. Moreover, when the same relationship was made between $SI_{\Psi leaf}$ and SI_{gs} but under SDI_{65} treatment (**Chapter 4**, Figure 4.3) it was observed that cv. Marta was again a cultivar more sensitive to water stress in physiological terms compared to cvs. Guara and Lauranne. These results, together with the greater yield in Marta under the 150-ETc treatment compared to the other two cultivar, seems to indicate a higher sensitivity of cv.Marta to water stress.

As an additional point of discussion, and within the hypothesis of the higher g_s sensitiveness observed in cv. Marta under DI strategies than in the other two cultivars; it was observed during field measurements that cv. Marta responded with a massive leaf abscission when water stress began (Figure. 8.2), compared to FI or 150-ETc treatments. This might be explained due to the inability of this cultivar to maintain adequate transpiration levels, as it is forced to pull leaves to maintain g_s levels.

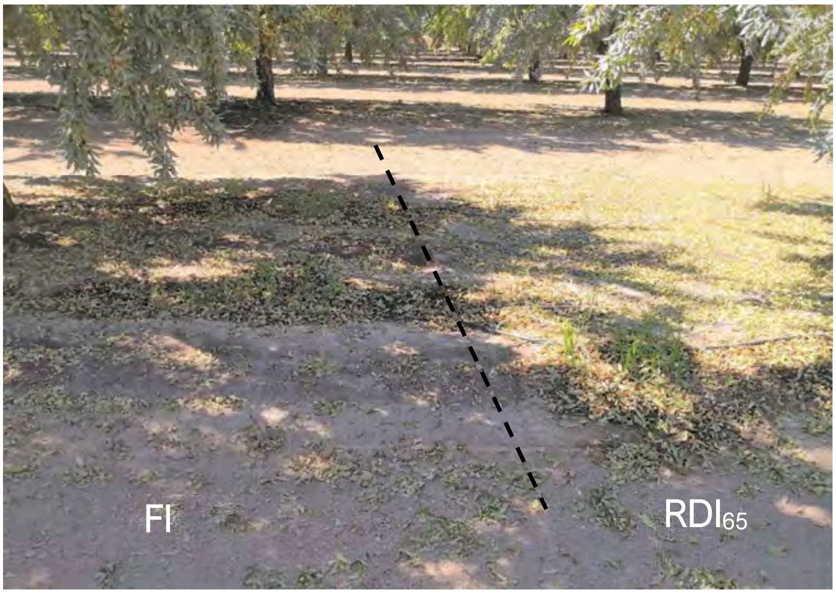

Figure 8.2 Significant leaf abscission from trees cv. Marta as a result of water stress during the kernel-filling stage.

This stomatal sensitivity of cv. Marta was further analysed in **Chapter 5** (Table 5.1) with the analysis of limitations carried out, which confirmed that this cultivar had a greater limitation at the diffusional level than the other two cultivars studied. This would explain the high capacity of this cultivar to apply stomatal control against a DI strategy compared to the remaining studied cultivars. Therefore, it seems that this greater sensitivity of cv. Marta to water stress compared to the other two studied cultivars, had a positive response in productive terms, under an irrigation treatment that provides more water (150-ETc), increasing significantly its production.

Additionally, with the water stress imposed in the DI treatments studied, g_s did not limit A_N. However, a down-regulation of photosynthetic activity was observed during the kernel-filling stage due to an excess of triose phosphate (TP) that limited J_{max}, but still was not enough to significantly limit A_N either (**Chapter 5**, Figure 5.3).

This behaviour in which there is a down-regulation of the photosynthetic capacity was evidenced by other authors such as McCormick et al.[145] or Paul and Foyer[144] but to the best of our knowledge this is the first time that this process has been described in almond trees. According to this, the kernel-filling stage would be the least sensitive stage to the application of DI strategies because it is the stage where the accumulation of TP begins and biochemical limitations take place. Based on the relationship between TPU and fruit growth (Figure 5.6), we hypothesize that this accumulation of photoassimilates in kernel-filling stage was produced by a decrease in the fruit growth rate compared to that observed during the vegetative stage. In this sense, as kernel growth rate is greater (coinciding with the vegetative stage) the TPU is much greater. As the cycle of the tree progresses and it enters the kernel-filling stage, the TPU decreases leading to their accumulation.

Therefore, the direct repercussion of the existence of a photosynthetic limitation (**Chapter 5**) and an accumulation of photoassimilates in the kernel-filling and postharvest stages means that the irrigation strategy that might provide better productive results in the almond tree is RDI because it would allow a tuning of irrigation based on the differential phenology of almond. In this agreement, higher irrigation amounts should be applied when TPU limitations are not relevant (vegetative development and fruit-growth period), but it could be decreased significantly during stage II when even an excess of photosynthate production could lead to A_N decrease, this fact not being water-stress dependent (**Chapter 5**).

4. **Is possible to compensate the yield reductions because of water stress in almond trees by improvements in the fruit quality?**

Although yield was barely affected by the water stress imposed, relevant effects on the nut's quality parameters were observed (**Chapter 6 and 7**). In general, the quality parameters were highly influenced by the cultivar and improved by the DI treatments. At the level of physicochemical parameters, the most representative in the samples studied were sugars (sucrose and glucose), organic acids (oxalic and citric acids) and fatty acids (ratio O/L, MUFA, PUFA).

About the influence of irrigation treatments on the concentration of sugars, it is worth noting the effect of the overirrigated treatment (Table 6.5). As expected, the sugar values were lower in this treatment than in the RDI_{65} treatment, which indicates that an extra amount of irrigation (>100%ETc) did not contribute anything to the final yield (**Chapter 3**, Figures 3.7 and 3.8), but it was not beneficial either for the concentration of sugars in almonds. This increase in sugars in RDI_{65} (Table 6.5) and SDI_{65} treatments (Table 7.5) was already discussed by other authors such as Nahar et al.[181], Lipan et al.[64], or Prgomet et al.[206] among others and associated this behaviour with solute concentration to maintain the osmotic balance of the leaf and increase the turgor that it lost due to lack of hydration. In this sense, this concentration of sugars would be related to the decrease in Ψ_{leaf} detected (**Chapters 3 and 4**) in the deficit treatments compared to the control in order to avoid a reduction of its photosynthetic capacity in the early stages of stress. This fact, has been discussed by Barzegar et al.[126], who in an experiment with six almond cultivars (Azar, Marcona, Mission, Nonpariel, Sahand, and Supernovoa) reported that Ψ_{leaf} decreased rapidly due to the osmotic adjustment of the plant that might be related to the concentration of sugars.

Regarding total sugars (essential for consumer acceptance), it had a very marked cultivar response. The cultivar with a higher concentration of total sugars in both DI strategies was Lauranne, while cvs. Guara and Marta depended on the applied deficit treatment. In the case of cv. Guara and in term of total sugars obtained a concentration of 49.2 $g \cdot kg^{-1}$ in RDC_{65} and 65.5 $g \cdot kg^{-1}$ in SDI_{65}. In the cv. Marta, the answer was different, obtaining higher values of total sugars in RDC_{65} than in SDI_{65} (51.7 $g \cdot kg^{-1}$ and 48.5 $g \cdot kg^{-1}$, respectively). If we compare this amount of sugar with the content of other healthy foods such as plums or bananas defined by Spanish food composition database (BEDCA), whose total sugar content is 120 $g \cdot kg^{-1}$ and 178 $g \cdot kg^{-1}$, respectively, we can assume that the total sugar content of almonds at a dietary level is low being only 5% of its weight[61].

As it happened with the sugars, the organic acids presented a characteristic cultivar response in both DI strategies (Tables 6.5 and 7.4, **Chapter 6 and 7** respectively). In this sense, cvs. Guara and Lauranne had similar concentrations of fatty acids in RDC_{65} while in SDI_{65} Guara had a higher amount. On the contrary, cv. Marta in both deficit treatment obtained the lowest concentration of fatty acids (9.43 $g \cdot kg^{-1}$ in

RDC_{65} and 7.29 g·kg^{-1} in SDI_{65}). Regarding the irrigation dose, all cultivars were positively influenced by the deficit treatment in both DI strategies. Although some authors[87,179] conclude that water stress has no relevant effects on these compounds, Lipan et al.[64] reported similar results to those found in this doctoral thesis but in other cultivar of almond. It is worth to remark the differences in the sugars profile between the studied cultivars in raw almonds, being the sucrose content significantly higher in Lauranne in comparison to the remaining studied cultivars. This difference that can be easily appreciated by the consumer could be a differential point in order to determine quality characteristics to discriminate among cultivars which are all grouped into a single group ("*comuna*") (Chapter 6, Table 6.5; Chapter 7, Table 7.5).

In relation with the fatty acids, the most important ones are oleic and linoleic acid, since the ratio between both defines the stability of the oil. In addition, linoleic acid is a fatty acid that the human body cannot produce, so it can only be obtained from ingestion of foods rich in it. Regarding the O/L ratio in both DI strategies, the values of the deficit treatments (RDI_{65} and SDI_{65}) are lower than the control, which gives the oil low stability. Taking into account the health properties, a high content of linoleic acid (a fatty acid not synthesized by the human body) is beneficial since this acid plays an important role in the human body, preventing, for example, the death of cardiac cells[205]. If we compare the DI strategies, the RDI_{65} had a higher content of MUFA, PUFA and O/L ratio than the SDI_{65} strategy in all cultivars. In other nuts, such as pistachio, these compounds were not affected by water stress[208]. However, significant differences were found with values higher than 50% of MUFA and 30% of PUFA depending upon the pistachio cultivar used.

The fact that the almond is rich in MUFA and PUFA gives it an added value since they contribute to preventing health problems of the cardiovascular type with its intake, as reported by Bitok and Sabaté[210]. At the level of fatty acids, it could be hypothesized that, as occurs at the agronomic and physiological level, the DI strategy that would provide the most nutritional benefits would be the RDI with the RDI_{65} treatment.

The data obtained at the physicochemical level was transferred to the sensory level in the ranking test (**Chapter 6**). In this sense, the dilution of sugars in the overirrigated treatment was perceived at a sensory level, since the almonds less sweet on the palate were those of these treatments (Figure 6.3). In terms of crispiness, this variable depended more on the cultivars than on the irrigation treatments because cv. Marta in RDI_{65} had the lower crispiness but Guara in RDI_{65} the highest one.

Another important aspect is that DI strategies not only improve the physical-chemical and sensory quality of the almond, as we have seen in **Chapter 6 and 7**, but also it has an added value to this product in the market, giving it the quality of being a hydrosustainable product[68,169,199.] By the contrast, a negative aspect associated to the DI strategies is the decrease in the final yield. However, in our study we demonstrate that an adequate water stress level did not cause a major yield reduction. Thus, as evidenced in **Chapters 6 and 7**, with the enhancement in the quality, it is possible to improve the marketability of this product and its acceptance by the consumer. Also, in **Chapters 3 and 4** it has been shown that deficit treatments increase the kernel unit weight, although this response was associated to a decrease in the fruit number per tree. In this sense, higher almond sizes would be related to improvements in terms of consumer acceptance, and hence higher market prices could be assumed by the consumers; offsetting the hypothetical yield reductions because of water withholdings; as it was suggested by García-Tejero et al.[199].

In this way, providing the almond obtained under these strategies a sustainability value (since it reduces the consumption of water in its production), could be an advantage in the market compared to the almond obtained through irrigation practices without water restrictions.

5. How to proceed for producing hydrosustainable almonds based on available irrigation water?

Before continuing with the irrigation recommendations, it would be worth deepening in the hydrosustainable concept and the strategies and taking decisions that should be considered. Recently, Corell et al.[215] reported the "HydroSOS" index for olive crop; considering 16 indicators that were grouped in 4 areas: hydraulic indicators; horticultural indicators related to irrigation scheduling, horticultural indicators related to when DI is applied (When); and horticultural indicators related to how DI strategies are applied (How). Within the first area would be included some questions such as the type of irrigation; the number and flow of drips; and the uniformity and frequency of irrigation (questions that have not been considered in this doctoral thesis). Regarding to the horticultural indicators that we have considered in this doctoral thesis; the IWP; the DI strategy and the crop water monitoring are key aspects that have been deeply studied. Thus, if our intention is promoting a proper almond orchard sustainability focusing our efforts in the water resources management, the most proper irrigation strategies should be those able to maximize the IWP, selecting the best DI treatment and with a proper crop-water monitoring when water stress is applied.

Table 8.1 includes the irrigation recommendations based on water availability per season and with the focus of obtaining hydrosustainable almonds. It must be taken into account that the data provided in this Table is conditioned by the local conditions of the framework for the development of this doctoral thesis, the climatic conditions of the area, and the hypothetical irrigation water available at the beginning of irrigation period.

Table 8.1 Irrigation recommendations based on water availability (mm·year^{-1}) per season for almond plantations.

Availability of irrigation water	Phenological stages of almond		
	Stage I *Vegetative*	*Stage II* *Kernel-filling*	*Stage III* *Postharvest*
600-400	100	65	100
400-300	100	50	75
300-200	75	40	50
<200	50	50	50

The water amounts recommended in the table corresponds to the percentage of irrigation applicable to the irrigation requirements (IR) of the crop. For example, with a water availability of 600 mm, 100% IR in stage I, 65% IR in stage II and 100% IR in stage III should be applied.

According to Table 8.1 and with the data found in the framework of this doctoral thesis, regardless of the amount of water available, the maximum possible IR must be provided in Stage I to ensure the appropriate vegetative development and fruit-growth because of the fast cell division that takes place in this period.

The maximum reduction to save water without harming the crop and improving the quality of almond might be carried out in Stage II. In this stage where a photosynthetic limitation exists due to the accumulation of photoassimilates (**Chapter 5**), higher water restrictions would not compromise the final yield. In addition, according to Moldero et al.[216] maintaining this crop under DI strategies would benefit the crop development in the long-term because the trees could better withstand constant DI than always being irrigated to meet maximum demand, which could lead to irreversible damage of plantation, particularly under a drought season, exacerbating the yield for many years.

Finally, the remaining water should be applied in Stage III to ensure that we do not negatively affect flower density and fruit set of the following year[57].

According the first scenario of Table 8.1 we have an availability of water without any type of restrictions for the farmer. In this case, it is recommended to provide to the cultivar 100% IR in stage I and stage III. However, it would be recommended to reduce the IR until 65%, in order to increase the quality of the almond and improve the adaptation of the crop to hypothetical water restrictions in further stages (this irrigation distribution was tested in **Chapters 3 and 5**; providing good yield results and improving the almond quality (**Chapters 6**).

In the second scenario, where the water supply is lower, it is advisable to continue providing 100% IR in stage I, reducing it by half in stage II and providing 75% IR in stage III, allowing a partial crop recovering. This strategy would allow us to keep a proper vegetative development and fruit growth; saving important water amounts during the kernel-filling period and hence, improving some almond quality parameters as it has been discussed in **Chapters 6 and 7**; and avoiding substantial damages during the postharvest, which would be observed in the following season.

In the third scenario, with supplies of 300-200 $mm \cdot year^{-1}$, we have considered a compromised situation for crop development. As it has already been previously discussed, the greatest amount possible should be provided in stage I, applying an irrigation dose of 75% IR. Later, in stage II we can reduce IR to 40% and to recover the crop in stage III with 50% IR. Obviously, this reduction during vegetative stage could promote a depletion of canopy volume and fruit setting; but we would avoid more serious damages because of the additional restrictions applied during the following stages (**Chapter 4**).

Finally, in the last scenario under severe drought conditions (<200 $mm \cdot year^{-1}$), the option of applying SDI strategy is proposed like the one tested in **Chapter 4**. In this sense, the maintenance at 50% IR irrigation throughout the whole season, trying to reduce at 40% during the Stage II. With this strategy we would be assuming significant reductions on yield as a crop adaptation to severe scenarios for the future seasons. In spite of this possible yield reduction, we would obtain improvements of almond marketability because of an increase on healthy and sensorial compounds (**Chapters 6 and 7**).

Chapter 9

Conclusions

1. Conclusions

In accordance with the objectives raised and results found in the framework of this doctoral thesis the conclusions are as follows:

1. A regulated deficit irrigation (RDI) strategy based of water withholdings during the kernel-filling stage around 35% of crop water requirements does not significantly penalize the final yield. This strategy reaches yields close to 3,000 $kg \cdot ha^{-1}$ and allow water saving around 1,400 $m^3 \cdot ha^{-1}$. In addition, this strategy would imply significant increases in the irrigation water productivity, improvements in the almond quality and size, and hence generating an added value on the market as a hydrosustainable product. By contrast, when a sustained deficit irrigation (SDI) strategy is applied, despite not having yield losses, the obtained benefits are not as remarkable as those obtained under RDI strategy.
2. At a physiological level, the study of the photosynthetic capacity in the different phenological stages of the crop allows us to conclude that in the kernel-filling stage there is a down-regulation of the photosynthetic rate due to the accumulation of photoassimilates, a process that does not occur in the vegetative stage. This fact would explain the best almond resilience to water stress during the kernel-filling stage, this being the best moment to apply water withholdings in this crop, through the use of a RDI strategy.
3. Furthermore, with water restrictions around 35% of the crop water requirement only Ψ_{leaf} was affected. Additionally, with the water stress imposed in the DI treatments studied, g_s did not limit A_N. This fact, joint to that A_N was not affected by the accumulation of photoassimilates, indicates that this crop can tolerate water restrictions of 35% of crop water requirements in kernel-filling stage without significant impact on yields.
4. Regarding to the effects of water stress imposed on almond quality parameters, deficit irrigation strategies on overall improved morphological, organoleptic and functional parameters of nuts. Besides, these strategies allowed to increase the sugars, fatty and organic acids, and the relation of some healthy parameters such as the monounsaturated and

polyunsaturated fatty acids. In addition, it was observed a strong dependence in the cultivar response in terms of quality parameters when applying a deficit irrigation strategy. For this reason, it would be advisable to reorganize the almond market in quality terms, providing the real commercial value to the cultivar and not including them in large heterogeneous groups.

As future perspectives, and taking into consideration the current and future scenarios of uncertainty in terms of water resources availability in the south Spain, it would be essential to continue with the almond cultivars characterization under adaptive and water-saving strategies; especially under alternative production systems (i.e., organic farming or conservation agriculture). Moreover, to improve our knowledge regarding to physiological processes involved in almond response to water stress and their relationships in productivity terms. This will enable farmers in water-scarce zones to adopt deficit irrigation strategies not only as a tool for saving water, improving IWP, and sustaining yield but also for producing almond nuts with enhanced nutritive and health characteristics. In this line, more research is needed to define the HydroSOS indexes in almonds that lead to boost the added value when it is subjected to water scarcity conditions, re-valorizing the almond production for a sustainable intensive production.

2. Conclusiones

De acuerdo con los objetivos planteados y los resultados obtenidos en el marco de la presente tesis doctoral las conclusiones son las siguientes:

1. Una estrategia de riego deficitario controlado (RDC) basada en recortes hídricos de un 35% durante el periodo de llenado de grano no penalizaría significativamente la producción final. Esta estrategia alcanza producciones cercanas a los 3.000 $kg \cdot ha^{-1}$ y nos permite un ahorro hídrico en torno a 1.400 $m^3 \cdot ha^{-1}$ respecto al tratamiento control. Además, esta estrategia supondría incrementos significativos en la productividad del agua de riego, mejoras en la calidad de la almendra, y su tamaño y por tanto generar un valor añadido en el mercado como producto hidrosostenible. Por el contrario, cuando se aplica una estrategia de riego sostenido (RDS), a pesar de no tener pérdidas de producción los beneficios obtenidos no son tan notables como los obtenidos bajo RDC.
2. A nivel fisiológico, el estudio de la capacidad fotosintética en los diferentes estados fenológicos del cultivo nos permite concluir que el periodo de llenado de grano hay una "*down-regulation*" de la capacidad fotosintética debido a la acumulación de fotoasimilados, un proceso que no ocurre en el periodo vegetativo. Este hecho explicaría la buena resiliencia del almendro al estrés hídrico durante el periodo de llenado de grano, convirtiéndose en el mejor momento para aplicar recortes hídricos en este cultivo mediante el uso de una estrategia RDC.
3. Además, con restricciones hídricas del 35% de las necesidades de riego solamente se ve afectado el Ψ_{hoja}. Adicionalmente, con el estrés hídrico impuesto en los RD estudiados, g_s no limitó A_N. Este hecho, unido a que A_N no se vio afectada por la acumulación de fotoasimilados, indica que este cultivo toleraría restricciones del 35% de las necesidades de riego en el periodo de llenado de grano sin tener un impacto significativo en la producción.

4. Respecto a los efectos del estrés hídrico impuesto sobre los parámetros de calidad de la almendra, las estrategias de riego deficitario, en rasgos generales, mejoran los parámetros morfológicos, organolépticos y funcionales de la almendra. Además, estas estrategias permitieron aumentar los azúcares, ácidos grasos y orgánicos, y la relación de algunos parámetros saludables como los ácidos grasos monoinsaturados y poliinsaturados. Asimismo, se observó una fuerte respuesta varietal en términos de los parámetros de calidad cuando se aplicaba una estrategia de riego deficitario. Por esta razón, sería aconsejable que el mercado de la almendra en términos de calidad se reorganizara, aportándole a cada una de las variedades el valor comercial que se merecen y no incluirlas a todas en un grupo heterogéneo.

Como perspectivas de futuro, y teniendo en cuenta los escenarios actuales y futuros de incertidumbre en cuanto a la disponibilidad de recursos hídricos en el sur de España, sería fundamental continuar con la caracterización de las variedades de almendro bajo estrategias adaptativas y de ahorro de agua; especialmente bajo sistemas de producción alternativos (es decir, agricultura ecológica o agricultura de conservación). Además, mejorar nuestro conocimiento sobre los procesos fisiológicos implicados en la respuesta del almendro al estrés hídrico y sus relaciones en términos productivos. Esto permitirá a los agricultores en zonas con escasez de agua adoptar estrategias de riego deficitario no solo como una herramienta para ahorrar agua, mejorar el IWP y mantener el rendimiento, sino también para producir almendras con características nutritivas y saludables mejoradas. En esta línea, se necesita más investigación para definir los índices HydroSOS en almendra que conduzcan a potenciar el valor añadido cuando se somete a condiciones de escasez de agua, revalorizando la producción de almendra para una producción intensiva sostenible.

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