

# Department of Plant Production Doctoral program in Agricultural Resources and Technologies

#### Ph.D. Thesis

## Effect of continued and regulated deficit irrigation on the productivity of four vegetable crops in open-field conditions in the Mediterranean area

Presented by

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Supervised by

Dr. Bernardo Pascual España

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Valencia, September 2019



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This thesis submitted in fulfillment of the requirements for the degree of Doctor of Philosophy in

**Agricultural Resources and Technologies** 

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#### **Abstract**

Water scarcity is becoming a critical problem in arid and semi-arid areas of the world, where part of the production of the main horticultural crops is located, as is the case of the Mediterranean area. Drought is one of the main limiting factors in agriculture and it is seriously affecting the production of horticultural crops. The improvement of water productivity in agriculture in general, and in horticulture in particular, can be achieved through the use of certain strategies. Deficit irrigation consists of the supply of water below the irrigation water requirements (IWR), so that there is a reduction in evapotranspiration. It can be done continuously (CDI) or regulated (RDI). With deficit irrigation, the irrigation water use efficiency can be improved, maintaining yield, and it could even lead to an improvement in the quality of the harvest. This study, carried out at the Cajamar in Paiporta Experimental Center (Valencia, Spain), analyzes the effect of deficit irrigation on four of the main cultivated horticultural crops, open field cultivated in the Mediterranean area: two of autumnal-winter crops (cauliflower and onion) and two spring-summer crops (pepper and watermelon). In the evaluation, the following parameters have been analyzed: plant growth and water status, yield, irrigation water use efficiency, quality of production and crop profitability. In the first season the CDI was tested, which allowed to establish the different growth stages for each crop, which were used in the following season for the RDI.

In the four crops, the control plants (100% IWR) have shown an adequate water status, in terms of both relative water content and membrane stability index, while those subjected to a severe CDI, have shown the lowest values of both indexes. The negative effect of deficit irrigation on yield has been less important in autumn-winter crops than in spring-summer crops, especially in cauliflower. The CDI at 50% IWR has drastically reduced the marketable yield and, consequently, the gross revenue, although it has supposed an improvement in the irrigation water use efficiency for the autumn-winter crops. From the individual analysis of the crops, it can be stated

that cauliflower yield obtained with CDI at 75% IWR or RDI at 50% IWR during the iuvenile phase, has remained at levels similar to the control, improving the irrigation water use efficiency. In relation to onion, in case of severe water restriction, it would be advisable to apply CDI with 75% IWR or RDI at 50% IWR during bulb ripening, since these strategies have slightly decreased yield, improving the irrigation water use efficiency. In less restrictive conditions, RDI at 75% IWR during the bulb maturation has led to a satisfactory yield, with an increase in the irrigation water use efficiency. In Italian sweet pepper, the application of RDI to 75% IWR during the harvesting has resulted in a considerable reduction of the yield, and therefore, of the gross income, although with important water savings and increasing the fruit soluble solids and phenolic compounds content. By shortening the cultivation cycle until the beginning of September, when most of the marketable yield has already been harvested, significant water savings would be achieved, and the land could be used in other crops. CDI at 75% IWR and 50% IWR, or RDI at 50% IWR at harvesting have resulted in a high incidence of fruit affected by blossom-end rot. In watermelon the RDI application can be recommended, both 75% and 50% IWR, during the fruit ripening, since it has led to acceptable marketable yields. In general terms, it can be affirmed that the application of CDI and RDI in the four crops has not significantly affected the product quality, in terms of the analyzed parameters.

#### Resumen

La escasez de agua se está convirtiendo en un problema crítico en zonas áridas y semiáridas del mundo, donde se localiza parte de la producción de los principales cultivos hortícolas, como es el caso del área mediterránea. La sequía es uno de los principales factores limitantes en la agricultura y está afectando gravemente a la producción de cultivos hortícolas. La mejora de la productividad del agua en la agricultura en general, y en la horticultura en particular, puede lograrse mediante la utilización de determinadas estrategias. El riego deficitario consiste en el aporte de agua por debajo de las necesidades de riego (NR) de los cultivos, de manera que se produce una reducción de la evapotranspiración. Puede realizarse de manera continua o sostenida (RDS) o controlada (RDC). Con el riego deficitario se puede mejorar la eficiencia del uso del agua de riego, manteniendo el rendimiento, e incluso en ocasiones, podría conducir a una mejora de la calidad de la cosecha. En este estudio, realizado en el Centro Experimental Cajamar de Paiporta (Valencia, España) se evalúa el efecto del riego deficitario en cuatro de los principales cultivos hortícolas cultivados al aire libre, en el área mediterránea: dos de cultivo otoñalinvernal (coliflor y cebolla) y dos de cultivo primaveral-estival (pimiento y sandía). En la evaluación se han analizado los siguientes parámetros: crecimiento y estado hídrico de las plantas, rendimiento, eficiencia del uso del agua de riego, calidad de la producción y rentabilidad de los cultivos. En la primera campaña se ensayó el RDS, lo que permitió establecer las diferentes etapas de crecimiento de cada cultivo, que se utilizaron en las siguientes campañas en el RDC.

En los cuatro cultivos, las plantas control (100% NR) han mostrado un adecuado estado hídrico, tanto en el contenido relativo de agua como en el índice de estabilidad de la membrana, mientras que las sometidas a un RDS severo, han mostrado los menores valores de ambos índices. El efecto negativo del riego deficitario sobre el rendimiento ha resultado menos importante en los cultivos de otoño-invierno que en los cultivos de primavera-verano, especialmente en la coliflor. El RDS del 50% NR

ha reducido drásticamente el rendimiento comercial y, consecuentemente, los ingresos brutos, aunque haya supuesto una mejora en la eficiencia del uso del agua de riego para los cultivos de otoño-invierno. Del análisis individual de los cultivos se deduce que el rendimiento en pellas de coliflor obtenidas con RDS al 75% NR o RDC al 50% NR durante la fase juvenil, se ha mantenido en niveles similares al control, mejorando la eficiencia del uso del agua de riego. En cebolla, en caso de restricción hídrica severa, sería aconsejable aplicar RDS con el 75% NR o RDC al 50% NR durante la maduración del bulbo, ya que estas estrategias han disminuido ligeramente el rendimiento, mejorando la eficiencia del uso del agua de riego. En condiciones menos restrictivas, RDC al 75% NR durante la maduración del bulbo ha dado lugar a un rendimiento satisfactorio, con un aumento de la eficiencia del uso del agua de riego. En pimiento dulce italiano, la aplicación de RDC al 75% NR durante la recolección ha dado lugar a una reducción considerable del rendimiento, y por tanto, de los ingresos brutos, aunque con importantes ahorros de agua y con un incremento en el contenido de sólidos solubles y de compuestos fenólicos de los frutos. Acortando el ciclo de cultivo hasta principios de septiembre, cuando ya se ha cosechado la mayor parte del rendimiento comercial, se conseguiría un importante ahorro de agua y permitiría utilizar la parcela en otros cultivos. El RDS al 75% y al 50% NR, o RDC al 50% NR durante la cosecha han dado lugar a una alta incidencia de frutos afectados por blossom-end rot. En sandía puede recomendarse la aplicación de RDC, tanto al 75% como al 50% NR durante la maduración del fruto, ya que ha conducido a rendimientos comerciales aceptables. De manera general se puede afirmar que la aplicación de RDS y de RDC en los cuatro cultivos, no ha afectado de manera importante a la calidad de la producción, en cuanto a los parámetros analizados.

#### Resum

L'escassesa d'aigua s'està convertint en un problema crític en zones àrides i semiàrides del món, on es localitza part de la producció dels principals cultius hortícoles, com és el cas de l'àrea mediterrània. La sequera és un dels principals factors limitants en l'agricultura i està afectant greument a la producció de cultius hortícoles. La millora de la productivitat de l'aigua en l'agricultura en general, i en l'horticultura en particular, es pot aconseguir mitjançant la utilització de determinades estratègies. El reg deficitari consisteix en l'aportació d'aigua per sota de les necessitats de reg (NR) dels cultius, de manera que es produeix una reducció de l'evapotranspiració. Es pot fer de manera contínua o sostinguda (RDS) o controlada (RDC). Amb el reg deficitari es pot millorar l'eficiència de l'ús de l'aigua de reg, mantenint el rendiment, i fins i tot de vegades, podria conduir a una millora de la qualitat de la collita. En aquest estudi, realitzat al Centre Experimental Cajamar de Paiporta (València, Espanya) s'avalua l'efecte del reg deficitari en quatre dels principals cultius hortícoles conreats a l'aire lliure, a l'àrea mediterrània: dos de cultiu de tardor-hivern (coliflor i ceba) i dues de cultiu primaveral-estival (pimentó i meló d'Alger). En l'avaluació s'han analitzat els següents paràmetres: creixement i estat hídric de les plantes, rendiment, eficiència de l'ús de l'aigua de reg, qualitat de la producció i rendibilitat dels cultius. A la primera campanya es va assajar el RDS, el que va permetre establir les diferents etapes de creixement de cada cultiu, que es van utilitzar en les següents campanyes en el RDC.

En els quatre cultius, les plantes control (100% NR) han mostrat un adequat estat hídric, tant en el contingut relatiu d'aigua com en l'índex d'estabilitat de la membrana, mentre que les sotmeses a un RDS sever, han mostrat els menors valors d'ambdós índexs. L'efecte negatiu del reg deficitari sobre el rendiment ha resultat menys important en els cultius de tardor-hivern que en els cultius de primavera-estiu, especialment en la coliflor. El RDS del 50% NR ha reduït dràsticament el rendiment comercial i, conseqüentment, els ingressos bruts, encara que hagi suposat una

millora en l'eficiència de l'ús de l'aigua de reg per als cultius de tardor-hivern. De l'anàlisi individual dels cultius es dedueix que el rendiment de coliflors obtingudes amb RDS al 75% NR o RDC al 50% NR durant la fase juvenil, s'ha mantingut en nivells similars al control, millorant l'eficiència de l'ús de l'aigua de reg. En ceba, en cas de restricció hídrica severa, seria aconsellable aplicar RDS amb el 75% NR o RDC al 50% NR durant la maduració del bulb, ja que aquestes estratègies han disminuït lleugerament el rendiment, millorant l'eficiència de l'ús de l'aigua de reg. En condicions menys restrictives, RDC al 75% NR durant la maduració del bulb ha donat lloc a un rendiment satisfactori, amb un augment de l'eficiència de l'ús de l'aigua de reg. En pimentó dolc italià, l'aplicació de RDC al 75% NR durant la recol·lecció ha donat lloc a una reducció considerable del rendiment, i per tant, dels ingressos bruts, encara que amb importants estalvis d'aigua i amb un increment en el contingut de sòlids solubles i de compostos fenòlics dels fruits. Retallant el cicle de cultiu fins a principis de setembre, quan ja s'ha collit la major part del rendiment comercial, s'aconseguiria un important estalvi d'aigua i permetria utilitzar la parcel·la en altres cultius. El RDS al 75% i al 50% NR, o RDC al 50% NR durant la collita han donat lloc a una alta incidència de fruits afectats per blossom-end rot. En meló d'Alger es pot recomanar l'aplicació de RDC, tant al 75% com al 50% NR durant la maduració del fruit, ja que ha conduït a rendiments comercials acceptables. De manera general es pot afirmar que l'aplicació de RDS i de RDC en els quatre cultius, no ha afectat de manera important a la qualitat de la producció, pel que fa als paràmetres analitzats.

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### **Abbreviations**

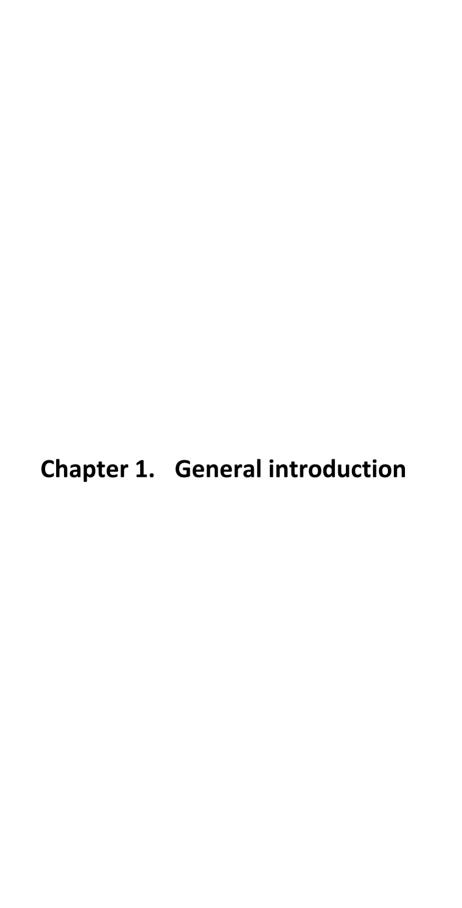
BER	Blossom end rot	IRWR	Internal renewable water resources
C*	Chroma	IS	Irrigation strategy
CDI	Continued deficit irrigation	IWA	Irrigation water applied
CI	Colour index	IWR	Irrigation water requirement
cv.	Cultivar	IWUE	Irrigation water use efficiency
DI	Deficit irrigation	Kc	Crop coefficient
DM	Dry matter	$\mathbf{K}_{\mathbf{p}}$	Pan coefficient
Df	Degree of freedom	$\mathbf{K}_{\mathbf{y}}$	Yield response factor
EC	Electrical conductivity	LSD	Least significant difference
ET	Evapotranspiration	MI	Maturity index
Eta	Actual crop evapotranspiration	MSI	Membrane stability index
ETm	Maximum crop evapotranspiration	MY	Marketable yield
ЕТо	Reference evapotranspiration	Pe	Effective precipitation
ЕТс	Crop evapotranspiration	RDI	Regulated deficit irrigation
Ef	Irrigation efficiency	RWC	Relative water content
$E_{pan}$	Evaporation from a class A pan	SSC	Soluble solids content
ERWR	External renewable water resources	TRWR	Total renewable water resources
FC	Field capacity	VSWC	Volumetric soil water content
FW	Fresh weight	WUE	Water use efficiency
GS	Growing season	Ya	Actual marketable yield
Н°	Hue angle	Ym	Maximum marketable yield
HI	Harvest index		

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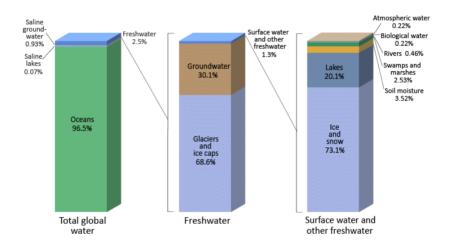
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#### 1.1. Global water availability

Water is at the core of sustainable development and considered as essential resource of food production around the world. Only 2.5 % of the water stored on earth is freshwater, the rest being oceans and other saline water. Glaciers and ice caps cover approximately 10% of the world land which concentrated in Greenland and Antarctica contain approximately 68.6 % of the world freshwater. Groundwater is the most abundant source of freshwater (30.1% of the freshwater), followed by ice, snow, lakes, rivers and reservoirs (together 1.3% of the freshwater; Figure 1). Unfortunately, most of the earth water resources are not readily accessible for human use, which illustrates that fresh water is a valuable resource (Aquastat, 2018).



**Figure 1.** Global water distribution and fresh water resources (Shiklomanov, 1993).

The worldwide average annual precipitation is approximately 814 mm, of which 56% is evapotranspired by forests and other natural landscapes, 5% is used by rainfed agriculture and 39% is the, theoretically, worldwide available annual renewable freshwater (Aquastat, 2018). There is a great variability of precipitation amount and distribution between the continents and the regions.

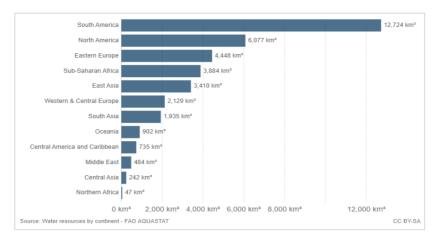
The total renewable water resources (TRWR) consists of internal renewable water resources (IRWR; refers to internal river flows and groundwater from rainfall) and external renewable water resources (ERWR; refers to water resources that enter from upstream countries through rivers or aquifers).

**Table 1.** Internal renewable water resources (IRWR), external renewable water resources (ERWR), total renewable water resources (TRWR) and total renewable water resources per capita in the world continents (Aquastat, 2018).

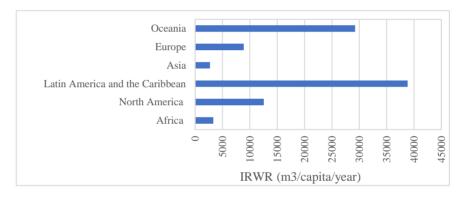
Continent	IRWR	ERWR	TRWR	TRWR
	(km <sup>3</sup> year <sup>-1</sup> )	(km <sup>3</sup> year <sup>-1</sup> )	(km <sup>3</sup> year <sup>-1</sup> )	(m³/capita/year)
Africa	3.931	1.700	5.631	606966
North America	6.077	356	6.433	93921
Latin America and the Caribbean	13.459	5.283	18.742	1185536
Asia	11.865	3.378	15.242	476450
Europe	6.576	1.058	7.788	901196
Oceania	902	0	902	239118
Total world	42.810	11775	54738	3503187

The worldwide TRWR is about 54.738 (km³ year⁻¹), The Latin America and Caribbean has the largest TRWR and ERWR (km³ year⁻¹), while the lowest is in Oceania. Asia has a larger TRWR (km³ year⁻¹) than Africa and Europe, with a lower TRWR per capita (m³/capita/year), due to the higher population in Asian countries, and the vice versa for Oceania (Table 1).

The world internal freshwater resources are estimated to be in the order of 42.810 km³ year⁻¹ (Table 1), Latin America and the Caribbean has the largest share of the world's total freshwater resources with 31%, followed by Asia with 28%, Europe, North America and Africa. South America countries present the largest share of IRWR followed by North America and Eastern Europe, while Middle east, North Africa and Central Asia have the lowest share of the world IRWR (Figure 2). In terms of resources per inhabitant per continent, Latin America and the Caribbean has 38.825 m³ year⁻¹, followed by Oceania 29.225 m³ year⁻¹, North America 12.537 m³ year⁻¹, Europe 8.875 m³ year⁻¹, Africa 3.319 m³ year⁻¹ and Asia 2.697 m³ year⁻¹ (Figure 3).



**Figure 2.** Internal renewable water resources by region (km<sup>3</sup> year<sup>-1</sup>; Ritchie and Roser, 2018, based on data of Aquastat, 2018).



**Figure 3.** Internal renewable water resources (IRWR) per capita (m³ year<sup>-1</sup>; based on data of Aquastat, 2018).

Global water withdrawal increased during the last century from less than 600 km<sup>3</sup> year<sup>-1</sup> in 1900 to approximately 4000 km<sup>3</sup> year<sup>-1</sup> in 2018 (Aquastat, 2018). The proportion of total renewable water resources withdrawn is the total volume of groundwater and surface water withdrawn from their sources for human activity use (agricultural, municipal and industrial sectors).

The countries known to be under water stress or scarcity per capita are those which are excessively using their renewable water resources as North Africa, Middle-East and central Asia including Afghanistan and Pakistan. Spain and South Asia are also under excessive withdrawal of renewable water resources (Figure 4).

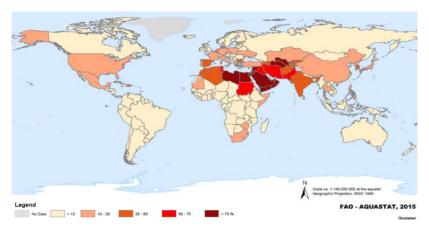
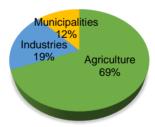


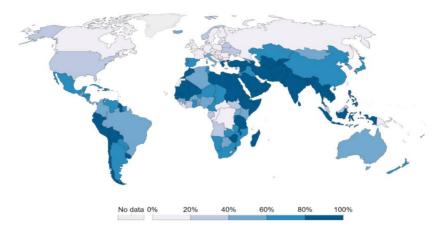
Figure 4. Renewable water resources withdrawn (%; Aquastat, 2018).

The major sectors that withdraw water are irrigated agriculture, industry and urban and municipal use. Worldwide, agriculture consumes approximately 2769 km<sup>3</sup> year<sup>-1</sup> of the available water, industry use approximately 786 km<sup>3</sup> year<sup>-1</sup> and urban and municipal use represents approximately 464 km<sup>3</sup> year<sup>-1</sup> of the total water withdrawal (4000 km<sup>3</sup> year<sup>-1</sup>; Figure 5).



**Figure 5.** Distribution of renewable water resources (%) per sector in the world (based on data of Aquastat, 2018).

There are some countries located in South and Central of Asia, Africa and Latin America where agriculture use represents more than 80% of water withdrawals. In African Mediterranean countries, agriculture consumes approximately 84% of total water withdrawal, while it consumes approximately 57% in European Mediterranean countries. In Spain, agriculture uses about 68%, industry 18% and municipal use is 14% of the available freshwater (Figure 6).



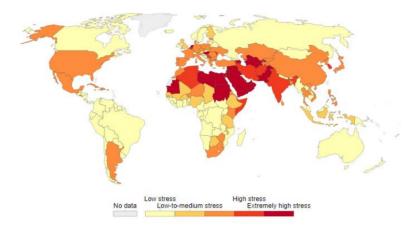
**Figure 6.** Agricultural water withdrawals (%) as a share of total water withdrawals (Ritchie and Roser, 2018, based on data of Aquastat, 2018).

## 1.2. Water scarcity and future challenges faces irrigated agriculture

Irrigation water is a crucial resource for sustainable agricultural development worldwide. In the arid and semiarid areas, including Mediterranean region, water is becoming increasingly scarce, increasing competition for water among agricultural, industrial and urban consumers (Chai et al., 2016). According to WHO (2017), approximately 2.1 billion people lack to safe drinking water. Nowadays, many river basins do not have enough water to meet all their demands, rising competition and conflicts among countries for scarce water resources.

Several regions across the Middle East, North Africa and South Asia have extremely high levels of water stress. Countries such as Saudi Arabia, Egypt, United Arab Emirates, Syria, Pakistan and Libya have water withdrawal rates that well exceed 100%, this means that these countries are either over extracting from existing aquifer sources or producing a large share of water from desalinization. Most of the African Mediterranean countries are under extremely to high water stress, while those in the European side are under medium water stress. Countries across South Asia are under high water stress; medium-to-high across East Asia, as well as the United States and

much of the Southern and Eastern Europe. Water stress is typically low or low-to-medium in Northern Europe, Canada, much of Latin America, Sub-Saharan Africa and Oceania (Figure 7; Ritchie and Roser, 2018).



**Figure 7.** Freshwater withdrawals as a share of internal renewable water resources (Ritchie and Roser, 2018, based on data of Aquastat, 2018).

Globally, irrigation and food production are closely linked, approximately 40% of global agricultural production is from irrigated croplands (Winter et al., 2017). During the last decades, global water withdrawal has increased by 1.7 times the population growth (Figure 8; Aquastat, 2018).

The scarcity of water can be attributed to different causes, from drought and natural aridity, to desertification and water shortage caused by the human being (Pereira et al., 2002). The irrigated agriculture area worldwide was approximately 40 million ha in 1990. It increased more than eightfold over the last century to approximately 325 million ha (Aquastat, 2018). Globally, the total water uses in crop production (evapotranspiration) was estimated in 7130 km³ in 2000, and is likely to rise to between 12,000 and 13,500 km³ by 2050. Forecast to 2050 estimates an increase of cropped area of 29%, with rainfed areas increasing from 549.812 million in 1998 to 698.743 million ha (27%; Bruinsma, 2009; Turral et al., 2011). Increasing the irrigated area in the world leads to an increase in the water requirements and in

the water withdrawal, and consequently, it increases the pressure on freshwater resources.

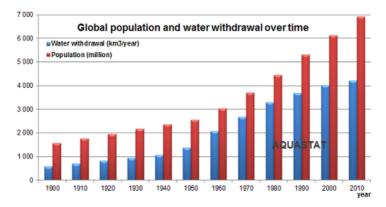


Figure 8. Global water withdrawal and world population over time (Aquastat, 2018).

The world's population is growing about 80 million people per year and is expected to reach 8.5 billion by 2030, 9.7 billion in 2050 and 11.2 billion in 2100 (UN DESA, 2015). Population growth, urbanization and industrialization, which increases in production and consumption, have generated increasing demands for freshwater resources. Feeding 9 billion people by 2050 will require a 70-90% increase in agricultural production and a 15% increase in water withdrawals (De Fraiture et al., 2007; Turral et al., 2011; WWAP, 2016). Besides this increasing demand, the resource is already scarce in many parts of the world. Estimates indicate that 40% of the world population live in water scarce areas. By 2025, about 1.8 billion people will be living in regions or countries with absolute water scarcity (UN-Water, 2007).

Climate change poses serious threats to global food security due to changes in water supply and demand (Kang et al., 2017). Forecasts indicate that climate change will affect the agriculture sector, increasing global temperature and potential evapotranspiration, reduce precipitation and snowmelt, alter precipitation distribution and pattern, sea-level and CO<sub>2</sub> concentration. Climate change have a negative effect on water resources, irrigated and dryland agriculture. Recent projections result in an increase of temperature about 1.5 to 4°C in 2050.

Precipitation patterns including rainfall and snow likely to decrease about 10 to 20%, depending on the season, in 2050. Foreseen indicates rising the potential evapotranspiration could double in the next 50 years, consequently, increasing water demand. In arid, semi-arid and sub-humid climates, the incidence of floods and droughts increases, also contributing to desertification (Turral et al., 2011; Wheeler and Braun, 2013; IPCC, 2014; Guiot and Cramer, 2016). The Mediterranean region is considered one of the arid and semiarid regions where irrigated agriculture expected to be strongly vulnerable to climate change, for this region important reductions in freshwater supplies from surface and groundwater resources are suggested, increasing the incidence of extreme drought events (Jiménez Cisneros et al., 2014; Kahil et al., 2015; Guiot and Cramer, 2016). At the same time, it can be expected an increase of the irrigation water withdrawal in the region (Daccache et al., 2014; Guiot and Cramer, 2016). Surging challenges associated with climate change will be difficult to manage in a context of rising world food demand and increasing competition between sectors of water uses (Elliott et al., 2014).

The existing drought risks are expected to intensify, particularly in regions where water scarcity is already a concern, as in the Mediterranean region (Iglesias and Garrote, 2015). The Mediterranean climate is characterized by mild winter temperatures and long, hot and dry summers, with precipitation subject to high interannual and seasonal variability; therefore, in this region irrigation is essential for crop production (Daccache et al., 2014).

To mitigate the foreseen future global changes and ensure food security, researchers are trying to increase water productivity through different approaches (Molden et al., 2010; Kang et al., 2017; Malek and Verburg, 2017; Galindo et al., 2018). Increasing water scarcity and irrigation costs are leading to develop watersaving irrigation strategies to improve productivity of water use for crop production (Jones, 2004; Fereres and Soriano, 2007; Ghazouani et al., 2019).

#### 1.3. Irrigation water requirements

Crop water requirement is defined as the amount of water required to compensate the evapotranspiration loss from the cropped field. For a given crop, water requirement principally depends on crop development and climate conditions. Irrigation water requirement (IWR) represents the difference between the crop water requirement and the effective precipitation (Pe). The IWR also includes additional water for leaching of salts and to compensate for the lack of uniformity of water application (Allen et al., 1998; Savva and Frenken, 2002; Zotarelli et al., 2018).

The evapotranspiration (ET) is the combination of two separate processes evaporation (E) and transpiration (T). Evaporation is the process whereby liquid water is converted to vapour and removed from the evaporating surface. Transpiration consists of the vaporization of liquid water that is contained in plant tissues and the vapour removal to the atmosphere, predominately through stomata. The main factors affecting evapotranspiration are climatic parameters, crop characteristics, crop management practices and environmental aspects. When the crop is small the evaporation is dominant, decreasing with the plant growth; once the crop is fully developed, completely covering the ground, transpiration becomes the dominant process (Allen et al., 1998; Savva and Frenken, 2002).

The term reference crop evapotranspiration or reference evapotranspiration (ETo) is the evapotranspiration from a reference surface not short of water. The reference surface is a hypothetical grass reference crop with an assumed crop height of 0.12 m, a fixed surface resistance of 70 s m<sup>-1</sup> and an albedo of 0.23. It resembles an extensive surface of green, well-watered grass of uniform height, actively growing and completely shading the ground. The fixed surface resistance of 70 s m<sup>-1</sup> implies a moderately dry soil surface resulting from about a weekly irrigation frequency (Allen et al., 1998). The concept of ETo was introduced to study the evaporative demand of the atmosphere independently of crop type, crop development stage and management practices.

The crop evapotranspiration (ETc) refers to the amount of water that is lost through evapotranspiration (ET) of a crop being disease-free, well-fertilized crops, grown in large fields under optimum soil water conditions and achieving full production under the given climatic conditions (Allen et al., 1998). ETc can be calculated from climatic data by directly integrating the effect of crop characteristics into the ETo. ETc can be determined as following (Allen et al., 1998):

$$ETc = ETo \times Kc$$

Where, ETc is the crop evapotranspiration (mm day<sup>-1</sup>), ETo is the reference crop evapotranspiration (mm day<sup>-1</sup>) and Kc is the single crop coefficient.

ETo can be directly measured with lysimetric stations, computed from meteorological data, or estimated from pan evaporation. Currently, the FAO Penman-Monteith is the recommended method to compute ETo from meteorological data.

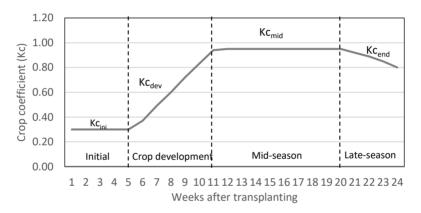
The pan evaporation method is still widely used, because it is very practical and simple. Evaporation from an open water surface provides an index of the combined effect of radiation, temperature, humidity and wind on evapotranspiration. The pans have been used successfully to estimate ETo by observing the water loss from the pan and using empirical coefficients to relate pan evaporation to ETo. Therefore, ETo is calculated according to the following equation (Allen et al., 1998):

$$ETo = E_{pan} \times K_{p}$$

where, ETo is the reference crop evapotranspiration (mm day<sup>-1</sup>),  $E_{pan}$  is the pan evaporation (mm day<sup>-1</sup>) and  $K_p$  is the pan coefficient.

Crop coefficient (Kc) integrates the effects of characteristics that distinguish field crops from grass, consequently, different crops have different Kc coefficients. The Kc varies during crop development due to the change in the crop characteristics over the growing season as the ground cover, crop height and leaf area. Crop growth period can be divided into four different growth stages (Allen et al., 1998): initial stage, crop development stage, mid-season stage and the late season stage (Figure 9):

- Initial (Kc<sub>ini</sub>); refers to early growth stage of the plant; from the planting date to the time when approximately 10% of the ground surface is covered by green vegetation.
- Crop development; runs from 10% of ground cover to effective full cover.
- Mid-season (Kc<sub>mid</sub>); runs from effective full ground cover to the start of maturity, as the beginning of the ageing (yellowing or senescence of leaves, leaf drop).
- Late season (Kc<sub>end</sub>); runs from the start of maturity to harvest or full senescence. The calculation of Kc and ETo is presumed to end when the crop is harvested, dries out naturally, reaches full senescence, or experiences leaf drop.



**Figure 9.** Crop coefficient of sweet pepper used in these experiments during the four crop growth stages.

#### 1.4. Irrigation scheduling by monitoring soil moisture

The purpose of irrigation scheduling is to determine the exact amount of water to apply and the exact timing for application (Dukes et al., 2010; Cahn and Johnson, 2017). Irrigation management directly affects the yield and quality vegetable crops production (Dukes et al., 2010; Garcia-Caparros et al., 2017; Cahn and Johnson, 2017).

Soil is a complex and heterogeneous system, composed of mixture of various solid, liquid and gaseous materials, which they form the three phases that integrate it: solid, liquid and gaseous. Under normal conditions, a part of the porous space

presented by the soils is occupied by water with dissolved salts, the so-called soil solution, and the rest of the porous space is occupied by a mixture of gases, called soil air. Soil water content refers to the amount of water that it is found in each position of a soil at a given moment and is generally expressed as a percentage, of mass or volume (Pascual-Seva, 2011). Soils water-holding are different depending on their texture and structure (Figure 10). The field capacity (FC) was defined as the amount of water left in the soil after the excess water has drained and the speed of the movement in depth has decreased significantly. The upper limit of water holding capacity is the FC, while the lower limit is called the permanent wilting point (PWP). The total amount of water available between FC and PWP is referred to the available water (AW).

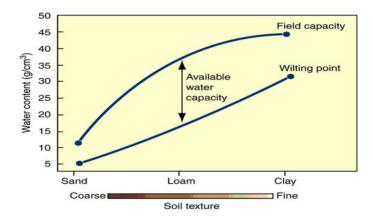


Figure 10. Relationship between soil water content and soil texture class.

The soil moisture can be measured as soil matric potential (kPa) or as volumetric soil water content (VSWC, m³ m⁻³). The VSWC can be made directly by gravimetry, with drying to constant weight in an oven at 105 °C, or by indirect methods (autmoatically) as neutron moisture probe, heat dissipation sensors (Pascual-Seva, 2011; Thompson and Voogt, 2016), and the latest generation of soil moisture measuring instruments is based on measuring the dielectric constant of the soil, which depends fundamentally on its water content (Gallardo and Thompson, 2018). A dielectric is an insulating material (or very little conductor below a certain electrical voltage, called breaking strain) that when placed between two charged surfaces (capacitor) allows a displacement of the load, but not a net flow of the load electrical

(Villar and Ferrer, 2005). There are three general types of di-electric sensor, TDR (Time Domain Refractometry), TDT (Time Domain Transmissometry), and capacitance, or FDR (Frequency Domain Refractometry).

The TDR system determines the speed of propagation of an electromagnetic wave through the closed circuit by two or three parallel steel rods. The TDR allows to measure a considerable volume of soil, does not need calibration and allows continuous measurements of humidity. TDT sensors are an adaptation of TDR sensors that are cheaper, electronically simpler and suitable for irrigation management in commercial farming. FDR or capacitance probes have been used commercially for the irrigation management of many herbaceous and woody crops, and also in research applications. The FDR system measures changes in soil capacitance in response to an electric field, which depend on the moisture content of the soil. It consists of several probes connected by cable to a data logger where readings are stored, there is currently a wireless version that transmits the signal by radio waves. The advantage of these equipment is to allow the characterization of the water dynamics in the soil and the extraction of water by the crop at different depths, making it possible to control the drainage in depth.

In these studies, the VSWC was continuously monitored using ECH<sub>2</sub>O EC-5 capacitance sensors (Figure 11a) connected to an Em50 data logger (Figure 11b), using the ECH<sub>2</sub>O Utility software (Figure 11c; Decagon Devices Inc., Pullman WA., USA). The EC-5 sensor determines VSWC in cylindric as shown in Figure 11d. The discharge data were measured and stored at 15 min intervals. Factory sensor calibration was included for mineral soils, provided  $\pm 3\%$  accuracy, and therefore was used directly in the experiments. In this research, to compare different irrigation strategies and depths, it was decided to present the VSWC variations throughout the growing season, as the ratio of the VSWC at each moment and FC.

Soil moisture sensors can contribute to crop irrigation sechduling by ensuring that crops have adequate water status and by limiting drainage which maximize water use efficiency in irrigated agriculture (Thompson and Voogt, 2016; Blanco et al., 2018).

#### Chapter 1. General introduction

These sensors can be used alone as "stand-alone" methods, or they can be used in combination with methods for estimating crop water requirements, or they can be used to complement irrigation management (Thompson et al., 2007; Thompson and Voogt, 2016; Dukes et al., 2010). Most suitable irrigation scheduling methods for vegetable production are estimating crop water requirements that takes into account plant stage of growth in combination with measuring soil water status (Thompson et al., 2007; Dukes et al., 2010; Thompson and Voogt, 2016).

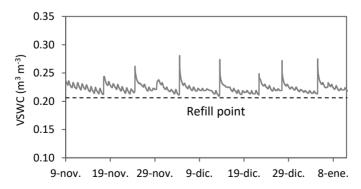


**Figure 11.** Capacitance probes unit used in these experiments; (a) EC-5 capacitance sensors; (b) Em50 data logger; (c) the ECH<sub>2</sub>O Utility software; (d) EC-5 sensor measurement dimensions.

Soil moisture sensors allow a precise adjustment of irrigation management such as the application of a controlled stress to improve quality, an exact control of the drainage for the management of salinity and the identification of a problem with the irrigation system (Gallardo and Thompson, 2018). In case of using DI strategies to save water, monitoring the soil or plant water status as an irrigation scheduling approach is even more critical for minimizing the risk of yield reduction, particularly

under the uncertainties in determining the exact water requirement (Fereres and Soriano, 2007). Pascual-Seva et al. (2015) stated that irrigation scheduling based on monitoring of the volumetric soil water content by capacitance probes sensores improved IWUE and considerably less deep percolation occurred and important water savings were achieved and could be an alternative to traditional irrigation management in tigernut crop.

Installing sensors correctly in situ is an important to provide effective measurement. Sensors should be placed in representative zones of the crop and the soil. One sensor should be placed in the root zone and additional sensors can be placed below the roots to control drainage (Thompson and Voogt, 2016). Interpretation of the soil moisture readings correctly is very important to assure aprppriate irrigation management and avoid over irrigation. Using volumetric soil water content for irrigation scheduling, have to determine in situ lower and upper limits values, which depend on a combination of crop and soil (Thompson and Voogt, 2016). These limits specify both the maximum permitted amount of soil moisture (Full point) and the minimum permitted amount of soil moisture (Refill point; Figure 12). Full point is an approximation of field capacity, while refill point is the amount of soil moisture close to but clearly in excess of that where the crop begins to suffer water stress. The Refill point identifies when to start irrigation, and the full point identifies when to stop (Thompson and Gallardo, 2005).



**Figure 12.** Example of maintaining soil water content between refill, monitored in onion crop under this experiment.

# 1.5. Deficit irrigation as water-saving strategy

Irrigation is the major agricultural use of water, and may be affected by the reduction of water supply. Therefore, innovation on irrigation technologies and management are necessary for achieving more-effective and rational water use (Kirda, 2002; Capra and Consoli, 2008; Levidow et al., 2014).

Improving irrigation water use efficiency in agriculture plays an important role in ensuring food and water security. Hence, sustainable practices and water-saving strategies to increase crop water productivity are gaining importance, especially in arid and semi-arid regions.

Deficit irrigation (DI) is a sustainable practice, which was proposed many years ago to improve water productivity, stabilizing yield and improving the product quality (Kirda, 2002; Costa et al., 2007; Fereres and Soriano, 2007). DI is an application of irrigation less than the optimum crop water requirements (Pereira et al., 2002; Costa et al., 2007; Capra et al., 2008; Chai et al., 2016; Galindo et al., 2018). It has been widely studied, particuluarly in regions where water is scarce (Pereira et al., 2002; Geerts and Raes, 2009).

The challenge is to establish DI on the basis of maintaining or even increasing crop productivity while saving irrigation water and, therefore, increasing the irrigation water use efficiency (Chai et al., 2016). For this reason, DI requires a precise knowladge of the crop yield response to water (Fereres and Soriano, 2007; Geerts and Raes, 2009). Nowadays, DI is a common practice throughout the world, especially in dry regions, where it is more important to maximize crop water productivity than to maximize the harvest per unit land (Ruiz-Sanchez et al., 2010). DI is simply a technique aimed at the optimization of economic output when water is limited, the reduction in the supply for irrigation to an area imposes many adjustments in the agricultural system. Thus, DI practices are multifaceted, inducing changes at the technical, socio-economical, and institutional levels (Fereres and Soriano, 2007).

The timing and extent of the water deficit are important for efficient water use and maximizing yield and they determine the successful application of DI (Chai et al., 201; Yang et al., 2017).

The crop response to water deficits depends on the pattern of stress imposed (Fereres and Soriano, 2007; Goldhamer et al., 2006). DI includes different strategies, in the present PhD thesis two strategies will be addressed: Contined deficit irrigation and Regulated deficit irrigation.

## 1.5.1. Continued deficit irrigation (CDI)

Continued deficit irrigation imposes the water deficit uniformly, proportionally to irrigation requirements, over the whole crop cycle to avoid applying of severe water stress at any particular moment that might affect marketable yield (Fereres and Soriano, 2007; Iniesta et al., 2009; Ruiz-Sanchez et al., 2010; Galindo et al., 2018). This approach allows the plants to adapt slowely to water stress (Fereres and Soriano, 2007).

## 1.5.2. Regulated deficit irrigation (RDI)

Regulated deficit irrigation is a stage-based DI, consist of imposing water restriction during a particular phenological stage when crops are less sensitive to water stress (non-critical period) and applying the full irrigation requirements during the sensitive phewnological stages (critical period), to reduce the amount of water applied (Geerts and Raes, 2009; Ruiz-Sanchez et al., 2010; Reddy, 2016). Chalmers et al. (1981) firstly proposed RDI to control vegetative growth in peach orchards. RDI approach is based on the fact that plant responses to water stress varies with growth stages and that less irrigation applied at non-critical stages may reduce the negative impact on marketable yield and be more beneficial in terms of saving water and improving the water use efficiency, even though it may reduce normal plant growth (Moutonnet et al., 2002; Álvarez et al., 2013; Chai et al., 2016). Hence, to apply RDI approach effectively, needed to identify the most critical growth stages for a specific

crop species and cultivar. Thererfore it is important to evalute the crop sensitivity to water deficit at various stages and to determine the optimal timming to apply RDI (Chai et al., 2016).

# 1.6. Irrigation water use efficiency and yield response factor

Water use efficiency (WUE) and Irrigation water use efficiency (IWUE) are common indicators employed to assess the efficiency of the use of irrigation water in crop production (Bos, 1980; Tolk and Howell, 2003), They are practical indexes in the assessment of plant responses to deficit irrigation (Geerts and Raes, 2009; Chai et al., 2016). The IWUE is defined as the ratio of the economically valuable yield (kg m<sup>-2</sup>) to the irrigation water applied (IWA), while WUE is the ratio of the economically valuable yield (kg m<sup>-2</sup>) to the volume of water consumed by the crop, including the effective precipitation. The main pathway for enhancing WUE and IWUE in irrigated agriculture is to increase the output per unit of water, reducing the water consumption and loss (ET, runoff and losses in depth), and reallocating water to higher priority uses (Howell, 2006; Leskovar et al., 2014; Kang et al., 2017).

The productive response of crops to water is used to increase the efficiency and the water productivity. Doorenbos and Kassam (1979) introduced a linear cropwater production function to describe the reduction in yield when crop is under water stress, being the yield response factor (K<sub>y</sub>) the factor that describes the relative reduction in yield according to the reduction in the crop evapotranspiration (ETc). The reduction of the soil water storage reduces the water availability for the crops and, consequently, it has an impact on actual ET and actual yield (Moutonnet et al., 2002). Yield response factor for a given crop can be determined from the FAO approach (Doorenbos and Kassam, 1979; Steduto et al., 2012), also called the water-production function, that is expressed as:

$$\left(1 - \frac{Y_a}{Y_X}\right) = K_y \left(1 - \frac{ET_a}{ET_X}\right)$$

where  $Y_x$  and  $Y_a$  are the maximum and actual yields,  $ET_x$  and  $ET_a$  are the maximum and actual evapotranspiration, and  $K_y$  is a yield response factor. Values of  $K_y$  greater than 1 indicate that the crop is sensitive to water deficit, and values lower than 1 indicate that it is tolerant (Doorenbos and Kassam, 1979; Steduto et al., 2012).

The  $K_y$  values are crop specific and vary over the growing season according to growth stages. The crop yield response factor for a crop at a particular stage is estimated by considering the deficit irrigation at a particular stage while keeping the crop fully irrigated at other stage. It can be also determined at seasonal level by exposing the crops at DI during whole growing season and finding out the relationship between relative yield reduction and seasonal relative ET reduction (Garg and Dadhich, 2014).

# 1.7. Plant water status under deficit irrigation

Plant leaf is a vital organ for transpiration and photosynthesis, and its anatomy plays a crucial role in plant development (Barbour and Farquhar, 2004; Chai et al., 2016). A short period of mild water deficit may promote plants to reduce leaf water content (Pérez-Pastor et al., 2014; Chai et al., 2016). Plant water status can be studied either in terms of water content or cell turgor, or in terms of water potential (Kramer, 1988). Water content and water potential have been used as indicators of leaf water status. The use of water content has been replaced by the relative water content (RWC) which is based on the maximum amount of water a tissue can hold (Yamasaki and Dillenburg, 1999). The RWC is considered an index that expresses the absolute amount of water that a plant requires to reach artificial full saturation (González and González-Vilar, 2001). This index reflects the metabolic activity in tissues, and it is used as a meaningful index for dehydration tolerance (Anjum et al., 2011; Kalariya et al., 2015). RWC correlates closely with plant's physiological activities, soil water status and it is used for screening the drought tolerance of different genotypes

(Kramer, 1988; Tanentzap et al., 2015). RWC is related to cell turgor, which is the process directly driving cell expansion (Jones, 2004). RWC is readily determined by obtaining the fresh weight plant tissue (either leaf discs or entire leaves) and then measuring its turgid weight after equilibration (floating tissue on water) for a prescribed period of time. The same tissue is oven-dried to a constant weight and RWC calculated from the following equation (Hayat et al., 2007):

RWC (%) = 
$$\frac{\text{Fresh wight - Dry weight}}{\text{Turgid weight - Dry weight}} \times 100$$

On the other hand, abiotic stresses modify membrane structure and composition, which cause leakage of ions (Taiz and Zeiger, 2002). The rate of damage to cell membranes by water stress might be assessed through estimation of electrolyte leakage from the cells (Blum and Ebercon, 1981). The cell membrane stability index (MSI) is a physiological aspect of detecting the integrity of cell membrane, which used to evaluate drought and heat tolerance. MSI is also widely used as an indicator of leaf desiccation tolerance (Chai et al., 2010), which detects the degree of cell membrane injury induced by water stress (Bajji et al., 2002). The MSI calculated using the following equation Rady (2011):

MSI (%) = 
$$\left(1 - \frac{C_1}{C_2}\right) * 100$$

where  $C_1$  is the electrical conductivity of the solution (samples submerged in distilled water) after 30 min in a water bath at  $40^{\circ}$ C, and  $C_2$  is the electrical conductivity of the solution after 10 min at  $100^{\circ}$ C.

# 1.8. Product quality

Consumers are increasingly interested in the fresh vegetables due to their nutritional value, functional properties and beneficial effects for human health, in addition to the sensory traits of taste and aroma (Maroto, 2008; Slavin and Lloyd, 2012; Rouphael and Kyriacou, 2018).

Fruit quality can be measured by multiple criteria, such as sensory attributes, textural properties, nutritive values, phytochemical constituents, functional properties and defects (Judith A, 1999; Camelo, 2004; Kyriacou and Rouphael, 2018). Fruit size and external appearance (form, colour, ...) determine fruit marketability. Firmness determine the product shelf life (Ripoll et al., 2014). Marketable products are commonly purchased on the basis of their fresh weight and sensorial attributes (texture, colour, aroma, and taste), these parameters which may be affected by water deficit (Nora et al., 2012).

The colour of the product is related to the product perception by the consumer, which influences his preference and choice. The colour is derived from the pigment concentration, which can change during maturation and ripening. (Barrett et al., 2010; Pathare et al., 2013). One of the most widely and appropriate measure for the product color is based on the CIELAB or CIE 1976 L\*a\*b\* color space (Figure 13), which was proposed by the Commission Internationale de l'Eclairage (CIE; CIE, 2007). These color coordinates can be determined by colorimeters; L\* quantify the surface product brightness, it is always positive, ranging between 0 (black) to 100 (white), a\* ranged between -100 to +100 that denote green (negative) or red (positive), and b\* ranged also between -100 to +100 and denote blue (negative) or yellow (positive). These CIELAB colour space coordinates (L\*a\*b\*) are used to calculate the color indices; Hue angle (H°), Chroma (C\*) and color index (CI; Figure 13). H° angle refers to the angle between the hypotenuse and 0° on the a\* axis; an angle of 0° (or 360°) indicates red-purple, 90°= vellow, 180°= green and 270°= blue (McGuire, 1992; Pathare et al., 2013). H° was calculated as described by McGuire (1992) as:

$$H^{\circ} = Arctang \left(\frac{b}{a}\right)$$

Chroma refers to the intensity or saturation of color, with greater C\* indicating greater intensity. C\* is used to define the degree of difference of a H° in comparison to grey colour with the same lightness. C\* is calculated as stated by Pathare et al. (2013):

$$C^* = \sqrt{(a^2 + b^2)}$$

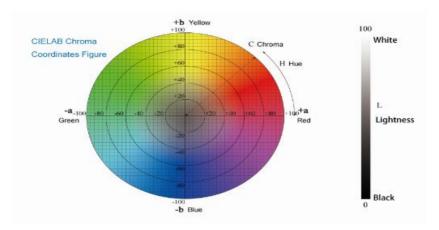


Figure 13. The CIELAB colour space coordinates (L\*a\*b\*) and color indices.

Color index reflect well the evolution of the fruit, and provides an excellent correlation between visual and instrumental appreciation (Jimenez-Cuesta et al., 1982; Martínez-Jávega et al., 2004). The CI is calculated described by Cristina (2014):

$$CI = \frac{a * 1000}{L * b}$$

A high product quality and taste is related to high sugar level and the appropriate sugar to acid ratio (maturity index). It may increase the product consumption (Kader, 2008; Ripoll et al., 2014). Usually, carbohydrate, protein, vitamin and phytochemical (polyphenolics, carotenoids, and glucosinolates) content in vegetables, determines their nutritional value (Camelo, 2004; Barrett et al., 2010). Currently, there is an increase in consumer interest for health benefits of the vegetable products, being an important source of polyphenols and vitamins, what is related to antioxidant activity (Ripoll et al., 2014).

Irrigation is one of the major factors affecting the product quality, since the quantity of water in the product determines the concentration of different elements, such as sugars and acids (Chen et al., 2014; Ripoll et al., 2014). Deficit irrigation

can improve the quality of the product, affecting its dry matter content and stimulating the production of secondary metabolites (Ripoll et al., 2014; Rao et al., 2016). Deficit irrigation effects on the quality of the vegetable products have been reported in several researches with different results (Barzegar et al., 2018). This might be related to the fact that crop response to deficit irrigation varies with location, stress patterns, species and cultivar, planting dates, and the quality traits evaluated (Fereres and Soriano, 2007). Some horticultural crops have a shallow root system, so they are sensitive to water stress (especially when stress is severe), leading to losses in both yield and quality attributes (Costa et al., 2007; Kyriacou and Rouphael, 2018).

# 1.9. Vegetable crops

Vegetables are grown worldwide and make are an important part of the diet of human in many parts of the world. Vegetables play an important role in human nutrition, and some of them are an important source of vitamins, minerals, fibre, proteins and carbohydrates, and also protective nutrients for human health (Maroto, 2008; Dias, 2011; Slavin and Lloyd, 2012). Drought stress is one of the major limiting factors for vegetable crops production. Currently, there is little available data of vegetable crops responses to different extends and timing of water deficits under Mediterranean conditions, particularly with the new developed hybrids. This doctoral thesis studies the productive response of four vegetable crops, which are part of the traditional crop rotations in the Mediterranean region: cauliflower, onion, sweet Italian pepper and watermelon:

### 1.9.1. Cauliflower

Cauliflower (*Brassica oleracea* var. *botrytis*) belongs to the *Brassicaceae family* also called *Cruciferae*, and it is an important vegetable crop. The origin of cauliflower and the *Brassica oleracea* group seems to be located in the Mediterranean basin, specifically in the Middle East. Cauliflower is a cool season vegetable (Dixon, 2007)

and is an annual plant that reproduces by seed. The edible part called "curd or head", is a mass of hypertrophied flower buds (Maroto, 2002, 2007).

Cauliflower have important benefits for human health, since it has medicinal and functional properties. Its caloric content is low, about 27-32 cal/100g. It has a high vitamin A concentration, which act as antioxidant strengthening its anti-cancer properties (Maroto, 2007, 2008). Most of *Brassicaceae* species have special organoleptic aspects (pungent and bitter-taste) due to their glucosinolate content. Moreover, it is folic acid rich, which intervenes in the formation and maturation of red and white blood cells (Maroto, 2007, 2008).

Cauliflower is an important vegetable crop worldwide, particularly in the Mediterranean area. Worldwide, the cultivated area of cauliflower and broccoli (considered together) is about 1.40\*10<sup>6</sup> ha, with a production of about 25.90\*10<sup>6</sup> Mg. The top 10 productive countries are presented in Table 2.

Table 2. Top	production c	countries of	cauliflower	and broccoli	(Faostat, 2018).

Ranking	Country	Production (Mg)
1	China	10180881
2	India	8199000
3	United States of America	1321060
4	Spain	605161
5	Mexico	583279
6	Italy	388281
7	Poland	314738
8	France	308488
9	Bangladesh	268484
10	Turkey	250330

Spain is an important producer of broccoli and cauliflower, classified in the fourth position, with a cultivated area about 32,977 ha, which produced approximately 60,5161 Mg. Furthermore, Spain is the world's first exporter of broccoli and cauliflower, followed by Mexico and USA, while United Kingdom, Canada and Germany are the most importing countries (MAPA, 2017; Faostat, 2018).

According to Maroto (2007) and Baixauli and Maroto (2017), the principal growth stages of the cauliflower plant are:

- Juvenility: it begins with germination, throughout this stage the plant only forms leaves and roots, the optimal temperature is set between 20 and 30 °C. The juvenile phase lasts between 5 and 8 weeks, depending on the cultivar (Figure 14).
- Curd induction: The cauliflower is considered an obligate vernalizing plant that need to the action of low temperature to produce flower. The duration and the value of vernalizing temperatures varies with the varieties and the seasons. The range between 6 and 15 °C and last for 5-15 weeks, this period can be shortened with lower temperatures, and lengthen in the opposite case.
- Curd growth: the plants after being induced to bloom, stop forming new leaves and those that had already formed have a low growth rate. The younger leaves progressively wrap the curd. Temperature plays a very important role in the curd's growth, since low temperatures (3-5°C) can lead to zero growth, while a temperature increase of 3-4°C can lead to an increase in yield, up to 80%.



Figure 14. Cauliflower crop at the juvenility stage.

## 1.9.2. Onion

Onion (*Allium cepa* L.) is the most important Allium vegetable crop, it belongs to family *Liliaceae*. Onion is one of the oldest cultivated plants, being originated in the regions around Central Asia in Iran and West Pakistan. Onion is a biannual plant

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that produces a large bulb in the first year of growth and blooms after vernalization (action of the low temperatures generally from 5 to 12°C at certain physiological conditions of the plant) in the second year. The onion plants are cultivated to use their bulbs (Maroto, 2002; Brewester, 2008; Miguel, 2017). Onions contain chemical groups that have significant nutritional and medicinal properties, namely the polyphenolics compounds (quercetin is the predominant) and the alk(en)yl cysteine sulfoxides (Leskovar et al., 2012; Shigyo et al., 2018).

Onion is one of the major vegetable crops around the world, its rank second only preceded by tomatoes. World production of onion about 98.0\*10<sup>6</sup> Mg, produced from an area about 5.20\*10<sup>6</sup> ha. Onion bulb production in Spain is about 1.25\*10<sup>6</sup> Mg, produced from area about 23,174 ha. The world's largest onion exporter countries are India, Netherlands, China, Egypt, Mexico and Spain, while the world's largest onion importer countries are Malaysia, United States of America and Saudi Arabia (MAPA, 2017; Faostat, 2018).

**Table 3.** The top countries of onion production (Faostat, 2018).

Ranking	Country	Production (Mg)
1	China	21803722
2	India	16086909
3	United States of America	3276361
4	Egypt	2089456
5	Iran	2032750
6	Turkey	1904853
7	Pakistan	1794126
8	Russian Federation	1790362
9	Brazil	1515421
10	Mexico	1342688

Onion plant development starts with germination, it forms a short stem and superficial root system extending only within the top 30 cm soil depth. During the vegetative growth stage onion plants form leaves every 7-10 days, up to a total of 13-18 at the beginning of the bulb formation (bulbing; Figure 15). The optimum temperature during vegetative development ranges between 13 and 24 °C. Bulbing is governed by the environmental condition, particularly long photoperiod, which

also interrelated with higher temperature (Rabinowitch and Brewster, 2018). Under favourable conditions of bulbing, the vegetative growth is gradually paralyzed, and the base of the inner leaves begin to thicken and forming the bulb (Maroto, 2002; Miguel, 2017). The bulbing starts when the ratio of the diameter of the bulb and neck (pseudostem stem) is greater than a certain value, that ranges between 1.5 (Miguel, 2017) and 2.0 (Brewester, 2008). During the bulb maturation, the outer leaves lose water and from one to three layers of thin skins that completely envelop them. Afterwards, the neck of the bulb weakens and bends, indicating that maturity started (Maroto, 2002; Miguel, 2017).



Figure 15. Onion plants during bulbing stage.

### 1.9.3. Sweet pepper

Pepper crop (*Capsicum annuum* L.) belongs to the *Solanaceae* family The origin of the sweet pepper is located in South America, more specifically in Bolivia and Peru (Maroto, 2002; Condés, 2017). Introduced initially in the Mediterranean area from America, then it was distributed throughout Africa, India, China, North America and Oceania. Sweet peppers have a high-water content, are rich in vitamins A1, C, B1, B2 and P. Red peppers are rich in vitamin A, while green peppers are rich in vitamin C. Its fiber content ranges from 20 to 24% dry matter. They are also rich in carbohydrates (Condés, 2017). In addition to the importance as a food, peppers have also received attention due to their high levels of phytochemicals with documented human health benefits. These include carotenoids, ascorbic acid,

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flavonoids, phenolic compounds (predominantly flavonoids and capsaicinoids), which are well known for their antioxidant activity (Howard et al., 2000; Naczk and Shahidi, 2006; Condés, 2017). Capsaicin prevents certain types of cancerous tumours (Prohens and Nuez, 2008).

The sweet pepper is one of the most important vegetable crop worldwide, classified in the seventh position among vegetable crops. The world total cultivated area of pepper in 2017 was approximately 1.99\*10<sup>6</sup> ha, with a production of approximately 36.0\*10<sup>6</sup> Mg. China is the largest producer of pepper, followed by Mexico and Turkey (Table 4). The production of pepper in Spain is approximately 1.08\*10<sup>6</sup> Mg, harvested from approximately 17823 ha. The main exporting countries are Mexico, Spain and Netherlands, while Germany, United Kingdom and France are the main importing countries (MAPA, 2017; Faostat, 2018).

**Table 4.** The top countries of pepper production (Faostat, 2018).

Ranking	Country	Production (Mg)
1	China	17435376
2	Mexico	2737028
3	Turkey	2457822
4	Indonesia	1961598
5	Spain	1082690
6	United States of America	921150
7	Nigeria	746157
8	Egypt	637760
9	Algeria	596670
10	Tunisia	437000

The root form a set of secondary roots branches, with higher density of secondary roots in the surface part. The stem is erect, in its first branch, originated when the seedling has reached a height of 15 to 20 cm, the first flower is produced. The flowers are hermaphrodite, they are attached to the stem by a peduncle of 10 to 20 mm in length. The fruit is in berry (Figure 16), constituted by a thick and juicy pericarp and an axis formed by a placental tissue, in which the seeds are found. The daily optimum temperature is around 25 °C, with a day-night thermal oscillation of

5 to 8 °C, greater intervals lead to greater plant development (Maroto, 2002; Condés, 2017).



**Figure 16.** Sweet pepper plants during harvesting stage.

#### 1.9.4. Watermelon

Watermelon is an important crop around the world. It receives different scientific names like *Citrullus lanatus* (Thunb.) Matsum. and Nakai, and *Citrullus vulgaris* Schrad, and belongs to the *Cucurbitaceae* family. Watermelon was originated in Africa and the Middle East. Watermelon has been cultivated in Egypt for 5000 years, from where it was spread to the rest of the Mediterranean area (Maroto, 2002; Gázquez, 2015; Baixauli, 2017).

Watermelon fruits are consumed almost exclusively in fresh. It is hydrating, remineralizing, diuretic, laxative and with a low caloric value (26 cal/100 g), which makes it advisable in weight loss diets, furthermore, considering its immediate sensation of satiety. It has high vitamin A and phytonutrients as lycopene and citrulline contents. Lycopene acts as antioxidant, reducing the risk of cancer and heart disease. Citrulline is a vasodilator and vasoprotector (Maroto, 2002; Gázquez, 2015; Baixauli, 2017).

Watermelon is an important crop around the world, with a production approximately of 118\*10<sup>6</sup> Mg from 3.48\*10<sup>6</sup> ha. Currently, the leading watermelon producing countries are China, Iran and Turkey (Table 5). Spain is the main producer

of watermelon for the European community, with 969327 Mg from 17,360 ha. The main exporting countries are Mexico, Spain and Italy, while United States of America, Germany and Canada are the main importing countries (MAPA, 2017; Faostat, 2018).

**Table 5.** The top countries of watermelon production (Faostat, 2018).

Ranking	Country	Production (Mg)
1	China	79043138
2	Iran	4059786
3	Turkey	4011313
4	Brazil	2314700
5	Uzbekistan	2030992
6	Algeria	1895074
7	United States of America	1842360
8	Egypt	1709964
9	Russian Federation	1699334
10	Mexico	1331508

Watermelon is an annual plant, which has an important aboveground system, and at the same time its main root is deep. The stems, which are covered with hairs and are provided with tendrils, extend along the ground in a crawling way, they can grow more than 3 m. To obtain a good pollination and a good fruit development, it requires between 500 to 1000 grains of pollen for each female flower. The fruit is a globular berry of variable size according to the cultivars (Figure 17). Watermelons are classified into two fundamental groups; diploid (cv. with seeds) and triploid (seedless cv.). Diploid cv. is used as pollinator to the seedless cv. It is a very sensitive to low temperatures, being the vegetative zero at 13 °C. The optimum interval for the growth between 21 and 30 °C, particularly at 25 °C, and it requires temperature between 18-25 °C, to produce flowers (Maroto, 2002; Gázquez, 2015; Baixauli, 2017).



Figure 17. Watermelon crop during fruit growth stage.

# 1.10. Experimental site description

The trails of this PhD thesis were carried out at the research field of the Cajamar Experimental Center in Paiporta, Valencia, Spain (39.4175 N, 0.4184 W; Figure 18).



**Figure 18.** The aerial view of the Cajamar Experimental Center. Detail of the two plots used in the experiments and the meteorological station (MS).

In the Cajamar Experimental Center there is a meteorological station, located in a plot adjacent to those used in the experimentation. The meteorological station includes a class A pan (Figure 19), which meet the standard consideration reported by Allen et al. (1998). The pan coefficient (K<sub>p</sub>) has been determined under the experimental site conditions, from which data ETo has been obtained.



**Figure 19.** Clase A Pan used in this research to estimate the daily evaporation.

# 1.11. Thesis objectives

Drought stress is one of the major limiting factors for vegetable crop production. Furthermore, in recent years, water is becoming increasingly scarce worldwide, and is seriously affecting agricultural production. Therefore, it is important to improve the irrigation water efficiency in agriculture, which could be achieved through using water-saving strategies, as deficit irrigation that is considered a sustainable technique in agriculture.

With the above analyzed background, a research line was proposed to study the effects of CDI and RDI on four vegetable crops, which are part of the traditional crop rotations in the Mediterranean region (Figure 20). Given that irrigation needs vary widely between crops, seasons and during the periods of each crop growth, in addition to the fact that the response of crops to deficit irrigation is different, the studies have been carried out in two autumn-winter cycle crops (cauliflower and onion) and other two crops in the spring-summer cycle (sweet pepper and watermelon).

To avoid the soil-borne fungal diseases resulting from serial cropping, the experiments were conducted in two subplots within the experimental centre (Figure 20).



**Figure 20.** Diagram illustrate crops rotation used: cauliflower (C), onion (O), pepper (P) and watermelon (W).

Within this context, the main objective of this PhD thesis is to analyse the response of the four vegetable crops to different deficit irrigation strategies. To achieve this goal, the following specific objectives are planned:

- To analyse the volumetric soil water content throughout the growing season.
- To study the plant water status at the different regulated deficit irrigation stages.
- To evaluate the biomass production and its partitioning.
- To analyse the productive response and yield quality.
- To determine the irrigation water use efficiency and crop profitability to check the most appropriate strategies for each crop.

In order to achieve these objectives, first of all, preliminary field studies were started in September 2015, testing CDI, which served to ascertain the crop behaviour under DI, in addition to determine the different growth stages of each crop, in order to be able to apply the RDI in the following seasons. Previous experiments had been carried out in the Experimental Center testing the application of 125% of the irrigation water requirements. with no positive results, so in this PhD thesis, the highest doses tested correspond to 100% of the irrigation water requirements.

This PhD thesis is presented in the form of a "compendium of publications", in which the articles are presented as they have been submitted to the journals, by crops, ordered according to the starting date for the respective experiments: cauliflower (Chapter 2), onion (Chapter 3), sweet Italian pepper (Chapter 4) and watermelon (Chapter 5).

### Chapter 2, Cauliflower: this chapter consists of two articles:

- The first one is entitled "Influence of irrigation rates on cauliflower yield" was presented at the VIII Iberian Congress of Horticultural Sciences, held in Coimbra (Portugal), and it was accepted for publication in Actas de Horticultura, on June 2017. It includes the results obtained in 2015 and 2016 growing seasons, using CDI strategies.
- The second one is entitled "Deficit irrigation as a sustainable practice to improve irrigation water use efficiency in cauliflower under Mediterranean conditions" is under review in Agronomy. It includes the results obtained in 2017 and 2018 growing seasons, applying both CDI and RDI strategies.

## Chapter 3, Onion: this chapter consists of two articles:

- "Influence of Deficit Irrigation on Productive Response of Drip-Irrigated
  Onion (*Allium cepa* L.) in Mediterranean Conditions". It includes the
  results obtained with CDI in 2016, 2017 and 2018. This article has been
  published in The Horticulture Journal. <a href="https://doi.org/10.2503/hortj.UTD-081">https://doi.org/10.2503/hortj.UTD-081</a>
- "Regulated Deficit Irrigation as a Water-saving Strategy for Onion Cultivation in Mediterranean Conditions", is a manuscript submitted on 08.07.2019 to Agronomy and pending decision after minor changes. It includes the results obtained with RDI.

### Chapter 4, sweet Italian pepper; this chapter consists of two articles:

• "Production response and irrigation water use efficiency of pepper (*Capsicum annuum* L.) to different deficit irrigation regimes" was presented at the *XXX International Horticultural Congress* (Istanbul, 2018) and it was accepted to be published in *Acta Horticulturae* on 31 October 2018. It includes the results obtained with CDI in 2016.

• "Effects of deficit irrigation on the yield and irrigation water use efficiency of drip-irrigated sweet pepper (*Capsicum annuum* L.) under Mediterranean conditions". This article includes the results obtained with both CDI and in 2017 and 2018. It is under review in Irrigation Science.

Chapter 5, watermelon: in this chapter is presented the article "Yield response of seedless watermelon to different drip irrigation strategies under Mediterranean conditions", published in Agricultural Water Management, 2019, 212, 99–110. https://doi.org/10.1016/j.agwat.2018.08.044

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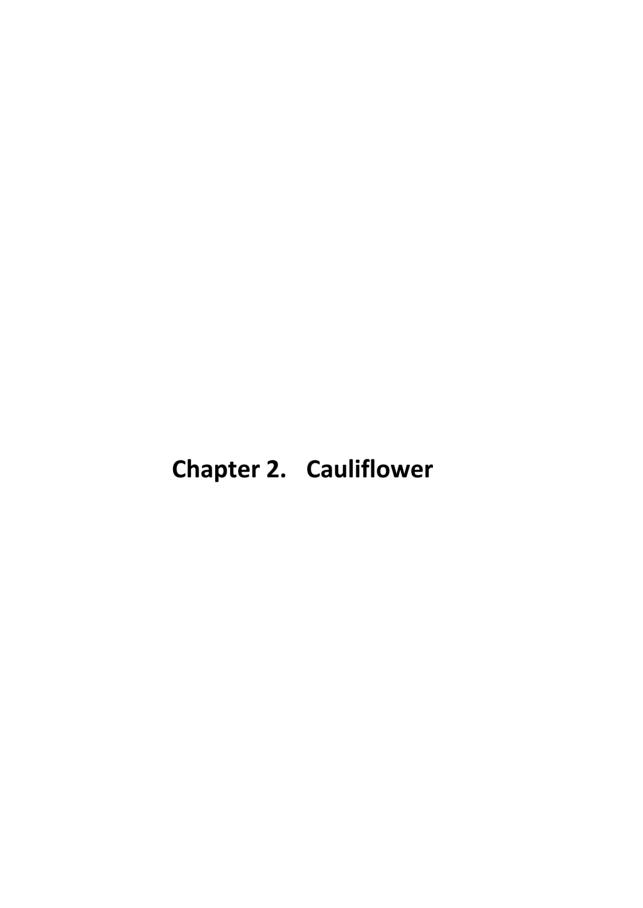
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# 2.1. Influence of irrigation rates on cauliflower yield

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### **2.1.1. Abstract**

Irrigation water is essential for food production in the Mediterranean area. In many cases, the irrigation water use efficiency could be improved by an adequate irrigation management.

In order to study the irrigation management in several horticultural crops, including cauliflower, a research line was initiated. Within this line, the productive response and the irrigation water use efficiency in response to three different irrigation managements (D1, D2 and D3) have been studied during two seasons, modifying the second year the doses according to the results obtained in the first year, applying 65, 90 and 115% of the water needs (ETc), respectively, in 2015 and 50, 75 and 100% ETc in 2016. The irrigation water applied was 139, 170 and 201 mm in 2015 and 90, 128 and 175 mm in 2016, at D1, D2 and D3, respectively. In 2015, D3 led to higher total yield (4.84 kg m⁻²; p≤0.05) than D2 (4.13 kg m⁻²); this difference was not observed in 2016, probably due to the important precipitations recorded during the season (497 mm). Marketable yield and averaged curd weight were not affected (p≤0.05) by the irrigation dose in either of the two seasons. Water use efficiency was neither affected by the irrigation strategy in any of the two years. In contrast, the highest irrigation water use efficiency (p≤0.01, 27.41 and 49.2 kg m⁻³ in 2015 and 2016, respectively) was obtained with D1.

**Key words:** Curd, water use efficiency, irrigation water use efficiency, water requirements.

### 2.1.2. Introduction

Cauliflower (*Brassica oleracea* var. *botrytis* L.) is an important vegetable crop, both worldwide (1.38\*106 ha and 24.18\*106 tons), as in Europe (1.37\*10<sup>5</sup> ha and 2.39\*10<sup>6</sup> tons) and in Spain (33,198 ha and 596,969 tons; Faostat, 2014).

Water is a limiting factor in agricultural crop production (Pomares et al., 2007). Agriculture consumes more than two-thirds of the planet's total fresh water. In recent

years, freshwater scarcity is becoming a major problem, especially in arid areas, increasing competition for water among agricultural, industrial and urban consumers (Chai et al., 2016). Agricultural water withdrawals are considered very high in relation to the other sectors (Bessembinder et al., 2005). Rapid population growth, increased incidence of drought caused by climate change and different human activities are factors that have increased this problem (World Bank, 2006). Widespread water constraints for agriculture have created a strong need to develop strategies aimed at improving the water use efficiency, which will be of great importance in order to compete with the water demand from other sectors (Fereres, 2008).

Probably, the irrigation water use efficiencies achieved nowadays can be improved through an adequate irrigation management. For this reason, a line of research has been initiated, to study the irrigation management in different vegetable crops, including cauliflower, in which this study is framed. The objective is to study the cauliflower productive behavior and irrigation water use efficiency in response to three different irrigation managements.

### 2.1.3. Materials and methods

The study was carried out during the 2015-2016 (2015) and 2016-2017 (2016) seasons at the Cajamar Experimental Center, located in Paiporta (Valencia; 39.4175 N, 0.4184 O). The plants, of cultivar 'Naruto F1' (Clause®) were obtained from seedbed in a greenhouse. They were transplanted in an open-field in a staggered pattern on 1 September 2015 and 9 September 2016, in beds of 1.0 m wide with distance between plants of 0.66 m, with a planting density of 3 plants m<sup>-2</sup>. The experimental plot, of 29.03 m<sup>2</sup>, consisted of four beds of 7.26 m length, considering the two of the extremes as a guard. The experiment consisted of applying three doses of high-frequency drip irrigation (D1, D2 and D3) corresponding to 65, 90 and 115% of water needs (ETc) in 2015, while in 2016 the doses were modified based on the results obtained in the first year, contributing 50, 75 and 100% ETc.

The ETc was determined from the reference evapotranspiration (ETo), calculated from the evaporation measured from a class-A evaporation pan installed in the Experimental Centre, with pan coefficient (Kp) of 0.815 (Doorenbos & Pruitt, 1977), and the single crop coefficient (Kc) of 0.7, 1.05 and 0.95 corresponding to initial stage (K<sub>c ini</sub>), mid-season (K<sub>c mid</sub>) and late-season stage (K<sub>c end</sub>; Allen et al., 2006), adapting the each stage length to the crop cycle, in this case an average cycle (theoretically 120 days). The irrigation efficiency (percolation and uniformity) was estimated to be 0.95 (Pomares et al., 2007). In the second season the volumetric soil water content (VSWC; m<sup>3</sup> m<sup>-3</sup>) was continuously monitored using ECH<sub>2</sub>O EC-5 capacitance sensors connected to an Em50 data logger, using the ECH2O Utility software (Decagon Devices Inc., Pullman WA., USA). In each treatment, one sensor was installed horizontally, at a depth of 0.05 m, in the middle of the beds below a dripline and equidistant between two adjacent emitters. Additionally, in T1, another sensor was placed at 0.10 m depth to verify that water losses in depth were nearly negligible. In 2016 season, the irrigation events for the all the IS began when the VSWC in T1 (sensor at depth 0.05 m) descended to 80% of field capacity, applying the corresponding irrigation dose.

The soil was of silt loam texture, pH =7.4, with an organic matter content of 1.89 %, EC (ext. 1:5) of 0.39 dS m<sup>-1</sup> with available phosphorous (43 mg kg<sup>-1</sup>; Olsen) and potassium (340 mg kg<sup>-1</sup>; ammonium acetate extract) concentrations]. Irrigation water was pumped from a well, with (on average) EC 2.16 dS m<sup>-1</sup> and 77 mg kg<sup>-1</sup> N-NO<sub>3</sub> content. The incorporation of nutrients (100-50-100 kg ha<sup>-1</sup> N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O) was performed by fertigation, following the criteria indicated by Pomares et al. (2007).

Plant growth parameters were determined on 21 December 2015 and 10 January 2017. Three plants of each plot were analyzed to determine their height and diameter, the relative chlorophyll index (SPAD) in three points of three fully developed leaves in each plant using a SPAD-502 m (Konica Minolta Sensing Inc., Tokyo, Japan). Aboveground parts of the plants were divided into two parts and analysed separately: vegetative, including stem and leaves (hereinafter referred to as shoots), and

reproductive, the curd. The fresh biomass of the different parts of the plant (shoots and curd) and their dry matter content (%) were determined in the  $2^{nd}$  and  $3^{rd}$  harvest passes in 5 plants.

The harvest was done in five passes between 18 December 2018 and 04 January 2016 in 2015 season, and from 04 January 2017 to 01 February 2017 in 2016 season. Yield components were determined from 5 m length (15 plants) of the central part of the bed, leaving the plants of each side to avoid the marginal effects. Curd yield was partitioned into marketable and non-marketable yield. The non-marketable yield included small curds (lower than 700 g) or with lack of compactness, or premature opening. No riceyness nor hollow stem was detected in any curd to be considered as non-marketable. In addition, in the second season width and height were measured and the width/height ratio was calculated for five curds per plot.

Based on the marketable yield and the irrigation water applied in each treatment, the irrigation water use efficiency (IWUE, kg m<sup>-3</sup>) and water efficiency (WUE, kg m<sup>-3</sup>) were determined, the last one considering, in addition to irrigation water, the effective precipitation. The experimental design was performed in a randomized complete block design in three replicates. The results were evaluated by analysis of variance (ANOVA) using Statgraphics Centurion XVI (StatPoint Technologies, 2013). Least significant difference (LSD) at a 0.05-probability level was used as the mean separation test.

### 2.1.4. Results and discussion

The duration of each of plant growth stage (Allen et al., 2006), initial, crop development, mid-season stage, and late-season stage, for Kc utilization were 21, 35, 42, 28 days, respectively in 2015, and 21, 35, 42, 46 days, respectively in 2016, with a total duration of 126 days in 2015 and 144 days in 2016.

Table 1 presents the Kc values used according to the growth stages (Allen et al., 2006). Table 2 presents the values of ETo, ETc, as well as irrigation water

### Chapter 2. Cauliflower

requirements and irrigation water applied in the three treatments. In 2015 season, 13 irrigations events were carried out, the first one being at planting, in which 58 mm were applied equally in the three treatments to ensure adequate plant establishment. In the other 12 irrigations, 81, 112 and 143 mm in D1, D2 and D3 were applied, respectively. In 2016 season, 14 irrigations were applied, applying 30 mm in the planting irrigation in the three doses, followed by 90, 128 and 175 mm in D1, D2 and D3, respectively. The effective precipitation was very different in the two years, 167 mm in 2015 and 497 mm in 2016.

**Table 1.** Dates corresponding to the crop growth stages and crop coefficient (Kc, dimensional) for each season.

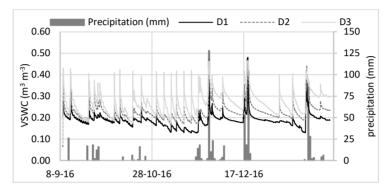
Growth stage	Kc	2015	2016
Initial	0.7	1-Sep15	9-Sep16
	0.75	8-Sep15	13-Sep16
	0.8	22-Sep15	30-Sep16
Crop development	0.85	29-Sep15	7-Oct16
	0.9	6-Oct15	14-Oct16
	0.95	13-Oct15	21-Oct16
3.61	1.05	20-Oct15	28-Oct16
Mid-season	1.05	27-Oct15	4-Nov16
	0.95	20-Nov15	28-Nov16
T. /	0.9	8-Dec15	16-Dec16
Late season	0.9	15-Dec15	24-Dec16
	0.9	18-Dec15	4-Jan17

**Table 2.** Total values of evaporation from Clase A pan (Epan, mm), reference evapotranspiration (ETo, mm), crop evapotranspiration (ETc, mm), effective precipitation (Pe, mm), irrigation water requirements [IWR = (ETc –Pe) efficiency<sup>-1,</sup> mm], irrigation water applied (IWA; mm) and total water received (Pe + irrigation applied, mm) for each irrigation regime (IR) and season (2015 and 2016).

Year	Epan	ЕТо	ETc	Pe	IWR	IR	IWA* (mm)	Total water received (mm)
						D1	139	306
2015	353	288	244	167	124	D2	170	337
						D3	201	368
						D1	120	617
2016	308	251	210	497	172	D2	158	655
						D3	205	702

<sup>\*</sup> includes planting irrigation, which involved 58 mm in 2015 and 30 mm in 2016.

The volumetric soil water content (Figure 1 and 2) in D1 was lower than in the other strategies, both at 0.05 and 0.10 m depth, especially from the second half of October. These differences disappeared in the sensor located 0.10 m deep with the rains during the second half of November, especially on the 28<sup>th</sup> day when the precipitation reached 128 mm, although they remained at 0.05 m.



**Figure 1.** Volumetric soil moisture content (VSWC) at 0.05 m depth and daily precipitation.

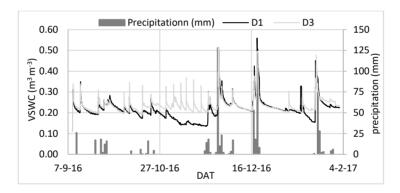


Figure 2. Volumetric soil moisture content (VSWC) at 0.10 m depth and daily precipitation.

In the analyzed years, none of the irrigation levels significantly affected ( $p\le0.05$ ) neither plant height, the SPAD index of the leaves (Table 3), nor fresh weight and dry matter content of the shoots nor curd fresh weight (Table 4). In contrast, in 2016 the plants irrigated with D3 produced plants with higher width ( $p\le0.01$ ; 107 cm) than those irrigated with D1 (103 cm), however, this difference ( $p\le0.05$ ) was not observed in 2015. As for the curd dry matter content, in 2015 (Table 4) it was higher ( $p\le0.01$ ) in plants irrigated with D2 (8.7%) than in plants irrigated with the other strategies,

without differences between them (7.8% in D1 and 7.7% in D3); in 2016 the highest dry matter content ( $p \le 0.05$ ) corresponded to the plants irrigated with D1 (8.5%) and the lowest was observed with D3 (8.0%).

**Table 3.** Effect of irrigation level on plant growth, in term of plant height and width and chlorophyll relative index (SPAD).

Irrigation		2015			2016	
level	Height (cm)	Width (cm)	SPAD (-)	Height (cm)	Width (cm)	SPAD (-)
D1	95.9	100.1	58.2	89.9	103.1 b	62.1
D2	92.7	96.6	57.3	90.3	104.8 ab	60.4
D3	93.1	102.1	60.5	91.1	107.0 a	61.2
(P≤ 0.5)	ns	ns	ns	ns	**	ns
LSD	3.0	6.0	4.6	1.3	2.3	1.6

Different letters in each column indicate significant differences at  $P \le 0.05$  using the LSD test ( $p \le 0.05$ ); no: Indicates not significant; \*\*: Indicates significant differences at  $P \le 0.01$ .

In 2015, the total yield obtained with D3 (4.84 kg m<sup>-2</sup>) has been higher (p $\leq$ 0.05; Table 5) than that obtained with D2 (4.13 kg m<sup>-2</sup>), but this difference is due to the non-marketable curds, which in D2 (0.26 kg m<sup>-2</sup>) was lower (p $\leq$ 0.05; data not shown) than those of D1 (0.47 kg m<sup>-2</sup>) and D3 (0.46 kg m<sup>-2</sup>), so that there have been no differences (p $\leq$ 0.05) in terms of marketable yield nor to the average weight of the curds.

**Table 4.** Effect of irrigation level on the fresh and dry weight, of the plants (total and decomposed into leaves and stem) and content in dry matter of leaves and stem, and curd.

		2015					2016			
Irrigation level		weight ant <sup>-1</sup> )	t Dry matter (%)		Fresh weight (g plant <sup>-1</sup> )		Dry matter (%)			
	Total	Curd	Total	Curd		Total	Curd	Total	Curd	
D1	4088.3	1363.9	7.9	7.8 b	•	3693.3	1607.7	8.4	8.5 a	
D2	4150.8	1403.9	8.3	8.7 a		3586.7	1628.8	8.5	8.3 ab	
D3	4269.2	1397.7	7.9	7.7 b		4087.9	1612.3	8.6	8.0 b	
(P≤ 0.5)	ns	ns	ns	**		ns	ns	ns	*	
LSD	668.3	364.5	0.5	0.5		638.6	216.5	0.9	0.4	

Different letters in each column indicate significant differences at  $P \le 0.05$  using the LSD test ( $p \le 0.05$ ); no: Indicates not significant; \*: Indicates significant differences at  $P \le 0.05$ ; \*\*: Indicates significant differences at  $P \le 0.01$ .

In 2016, the irrigation levels were not significantly influenced either total yield, marketable yield, the average curd weight (Table 5). The differences observed in 2015 in favor of D3 were not observed in 2016, which could be related to the high rainfall

recorded during the growing cycle (497 mm). The characteristics of the curds (width, height and width/height ratio; Table 6) were not affected (p≤0.05) by the dose of irrigation applied.

**Table 5.** Effect of irrigation level on production parameters: yield, total and marketable and average marketable curd weight (ACW).

		2015		2016				
Imiaation	7	7 ield			Yield	_		
Irrigation level	level Total Marketable (kg m <sup>-2</sup> ) (kg m <sup>-2</sup> )	Marketable (kg m <sup>-2</sup> )	ACW	Total (kg m <sup>-2</sup> )	Marketable (kg m <sup>-2</sup> )	ACW		
D1	4.27 ab	3.80	1.69	4.73	4.43	1.80		
D2	4.13 b	3.87	1.72	4.50	4.13	1.80		
D3	4.84 a	4.38	1.74	4.13	3.87	1.70		
$(P \le 0.5)$	*	ns	ns	ns	ns	ns		
LSD	0.58	0.55	0.06	0.84	0.86	0.20		

Different letters in each column indicate significant differences at  $P \le 0.05$  using the LSD test ( $p \le 0.05$ ); no: Indicates not significant; \*: Indicates significant differences at  $P \le 0.05$ .

These results are in accordance with those obtained by Pomares et al. (2007) in several experiments carried out in different seasons with autumn planting using medium-cycle cultivars (Nautilus, Arfak, Balmoral and Lara) and early cycle (Barcelona) in which they applied three irrigation levels, corresponding to 75, 100 and 125% of the ETc; these researchers found no significant differences in the yield obtained in response to the different water inputs. The irrigation water applied corresponding to 75% ETc in the various years ranged from 122 to 257 mm, while 100% ETc ranged from 212 to 446 mm.

**Table 6.** Effect of irrigation level on the characteristics of the curd: width, height, width/height ratio in 2016.

Irrigation level	Curd width (cm)	Curd height (cm)	Width/height
D1	16.26	11.04	1.48
D2	16.63	11.57	1.44
D3	16.72	11.25	1.49
$(P \le 0.5)$	ns	ns	ns
LSD	0.87	0.56	0.07

Different letters in each column indicate significant differences at  $P \le 0.05$  using the LSD test ( $p \le 0.05$ ); no: Indicates not significant.

The irrigation levels influenced (p≤0.01) on the IWUE (Table 7), with higher values corresponded to D1 (27.4 and 49.2 kg m<sup>-3</sup> in 2015 and 2016, respectively), followed by D2 (22.8 and 32.2 kg m<sup>-3</sup> in 2015 and 2016, respectively) and D3 (21.8 and 22.2 kg m<sup>-3</sup> in 2015 and 2016 respectively). The low values of IWUE obtained in 2015 motivated the change in irrigation thresholds for the 2016 season. On the other hand, the WUE was not affected by the irrigation level, contrary to the results obtained by Pomares et al. (2007), which obtained WUE values that decreased from 18.7 kg m<sup>-3</sup> (75% ETc) to 11.2 kg m<sup>-3</sup> (125% ETc). Obviously, the water inputs depend on the effective precipitation, which in turn depends on the climate of the area and the planting date. In this sense, although rains in the Valencian Community are relatively common in the autumn-winter season, they have a very variable intensity, so the irrigation needs also vary in the different seasons, affecting the IWUE and the WUE.

**Table 7.** Effect of irrigation level on water use efficiency (WUE; Kg m<sup>-3</sup>) and irrigation water use efficiency (IWUE; Kg m<sup>-3</sup>).

_	2015	i	2016		
Irrigation level	WUE (Kg m <sup>-3</sup> )	IWUE (Kg m <sup>-3</sup> )	WUE (Kg m <sup>-3</sup> )	IWUE (Kg m <sup>-3</sup> )	
D1	12.44	27.41 a	7.5	49.2 a	
D2	11.48	22.78 b	6.6	32.3 b	
D3	11.91	21.80 b	5.8	22.2 c	
$(P \le 0.5)$	ns	**	ns	**	
LSD	1.63	2.65	1.4	8.2	

Different letters in each column indicate significant differences at  $P \le 0.05$  using the LSD test ( $p \le 0.05$ ); no: Indicates not significant; \*\*: Indicates significant differences at  $P \le 0.01$ .

Bozkurt et al. (2011) studied in the cauliflower crop (cv. Tetris F1) planted in winter in Turkey, four levels of irrigation (Kcp) derived from evaporation accumulated in a Class A pan between two irrigations; the four levels tested were: full irrigation (100%; Kcp = 1.0), 75% of full irrigation (Kcp = 0.75), 125% of full irrigation (Kcp = 1.25) and control (Kcp = 0). These researchers found that Kcp = 1.0 led to the highest yield and average curd weight. In addition, in that experiment WUE was increased with the decrease in total irrigation water applied; these results are different from those obtained in this study, especially in the second year, probably due to abundant rainfall registered throughout the cycle, particularly in 2016 (in which the effective

precipitation was 497 mm, while it was 167 mm in 2015) compared to the effective precipitation in their experiments, 263, 318, 178 mm in the three years. Instead, the same trends were observed in relation to the IWUE.

The results suggest that D3 is excessive, which could be due to an overvaluation of pan and/or crop coefficients, so it would be interesting to determine them in future studies, as it would be of great importance to study the plant productive response to water deficit at each of the different growth stages.

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# 2.2. Deficit Irrigation as a Sustainable Practice to Improve Irrigation Water Use Efficiency in Cauliflower under Mediterranean Conditions

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### **2.2.1. Abstract**

Water shortage is one of the major constraints to vegetable production. Deficit irrigation is a sustainable technique that improves irrigation water use efficiency. Field studies were conducted during two growing seasons to evaluate the effects of deficit irrigation on cauliflower growth, curd yield, irrigation water use efficiency, and profitability. Nine irrigation treatments were used, applying 100%, 75%, or 50% of the irrigation water requirements (IWR) during the entire growing season (CDI). or 75% and 50% IWR during one of the following stages (RDI): juvenility, curd induction, and curd growth. Severe deficit irrigation applied during juvenility and curd induction reduced the plant size, but it only led to a significant reduction of marketable yield (22%) and average curd size and weight when it was maintained throughout the crop cycle. However, it supposed the highest WUE and IWUE values (20.3 kg m<sup>-3</sup> and 43.6 kg m<sup>-3</sup>, respectively). CDI applying 75% IWR or reducing water applied to 50% IWR during juvenility resulted in a not statistically different curd yield compared to fully irrigated plants (4.4 kg m<sup>-2</sup>), and therefore similar gross revenues (16859 € ha<sup>-1</sup>) with important water savings (23.5%), improving IWUE (34.1 kg m<sup>-3</sup>). Thus, these strategies can be recommended for further use.

**Keywords:** continued and regulated deficit irrigation; volumetric soil water content; curd quality traits; harvest index; gross revenue; water economic value.

### 2.2.2. Introduction

The cauliflower (*Brassica oleracea* var. *botrytis* L.) is an important vegetable crop worldwide, particularly in the Mediterranean area, being originally from the eastern Mediterranean [1]. It has an important role in the human diet, with medicinal and functional properties [2]. Cauliflower has a great economic importance; the global cultivated area of cauliflower and broccoli in 2017 was about 1.40×10<sup>6</sup> ha and approximately 25.98×10<sup>6</sup> tons of curds was produced. Worldwide, China is the largest producer of cauliflower, followed by India and the USA [3]. Spain is ranked

fourth worldwide and first in Europe for cauliflower and broccoli production, and it was the world's first exporter [3].

Sustainable water management is a key objective of sustainable agricultural practices, given that agriculture accounts for the major share of total water use—approximately 69% of the global freshwater withdrawals. Drought stress is one of the major constraints that threatens crop production [4]. Water shortage is becoming a critical issue in arid and semi-arid areas of the world, including the Mediterranean area [5,6]. The demand for water is expected to increase in the future, particularly with the foreseen growth of the world population, the increase of the irrigated agriculture area, and climate change [7–9].

Deficit irrigation (DI) is considered to be a sustainable practice, and was developed to improve water productivity, maintain yield, and even improve the product quality [10,11]. Deficit irrigation consists of applying irrigation below the optimum crop water requirements, either during the whole growing season (continued DI; CDI) or at specific phenological stages, when the crop is less sensitive to water stress (regulated DI; RDI) [5,10,12,13].

The plant response to DI depends upon the timing, duration, and the magnitude of water restriction [4,5,13], and it is crop-specific. Therefore, DI requires a precise knowledge of the crop yield and quality response to water stress [14]. At present, the aim of researchers and growers is not only to increase crop yield, but also to maximize irrigation water use efficiency [15].

Cauliflower is considered a sensitive crop to water stress, and such susceptibility has been documented in several reports, such as by Kochler et al. [16] in Germany, Sarkar et al. [17,18] in India, and Pereira et al. [19] in Brazil. Bozkurt et al. [20] and Souza et al. [21] studied different irrigation levels below and above optimum irrigation, and they obtained the highest yield with full irrigation, while excess water applications had a negative effect on the yield of cauliflower. According to Latif et al. [22], water stress reduced plant growth, leaf chlorophyll concentration, relative water

content, and protein content of cauliflower cultivated under net-house conditions in Iran.

In contrast, Seciu et al. [23] pointed to cauliflower having an intermediate susceptibility to water deficits. In the autumn or winter cultivation seasons, with different cultivars of cauliflower in Spain, Pomares et al. [24] did not observe important differences in curd yield with irrigation rates of 75%, 100%, and 125% ETc. Cauliflower yield losses observed by Thompson et al. [25] during three field experiments in southern Arizona were the consequence of, to a great extent, excessive irrigation rather than of deficit irrigation.

Obviously, plant response to water deficits varies with season, region, cultivar, and stress patterns. Currently, there is little available data on cauliflower response to different levels and timing of water deficits under Mediterranean conditions, especially for developed hybrids. Therefore, it is important to evaluate the sensitivity of cauliflower to water deficits at various stages, in order to determine the optimal timing to apply water reductions.

The main aim of this research is to evaluate the effects of continued and regulated deficit irrigation on plant growth, plant water status, and productive response of cauliflower grown under Mediterranean conditions.

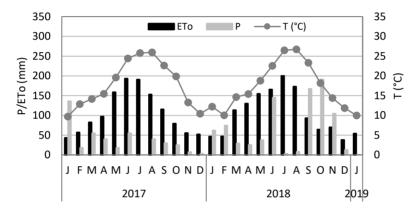
### 2.2.3. Materials and Methods

### 2.2.3.1. Experimental site conditions

Two experiments were conducted during two successive growing seasons (2017 and 2018) at the Cajamar Experimental Centre in Paiporta, Valencia, Spain (39.4175 N, 0.4184 W). To avoid soil replanting disorders resulting from serial cauliflower cropping, two subplots within the experimental plot were used. The soils were deep, with a medium (silt loam) texture, and were classified as Petrocalcic Calcixerepts according to the USDA Soil Taxonomy [26]. The soil analyses indicated that the soil of the two subplots were similar, being very slightly alkaline (pH = 7.6–7.7), and

were highly fertile [organic matter = 2.0-2.1% and high available phosphorous ( $42-43 \text{ mg kg}^{-1}$ ; Olsen) and potassium ( $445-503 \text{ mg kg}^{-1}$ ; ammonium acetate extract) concentrations]. Irrigation water was pumped from a well, with (on average) EC  $2.16 \text{ dS m}^{-1}$  and  $77 \text{ mg kg}^{-1} \text{ N-NO}_{3-}$  content.

Figure 1 shows the most significant climatological data of the growing seasons. According to Papadakis's agro-climatic classification [27], the climate is subtropical Mediterranean (Su, Me), with hot and dry summers and an average annual rainfall of approximately 450 mm, irregularly distributed throughout the year.



**Figure 1.** Monthly reference evapotranspiration (ETo; mm), precipitation (P; mm) and average temperature (T; °C) during the two growing seasons.

### 2.2.3.2. Crop management and plant material

The cauliflower 'Naruto F1' (Clause®) was used in the experiments, due to its adaptation to the soil and climate conditions in the area and to its high productivity, as evaluated at Cajamar Experimental Centre [28]. The curds are round, uniform, dense, and bright white, with an excellent behavior for both the fresh market and for the industry. Plants are vigorous, with a strong foliage that protects the curd.

Seeds were sowed on 8 August 2017 and 11 August 2018, in polystyrene trays of 126 cells, in a peat moss based substrate (70% blonde and 30% dark) recommended for vegetable seedbeds (Pindstrup Mosebrug S.A.E., Sotopalacios, Spain), and they were maintained in a Venlo-type greenhouse. Seedlings were

transplanted on 12 September 2017 and 24 September 2018, when they reached the four-leaf stage, in an open-field in a staggered pattern at a spacing of 0.66 m apart. The row length was 7.25 m, and the distance between the centers of the flat raised beds was 1.0 m, being that the raised bed was 0.6 m wide. The incorporation of nutrients (200-80-200 kg ha<sup>-1</sup> N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O) was performed by fertigation, following the criteria indicated by Pomares et al. [24].

# 2.2.3.3. Deficit irrigation strategies and growth stages

The cauliflower growth period was divided into four stages [1,29]: (1) initial, from transplanting to plant establishment; (2) juvenility, from establishment until the plant forms a critical number of leaves (12–15 leaves for autumn genotypes); (3) curd induction, until appearance of the curd (until approximately 4 cm diameter; cauliflower responds to relatively low temperatures from this physiological age, inducing curd formation); and (4) curd growth, extending from curd growth until the end of the harvest. These four growth stages coincide with those defined by Allen et al. [30]: (1) initial, (2) crop development, (3) mid-season stage, and (4) late-season stage. During the initial period, all plants were irrigated without restriction to ensure correct plant establishment. Then, different irrigation strategies were initiated.

The experiments consisted of nine irrigation strategies (ISs) in the two growing seasons (GS). The analyzed ISs included T1, T2, and T3 applying 100%, 75%, and 50% of the irrigation water requirements (IWR), respectively, throughout the GS; T4, T5, and T6 reduced the irrigation water applied (IWA) to 75% of the IWR during the crop growth stages, 2, 3, and 4, respectively; T7, T8 and T9 reduced the water applied to 50% of the IWR, at the same growth stages.

### 2.2.3.4. Irrigation scheduling and system

The IWR was determined using the following equation:

$$IWA = \frac{ET_C - Pe}{Ef}$$
 (1)

where ETc (mm) is the crop evapotranspiration, Pe is the effective precipitation (mm) determined from rainfall data using the method of the U.S. Bureau of Reclamation [31], as presented by Pascual-Seva et al. [32], and Ef is the irrigation efficiency of 0.95 [considering DU (distribution uniformity) = 0.97; deep percolation ratio (DPr) = 0.98; LR (leaching requirement) is negligible, as it has been stated for cauliflower cultivars grown in the Experimental Centre].

The ETc (mm) was calculated from the ETo and a single crop coefficient (Kc) proposed for local conditions by the IVIA [33], adapting the duration of each stage to the growing cycle.

$$ET_C = ET_0 \times Kc \tag{2}$$

where ETo is the reference evapotranspiration and Kc is the crop coefficient, which are 0.7, 1.0 and 0.9, corresponding to initial, mid-season and late season stages. ETo was determined according to Allen et al. [30], as follows:

$$ET_0 = E_{pan} \times K_p \tag{3}$$

where  $E_{pan}$  (mm day<sup>-1</sup>) is the evaporation from a class A pan installed adjacent the Experimental Center and  $K_p$  (0.815) is the pan coefficient determined according to Allen et al. [30].

The irrigation water was supplied by a drip irrigation system with one lateral line per bed, using a turbulent flow dripline (16 mm; AZUDRIP Compact; Sistema Azud S.A., Murcia, Spain) with emitters spaced 0.33 apart and a discharge rate of 2.2 L h<sup>-1</sup>. An irrigation controller programmer (NODE-100 single station controller, Hunter, California, USA) was connected to the irrigation system for programming the irrigation events. A water flow meter (MJ-SDC TYP E, Ningbo Water Meter Co., Ltd., Ningbo, China) was connected to each IS, to record the IWA.

#### 2.2.3.5. Volumetric soil water content

The volumetric soil water content (VSWC; m<sup>3</sup> m<sup>-3</sup>) was continuously monitored using ECH<sub>2</sub>O EC-5 capacitance sensors connected to an Em50 data logger, using

the ECH<sub>2</sub>O Utility software (Decagon Devices Inc., Pullman WA., USA). Following the recommendations described by Sarkar et al. [18] and Pereira et al. [19], in each treatment, one sensor was installed horizontally at a depth of 0.15 m, in the middle of the beds below a dripline and equidistant between two adjacent emitters. Additionally, in T1, another sensor was placed at 0.30 m depth to verify that water losses at depth were nearly negligible. The VSWC was measured and stored at 15 min intervals, and the variations in the VSWC were used to determine the in-situ field capacity (FC). To compare the VSWC corresponding to the different IS and GS, their values are presented as the ratio of the VSWC compared with the VSWC at FC (% FC). The irrigation events for the ISs began when the VSWC in T1 descended to 80% of FC, applying the corresponding IWA.

#### 2.2.3.6. Relative water content and the membrane stress index

The relative water content (RWC; %) and the membrane stress index (MSI; %) were evaluated at the end of each stage. Leaf RWC was determined in fresh leaf discs of 2 cm diameter using the method developed by Barrs [34], and it was calculated using the following equation from Hayat et al. [35]:

RWC (%) = 
$$\frac{(FW - DW)}{(TW - DW)} * 100$$
 (4)

where FW, DW and TW are the disc fresh weight, dry weight and turgor weight, respectively.

The MSI was determined using 0.2 g samples of fully expanded leaf tissue, following the methodology described by Rady [36], and it was calculated as

MSI (%) = 
$$\left(1 - \frac{C_1}{C_2}\right) * 100$$
 (5)

where  $C_1$  is the electrical conductivity of the solution after the samples were heated at 40 °C in a water bath for 30 min, and  $C_2$  is the electrical conductivity of the solution after the samples were boiled at 100 °C for 10 min.

### 2.2.3.7. Plant growth and the harvest index (HI)

Growth parameters were evaluated at the end of plant growth. Plant height and diameter and leaf number per plant were determined in the field, with four plants each plot. The chlorophyll index (SPAD) allows the indirect and non-destructive evaluation of the content of leaf chlorophyll by light intensity absorbed by the tissue sample. The SPAD was measured at three points in three fully developed leaves in each plant using a SPAD-502 m (Konica Minolta Sensing Inc., Tokyo, Japan). Aboveground parts of the plants were divided into two parts and analyzed separately: vegetative, including stem and leaves (hereinafter referred to as shoots), and reproductive—the curd. Each sampled plant part (shoots and curd) was weighed with a precision analytical balance (Mettler Toledo AG204; Switzerland); thereafter, they were dried at 65 °C in a forced-air oven (Selecta 297, Barcelona, Spain) until they reached a constant weight, to obtain the dry weights. The harvest index (HI) was determined as the ratio of curd to total aboveground biomass on a dry mass basis (g g-1; [37]).

# 2.2.3.8. Curd yields, irrigation water use efficiency (IWUE) and yield response factor $(K_y)$

In 2017, the harvest was completed in five passes between 12 and 29 January 2018, and in 2018 it started on 17 January 2019 and lasted until 04 February, requiring six passes. The yield components were determined from a 5 m length (15 plants) of the central part of the bed, leaving the plants on each side to avoid marginal effects. Total curd yield was partitioned into marketable (MY) and non-marketable yield. The MY was considered "with leaves" [38]. The non-marketable yield included curds that were small (lower than 700 g) or that presented defects in shape (lack of compactness, or premature opening), that were the only culls found.

The IWUE was calculated as the ratio of marketable yield (fresh mass; kg m $^{-2}$ ) to IWA (m $^{3}$  m $^{-2}$ ; [39]). The WUE was calculated as the ratio of marketable yield (kg m $^{-2}$ ) and IWA + Pe (m $^{3}$  m $^{-2}$ ; [40]). The yield response to water deficits during the

crop cycle-was determined according to Doorenbos and Kassam [41], using the following equation:

$$\left(1 - \frac{Y_a}{Y_m}\right) = Ky \left(1 - \frac{ET_a}{ET_m}\right) \tag{6}$$

where  $Y_a$  and  $Y_m$  are the actual and maximum marketable yield (kg m<sup>-2</sup>), respectively;  $ET_a$  and  $ET_m$  are the actual and maximum ET (mm), respectively; and  $K_y$  is the yield response factor.  $ET_a$  and  $ET_m$  were calculated as ET = IWA + Pe, considering both the drainage and the variation in the volumetric soil water content to be negligible.

### 2.2.3.9. Physical properties and colour indices of the curds

Curd physical attributes and color indices were assessed during the second harvest. Three representative curds per plot were selected to determine perimeter with a flexible measuring tape. Then, curd color was measured using a chroma meter (Minolta CR-300; Konica Minolta Sensing Inc., Tokyo, Japan) and the CIELAB (CIE 1976 L\*a\*b\*) color space coordinates were obtained from three readings performed at the curd surface. The average values were used to calculate the following color indices. L represented curd brightness. Hue angle (H°) was calculated as described by McGuire [42]:

$$H^{\circ} = \arctan\left(\frac{b}{a}\right) + 180 \tag{7}$$

Chroma (C\*) was calculated as stated by Pathare et al. [43]:

$$C *= \sqrt{(a^2 + b^2)} \tag{8}$$

Curd firmness was determined using a digital penetrometer with a tip of 8 mm diameter (Penefel DFT 14, Agro Technologies, Forges les Eaux, France). Later, these curds were cut to determine curd size (height and diameter) using a measuring tape, and the trunk width was measured with a digital caliber model TOP CRAFT (Ovibell GmbH & Co., Mülheim an der Ruhr, Germany).

### 2.2.3.10. Profitability

The gross revenue and economic value of water were calculated considering the average values of MY and IWUE, alongside the average cauliflower curd price corresponding to the last three years  $(0.38 \in \text{kg}^{-1})$  [44].

### 2.2.3.11. Experimental layout and statistical analysis

The experiment was performed in a randomized complete block design in three replicates. Each experimental plot area was approximately 14.5 m<sup>2</sup> and the plots were separated with a blank bed. The results were evaluated by analysis of variance (ANOVA) using Statgraphics Centurion XVII [45]. Least significant difference (LSD) at a 0.05-probability level was used as the mean separation test.

### **2.2.4.** Results

# 2.2.4.1. Growth stages and irrigation water applied

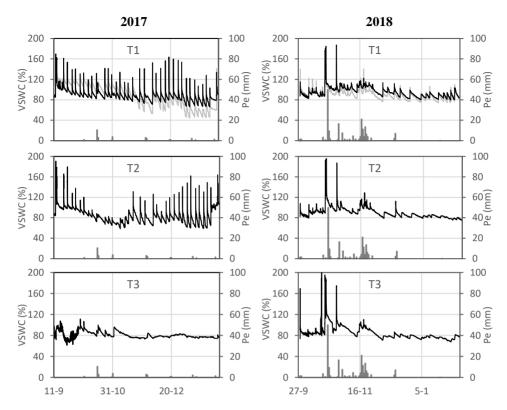
The duration and the IWA of each growth stage (initial, juvenility, curd induction and curd growth) are presented in Table 1. The total growth cycle period (including the initial period) was 140 days in 2017 and 134 days in 2018. The total pan evaporation and ETo were higher in 2017 (326 and 266 mm, respectively) than in 2018 (226 and 184 mm, respectively). The effective precipitation varied between GS, being much higher in 2018 (177 mm) than in 2017 (30 mm). Initially, all treatments were irrigated with 30 and 26 mm in 2017 and 2018, respectively, to ensure adequate plant establishment. The IWA values during the differential irrigation periods ranged from 113 (T3) to 224 mm (T1) in 2017, and from 57 (T3) to 113 mm (T1) in 2018 (Table 1).

**Table 1.** Duration (days) and irrigation water applied (mm) per irrigation strategy in each growth stage, from establishment and during the 2017 (12 September – 29 January) and 2018 (24 September – 4 February) growing seasons (GS).

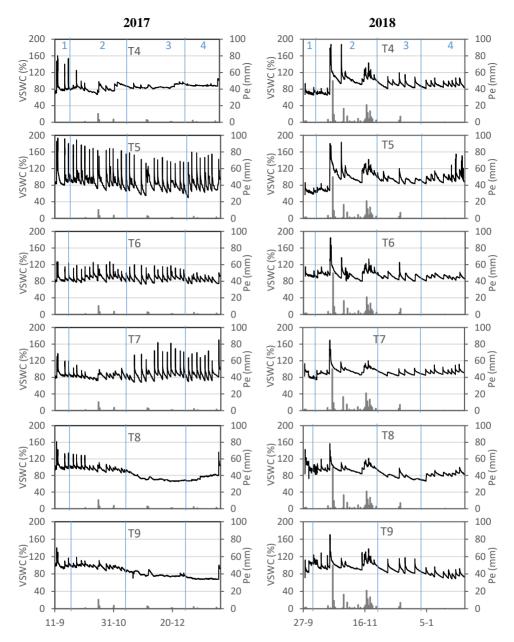
GS	Stages	Days									
			T1	T2	Т3	T4	T5	T6	T7	T8	Т9
2017											
	Juvenility	50	106	81	54	81	106	106	54	106	106
	Curd induction	48	60	45	30	60	45	60	60	30	60
	Curd growth	29	58	44	29	58	58	44	58	58	29
	Total	127	224	170	113	199	209	209	172	194	195
2018											
	Juvenility	50	51	38	25	38	51	51	25	51	51
	Curd induction	38	28	21	14	29	21	29	29	14	29
	Curd growth	35	33	26	18	34	34	26	34	34	18
	Total	123	113	85	57	101	107	105	88	99	98

#### 2.2.4.2. Volumetric soil water content

Figures 2 and 3 show the VSWC for the different ISs at 0.15 m (and 0.30 m for T1) depths, as well as the daily rainfall during the growing seasons. The VSWC at 0.15 m depth varied between the GS, with higher values in 2018 (on average 91.5% FC) than in 2017 (on average 85.1% FC). The average VSWC at 0.15 m depth in 2017 ranged between 88.2% (T1) and 81.0% FC (T3), and in 2018 between 95.2% (T1) and 84.8% FC (T3). Regarding the RDI strategies, in both GSs, a slight reduction in VSWC at 0.15 m was registered in the phases when the restriction was applied, particularly with severe water deficits (50% IWR).



**Figure 2.** Relative soil water content [%; volumetric soil water content/volumetric soil water content at field capacity at a 0.15 m (–) and 0.30 m (–) depth] for T1, T2 and T3 irrigation strategies and daily rainfall (vertical bars) during each growing season.



**Figure 3.** Relative soil water content [%; volumetric soil water content/volumetric soil water content at field capacity at a 0.15 m] for T4, T5, T6, T7, T8 and T9 irrigation strategies and daily rainfall (vertical bars) during each growing season. Crop growth stages: (1) Initial; (2) Juvenility; (3) Curd induction; (4) Curd growth.

# 2.2.4.3. Relative water content (RWC) and the membrane stability index (MSI)

The effects of the GS and the IS on cauliflower RWC and MSI indices are presented in Table 2. At the end of juvenility, both parameters were affected by both the GS ( $P \le 0.01$ ) and the IS ( $P \le 0.01/P \le 0.05$ ), in the sense that higher values were found during 2018 than 2017, but this difference was not observed in the following stages. At the end of juvenility, lower values were obtained with the IS that had been exposed to water restriction in that stage (particularly with the most severe strategies; T3 and T7).

**Table 2.** Effect of the growing season and the irrigation strategy on relative water content (RWC) and membrane stability index (MSI) at the end of each growth stage: juvenility (2), curd induction (3) and curd growth (4).

		RWC (%	)		MSI (%)	)
	2	3	4	2	3	4
Growing season (GS)	)					
2017	85.9 b	84.6	83.6	84.1 b	82.8	80.8
2018	87.9 a	85.3	84.1	85.7 a	83.5	81.5
LSD	0.89	0.88	0.85	0.94	0.81	1.07
Irrigation strategy (IS	S)					
T1	88.0 a	87.2 a	86.6 a	86.3 a	85.2 a	83.8 a
T2	86.4 abc	83.9 c	83.2 de	84.5 abc	82.5 cd	80.7 bc
T3	84.9 c	80.8 e	78.3 f	83.2 c	80.0 e	77.1 d
T4	86.6 abc	86.4 ab	86.3 a	85.0 abc	83.8 abc	82.6 ab
T5	88.2 a	83.5 cd	85.7 ab	85.5 a	82.6 cd	82.0 ab
T6	88.0 a	88.1 a	85.0 abc	85.3 ab	84.9 a	82.1 ab
T7	85.0 c	85.1 bc	84.0 bcd	83.3 bc	83.2 bcd	81.0 bc
T8	87.7 ab	82.0 de	83.3 cde	85.6 a	81.5 de	81.5 bc
Т9	88.0 a	87.1 a	82.1 e	85.8 a	84.7 ab	79.6 с
LSD	1.89	1.88	1.80	1.99	1.71	2.26
ANOVA (df)			Percentage of	f sum of squa	res	
GS (1)	21.5 **	1.6 ns	1.0 ns	16.4 **	3.0 ns	1.7 ns
IS (8)	33.9 **	73.5 **	74.2 **	26.8 *	62.0 **	54.6 **
GS*IS (8)	7.8 ns	2.3 ns	5.1 ns	6.7 ns	1.1 ns	3.1 ns
Residuals (36)	36.8	22.5	19.8	50.2	33.9	40.5
Standard deviation	1.6	1.6	1.5	1.7	1.5	1.9

df: degrees of freedom. Mean values followed by different lower-case letters in each column indicate significant differences at  $P \le 0.05$  using the LSD test. \*\* (\*): Indicates significant differences at  $P \le 0.01$  ( $P \le 0.05$ ). ns: Indicates no significant difference.

At the end of the curd induction and curd growth stages, both RWC and MSI were negatively affected ( $P \le 0.01$ ) by the IS, representing 74%, 74%, 62%, and 55% of the sum of squares for RWC and MSI at the same stages, respectively (Table

2). The lowest values were obtained with the most restrictive CDI (T3), followed by the plants that were exposed to severe water restrictions at the corresponding stage (T8 in stage 3 and T9 in stage 4).

# 2.2.4.4. Plant growth and harvest index (HI)

Cauliflower growth was significantly ( $P \le 0.05$ ) affected by the GS and the IS (Table 3), but not by their interaction. The plants grown in 2017 were higher and wider ( $P \le 0.01$ ) than those grown in 2018, but they presented a similar number of leaves.

**Table 3.** Effect of the growing season and the irrigation strategy on plant height and diameter, leaf number per plant, leaf chlorophyll index (SPAD), shoots fresh weight (SFW), shoots dry weight (SDW), curd dry weight (CDW) and harvest index (HI).

	Height	Diameter	Leaf no.	SPAD	SFW	SDW	CDW	HI
	(cm)	(cm)	plant <sup>-1</sup>	(-)	$(kg m^{-2})$	$(kg m^{-2})$	$(kg m^{-2})$	(-)
Growing season (G	S)							
2017	83.0 a	103.0 a	14.47	64.64 a	7.775 a	0.729 a	0.310 a	0.30 b
2018	73.3 b	94.1 b	14.42	60.77 b	4.905 b	0.525 b	0.277 b	0.35 a
LSD	1.21	1.41	0.21	1.32	0.490	0.044	0.016	0.01
Irrigation strategy (IS)								
T1	80.7 a	102.3 a	15.25 a	65.14	6.856	0.630	0.323 a	0.35
T2	76.6 c	97.9 bcd	13.98 de	62.52	6.216	0.643	0.294 bc	0.32
T3	73.0 d	94.4 e	13.69 e	60.63	5.633	0.584	0.265 c	0.31
T4	78.7 abc	99.5 abcd	14.40 cd	62.55	6.492	0.678	0.294 abc	0.31
T5	79.0 abc	99.0 bcd	14.69 bc	62.40	6.248	0.634	0.294 abc	0.33
T6	79.8 ab	99.79 abc	15.03 ab	64.25	6.660	0.648	0.303 ab	0.32
T7	77.4 bc	96.92 de	14.27 cd	61.69	6.220	0.595	0.296 abc	0.34
T8	78.1 bc	96.75 cde	14.27 cd	61.55	6.215	0.617	0.272 bc	0.31
T9	79.96 ab	100.42 ab	15.04 ab	62.63	6.524	0.618	0.297 abc	0.33
LSD	2.57	2.99	0.44	2.81	1.040	0.093	0.033	0.03
ANOVA (df)			Percer	ntage of s	sum of squ	ares		
GS (1)	49.9 **	38.3 **	0.1 ns	13.5 **	57.7 **	45.3 **	13.2 **	29.7 **
IS (8)	10.1 **	9.5 **	27.4 **	5.6 ns	3.0 ns	2.9 ns	13.8 *	8.0 ns
GS*IS (8)	1.3 ns	3.3 ns	3.0 ns	0.6 ns	0.8 ns	3.6 ns	4.9 ns	4.9 ns
Residuals (198/90)	38.8	48.9	69.5	80.3	38.1	48.2	68.1	57.5
Standard deviation	4.5	5.3	0.8	4.9	423.4	38.1	13.5	0.0

df: degrees of freedom (198 for plant height, diameter, leaf number and SPAD; 90 for shoot and curd biomass and HI). Mean values followed by different lower-case letters in each column indicate significant differences at  $P \le 0.05$  using the LSD test. \*\* (\*): Indicates significant differences at  $P \le 0.01$  ( $P \le 0.05$ ). ns: Indicates no significant difference.

Regarding the IS, the shortest plants were obtained with the most restrictive CDI (T3) followed by the moderate CDI (T2), not differing from plants exposed to water restriction during the juvenility and curd induction stages (T4, T5, T7 and T8). Plants with lower width were also obtained with T3, not differing from T7 and T8. The lowest number of leaves per plant was found in CDI (T3 and T2). Higher values of SPAD ( $P \le 0.01$ ; Table 3) were reported in 2017 than in 2018, not being affected by the IS. Heavier plants were obtained in 2017 than in 2018 ( $P \le 0.01$ ), considering both the shoot and the curd, fresh and dry weight. Shoot fresh and dry weight were not affected by the IS, but it did reduce the curd dry weight ( $P \le 0.05$ ). The curd dry weight decreased significantly ( $P \le 0.05$ ) with CDI and severe water stress at curd induction (T8). The GS affected ( $P \le 0.01$ ) the HI, with the highest value obtained in 2018, while the IS did not affect it ( $P \le 0.05$ ).

# 2.2.4.5. Curd yields, irrigation water use efficiency (IWUE) and yield response factor $(K_y)$

Cauliflower curd yield was affected ( $P \le 0.01$ ; Table 4) by the GS, representing 73%, 63% and 72% of the sum of squares of total yield, MY and average curd weight, respectively, with higher values in 2017. Yield was not affected ( $P \le 0.05$ ) by IS; regarding MY, T3 led to the lowest value ( $P \le 0.01$ ), whereas the other strategies did not differ from full irrigation (T1), as observed for the average curd weight ( $P \le 0.05$ ). The non-marketable yield was not affected ( $P \le 0.05$ ) by the GS or by the IS.

WUE was affected by the GS, IS and by their interaction ( $P \le 0.01/P \le 0.05$ ). T3 led to the highest WUE in 2017, while in 2018 all irrigation strategies recorded similar values (data not shown). IWUE was significantly ( $P \le 0.01$ ) influenced by both analyzed factors, with the greatest IWUE in 2018 and severe water stress during the whole cycle (T3), followed by moderate CDI (T2) and reducing IWA to 50% during juvenility (T7).

**Table 4.** Effect of the growing season and the irrigation strategy on the total yield (Yield), marketable yield (MY; in kg m<sup>-2</sup> and in % Yield on a fresh weight basis), average curd weight (ACW), water use efficiency (WUE) and irrigation water use efficiency (IWUE).

Yield	MY	MY	ACW	NMY	WUE	IWUE
(kg m <sup>-2</sup> )	(kg m <sup>-2</sup> )	(%)	(kg curd <sup>-1</sup> )	(kg m <sup>-2</sup> )	(kg m <sup>-3</sup> )	$(kg m^{-3})$
)						
5.12 a	4.74 a	92.7	1.77 a	0.38	22.14 a	25.93 b
3.75 b	3.54 b	94.4	1.32 b	0.21	13.03 b	38.28 a
0.23	0.25	3.5	0.08	0.17	1.02	2.23
S)						
4.56	4.44 a	97.5	1.60 a	0.13	16.54 b	28.30 c
4.23	4.07 a	96.3	1.50 ab	0.16	18.27 ab	34.06 b
3.96	3.47 b	88.6	1.41 b	0.49	20.26 a	43.58 a
4.64	4.32 a	93.1	1.66 a	0.32	17.45 b	30.80 bc
4.64	4.35 a	93.8	1.65 a	0.30	16.93 b	29.50 bc
4.62	4.33 a	94.1	1.57 ab	0.29	16.90 b	29.46 bc
4.40	4.18 a	94.7	1.56 ab	0.22	18.61 ab	34.17 b
4.48	4.06 a	90.9	1.51 ab	0.42	16.64 b	29.33 с
4.42	4.06 a	92.8	1.52 ab	0.36	16.67 b	29.75 bc
0.50	0.52	7.4	0.16	0.37	2.16	4.72
		Percentag	ge of sum of	squares		
73.1 **	63.1 **	2.0 ns	71.9 **	7.8 ns	79.6 **	54.6 **
7.1 ns	13.2 *	18.3 ns	8.1 *	13.9 ns	5.3 *	29.1 **
0.3 ns	0.2 ns	2.4 ns	1.7 ns	3.3 ns	6.4 **	0.8 ns
19.5	23.5	77.3	18.3	74.9	8.7	15.5
0.4	0.4	6.3	0.1	0.3	1.8	4.0
	(kg m <sup>-2</sup> )  5.12 a 3.75 b 0.23  8)  4.56 4.23 3.96 4.64 4.62 4.40 4.48 4.42 0.50  73.1 ** 7.1 ns 0.3 ns 19.5	(kg m <sup>-2</sup> ) (kg m <sup>-2</sup> )  5.12 a 4.74 a 3.75 b 0.23 0.25  8)  4.56 4.44 a 4.23 4.07 a 3.96 3.47 b 4.64 4.32 a 4.64 4.35 a 4.62 4.33 a 4.40 4.18 a 4.48 4.06 a 4.42 4.06 a 0.50 0.52  73.1 ** 63.1 ** 7.1 ns 13.2 * 0.3 ns 0.2 ns 19.5 23.5	(kg m <sup>-2</sup> ) (kg m <sup>-2</sup> ) (%)  5.12 a 4.74 a 92.7 3.75 b 3.54 b 94.4 0.23 0.25 3.5  4.56 4.44 a 97.5 4.23 4.07 a 96.3 3.96 3.47 b 88.6 4.64 4.32 a 93.1 4.64 4.35 a 93.8 4.62 4.33 a 94.1 4.40 4.18 a 94.7 4.48 4.06 a 90.9 4.42 4.06 a 92.8 0.50 0.52 7.4  Percentag  73.1 ** 63.1 ** 2.0 ns 7.1 ns 13.2 * 18.3 ns 0.3 ns 0.2 ns 2.4 ns 19.5 23.5 77.3	(kg m <sup>-2</sup> ) (kg m <sup>-2</sup> ) (%) (kg curd <sup>-1</sup> )  5.12 a 4.74 a 92.7 1.77 a 3.75 b 3.54 b 94.4 1.32 b 0.23 0.25 3.5 0.08  8)  4.56 4.44 a 97.5 1.60 a 4.23 4.07 a 96.3 1.50 ab 3.96 3.47 b 88.6 1.41 b 4.64 4.32 a 93.1 1.66 a 4.64 4.35 a 93.8 1.65 a 4.62 4.33 a 94.1 1.57 ab 4.40 4.18 a 94.7 1.56 ab 4.48 4.06 a 90.9 1.51 ab 4.42 4.06 a 92.8 1.52 ab 0.50 0.52 7.4 0.16  Percentage of sum of 73.1 ** 63.1 ** 2.0 ns 71.9 ** 7.1 ns 13.2 * 18.3 ns 8.1 * 0.3 ns 0.2 ns 2.4 ns 1.7 ns 19.5 23.5 77.3 18.3	(kg m <sup>-2</sup> ) (kg m <sup>-2</sup> ) (%) (kg curd <sup>-1</sup> ) (kg m <sup>-2</sup> )  5.12 a 4.74 a 92.7 1.77 a 0.38 3.75 b 3.54 b 94.4 1.32 b 0.21 0.23 0.25 3.5 0.08 0.17  8)  4.56 4.44 a 97.5 1.60 a 0.13 4.23 4.07 a 96.3 1.50 ab 0.16 3.96 3.47 b 88.6 1.41 b 0.49 4.64 4.32 a 93.1 1.66 a 0.32 4.64 4.35 a 93.8 1.65 a 0.30 4.62 4.33 a 94.1 1.57 ab 0.29 4.40 4.18 a 94.7 1.56 ab 0.22 4.48 4.06 a 90.9 1.51 ab 0.42 4.42 4.06 a 92.8 1.52 ab 0.36 0.50 0.52 7.4 0.16 0.37  Percentage of sum of squares  73.1 ** 63.1 ** 2.0 ns 71.9 ** 7.8 ns 7.1 ns 13.2 * 18.3 ns 8.1 * 13.9 ns 0.3 ns 0.2 ns 2.4 ns 1.7 ns 3.3 ns 19.5 23.5 77.3 18.3 74.9	(kg m²)         (kg m²)         (%)         (kg curd¹)         (kg m²)         (kg m³)           )         5.12 a         4.74 a         92.7         1.77 a         0.38         22.14 a           3.75 b         3.54 b         94.4         1.32 b         0.21         13.03 b           0.23         0.25         3.5         0.08         0.17         1.02           8)         4.56         4.44 a         97.5         1.60 a         0.13         16.54 b           4.23         4.07 a         96.3         1.50 ab         0.16         18.27 ab           3.96         3.47 b         88.6         1.41 b         0.49         20.26 a           4.64         4.32 a         93.1         1.66 a         0.32         17.45 b           4.64         4.35 a         93.8         1.65 a         0.30         16.93 b           4.62         4.33 a         94.1         1.57 ab         0.29         16.90 b           4.40         4.18 a         94.7         1.56 ab         0.22         18.61 ab           4.48         4.06 a         90.9         1.51 ab         0.42         16.64 b           4.42         4.06 a         92.8         1.52 ab<

df: degrees of freedom. Mean values followed by different lower-case letters in each column indicate significant differences at  $P \le 0.05$  using the LSD test. \*\* (\*): Indicates significant differences at  $P \le 0.01$  ( $P \le 0.05$ ). ns: Indicates no significant difference.

Considering the average MY and IWA values at the total cycle (CDI) or at each stage separately in both GSs, MY increased linearly with increasing IWA either during the whole GS (CDI) or at each stage when the water restriction was applied, as presented in the following equations:

CDI: MY = 2.4080 + 0.0126 IWA (r = 0.90;  $P \le 0.01$ )

Juvenility: MY = 2.5289 + 0.0119 IWA (r = 0.88; P  $\leq 0.01$ )

Curd induction: MY = 2.4596 + 0.0115 IWA (r = 0.84; P  $\leq 0.01$ )

Curd growth:  $MY = 2.4463 + 0.0116 \text{ IWA } (r = 0.69; P \le 0.01).$ 

The IWUE decreased linearly with increasing IWA, following these equations:

CDI: IWUE = 55.7151 - 0.1606 IWA (r = -0.91; P  $\leq 0.01$ )

Juvenility: IWUE = 49.5329 - 0.1234 IWA (r = -0.88; P  $\le 0.01$ )

Curd induction: IWUE = 46.2573 - 0.1092 IWA (r = -0.89; P  $\le 0.01$ )

Curd growth: IWUE = 46.6245 - 0.1109 IWA (r = -0.91; P  $\leq 0.01$ ). Regarding  $K_v$ , considering the CDI for the two GS, its value was 0.56.

# 2.2.4.6. Physical and colour indices of the curds

The physical properties and color indices of cauliflower curds in response to the IS and GS are shown in Table 5. Curd size (height, diameter and perimeter) was significantly affected ( $P \le 0.01$ ) by the two analyzed factors, GS and IS.

**Table 5.** Effect of the growing season and the irrigation strategy on curd size (height, diameter and perimeter), color indices [hue angle (H°), Chroma (C\*) and brightness (L)], dry matter content (DM) and firmness.

	Length	Width	Perimeter	H°	C*	L	DM	Firmness
	(cm)	(cm)	(cm)				(%)	(N)
Growing season (GS)								
2017	11.99 a	16.38 a	50.28 a	97.75 b	16.91 b	82.66 a	8.12 a	5.81 b
2018	11.16 b	15.70 b	47.95 b	98.81 a	17.75 a	79.31 b	7.58 b	8.78 a
LSD	0.19	0.29	0.80	0.50	0.68	1.80	0.46	0.37
Irrigation strategies (IS)								
T1	11.85 a	16.42 a	50.50 a	98.07	17.46	80.40	7.30	7.06
T2	11.47 abc	15.96 ab	48.83 ab	97.81	17.61	80.01	7.89	7.09
T3	11.14 c	14.79 c	46.67 c	98.05	17.32	80.31	7.84	6.93
T4	11.75 ab	16.50 a	49.33 ab	99.11	17.51	79.14	7.62	7.16
T5	11.78 ab	16.46 a	49.46 ab	98.49	17.06	81.42	7.62	7.56
T6	11.69 ab	16.36 a	50.08 ab	98.28	18.12	82.77	7.97	7.90
T7	11.57 ab	16.18 ab	49.50 ab	98.37	17.01	81.51	8.11	7.17
T8	11.52 abc	15.88 ab	48.58 ab	98.70	16.44	82.20	7.81	7.03
T9	11.46 abc	16.14 ab	49.08 ab	97.65	17.43	81.12	8.47	7.78
LSD	0.41	0.62	1.69	1.06	1.44	3.82	0.98	0.79
ANOVA (df)	Percentage of sum of squares							
GS (1)	29.2 **	9.3 **	21.3 **	23.2 **	12.2 *	20.3 *	10.3 *	83.1 *
IS (8)	7.4 *	21.9 **	16.7 **	15.0 ns	13.2 ns	8.3 ns	13.6ns	4.2 ns
GS*IS (8)	4.4 ns	4.6 ns	4.7 ns	16.7 ns	5.6 ns	20.1 ns	10.1ns	1.3 ns
Residuals (144)	59.0	64.3	57.3	45.1	69.0	51.3	65.9	11.4
SD	0.6	0.9	2.1	0.9	1.2	3.3	0.8	0.7

df: degrees of freedom. SD: Standard deviation. Mean values followed by different lower-case letters in each column indicate significant differences at  $P \le 0.05$  using the LSD test. \*\* (\*): Indicates significant differences at  $P \le 0.01$  ( $P \le 0.05$ ). ns: Indicates no significant difference.

Higher values were obtained in 2017, and regarding the IS, plants cultivated under the severe CDI strategy (T3) recorded the lowest values, while those subjected to full irrigation (T1) recorded the highest values, without significant differences with the other ISs. Curd dry matter content and curd firmness were affected ( $P \le 0.05$ ) by GS, with 2017 showing the highest values of dry matter content and the

lowest of firmness. Color indices (H°, C\* and brightness) were influenced (P  $\leq$  0.05/0.01) by the GS, with higher H° and C\* and lower L values in 2018, but none of these parameters were affected (P  $\leq$  0.05) by the IS.

# 2.2.4.7. Profitability

The gross revenue and water economic value were affected by the GS ( $P \le 0.01$ ) and IS ( $P \le 0.01/0.05$ ; Table 6). The highest ( $P \le 0.01$ ) gross revenue and lowest water economic value were obtained in 2017. Severe CDI (T3) led to the lowest gross revenue ( $P \le 0.05$ ) (13,161  $\in$  ha<sup>-1</sup>) and the highest ( $P \le 0.01$ ) water economic value (16.56  $\in$  m<sup>-3</sup>).

**Table 6.** Effect of the growing season and the irrigation strategy on the gross revenue, economic value and crop profits.

	The gross revenue (€ ha <sup>-1</sup> )	Economic value (€ m <sup>-3</sup> )			
Growing season (GS)					
2017	18004 a	9.85 b			
2018	13460 b	14.54 a			
LSD	938	0.84			
Irrigation strategy (IS)					
T1	16859 a	10.76 c			
T2	15465 a	12.94 b			
Т3	13161 b	16.56 a			
T4	16427 a	11.70 ab			
T5	16506 a	11.21 ab			
T6	16441 a	11.20 ab			
T7	15885 a	12.98 b			
Т8	15414 a	11.14 c			
Т9	15433 a	11.31 ab			
LSD	1991	1.79			
ANOVA (df)	Percentage of sum of squares				
GS (1)	63.1 **	54.6 **			
IS (8)	13.2 *	29.1 **			
GS*IS (8)	0.2 ns	0.8 ns			
Residuals (36)	23.5	15.5			
Standard deviation	1700.4	1.5			

df: degrees of freedom. Mean values followed by different lower-case letters in each column indicate significant differences at  $P \le 0.05$  using the LSD test. \*\* (\*): Indicates significant differences at  $P \le 0.01$  ( $P \le 0.05$ ). ns: Indicates no significant difference.

### 2.2.5. Discussion

Juvenility is usually the longest stage in cauliflower autumn and winter production, occurring during September and October, when temperature and evapotranspiration are higher compared to the later stages, and therefore the IWRs (IWA for T1; Table 1) were higher at this stage. The lower IWA in 2018 was due to both the lower evaporative demands and the higher incidence of precipitation (Figure 1). Differences between years were also reported by Bozkurt et al. ([20]; 270, 212 and 507 mm for 2005, 2006 and 2007, respectively), and the consequences of the lower precipitations were registered in 2007. The IWA full irrigation strategy (T1) in 2017 is similar to that applied in 2006 by Bozkurt et al. [20] in Turkey, while the IWA in 2018 is similar to that applied by Kałużewicz et al. ([46]; 114 mm) in Poland.

The VSWC recorded at 0.15 m depth in 2018 (on average 88.1% FC) was slightly higher than that in 2017 (on average 87.3% FC); this difference may be mainly related to the higher precipitation registered in 2018, particularly that which occurred in October and November. The full irrigation strategy (T1) recorded the greatest average VSWC over the two years. In both GSs, a decrease in VSWC values was observed during the corresponding restriction phase, particularly for severe water reductions, recovering when full irrigation was restored. Costa et al. [10] and Du et al. [15] have reported that an early plant response to soil water drying is stomatal closure (which may start during moderate water shortages), and is regulated (among other factors) by hormonal signals (ABA) which are transported from dehydrated roots to the leaves, impacting directly on plant water status and carbon exchange.

Irrigation management (starting each event when the VSWC dropped to 80% of FC) has been proved to be adequate in a preliminary study carried out it the same Experimental Centre with the same cultivar by Abdelkhalik et al. [47]. The MY obtained in the present study (5.02 kg m<sup>-2</sup> in 2017 and 3.85 kg m<sup>-2</sup> in 2018) is similar to that obtained (3.48 kg m<sup>-2</sup> in 2017 and 4.08 kg m<sup>-2</sup> in 2018) in other experiments

conducted with standard conditions [30] and full irrigation in the Experimental Centre [28].

Leaf water status depends upon the VSWC, which can become a stressor [48]. At the end of juvenility both RWC and MSI were higher in 2018 than 2017, but this difference was not significant ( $P \le 0.05$ ) in the later stages due to the small VSWC differences between GSs, as previously indicated. Regarding IS, fully irrigated plants during the whole growing period (T1) showed the highest RWC and MSI, while the lowest values in each phase were obtained for the strategies that reduced the water applied in the corresponding phase, particularly at severe levels (50% of the IWR). Results indicate that the negative effects for plants exposed to water deficits at juvenility were counteracted by full irrigation in the later stages, suggesting that early water stress, particularly moderate water stress (75% of the IWR), can be compensated for with an adequate water supply in the later growth stages. The RWC and MSI obtained in this study were in agreement with those reported by Wu et al. [49] and Latif et al. [22]. Differences in RWC and MSI between the full irrigation strategy (T1) and the most restricted CDI (T3) could explain the reduction of the CDI in the growth and MY.

As previously cited, an initial effect of decreased soil water availability is stomatal closure, which reduces carbon uptake by leaves, and limits photosynthetic activity, consequently leading to a reduction of plant growth [48,50]. Severe water stress leads to RWC and MSI reductions, inducing modifications in the relative rates of photosynthesis and respiration, and even leading to photosynthesis ceasing, respiration increasing, and abscisic acid accumulation [51].

Plant growth, expressed as plant size (height and diameter) and leaf number, was negatively affected ( $P \le 0.01$ ) by water deficits, corresponding with lower values of CDI (T3 and T2) and severe water restriction at juvenility (T7) and curd induction (T8). Similarly, Souza et al. (2018) recorded lower values of plant height and leaf number of cauliflower grown under water stress at 40% ETc, compared to higher irrigation levels.

Shoot biomass was not significantly reduced by deficit irrigation, although the lowest values were obtained in T3, which it is in agreement with Latif et al. [22], who found that the shoot fresh and dry weight of two cauliflower cultivars ('Local' and 'S-78'), decreased when the soil moisture was maintained at 60% FC, conditions that were, evidently, more severe than those herein presented. Plants subjected to severe CDI (T3) recorded the lowest curd dry weight. These results are in agreement with Bozkurt et al. [20], in the sense that the highest and lowest values of yield were obtained applying 100% and 0%, respectively, of the cumulative evaporation in a Class-A pan.

In this study, IS did not alter HI. Similar results were reported by Bozkurt et al. [20], who only observed lower HI values with non-irrigated cauliflower, compared to applying 0.75 and 1.0 of evaporation from a Class A pan. According to Fereres and Soriano [11], mild water deficits lead to reduced biomass production, while dry matter partitioning is usually not affected and the HI is maintained [11], although more severe stress can affect the dry matter partitioning, reducing HI.

Temperature has an important effect on growth and development of cauliflower plants. As Dixon [2] stated, initially the leaf initiation rate during juvenility is related to temperature; after juvenility, during curd induction, relatively low temperatures are required; but later, during curd growth, the diameter of the curd increases with temperature up to a maximum. The greater plant size, shoot biomass and curd weight obtained in 2017 compared to 2018 could be related to the slightly higher temperatures registered during the juvenility and curd growth phases in 2017.

Severe CDI (T3) caused a significant reduction in the MY (22%) compared to full irrigation. Further, although it was not significant ( $P \le 0.05$ ), both T8 and T9 caused a 9% reduction in the MY compared to full irrigation. This reduction was probably related to the increase of the non-marketable curds due to their small weight or defects in shape [although this was not statistically ( $P \le 0.05$ ) significant]. Sarkar et al. [18] recorded the maximum cauliflower curd yield under irrigation at -0.03 Mpa soil matric potential, and it decreased by 10.4% and 31.4% under -0.05 Mpa

and -0.07 Mpa, respectively. In this research MY decreased linearly with decreasing IWA for water restriction at the total growth cycle or at the different water restriction stages. Bozkurt et al. [20] found a significant second-degree polynomial relationship between the cauliflower yield and IWA, but they evaluated different irrigation levels from 0 to 1.25 of pan evaporation, exceeding the maximum crop water needs.

WUE was greatly affected by GS (representing 80% of sum of the square), IS (representing the 5% of sum of the square), and by their interaction (6% of sum of the square), so that only in 2017 (with higher WUE values the consequence of the higher rainfall registered in 2018) were differences found ( $P \le 0.01$ ) between ISs (data not shown), with the highest value obtained with T3. In contrast, the greatest IWUE was obtained in 2018, mainly because the IWA in 2018 was approximately 50% that of 2017. Evidently, IWUE depends, among other factors, on the crop cycle and particularly on meteorological conditions. In relation to IS, the greatest IWUE was obtained with T3, which implies that the water savings (50%) were greater than the reduction of MY (22%) compared to full irrigation (T1). Furthermore, the CDI with 75% of the IWR (T2) and water deficits of 50% of the IWR during juvenility (T7) increased IWUE by 20% and 21%, respectively, because of the important water savings in relation to T1—approximately 24% and 23%, respectively. According to Tolk and Howell [52], the greatest IWUE usually occurs at an ET that is generally less than the maximum ET, suggesting that irrigating to achieve the maximum yield would not correspond to the most efficient use of irrigation water, as found in this study. The WUE and IWUE obtained in the present research are consistent with those obtained by Bozkurt et al. [20], who reported that both WUE and IWUE values in cauliflower 'Tetris F1' increased with decreasing irrigation rate.

 $K_y$  values lower than 1 indicate that the crop is tolerant to water deficits [41,53]. The Ky obtained for CDI (on average for both GS; 0.56) indicates that it is not very sensitive to water deficits. Similar  $K_y$  values were reported by Sarkar et al. [18] in India, of 0.65, 0.86 and 0.77 for irrigation at -0.03 Mpa, -0.05 Mpa and -0.07 Mpa of the soil matric potential, respectively.

In this study, the curds produced by fully irrigated plants (T1) were the highest, and with the greatest diameter and perimeter, while severe CDI (T3) was the only strategy that reduced these dimensions ( $P \le 0.05$ ), which is related to the decrease of average curd weight, and consequently to the decrease of MY. These results agree with those obtained by Bozkurt et al. [20] and Souza et al. [21].

Cauliflower curd marketability improves with its whiteness. Curds produced in 2017 had higher values of L and lower values of H° and C\* than those in 2018, indicating that they were whiter. In agreement with Wang et al. [54], the slight yellowish color of curds produced in 2018 may be mainly related to the accumulation of chlorophylls, carotenoids and anthocyanins, which in turn, may be related to the greater radiation registered during the curd growth stage (on average 9.7 MJ m<sup>-2</sup> day<sup>-1</sup> in 2018 and 7.0 MJ m<sup>-2</sup> day<sup>-1</sup> in 2017). These color indices were not affected by IS, and their values are consistent with those obtained by Gu et al. [55] and Wang et al. [54].

Adequate deficit irrigation management requires evaluation of the economic impact of the yield reduction produced by water stress [14]. This enables growers to decide on whether or not to implement water reduction. The potential profitability of the deficit irrigation could be achieved through increasing the IWUE or reducing irrigation costs [56]. Moderate CDI (T2) or severe water restriction at juvenility (T7) would led to a slight decrease in the gross revenue (8 and 6%, respectively), even though they increase the IWUE, therefore increasing the water economic value (20 and 21%, respectively) compared to full irrigation (T1). Moderate water stress at juvenility (T4) caused a low reduction in the gross revenue (3%), whilst saving 11% of water, increasing the water economic value by 9% in relation to full irrigation (T1). In the other side, severe CDI (T3) increased the water economic value (54%), but it led to a noticeable reduction of the gross revenue and profits (22%) compared to full irrigation (T1), seriously reducing the economic viability of the crop.

### 2.2.6. Conclusions

This research analyzed the response of cauliflower "Naruto F1", in terms of plant growth and water status, productive response, curd characteristics and profitability, to different strategies of deficit irrigation.

Severe deficit irrigation applied during the first crop phases (juvenility and curd induction) reduces the plant height and diameter, as well as the number of leaves per plant, but only leads to a reduction of marketable yield and average curd size and weight when it is maintained throughout the crop cycle, despite leading to the highest WUE and IWUE values.

Continued deficit irrigation, applying 75% of the water requirements, or regulated deficit irrigation, applying 50% of the water requirements during juvenility, result in similar curd yields as fully irrigated plants, and therefore similar gross revenues with important water savings, improving IWUE. Therefore, these strategies are recommended.

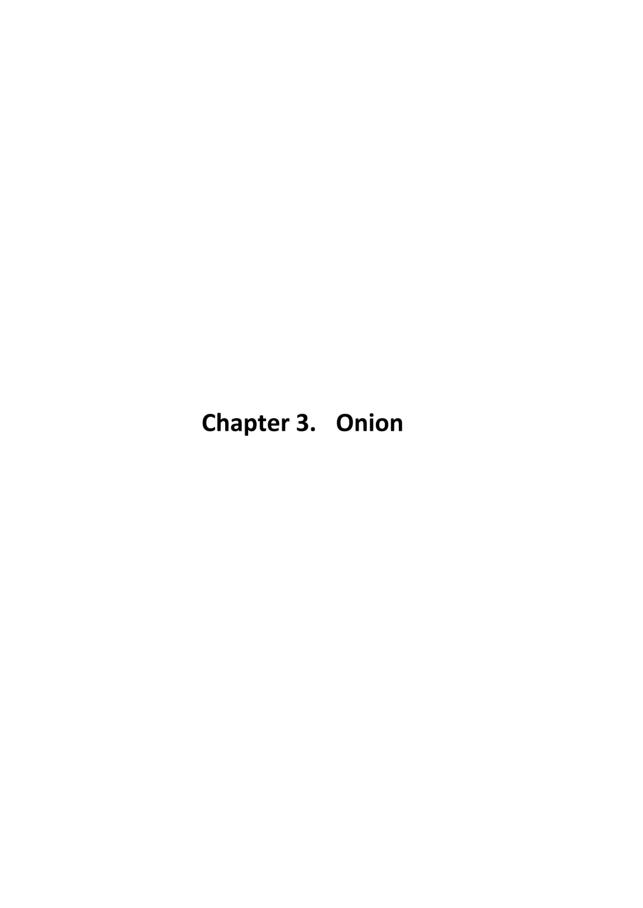
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# 3.1. Influence of Deficit Irrigation on Productive Response of Drip-Irrigated Onion (*Allium cepa* L.) in Mediterranean Conditions

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#### **3.1.1. Abstract**

Water is an essential resource for food production, and agriculture consumes close to 69% of total freshwater use. Water shortage is becoming critical in arid and semiarid areas worldwide; therefore, it is vital to use water efficiently. The objective of this research was to evaluate the response of onion growth, plant water status, bulb yield, irrigation water use efficiency and bulb quality using three continued deficit strategies, applying 100, 75, and 50% of the irrigation water requirements during three seasons. The yield response factor was 0.71, indicating that in the analysed conditions the crop was tolerant to a water deficit. Compared to full irrigation, deficit irrigation with 75% of the irrigation water requirements resulted in a low yield and profit reduction for the growers (10.3% and 10.9%, respectively), but also important water savings (26.6%), improving both the irrigation water use efficiency and water use efficiency. However, onion exposure to severe water deficits at 50% of the irrigation water requirements drastically reduced plant growth and bulb yield and growers' profits, although it did increase their soluble solid content. Irrigating at 75% of the irrigation water requirements could be an actionable strategy for onion production under water-limited conditions.

**Keywords:** bulb quality, bulb yield, irrigation water use efficiency, plant growth, plant water status.

#### 3.1.2. Introduction

Onions (*Allium cepa* L.) are an important vegetable crop around the world, ranking second behind tomatoes. Worldwide onion production in 2016 was approximately 93.2 million tonnes produced from 4.95 million ha. The major producing countries in 2016 were China, India, and the United States of America (Faostat, 2018).

Globally, water is at the core of sustainable development and considered an essential resource for food production (Howell, 2001). Agriculture uses large amounts of water; approximately 69% of the total consumption of freshwater around the world and in the Mediterranean region (Aquastat, 2018). The area of irrigated agriculture

leading to an increase in water requirements and in pressure on freshwater resources (Aquastat, 2018). Climate change will affect the agriculture sector as it will increase global temperatures. This will lead to potential evapotranspiration, reduced precipitation and alterations in precipitation distribution and patterns (Turral et al., 2011; Kang et al., 2017). Increasing water scarcity and irrigation costs have heightened interest in improving the productivity of water use in agriculture (Bessembinder et al., 2005; Fereres and Soriano, 2007) by using efficient irrigation management approaches and appropriate strategies that increase water productivity (Molden et al., 2010; Malek and Verburg, 2017). Irrigation water use efficiency (IWUE) and water use efficiency (WUE) are common indicators used to assess the efficiency of irrigation water use in crop production (Tolk and Howell, 2003; Pascual-Seva et al., 2016). Currently, the main aim is to increase crop production by maximizing IWUE and increasing crop production per unit of water applied. Within this context, the use of the deficit irrigation technique applies less irrigation than the optimum crop water requirements in order to improve water use efficiency (Pereira et al., 2002; Costa et al., 2007; Geerts and Raes, 2009; Galindo et al., 2018). The real challenge is to establish deficit irrigation while maintaining or even increasing crop production and saving irrigation water, thereby increasing the IWUE (Chai et al., 2016). For this reason, deficit irrigation requires accurate knowledge of the crop yield response to the water applied (Fereres and Soriano, 2007). Currently, deficit irrigation is a common practice throughout the world, especially in dry regions, where it is just as important to maximize crop water productivity as increase the harvest per unit of land (Kirda, 2002). The effects of deficit irrigation on yield and harvest quality are crop-specific; therefore, knowledge about how different crops respond to water deficits is essential for the optimal application of deficit irrigation (Costa et al., 2007). Furthermore, the extent of the water deficit is important not only for efficient water use and maximizing yield (Yang et al., 2017), but also for increasing farmers' profits (Fereres and Soriano, 2007). Doorenbos and Kassam (1979) introduced a linear crop-water production function to describe the reduction in yield when a crop is

increased worldwide from 196 million ha in 1973 to 325 million ha in 2013, naturally

under stress due to a shortage of soil water. The yield response factor  $(K_y)$  is a factor that describes the reduction in relative yield according to the reduction in crop evapotranspiration (ETc). Determining farmers' potential profits would help growers and technicians in decision-making regarding irrigation management.

Monitoring soil moisture by sensors is a technique that can contribute to crop irrigation scheduling, ensuring an adequate water status for the crop and limiting drainage, which in turn maximizes the water use efficiency in irrigation agriculture (Blanco et al., 2018; Gallardo and Thompson, 2018). Moreover, soil moisture monitoring can minimize the risk of yield reduction when using deficit irrigation strategies that are very restrictive in terms of using water (Fereres and Soriano, 2007).

The relative water content and membrane stability index are indicators of plant water status (Semida et al., 2017); the relative water content refers to the plant water content, and it has been used as a meaningful index of dehydration tolerance, while the membrane stability index indicates the integrity of cell membranes. It has also been widely used as an indicator of leaf desiccation tolerance (Abdelkhalik et al., 2019).

Onion roots are fasciculate, slightly ramified, short, and generally do not exceed a depth of 0.20–0.25 m in soil (Miguel, 2017). Due to this shallow root system, onions are very sensitive to water stress. Therefore, frequent and adequate irrigation management is required to achieve a good yield (Zheng et al., 2013; Temesgen et al., 2018). These characteristics have led to studies in different conditions, such as those carried out in a spring-summer cycle in a temperate Mediterranean climate (in Albacete, Spain, by Martín de Santa Olalla et al., 2004), in an arid climate (in Gansu, Norwest China, by Zheng et al., 2013), in an autumn-winter cycle in a humid subtropical climate (Uvalde, Texas, by Leskovar et al., 2012) and in an arid climate (in Fayoum, Egypt, by Semida et al., 2017). Results of these studies showed differences in the yield and bulb characteristics when using different deficit irrigation strategies, and the irrigation strategy can also affect the bulb quality. Onions have significant nutritional and medicinal properties, and are an important source of polyphenolic flavonoids (Leskovar et al., 2012). The effect of deficit

irrigation on onion phenolic content is still largely unknown (Leskovar et al., 2012). Specialized literature usually addresses different parameters separately, while the present study aims to analyse together the different parameters that reflect the onion response to deficit irrigation. The crop response to deficit irrigation varies with location, stress pattern, cultivar, planting date, and other factors (Fereres and Soriano, 2007); therefore, this response must be determined for the particular conditions in each cultivation area, in this case, the Mediterranean area. The objective of this research was to evaluate the vegetative and productive responses of onion plants, including plant water status, bulb quality and K<sub>y</sub>, IWUE and farmers' profit, using two deficit irrigation strategies in an autumn-winter cycle under Mediterranean conditions.

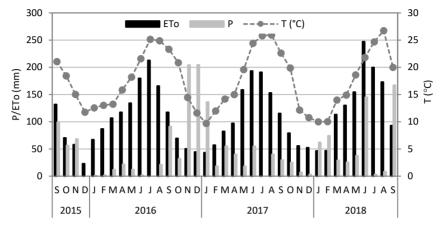
#### 3.1.3. Materials and Methods

# 3.1.3.1. Experimental site and the deficit irrigation strategies

The experiments were conducted during three growing seasons in 2016, 2017, and 2018 at the Cajamar Experimental Centre in Paiporta, Valencia, Spain (39.4175 N, 0.4184 W). To avoid soil replanting disorders resulting from serial onion cropping, two subplots within the experimental plot were used: plot 1 in 2016 and 2018, and plot 2 in 2017. The soils were deep, with a medium (silt loam) texture and classified as Petrocalcic Calcixerepts according to the USDA Soil Taxonomy (Soil Survey Staff, 2014). Soil analyses indicated that the soils of the two subplots were similar, being very slightly alkaline (pH = 7.4–7.5) and highly fertile [organic matter = 1.9–2.1% with high available phosphorous (43–45 mg kg<sup>-1</sup>; Olsen) and potassium (340 – 371 mg kg<sup>-1</sup>; ammonium acetate extract) concentrations].

Irrigation water was pumped from a well, with (on average) electrical conductivity of 2.16 dS m<sup>-1</sup> and 77 mg kg<sup>-1</sup> N-NO<sub>3-</sub> content. According to Papadakis's agro-climatic classification (Verheye, 2009), the climate is subtropical Mediterranean (Su, Me), with hot dry summers and an average annual rainfall of approximately 450 mm, irregularly distributed throughout the year, falling mostly

during the autumn and/or the end of winter/beginning of spring. Figure 1 shows the most significant climatological data in the growing seasons.



**Figure 1.** Monthly precipitation (P, mm), reference evapotranspiration (ETo, mm) and average temperature  $(T, {}^{\circ}C)$  during the three growing seasons.

The period from transplanting until establishment was considered as the initial period, and all the plants were irrigated without restrictions. Then, different irrigation strategies were initiated. This study comprised three irrigation strategies corresponding to 100% (*I100*), 75% (*I75*) and 50% (*I50*) of the irrigation water requirement (mm) during three growing seasons in 2016, 2017, and 2018.

#### 3.1.3.2. Irrigation scheduling

The irrigation water requirements were determined using the following equation:

$$IWR = \frac{ETc - Pe}{Ef}$$

where ETc is the crop evapotranspiration (mm), Pe is the effective precipitation (mm) determined from rainfall data using the method of the U.S. Bureau of Reclamation (Stamm, 1967) as presented by Pascual-Seva et al. (2016), and Ef is the irrigation efficiency (including percolation and uniformity), which was considered to be 0.95 (as stated for onion cultivars grown in the Experimental Centre). The ETc was calculated from the

reference evapotranspiration (ETo; mm) and a single crop coefficient proposed for local conditions by the Instituto Valenciano de Investigaciones Agrarias (IVIA, 2011), adapting the duration of each stage to the growing cycle. The crop coefficient values were 0.3, 0.95, and 0.8, corresponding to initial, mid-season, and late season stages, respectively.

$$ETc = ETo \times Kc$$

ETo was determined according to Allen et al. (1998) as follows:

$$ETo = E_{pan} \times K_p$$

where  $E_{pan}$  (mm) is the evaporation from a class A pan installed adjacent the experimental plot in the Experimental Centre, and  $K_p$  (0.815) is the pan coefficient determined according to Allen et al. (1998).

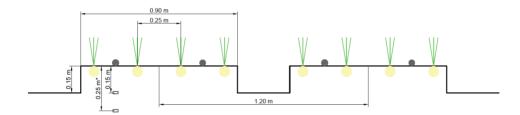
The irrigation water was supplied by a drip irrigation system, with two turbulent flow surface driplines of 16 mm per bed, with emitters spaced 0.33 m apart, and a discharge rate of 2.2 L h<sup>-1</sup>. The amount of irrigation water applied (IWA) for each irrigation event was recorded using total water flow meters connected to the irrigation system.

The volumetric soil water content (m³ m⁻³) was continuously monitored using ECH₂O EC-5 capacitance sensors connected to an Em50 data logger, using the ECH₂O Utility software (Decagon Devices Inc., Pullman, WA, USA). Given that most roots were concentrated in the top 0.20 m of the soil, in each treatment one sensor was installed horizontally at a depth of 0.15 m below a dripline and equidistant between two adjacent emitters following the methodology described by Enciso et al. (2009), (Figure 2). Additionally, in 1100, another sensor was placed at a depth of 0.25 m to verify that water losses at depth were almost negligible. The volumetric soil water content was

measured and stored at 15 min intervals, and its variations were used to determine the in-situ field capacity. To compare the soil moisture level with the different irrigation strategies, it is presented as the ratio of the volumetric soil water content at each moment to that at field capacity (%). The irrigation events of *I100* began when the volumetric soil water content fell to a value of 80% field capacity, and in the other irrigation strategies they started at the same time with corresponding reductions in IWA.

# 3.1.3.3. Plant material and cropping system

The onion 'Hamaemi' (Agriseeds Ibérica S.L., Valencia, Spain) was used in the experiments. It is one of the most grown cultivars in the area because it is a tender onion that is appreciated by the local market, and because of its adaptation to the soil and climatic conditions in the area, as evaluated by Cajamar Experimental Centre (Fundación Ruralcaja; 2005, 2006). The bulbs are medium size, with a flattened globose shape and a straw yellow color.



**Figure 2.** Schematic representation of the irrigation system and location of the sensors installed. \*: only in full irrigation.

Plants were transplanted in an open field when they reached the two-leaf stage on 19 November 2015, 4 November 2016, and 30 October 2017. Seeds were sown 45 days before transplanting in 448 cell flexible polyethylene trays in a peat moss based substrate (70% blonde and 30% dark) recommended for vegetable seedbeds (Pindstrup Mosebrug S.A.E., Sotopalacios, Spain). They were maintained in a Venlo-Type greenhouse. Transplanting was done with

a four row onion transplanter (Minoru Industrial co., ltd., Okayama, Japan), with plant and row spacing of  $0.11~\text{m}\times0.25~\text{m}$  and with four plant rows per bed. The top of the flat raised bed was 0.90~m wide (the distance from the bed centre-to-centre was 1.20~m; Figure 2). The raised bed had a length of 7.25~m and a height of 0.15~m. The incorporation of nutrients ( $200\text{-}100\text{-}250~\text{kg ha}^{-1}$  N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O) was performed by fertigation following the criteria described by Miguel (2017).

#### 3.1.3.4. Measurements

#### 3.1.3.4.1. Plant growth, relative water content and membrane stability index

Three onion plants per plot were selected a week before harvest to determine the plant growth parameters: plant height, leaf number per plant, bulb diameter, neck (pseudostem) diameter and bulbing ratio (bulb diameter/neck diameter). The leaf chlorophyll index (SPAD) was measured at three points in three fully developed leaves of each plant using a SPAD-502 m (Konica Minolta Sensing Inc., Tokyo, Japan). Next, these plants were separated into leaves and bulbs and weighed (fresh weight). Then, these parts were dried at 65°C in a forced-air oven (Selecta 297, Barcelona, Spain) until reaching a constant weight to obtain the dry weight and dry matter content. The harvest index was determined as the ratio of yield to total biomass (leaves + bulbs) on a dry mass basis (g g-1; Turner, 2004).

Leaf relative water content (%) was determined in fresh leaf discs of 2 cm diameter using the method developed by Hayat et al. (2007). The membrane stability index (%) was estimated using 0.2 g samples of fully expanded leaf tissue following the methodology described by Rady (2011). Relative water content and membrane stability index were evaluated every 30 days during the crop cycle.

# 3.1.3.4.2. Bulb yields, irrigation water use efficiency and yield response factor

Yield was determined from 3 m lengths of the two central plant rows, leaving a plant row on each side of the bed to avoid marginal effects. Bulbs were harvested

two weeks after 50% of the leaves (by the pseudostems) were bent over, on 2 May 2016, 20 April 2017, and 7 May 2018. Total bulb yield was separated into marketable and non-marketable yield. For the marketable yield, the average bulb weight was determined. The non-marketable yield was separated according to the nature of blemishes, small bulbs (diameter less than 55 mm), deformed bulbs and bolting plants, according to Leskovar et al. (2012).

The IWUE was calculated as the ratio of marketable yield (fresh mass; kg m<sup>-2</sup>) to the IWA (m<sup>3</sup> m<sup>-2</sup>; Cabello et al., 2009). The WUE was calculated as the ratio of marketable yield (kg m<sup>-2</sup>) to IWA + effective precipitation (m<sup>3</sup> m<sup>-2</sup>; Ko and Piccinni, 2009). The yield response to water deficits during the growing season was determined according to Doorenbos and Kassam (1979), using the following equation:

$$\left(1 - \frac{Y_a}{Y_m}\right) = K_y \left(1 - \frac{ET_a}{ET_m}\right)$$

where Ya and Ym are the actual and maximum (fully irrigated) marketable yield (kg m $^{-2}$ ), respectively; ETa and ETm are the actual and maximum ET (mm), respectively; and  $K_y$  is the yield response factor. ETa and ETm were calculated using the soil water balance as follows: ET = IWA + effective precipitation, considering both the drainage and the variation in the volumetric soil water content to be negligible.

# 3.1.3.4.3. Onion bulb quality

Three representative marketable bulbs per plot were selected to determine bulb size (diameter and height) and shape (relation of diameter/height). Afterwards, these bulbs were used to determine bulb external firmness using a digital penetrometer with a tip 8 mm in diameter (Penefel DFT 14, Agro Technologies, Forges les Eaux, France). The soluble solids content (°Brix) was determined with bulb juice using a digital refractometer (PAL-1; Atago, Tokyo, Japan). Acidity was determined as citric acid (%) by titration with 0.1 M NaOH. The polyphenol content in bulbs was determined as described by Domene et al. (2014). The total carbohydrates of bulbs

were determined according to BeMiller (2014). Proteins were determined by the Kjeldahl method, as described by Chang (2003).

# 3.1.3.5. Experimental design and statistical analysis

The experiments were performed in a randomized complete block design with three replicates. Each experimental plot area was 26.1 m<sup>2</sup>, and each plot included three beds. The results were analysed using analysis of variance (ANOVA) with Statgraphics centurion XVII (Statistical Graphics Corporation, 2014) software. Percentage data were arcsin transformed before analysis. The least significant difference (LSD) at a 0.05-probability level was used as the mean separation test.

#### **3.1.4.** Results

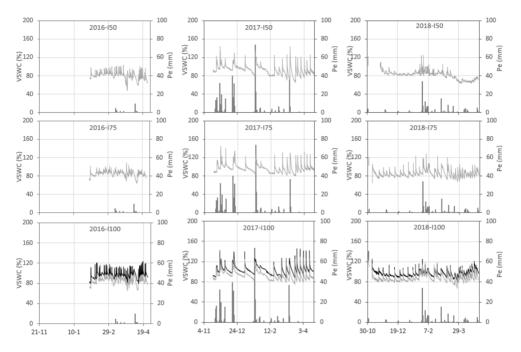
# 3.1.4.1. Growth periods and irrigation water applied

The total growth cycle periods were 166, 168, and 190 days in 2016, 2017, and 2018, respectively. The total pan evaporation and reference evapotranspiration were lower in 2017 (334 and 272 mm, respectively) than in 2016 (498 and 406 mm) and 2018 (576 and 469 mm). Effective precipitation was higher in 2017 (387 mm) than in 2016 (28 mm) and 2018 (148 mm). Initially, all treatments were irrigated with 40, 28, and 37 mm in 2016, 2017 and 2018, respectively, as initial irrigation amounts to ensure adequate plant establishment. The IWA volumes during the differential irrigation periods of *I100*, *I75*, and *I50* were 356, 261, 180 mm, respectively, in 2016, 167, 120, 79.5 mm, in 2017, and 344, 260, 172 mm, in 2018.

#### 3.1.4.2. Volumetric soil water content

Figure 3 shows the volumetric soil water content for the three irrigation strategies, as well as the effective precipitation, in 2016, 2017, and 2018. Generally, the volumetric soil water content values of the three treatments at a depth of 0.15 m were higher in 2017 (on average, 92.9% of the field capacity) than in 2018 (on average, 84.6% of the field capacity) or 2016 (on average, 83.6% of the field capacity) due to

the higher precipitation in 2017. Soil moisture was higher in *I100* than in *I75*, which in turn was higher than that in *I50* (on average 88.8, 87.6, and 84.7% of the field capacity, respectively), as expected. Volumetric soil water content at 0.25 m soil depth in *I100* in each season did not show any increase in the average values, indicating that deep percolation below the root zone was negligible.



**Figure 3.** Volumetric soil water content (VSWC, -15 cm, -25 cm) for each irrigation strategy and daily effective rainfall (Pe, -15) during the 2016, 2017 and 2018 seasons.

#### 3.1.4.3. Plant growth, relative water content and the membrane stability index

The higher effective precipitation registered in 2017 compared with that in 2016 and 2018, reduced the number of irrigation events, as well as the corresponding IWA, minimizing the effect of the irrigation strategies on the different parameters in 2017, unlike what happened in 2016 and 2018. This fact is responsible for the statistical significance of the interaction between irrigation strategy and growing season in most of the studied parameters (Tables 1, 3, 4;  $P \le 0.01/0.05$ ), so no further comments on the interactions will be made. Lower values for plant growth traits (P)

 $\leq$  0.01; Table 1), yield components (total and marketable yield and average bulb weight; Table 3), WUE, bulb size (bulb diameter) and shape (diameter/height), and bulb quality (soluble solids content, acidity, and carbohydrates; Table 4) were obtained in 2017 compared to 2016 and 2018. In contrast, the IWUE, relative water content, membrane stability index and bulbing ratio were higher ( $P \leq$  0.01) in 2017. The lowest percentage of non-marketable yield (Table 3;  $P \leq$  0.01) was obtained in 2016 because of the absence of deformed bulbs and bolting plants, while in the other years these discards represented 1.5% and 7.4% (on average), respectively.

**Table 1.** Effect of the growing season and the irrigation strategy on plant height, leaf number per plant (LN), leaf chlorophyll content (SPAD), leaf fresh weight (LFW), leaf dry matter content (LDMC), neck diameter (ND), bulbing ratio, and harvest index (HI).

	Plant height	LN	SPAD	LFW	LDMC	ND	Bulbing ratio	HI
	(cm)		(-)	(g plant <sup>-1</sup> )	(%)	(mm)	(-)	(-)
Growing season (GS)								
2016	73.51 a	7.59 a	62.84	79.61 a	8.70 c	15.68 a	6.12 b	0.75 c
2017	47.67 c	6.81 b	61.13	21.42 c	14.75a	10.39 c	7.29 a	0.80 b
2018	55.56 b	7.30 a	63.64	36.70 b	10.42b	13.23 b	6.47 b	0.86 a
LSD	2.20	0.47	2.83	5.86	1.21	0.90	0.48	0.02
Irrigation strategies (IS)								
I100	63.37 a	7.67 a	62.80	59.20 a	10.67	14.33 a	6.59	0.80
I75	57.93 b	7.04 b	61.21	42.16 b	11.45	12.97 b	6.57	0.81
I50	55.44 c	7.00 c	63.59	36.37 c	11.42	12.01 c	6.72	0.81
LSD	2.20	0.47	2.83	5.91	1.21	0.90	0.48	0.02
ANOVA (df)	% sum of squares							
GS (2)	77.9 **	10.6 **	4.1 ns	72.1 **	57.1**	52.7 **	23.3 **	55.2**
IS (2)	7.3 **	9.7 **	3.7 ns	8.8 **	1.5 ns	10.2 **	0.4 ns	1.5
GS*IS (4)	5.1 **	12.1 **	1.5 ns	8.6 **	1.7 ns	9.6 **	8.5 ns	4.2
Residuals (72)	9.7	67.6	90.7	10.5	39.6	27.6	67.9	39.0
Standard deviation	4.1	0.9	5.2	10.9	2.2	1.7	0.9	0.0

df: degrees of freedom. Mean values followed by different lower-case letters in each column indicate significant differences at  $P \le 0.05$  using the LSD test. \*\*: significant differences at  $P \le 0.01$ . ns: no significant difference.

Deficit irrigation regimes reduced ( $P \le 0.01$ ) onion plant growth (Table 1), and plant height, number of leaves/plant, neck diameter and leaf fresh weight decreased with increasing water restriction. The leaf chlorophyll index (SPAD), bulbing ratio, leaf dry matter content and harvest index were not affected ( $P \le 0.05$ ) by the irrigation strategy. The 2018 season resulted in a higher harvest index, followed by 2017, and finally, 2016.

The lowest average relative water content was recorded in 2016, while the highest membrane stability index was recorded in 2017, probably due to the higher volumetric soil water content (on average 93% of the field capacity; Table 2) monitored in this season. The irrigation strategy did not affect ( $P \le 0.05$ ) the relative water content or the membrane stability index values at 70 days after transplanting; however, from the analysis performed at 100 days, both parameters decreased ( $P \le 0.05$ ) with increasing deficit irrigation, showing the biggest differences between strategies at 160 days after transplanting (Table 2).

**Table 2.** Effect of the growing season and the irrigation strategy on the relative water content (RWC) and membrane stability index (MSI) at different days after transplanting (DAT).

	70 D	AT	160	DAT		
	RWC (%)	MSI (%)	RWC (%)	MSI (%)		
Growing season (GS)						
2016	79.27 b	62.94 b	73.34 b	53.21 b		
2017	81.12 a	69.80 a	80.39 a	67.93 a		
2018	81.24 a	68.70 a	80.69 a	58.05 b		
LSD	1.82	2.28	2.51	5.03		
Irrigation strategies (IS)						
1100	79.34	68.51	81.77 a	65.73 a		
<i>175</i>	78.38	66.97	78.72 b	60.07 b		
<i>I50</i>	77.21	65.95	73.93 с	53.38 c		
LSD	1.82	3.08	2.51	5.03		
ANOVA (df)	% sum of squares					
GS (2)	70.9 **	61.7 **	43.1 **	43.8 **		
IS (2)	6.6 ns	7.6 ns	39.0 **	29.7 **		
GS*IS (4)	3.1 ns	6.6 ns	1.9 ns	6.4 ns		
Residuals (18)	19.5	24.1	16.0	20.1		
Standard deviation	1.8	2.3	2.5	5.1		

df: degrees of freedom. Mean values followed by different lower-case letters in each column indicate significant differences at  $P \le 0.05$  using the LSD test. \*\*: significant differences at  $P \le 0.01$ . ns: no significant difference.

# 3.1.4.4. Bulb yield, irrigation water use efficiency and yield response factor

Total bulb yield, marketable yield and average bulb weight significantly decreased as IWA decreased. *I100* resulted in the highest values, while *I50* led to the lowest, with intermediate values for *I75* ( $P \le 0.01$ ; Table 3).

In general, reducing IWA down to 75% of the irrigation water requirements led to an improvement in the IWUE because of the water savings (on average 26.6%) and, at the same time, a low yield reduction (on average 11.1%) compared to *I100*. Applying the 50% irrigation water requirement resulted in a drastic reduction in bulb yield (on average 29.4%), but the water savings (on average 50.6%) resulted in the highest IWUE.

**Table 3.** Effect of the growing season and the irrigation strategy on the total yield (TY), non-marketable yield (NMY), marketable yield (MY), average bulb weight (ABW), water use efficiency (WUE), and irrigation water use efficiency (IWUE).

	TY	NMY	MY	ABW	WUE	IWUE
	(kg m <sup>-2</sup> )	(%)	(kg m <sup>-2</sup> )	(g bulb <sup>-1</sup> )	(kg m <sup>-3</sup> )	(kg m <sup>-3</sup> )
Growing season (GS)						
2016	7.98 a	4.72 b	7.60 a	304.36 a	27.1 a	29.52 b
2017	4.71 b	14.94 a	4.01 c	172.25 b	7.90 c	35.48 a
2018	8.04 a	15.47 a	6.86 b	311.30 a	16.84 b	27.24 b
LSD	0.43	3.99	0.46	12.52	1.33	2.61
Irrigation strategies (IS)						
1100	7.80 a	9.43	7.12 a	292.96 a	16.03	24.76 c
<i>175</i>	7.09 b	11.76	6.32 b	260.05 b	17.64	30.28 b
<i>I50</i>	5.85 c	13.93	5.02 c	234.90 с	18.21	37.19 a
LSD	0.66	3.99	0.66	17.22	2.12	4.09
ANOVA (df)		% sun	n of squares			
GS (2)	62.7 **	49.5 **	59.5 **	77.6 **	88.9 **	17.9 **
IS (2)	16.8 **	6.8 ns	18.6 **	10.7**	1.2 ns	38.3 **
GS*IS (4)	5.9 **	21.7 *	6.3 **	2.7**	2.2 **	13.2 **
Residuals (18)	14.5	21.9	15.6	9	7.7	30.6
Standard deviation	0.8	4	0.8	23.1	2.4	4.8

df: degrees of freedom. Mean values followed by different lower-case letters in each column indicate significant differences at  $P \le 0.05$  using the LSD test. \*\*: significant differences at  $P \le 0.01$ . ns: no significant difference.

Marketable bulb yield increased linearly ( $P \le 0.01$ ) with IWA, and the positive linear relationships were expressed as follows: marketable yield = 1.929 + 0.0196 IWA, with a high correlation coefficient (r = 0.96). IWUE decreased linearly ( $P \le 0.01$ ) with increasing IWA, following the expression IWUE = 43.165 - 0.057 IWA (r = -0.63), while the opposite was observed for WUE, and the relationship was expressed as WUE = 7.022 + 0.047 IWA (r = 0.51). The fitted linear regression of the onion yield response to water deficits during the three seasons was significant (P)

 $\leq$  0.01) as follows: 1 - (Ya/Ym) = 0.71 [1 - (ETa/ETm)], producing a high correlation coefficient (r=0.96). The yield response factor ( $K_y$ ) was 0.71 considering all three years together; if the growing seasons were considered separately, the values were 0.67, 0.71, and 0.76 for 2016, 2017, and 2018, respectively.

### 3.1.4.5. Onion bulb quality

The bulb size was affected ( $P \le 0.01$ ; Table 4) by the irrigation strategy. The lowest bulb size (diameter and height) corresponded to I75 and I50. Bulb shape and dry matter content were not significantly ( $P \le 0.05$ ) affected by the irrigation strategy (Table 4).

**Table 4.** Effect of the growing season and the irrigation strategy on bulb characteristics: size [diameter (D) and height (H) and shape (D/H)], dry matter content (DMC), firmness, soluble solid content (SSC), acidity, and polyphenol (Pph), carbohydrate (Ch) and protein (Pr) content.

	D (mm)	H (mm)	D/H	DMC (%)	Firmness (N)	SSC (°Brix)	Acidity (%)	Pph (mg GA/100 g)	Ch (mg/100g)	Pr (g/100 g)
Growing seaso	n (GS)									
2016	88.44 a	70.94	1.25 a	6.54 c	29.73	8.07 a	0.10 a	177.70	50.66 b	0.927 b
2017	74.46 c	70.72	1.06 b	7.41 b	26.86	5.18 c	0.08 b	168.92	38.68 c	1.161 a
2018	84.95 b	69.30	1.23 a	8.39 a	29.67	7.64 b	0.10 a	183.02	56.34 a	0.924 b
LSD	3.26	2.80	0.05	0.41	3.28	0.31	0.01	29.77	4.86	0.87
Irrigation strate	egies (IS)									
1100	87.13 a	73.24a	1.19	7.47	28.64	6.85 b	0.10 a	166.57	48.02	1.049
<i>175</i>	81.21 b	69.88b	1.17	7.36	28.66	6.79 b	0.09 ab	187.36	48.70	0.986
<i>I50</i>	79.52 b	67.84b	1.18	7.51	28.95	7.25 a	0.08 b	175.72	48.97	0.976
LSD	3.26	2.80	0.05	0.41	3.28	0.31	0.01	29.77	4.86	0.87
ANOVA (df)	% sum of squares									
GS (2)	41.9 **	1.5ns	46.0**	49.6**	19.0 ns	89.9 **	33.3 **	4.6 ns	69.8 **	64.1 **
IS (2)	12.6 **	14.1**	0.6 ns	0.4 ns	0.2 ns	2.3 *	20.9 *	9.9 ns	0.2 ns	5.3 ns
GS*IS (4)	7.3 *	16.9**	2.8 ns	5.7 ns	3.4 ns	4.2 **	8.4 ns	3.2 ns	9.4 ns	3.8 ns
Residuals (18)	38.1	67.5	50.5	44.4	77.3	3.5	37.4	82.3	20.7	26.8
SD	6.0	5.2	0.1	0.8	3.3	0.3	0.0	30.1	4.9	0.9

df: degrees of freedom. SD: standard deviation. Mean values followed by different lower-case letters in each column indicate significant differences at  $P \le 0.05$  using the LSD test. \*\* (\*): Indicates significant differences at  $P \le 0.01$  ( $P \le 0.05$ ). ns: Indicates no significant difference.

The irrigation strategy affected ( $P \le 0.05$ ) bulb soluble solids content and acidity; I50 produced the highest soluble solids content and the lowest acidity (Table 4). The irrigation strategy did not affect ( $P \le 0.05$ ) bulb firmness or polyphenol,

carbohydrate or protein contents (Table 4). Although not significantly, water deficit regimes increased the polyphenol content and reduced the protein content.

#### 3.1.5. Discussion

Doorenbos and Kassam (1979) and Ortolá and Knox (2015) reported that onion irrigation water requirements ranged from 350 and 550 mm, representing the lowest threshold of the water requirements in 2016 and 2018, respectively. The lower requirements in 2017 were related to the higher effective precipitation and lower evaporative demand that were recorded in that year compared with the other two years. The recorded IWA values were similar to those found by Leskovar et al. (2012) in a similar onion growing cycle in a humid subtropical climate with similar irrigation strategies. The volumes applied in the full irrigation strategy were similar to those applied by Martín de Santa Olalla et al. (1994) and Zheng et al. (2013).

At the beginning of the crop cycle, the plant water status did not show any difference between treatments, either for the relative water content or the membrane stability index. However, at the end of the cycle, plants under deficit irrigation strategies showed lower values of both parameters, indicating that they had poorer water status than the fully irrigated plants. This was probably related to the lower soil moisture in the deficit irrigated plants, and was also reported by Semida et al. (2017) and Wakchaure et al. (2018). Leskovar et al. (2012) and Semida et al. (2017) observed that onion plant growth and bulb yield decreased with deficit irrigation as found in this study. Stomata are sensitive to changes in soil water potential and they close in response to drying soil (Costa et al., 2007). This fact is particularly important for plants that have a shallow root system that is very sensitive to water stress, as is the case for onions. Stomatal closure decreases the internal CO<sub>2</sub> availability, and this directly affects the rate of photosynthesis and overall plant growth (Osakabe et al., 2014), leading to a reduction in plant yield.

The regulation of stomatal apertures is a central process to determine the WUE. Given the linear relationship that exists between stomatal conductance and

transpiration under a constant vapour pressure deficit of air, and the non-linear relationship between stomatal conductance and the photosynthetic rate, lower stomatal apertures may improve water use efficiency (Chaves et al., 2002). These relationships explain the higher IWUE values obtained with deficit irrigation than with the fully irrigated plants.

Full irrigation led to the highest bulb average weight (and size - diameter and height), and these decreased with water reduction. These results agree with those described by Kumar et al. (2007) and Leskovar et al. (2012).

Values of Ky lower than 1 indicate that under those conditions a crop is tolerant to a water deficit, while values of  $K_y$  greater than 1 indicate that a crop is sensitive to a water deficit (Doorenbos and Kassam, 1979; Steduto et al., 2012). In this study, Ky values were lower than 1; therefore, this can be considered as tolerant to the water deficit, in contrast to the result obtained by Kadayifci et al. (2005), which was 1.50, probably because they carried out the experiment in a summer-autumn cycle in Turkey, with more limiting conditions.

Average values of the bulb quality parameters analysed in this study are in agreement with those presented in the literature. Particularly, the soluble solids content values are in agreement with those presented by Leskovar et al. (2012); the protein and carbohydrate contents are in agreement with those presented by the Spanish database of food composition (BEDCA), which was set up according to the European standards of the European network of excellence EuroFir (BEDCA, 2006); the phenolic contents are in accordance with those obtained by Leskovar et al. (2012) and Wakchaure et al. (2018).

Reducing IWA to 50% of the irrigation water requirements increased the soluble solids content and reduced the acidity of the bulbs, probably due to the earlier bulb maturity caused by this strategy. This fact is related to comments by Zheng et al. (2013), who indicated that plants accelerated their growth process in response to water deficit, decreasing cell multiplication and expansion and thus reducing bulb

yield. An increase in the soluble solids content and a decrease in acidity occurs during the ripening process, and leaves in *I50* were bent over a week earlier than those in full irrigation, in agreement with Zheng et al. (2013) and Wakchaure et al. (2018), who observed that early bulb maturity corresponded with the most restrictive treatment. Similar results were reported by Semida et al. (2017), who obtained higher soluble solids content with the most severe drought stress.

Water deficit strategies did not significantly affect the total phenolic and protein contents (or the carbohydrate content) as found by Leskovar et al. (2012; who analysed the quercetin content).

In average Mediterranean climatic conditions, if water is not the limiting factor, *I100* may be recommended, since it leads to the maximum yield and maximum profit for the grower without differences in bulb quality. However, if water is scarce, *I75* could be applied because although yield and grower profits may be reduced, it will lead to important water savings. In rainy seasons, *I50* can be also recommended, since yield and bulb quality are not negatively affected by the irrigation decrease.

#### 3.1.6. Conclusions

The present study analysed the effects of continued deficit irrigation on the growth, plant water status, productive response, and irrigation water use efficiency

of the onion 'Hamaemi'. Taking into account that the productive response depends on the climate, and particularly on rainfall, under average conditions the marketable yield linearly increased with more irrigation water applied, while the irrigation water use efficiency decreased, and both had high correlation coefficients. The yield response factor was 0.71, indicating that under the analysed conditions, the crop is tolerant to water deficits. Reducing the water applied to 50% of the water requirements led to the highest irrigation water use efficiency, and resulted in important water savings. Nevertheless, it drastically reduced bulb yield and growers' profits. Reducing the water applied to 75% of the water requirements resulted in a low yield and profit reduction, but important water savings compared with full irrigation, improving the irrigation water use efficiency. This is the recommended strategy for onion production under Mediterranean conditions.

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# 3.2. Regulated Deficit Irrigation as a Water-saving Strategy for Onion Cultivation in Mediterranean Conditions

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Pending editor decision after minor changes in Agronomy

#### **3.2.1. Abstract**

Field experiments were performed for two growing seasons in Spain under Mediterranean conditions to evaluate the response of onion growth, plant water status, bulb yield, irrigation water use efficiency (IWUE) and gross revenue to regulated deficit irrigation strategies (RDI). Seven irrigation treatments were utilized, including the application of 100% irrigation water requirements (IWR) during the entire growing season and the application of 75 or 50% of the IWR during one of the following growth stages: the vegetative growth, bulbing, and bulb ripening stages. The deficit irrigation strategies tested decreased marketable yields to greater or lesser extents; therefore, if water is readily available, full irrigation would be recommended. The RDI with 50% of the IWR during the bulb ripening stage led to important water savings (22%) and to slight decreases in yield (9%), improving IWUE (20%) compared to full irrigation, and this strategy can be recommended under a severe water shortage. Satisfactory bulb yield was obtained with RDI with 75% of the IWR during the bulb ripening stages, resulting in a lower reduction in yield (4%) and in an increased IWUE (9%); this strategy is an advisable strategy for onion production under a mild water shortage in Mediterranean conditions.

**Keywords:** Irrigation water requirements; irrigation water applied; irrigation water use efficiency; volumetric soil water content; relative water content; membrane stability index; plant growth; bulb yield; yield response factor; gross revenue.

# 3.2.2. Introduction

Onion (*Allium cepa* L.) is the second most important vegetable crop worldwide, producing approximately 98 million tons on 5.20 million ha in 2017. Globally, China, India and the USA were the major onion-producing countries in 2017, whereas Russia, the Netherlands and Spain are the principle onion producers in the European Union [1]. Onions are traditionally cultivated in Valencia (Spain) in winter within the traditional crop rotations.

Irrigation water is a crucial resource for sustainable agricultural development worldwide. In arid and semiarid areas, including the Mediterranean region, water scarcity is becoming critical, increasing competition for water among agricultural, industrial and urban consumers [2,3]. Agriculture is the largest user of water worldwide, accounting for approximately 69% of the total consumption of freshwater [4]. The total irrigated agricultural area was approximately 40 million ha in 1900, and it has increased more than eightfold worldwide over the last century to approximately 325 million ha; consequently, water withdrawal has increased from less than 600 km³ year¹ to approximately 4,000 km³ year¹ [4,5]. Population growth, urbanization, the increase of irrigated agriculture and the greater incidence of drought caused by climate change, particularly in the Mediterranean area, indicate that irrigation water demand, as well as irrigation costs, will continue to increase in the future [6]. Furthermore, the Mediterranean area has low water resources per habitant and is thus considered a water-stressed area and faces a great challenge to cope with water scarcity [7,8].

The increase in irrigation costs and water scarcity have increased interest in improving water productivity for irrigated agriculture [9,10], which can be achieved by both efficient irrigation design and appropriate irrigation management [11,12]. Within this context, a deficit irrigation strategy is a sustainable practice of applying irrigation levels that are below the optimum crop water requirements, improving water productivity [10,13–17]. Plants respond differently to water reductions applied at different development stages; therefore, their yield responses vary depending on their sensitivity at each growth stage [2,18]. Regulated deficit irrigation (RDI) is a stage-based DI and consists of imposing water deficits at particular phenological stages, when the crop is less sensitive to water stress [10,17,19]. Therefore, to apply the RDI approach effectively, identifying the most critical growth stages for a specific crop species and cultivar is needed [16].

Irrigation water use efficiency (IWUE) is a key variable used to assess the efficiency of irrigation water use in crop production [20] and is a practical index for

the assessment of plant responses to deficit irrigation [16,19]. Enhancing IWUE in irrigated agriculture increases the yield per unit of water applied [21]. Under limited water conditions, one of the main goals of farmers and researchers is to maximize IWUE rather than to increase yields [19]. The yield response factor (K<sub>y</sub>) represents the relationship between a relative yield decrease and a relative water deficit, providing quantitative evaluation of yield responses to soil water deficits during the growing season [22,23]. The relative water content (RWC) and membrane stability index (MSI) are indicators of plant water status [24]. The RWC refers to the plant water content, and it has been used as a meaningful index for dehydration tolerance, while the MSI detects the integrity of cell membranes, and it has also been widely used as an indicator of leaf desiccation tolerance [25].

Onion plants possess shallow-root systems with most parts of the roots in the top 0.20 m of the soil [26,27]; therefore, onions require frequent and light water applications to avoid incurring large soil water deficits [28,29]. Hence, onions are very sensitive to water stress, requiring adequate irrigation management to achieve high commercial yields. Such sensitivity has been observed by Leskovar et al. [30] in Texas, USA, Zheng et al. [28] in northwestern China, Semida et al. [24] in Egypt and Rop et al. [31] in Kenya. These studies observed reductions in bulb yield and size under water deficits. Compared to the bulb ripening stage, onions are more sensitive to soil water deficits at the bulbing [32] and vegetative growth [28] stages. Water restriction during the vegetative growth and bulbing stages result in the highest percentages of small size bulbs in Spain [33].

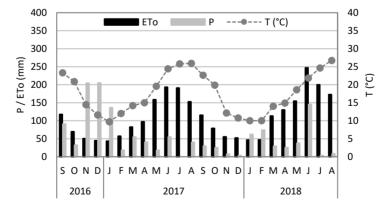
The crop response to deficit irrigation varies with location, stress patterns, cultivar, planting dates, and other factors [10], and it is therefore important to determine the onion response to deficit irrigation for the particular conditions within a traditional Valencian crop rotation. The objective of this study was to determine the effects of RDI on the growth, plant water status, yield, bulb quality, IWUE and crop profitability of onions cultivated under Mediterranean conditions.

#### 3.2.3. Materials and methods

# 3.2.3.1. Experimental site conditions

Two field experiments were carried out during the 2017 and 2018 seasons at the Cajamar Experimental Centre in Paiporta, Valencia, Spain (39.4175 N, 0.4184 W). These experiments were performed in two different plots to avoid soil diseases caused by repeated onion cultivation [26]. Both soil plots are deep with a medium (silt loam) texture and are classified as Petrocalcic Calcixerepts according to the USDA Soil Taxonomy [34]. The soils of the two plots are similar, being very slightly alkaline (pH = 7.4 - 7.5) and highly fertile [organic matter = 1.9 - 2.1%, with highly available phosphorous (43 - 45 mg kg<sup>-1</sup>; Olsen) and potassium (340 - 371 mg kg<sup>-1</sup>; ammonium acetate extract) concentrations]. Irrigation water was pumped from a well, with (on average) an EC of 1.16 dS m<sup>-1</sup> and a 77 mg kg<sup>-1</sup> N-NO<sub>3</sub><sup>-</sup> content.

According to the Papadakis agro-climatic classification [35], the climate is subtropical Mediterranean (Su, Me) with hot dry summers and an average annual rainfall of approximately 450 mm, irregularly distributed throughout the year, falling mostly during the autumn and at the end of winter/beginning of spring. Figure 1 shows the most significant climatological data of the growing seasons.



**Figure 1.** Monthly precipitation (P, mm), reference evapotranspiration (ETo, mm) and average temperature  $(T, {}^{\circ}C)$  during the two growing seasons.

#### 3.2.3.2. Plant material and growth conditions

The onion 'Hamaemi' was used in these experiments. This onion produces medium size bulbs, with a straw yellow colour and flattened globose shapes. This cultivar is suitable for producing tender onions, which are appreciated by the local market, and it is well adapted to the soil and climatic conditions in the area [36].

Seeds were sowed on 20 September 2016 and 15 September 2017, in 448 cell flexible polyethylene trays in a peat moss based substrate (70% blonde and 30% dark; Pindstrup Mosebrug S.A.E., Sotopalacios, Spain) and placed in a Venlo-type greenhouse. Seedlings were transplanted to an open field when the plants had reached the two-leaf stage on 4 November 2016 and 30 October 2017. The transplantation was accomplished with a four-row onion transplanter (Minoru, Fukui, Japan), with plant and row spacings of 0.11 m × 0.25 m and with four plant rows per bed. The top of the flat raised bed was 0.90 m wide (the distance from the bed centre-to-centre was 1.20 m). The flat raised bed had a length of 7.25 m and a height of 0.15 m, with north-south orientation. Each experimental plot consisted of a bed (8.7 m² and 264 plants). The incorporation of nutrients (200-100-250 kg ha¹¹ N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O) was performed by fertigation with a nutrient solution based on the Sonneveld and Straverd [37] solution, following the criteria described by Miguel [38].

# 3.2.3.3. Deficit irrigation strategies and growth stages

The onion 'Hamaemi' was used in these experiments. This onion produces medium size bulbs, with a straw yellow colour and flattened globose shapes. This cultivar is suitable for producing tender onions, which are appreciated by the local market, and it is well adapted to the soil and climatic conditions in the area [36].

Seeds were sowed on 20 September 2016 and 15 September 2017, in 448 cell flexible polyethylene trays in a peat moss based substrate (70% blonde and 30% dark; Pindstrup Mosebrug S.A.E., Sotopalacios, Spain) and placed in a Venlo-type greenhouse. Seedlings were transplanted to an open field when the plants had

reached the two-leaf stage on 4 November 2016 and 30 October 2017. The transplantation was accomplished with a four-row onion transplanter (Minoru, Fukui, Japan), with plant and row spacings of  $0.11 \text{ m} \times 0.25 \text{ m}$  and with four plant rows per bed. The top of the flat raised bed was 0.90 m wide (the distance from the bed centre-to-centre was 1.20 m). The flat raised bed had a length of 7.25 m and a height of 0.15 m, with north-south orientation. Each experimental plot consisted of a bed ( $8.7 \text{ m}^2$  and 264 plants). The incorporation of nutrients ( $200-100-250 \text{ kg ha}^{-1} \text{ N-P}_2\text{O}_5\text{-K}_2\text{O}$ ) was performed by fertigation with a nutrient solution based on the Sonneveld and Straverd [37] solution, following the criteria described by Miguel [38].

**Table 1.** Duration (days) and irrigation water applied (mm) at vegetative growth (2), bulbing (3) and bulb ripening (4) stages in each irrigation strategy (T1-T7) during the 2017 (4 November – 27 April) and 2018 (30 October – 30 April) growing seasons (GS).

GS	Stores	C4 D	Irrigation water applied (mm)						
US	Stages	Days	T1	T2	T3	T4	T5	T6	T7
	2	84	17	12	17	17	8	17	17
2017	3	43	70	70	52	70	70	35	70
	4	37	80	80	80	60	80	80	40
	Total	164	167	162	149	147	158	131	127
	2	87	131	98	131	131	65	131	131
2018	3	39	61	61	46	61	61	31	61
	4	43	139	139	139	104	139	139	69
	Total	169	331	298	316	296	265	300	262

#### 3.2.3.4. Irrigation scheduling and system

The IWR values were determined using the following equation:

$$IWR = \frac{ET_C - Pe}{Ef}$$
 (1)

where ETc (mm) is the crop evapotranspiration; Pe is the effective precipitation (mm), determined from rainfall data using the method of the U.S. Bureau of Reclamation [40], as presented by Pascual-Seva et al. [41]; and Ef is the irrigation efficiency, being 0.95 [considering that the uniform distribution = 0.98; deep percolation ratio = 0.97;

the leaching requirement is negligible, as has been stated for onion cultivars grown in the Experimental Centre].

The ETc (mm) was calculated from the ETo, and a single crop coefficient (Kc) was proposed for local conditions by the IVIA [42], adapting the duration of each stage to the growing cycle (Table 1). The Kc values used were 0.3, 0.95 and 0.8, corresponding to the initial, mid-season and late season stages, respectively.

$$ETc = ETo \times Kc \tag{2}$$

where ETo is the reference evapotranspiration and Kc is the crop coefficient. The ETo was determined according to Allen et al. [39], as follows:

$$ETo = E_{pan} \times K_{p} \tag{3}$$

where  $E_{pan}$  (mm day<sup>-1</sup>) is the evaporation from a class A pan installed adjacent to the experimental plot, and  $K_p$  (0.815) is the pan coefficient determined according to Allen et al. [39].

The irrigation water was supplied by a double lateral line for each bed using a turbulent flow dripline (16 mm; AZUDRIP Compact; Sistema Azud S.A., Murcia, Spain) with emitters (2.2 L h<sup>-1</sup>) spaced 0.33 m apart. An irrigation controller programmer (NODE-100 single station controller, Hunter, California, USA) was used to control the time of each irrigation event, and a water flow meter (MJ-SDC TYP E, Ningbo Water Meter Co., Ltd., Ningbo, China) was installed in each IS to record the IWA.

#### 3.2.3.5. Volumetric soil water content

The volumetric soil water content (VSWC; m<sup>3</sup> m<sup>-3</sup>) was continuously monitored using ECH<sub>2</sub>O EC-5 capacitance sensors connected to an Em50 data logger using the ECH<sub>2</sub>O Utility software (Decagon Devices Inc., Pullman WA., USA). Onions have shallow root systems, with most roots being concentrated in the upper 0.20 m of the soil; therefore, following the methodology described by Enciso et al. [43], in each treatment, one sensor was installed horizontally, at a depth of 0.15 m, in the middle of

the beds below a dripline and equidistant between two adjacent emitters. Additionally, for T1, another sensor was placed at a 0.25 m soil depth to verify that water losses at that depth were nearly negligible. The VSWC was measured and stored at 15 min intervals, and variations in the VSWC were used to determine the in situ field capacity (FC). To compare the VSWCs corresponding to the different IS and GS, their values are presented as the ratio of the VSWC compared with the VSWC at FC (% FC). The irrigation events for all the IS began when the VSWC in T1 dropped to 80% of the FC, following the criteria used in prior experiments at the Experimental Centre, and lasted the time necessary for applying the corresponding IWA.

## 3.2.3.6. Relative water content and membrane stability index, plant growth and harvest index

The RWC and MSI were evaluated at the end of each growth stage. Leaf RWC was determined in fresh leaf discs of 2 cm diameter using the method developed by Hayat et al. [44], and it was calculated using the following equation:

$$RWC (\%) = \frac{FW - DW}{TW - DW} * 100$$
 (4)

where FW, DW and TW are the disc fresh weight, dry weight and turgor weight, respectively.

The MSI was determined using 0.2 g samples of fully expanded leaf tissue following the methodology described by Rady [45], and it was calculated as:

MSI (%) = 
$$\left(1 - \frac{C_1}{C_2}\right) * 100$$
 (5)

where  $C_1$  is the electrical conductivity of the solution after the samples were heated at  $40^{\circ}$ C in a water bath for 30 min, and  $C_2$  is the electrical conductivity of the solution after the samples were boiled at  $100^{\circ}$ C for 10 min.

Three onion plants per plot were selected at harvest (two weeks after 50% of the leaves near the pseudostems were bent over, 27 and 30 April of 2017 and 2018) to measure the following plant growth parameters: plant height, leaf number per plant,

bulb diameter and height. The leaf chlorophyll index (SPAD) was measured at three points in three fully developed leaves from each plant using a SPAD-502 m leaf chlorophyll meter (Konica Minolta Sensing Inc., Tokyo, Japan). Thereafter, these plants were separated into leaves and bulbs and each part was weighed (fresh weight) with a precision analytical balance (Mettler Toledo AG204, Switzerland), and dried at 65°C in a forced-air oven (Selecta 297, Barcelona, Spain) until a constant weight was reached to obtain dry weights and bulb dry matter content. The harvest index (HI) was determined as the ratio of total yield (TY) to total biomass (leaves + bulbs) on a dry mass basis (g g<sup>-1</sup>; [46]).

#### 3.2.3.7. Yield, irrigation water use efficiency and yield response factor

The yield components were determined from a 3 m length in the two central plant rows, leaving a plant row on each side of the bed to avoid marginal effects. The bulb yield was partitioned into marketable (MY) and non-marketable yield. The average bulb weight of MY was determined. The non-marketable yield was, in turn, classified according to the nature of blemishes, including small bulbs, bulbs with defects in shape and bolting plants, in accordance with Leskovar et al. [30].

The IWUE was calculated as the ratio of MY (fresh mass; kg m<sup>-2</sup>) to IWA (m<sup>3</sup> m<sup>-2</sup>; [47]). The yield response to water deficits  $(K_y)$  during the growing season and at each growth stage were determined according to Doorenbos and Kassam [48] using the following equation:

$$\left(1 - \frac{Y_a}{Y_m}\right) = K_y \left(1 - \frac{ET_a}{ET_m}\right) \tag{6}$$

where  $Y_a$  and  $Y_m$  are the actual MY (corresponding to the different RDI strategies at each stage) and maximum MY (fully irrigated plants), respectively;  $ET_a$  and  $ET_m$  are the actual (RDI) and maximum (full irrigated) ET (mm), respectively; and  $K_y$  is the yield response factor, which was obtained by lineal regression for each stage.  $ET_a$  and  $ET_m$  were calculated as ET = IWA + Pe, considering both the drainage and the variations in volumetric soil water content to be negligible.

#### 3.2.3.8. Onion bulb quality traits

Three representative (in size and shape) marketable bulbs per plot were used to determine the bulb size (height and diameter) and shape (diameter/height ratio). Then, these bulbs were used to determine the external bulb firmness using a digital penetrometer with an 8 mm diameter tip (Penefel DFT 14, Agro Technologies, Forges les Eaux, France). Then, the bulbs were liquefied with a domestic blender to obtain their juice, which was filtered. The soluble solids content (SSC, °Brix) was determined from the bulb juice using a digital refractometer (PAL-1, Atago, Tokyo, Japan). Acidity (grams of citric acid/100 g FW) was determined by titration with 0.1 M NaOH. The maturity index (MI) was calculated as the ratio of SSC (° Brix) and acidity (g citric acid 100 g-1 FW).

#### 3.2.3.9. Crop profitability

The determination of the profitability of the RDI, as presented in Pascual-Seva et al. [49], under the conditions of this study can help to make decisions that reduce water consumption. The gross revenue and the water economic value have been determined, taking into account the MY and the IWUE obtained in this study, and the average price of the onion bulbs over the previous three years  $(0.21 \, \epsilon \, \text{kg}^{-1} \, [51])$ .

#### 3.2.3.10. Experimental design and statistical analysis

The experiment was performed using a randomized complete block design with three replicates. The results for the different parameters were evaluated by analysis of variance (ANOVA) using Statgraphics Centurion XVII [50]. Percentage data were arcsin transformed before analysis. Least significant differences (LSD) at a 0.05-probability level were used as the mean separation test. MY and IWUE were related with IWA using Statgraphics Centurion XVII [50].

#### **3.2.4.** Results

The rainfall registered in the 2017 season was higher (618 mm) than in 2018 (203 mm), and most of the rainfall during 2017 occurred at the vegetative growth

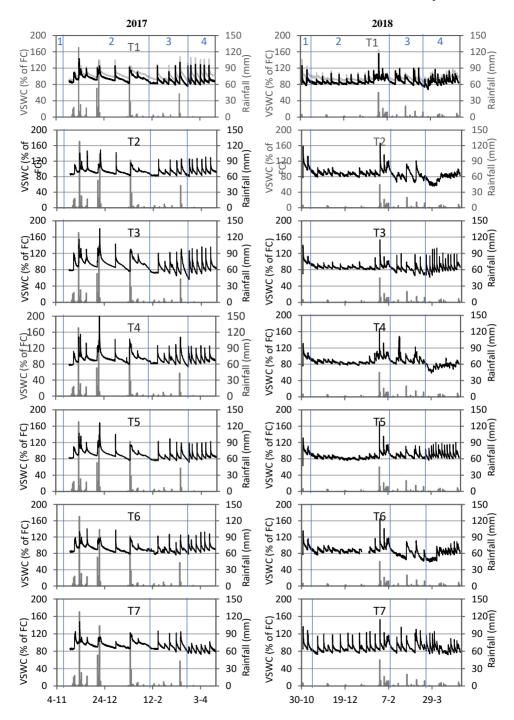
stage, with lower values of rainfall occurring at the bulb ripening stage in both GS (Figure 1). These facts are responsible for the significant interactive effect ( $P \le 0.01/0.05$ ) between the IS and GS on many of the studied parameters. When the GS x IS interaction was not significant ( $P \le 0.05$ ), the mean values of the two factors were analysed separately, but when the interaction was significant ( $P \le 0.05$ ), the two factors were analysed jointly.

#### 3.2.4.1. Growth stages and irrigation water applied

The total crop cycle period (including the initial period) was shorter in 2017 than in 2018, lasting 175 and 183 days, respectively. The total pan evaporation and ETo during the growing season were lower in 2017 (334 and 272 mm, respectively) than in 2018 (576 and 469 mm, respectively). The values of Pe during the growing season were higher during 2017 (387 mm) than during 2018 (148 mm).. In 2017, there were 10 irrigation events, while in 2018 the number of irrigation events increased to 27. The IWA values during the different irrigation periods ranged from 127 mm (T7) to 167 mm (T1) in 2017 and from 262 mm (T7) to 331 mm (T1) in 2018 (Table 1).

#### 3.2.4.2. Volumetric soil water content

The VSWC for the different IS and GS at 0.15 m depth (in addition 0.25 m depth in T1), as well as the daily rainfall during both GS, are presented in Figure 2. The high rainfall in 2017 led to a high VSWC for all the IS, being higher in 2017 (on average 92.0% of FC) than in 2018 (on average 86.4% of FC). Therefore, during 2017, there were no considerable differences in the VSWC among the different IS; however, the average VSWC in 2018 at a 0.15 m depth ranged between 87.6% of the FC for T1 and 84.7% of the FC for T6, and the VSWC decreased slightly over time. Lower variations in the VSWC at a 0.25 m depth were recorded in 2018 than in 2017.



**Figure 2.** Volumetric soil water content (VSWC in percentage of field capacity (FC), — 15 cm, — 25 cm) for each irrigation strategy and daily rainfall during the two growing seasons. Crop growth stages: (1) Initial; (2) Vegetative growth; (3) Bulbing; (4) Bulb ripening.

## 3.2.4.3. Relative water content, membrane stability index, onion growth and harvest index

The RWC and MSI were affected ( $P \le 0.05/0.01$ ) by the GS, and both parameters at each analysed time in 2017 were higher than in 2018 (Table 2). The RWC and MSI at establishment were not affected by the IS (P > 0.05; data not shown), but at the end of the vegetative growth (stage 2), both parameters were affected ( $P \le 0.01$ ) by the GS, IS, and by their interaction (Table 2). There were no differences between the IS in 2017, while in 2018, T2 and T5 led to lower values ( $P \le 0.01$ ; Table 3). At the bulbing and bulb ripening stages, the interactions were not significant (Table 2) for the RWC or MSI, and these lower values corresponded to the strategies of severe water restriction (50% IWR) in the corresponding stages (T6, T7).

**Table 2.** Effects of the growing season and the irrigation strategy on the relative water content (RWC) and membrane stability index (MSI) at the end of the vegetative growth (2), bulbing (3) and bulbing ripening (4) stages.

	RWC (%)			MSI (%)		
	2	3	4	2	3	4
Growing season (GS)				'		
2017	79.7 a	81.8 a	82.6 a	70.1 a	71.5 a	69.4 a
2018	76.8 b	79.8 b	80.1 b	61.7 b	59.7 b	58.9 b
LSD	1.5	2.1	1.9	1.4	1.7	1.9
Irrigation Strategies (IS)						
T1	79.4 a	81.8 ab	83.9 a	67.2 a	68.0 a	67.6 a
T2	76.1 bc	81.4 abc	82.8 a	63.6 bc	65.2 ab	65.1 ab
Т3	78.5 ab	78.9 bc	80.3 ab	67.0 a	65.9 ab	65.5 ab
T4	79.2 a	83.9 a	82.7 a	67.9 a	67.0 a	64.9 ab
T5	74.9 c	81.6 ab	80.6 ab	63.0 c	63.7 c	62.6 bc
T6	79.7 a	77.7 c	80.8 ab	65.7 ab	63.2 c	62.5 bc
T7	80.1 a	80.5 abc	78.5 b	67.0 a	66.0 ab	61.0 c
LSD	2.9	3.8	3.6	2.5	3.23	3.58
ANOVA (df)			% sum o	f squares		
GS (1)	14.2 **	7.1 ns	13.8 *	64.6 **	79.9 **	69.0 **
IS (6)	23.9 **	25.3 *	27.2 *	11.3 **	5.8 *	10.9 *
GS*IS (6)	34.5 **	17.9 ns	2.8 ns	13.1 **	3.0 ns	4.7 ns
Residuals (28)	27.4	49.6	56.1	11.0	11.3	15.5
Standard deviation	2.4	3.2	3.0	2.1	2.7	3.0

df: degrees of freedom. Mean values followed by different lower-case letters in each column indicate significant differences at  $P \le 0.05$  using the LSD test. \*\* (\*): Indicates significant differences at  $P \le 0.01$  ( $P \le 0.05$ ). ns: Indicates no significant difference.

**Table 3.** Growing season - irrigation strategy interaction for the relative water content (RWC) and membrane stability index (MSI) at the end of the vegetative growth, bulb dry weight (BDW), total yield (Yield), marketable yield (MY) and average bulb weight (ABW).

	RWC (%)	MSI (%)	BDW (kg m <sup>-2</sup> )	Yield (kg m <sup>-2</sup> )	MY (kg m <sup>-2</sup> )	ABW (g bulb <sup>-1</sup> )
2017						
T1	79.6 a	68.9 a	0.44 d	4.91 f	4.24 f	183.9 e
T2	80.5 a	68.9 a	0.40 d	4.80 f	4.28 f	184.8 e
Т3	78.6 a	69.4 a	0.38 d	4.87 f	4.20 f	185.6 e
T4	78.9 a	71.5 a	0.46 d	4.74 f	4.15 f	181.4 e
T5	80.2 a	70.8 a	0.43 d	4.80 f	4.13 f	185.4 e
T6	79.4 a	70.8 a	0.38 d	4.67 f	3.93 f	174.7 e
T7	80.5 a	70.7 a	0.38 d	4.72 f	4.12 f	185.4 e
2018						
T1	79.2 a	65.5 b	0.88 a	8.93 a	8.04 a	340.5 a
T2	71.7 b	58.2 d	0.78 b	8.40 bc	7.35 bc	318.0 bc
Т3	78.5 a	64.7 b	0.75 b	8.74 ab	7.77 ab	327.1 a
T4	79.4 a	64.3 b	0.76 b	8.97 a	7.66 ab	327.0 ab
T5	69.5 b	55.2 e	0.59 c	7.22 e	6.06 e	296.9 d
T6	80.0 a	60.5 cd	0.72 b	7.66 de	6.55 de	303.0 d
T7	79.6 a	63.3 bc	0.74 b	7.99 cd	7.03 cd	310.4 cd
LSD	3.3	2.97	0.09	0.49	0.63	15.5

Mean values followed by different lower-case letters in each column indicate significant differences at  $P \le 0.05$  using the LSD test.

The values of plant growth traits (except for SPAD; Table 4) were affected ( $P \le 0.01$ ) by the GS, with lower values obtained in 2017 than in 2018, except for the bulbing ratio, which was higher in 2017. In both GS, the bulbing ratios usually increased during the crop cycle, being considered as a measure of bulb formation; the bulbing ratios reached their highest values at harvest. The data for the bulbing ratios at the end of vegetative growth are presented in Table 4, and they were not affected by the IS. The plant heights and the numbers of leaves per plant were not affected by the IS (P > 0.05; Table 4); nevertheless, the lower values were obtained for plants that were exposed to water stress during the vegetative growth stage (T2 and T5). The SPAD index was not affected ( $P \le 0.05$ ) by the GS nor by IS. Water restrictions negatively affected the production of biomass, both for the leaves (fresh

and dry weight;  $P \le 0.05$ , Table 4) and for the bulbs (fresh  $P \le 0.05$ , Table 5; dry  $P \le 0.01$ , Table 4), with the greatest values (in these parameters) corresponding to full irrigation (T1) and moderate deficit irrigation during bulb ripening (T4). The bulb dry weights were affected by the IS only in 2018 ( $P \le 0.01$ ), when the highest values were obtained for the fully irrigated plants (T1;  $P \le 0.05$ ; Table 3), reducing in value with the water deficit, and this phenomenon occurred to a greater extent with the severe deficit and in the earlier stages. Greater values of HI ( $P \le 0.05$ ) were obtained in 2018 than in 2017 and were not affected by IS (P > 0.05).

**Table 4.** Effects of the growing season and the irrigation strategy on the bulbing ratio at the end of stage 2, and on the plant height, leaf number per plant, leaf chlorophyll index (SPAD), leaf fresh weight (LFW), leaf dry weight (LDW), bulb dry weight (BDW) and harvest index (HI) at harvesting.

	Bulbing ratio	Plant height	Leaf	SPAD	LFW	LDW	BDW	HI
	(-)	(cm)	(cm) number	(-)	(kg m <sup>-2</sup> )	(kg m <sup>-2</sup> )	(kg m <sup>-2</sup> )	(-)
Growing season	(GS)							
2017	1.79 a	47.14 b	6.71 b	62.95	0.67 b	0.085 b	0.411 b	0.83 b
2018	1.63 b	57.78 a	7.40 a	64.56	1.20 a	0.119 a	0.747 a	0.86 a
LSD	0.09	1.72	0.27	2.20	0.07	0.008	0.035	0.01
Irrigation strateg	ies (IS)							
T1	1.71	53.94	7.22	62.60	1.04 a	0.121 a	0.661 a	0.84
T2	1.75	50.33	6.94	65.14	0.90 bc	0.100 b	0.591 b	0.85
T3	1.68	52.39	7.11	64.67	0.96 abc	0.095 b	0.565 bc	0.85
T4	1.74	53.89	7.17	65.62	0.99 ab	0.105 ab	0.609 ab	0.85
T5	1.64	50.72	6.78	63.86	0.86 bc	0.099 b	0.512 c	0.84
T6	1.73	52.33	7.06	63.11	0.89 bc	0.096 b	0.551 bc	0.85
T7	1.74	53.61	7.11	61.28	0.89 bc	0.098 b	0.564 bc	0.84
LSD	0.17	3.21	0.50	4.11	0.13	0.016	0.066	0.02
ANOVA (df)			% s	um of squ	ares			
GS (1)	9.2 **	54.2 **	17.3 **	1.7 ns	65.1 **	31.2 **	69.2 **	22.1 **
IS (6)	2.0 ns	3.6 ns	2.9 ns	5.3 ns	3.2 *	7.4 *	4.7 **	2.7 ns
GS*IS (6)	6.1 ns	1.8 ns	3.6 ns	3.9 ns	2.1 ns	1.4 ns	3.9 **	6.4 ns
Residuals (112)	82.8	40.4	76.2	89.2	29.6	60.0	22.2	68.8
SD	0.3	4.9	0.8	6.2	0.2	0.0	0.1	0.0

df: degrees of freedom. SD: Standard deviation. Mean values followed by different lower-case letters in each column indicate significant differences at  $P \le 0.05$  using the LSD test. \*\* (\*): Indicates significant differences at  $P \le 0.01$  ( $P \le 0.05$ ). ns: Indicates no significant differences.

#### 3.2.4.4. Yield, irrigation water use efficiency and yield response factor

The GS had an important impact on yield (representing 92% and 87% of the sum of squares of yield and MY, respectively, Table 5). The MY losses corresponding to the most restricted strategies at the vegetative growth (on average 17% for T5) and bulbing (15% for T6) stages were greater than those obtained when the restriction was applied at the bulb ripening (9% for T7) stage. Yields and MY obtained in 2017 were lower than those in 2018 ( $P \le 0.05$ ), not differing between IS. In 2018, lower yields and MY were obtained with 50% IWR reduction, when it was applied both in the vegetative growth (T5) and bulbing stages (T6; Table 3).

**Table 5.** Effects of the growing season and the irrigation strategy on the total yield (Yield), marketable yield, average bulb weight (ABW), non-marketable yield, and its partitioning in small, deformed and bolting bulbs, and irrigation water use efficiency (IWUE).

	Yield	Marketable yield		Non-r	Non-marketable yield (% of yield)			
	(kg m <sup>-2</sup> )	(kg m <sup>-2</sup> )	ABW (g bulb-1)	Total	Small	Deformed	Bolting	(kg m <sup>-3</sup> )
Growing season	n (GS)							
2017	4.79 b	4.15 b	183.0 b	13.3	3.0 b	0.8 b	9.5 a	28.15 a
2018	8.27 a	7.21 a	317.6 a	13.0	8.3 a	1.7 a	3.0 b	24.42 b
LSD	0.22	0.29	7.04	2.6	1.7	0.9	2.2	1.20
Irrigation Strate	egies (IS)							
T1	6.92 a	6.14 a	262.2 a	11.7	4.4	1.3	6.1	24.85 bc
T2	6.60 ab	5.82 ab	251.4 abcd	11.5	4.1	1.7	5.8	25.58 bc
T3	6.80 a	5.99 ab	256.3 ab	12.5	4.7	0.8	7.1	26.43 bc
T4	6.86 a	5.91 ab	254.2 abc	13.6	5.6	1.8	6.3	27.07 b
T5	6.01 c	5.10 c	241.1 cd	14.9	8.4	1.7	4.8	24.49 c
T6	6.17 c	5.24 c	238.8 d	15.4	6.5	1.0	7.9	25.88 bc
T7	6.36 bc	5.58 bc	247.9 bcd	12.5	5.9	0.8	5.9	29.70 a
LSD	0.41	0.53	13.17	4.9	3.2	1.7	4.0	2.25
ANOVA (df)			% su	m of squar	es			
GS (1)	91.6. **	86.8 **	96.0 **	0.1 ns	46.8 **	11.7 *	52.5 **	35.6 **
IS (6)	3.3 **	4.9 **	1.3 *	13.9 ns	13.3 ns	8.7 ns	4.2 ns	26.7 **
GS*IS (6)	2.6 **	3.3 *	1.0 *	8.1 ns	6.9 ns	3.6 ns	4.2 ns	12.9 ns
Residuals (28)	2.5	5.0	1.8	77.9	33.0	75.9	39.0	24.7
SD	0.3	0.6	11.1	4.1	2.7	1.5	3.4	1.9

df: degrees of freedom. Mean values followed by different lower-case letters in each column indicate significant differences at  $P \le 0.05$  using the LSD test. \*\* (\*): Indicates significant differences at  $P \le 0.01$  ( $P \le 0.05$ ). ns: Indicates no significant differences.

The MY accounted (on average) for 87% of the yield; this MY proportion was not affected by the GS, IS or by their interaction (data of non-marketable yield shown in Table 5). The growing season had a greater influence on average bulb weight (ABW; 96% of the sum of squares) than IS (1.3%) and their interaction (1%); the bulbs obtained in 2017 were heavier than those in 2018, with no IS differences. However, in 2018, ABW was reduced with the water deficit ( $P \le 0.05$ ), and this phenomenon occurred to a greater extent with the severe deficit (Table 3). In 2017, there was a higher ( $P \le 0.01$ ) incidence of bolting bulbs, and a lower number of small bulbs ( $P \le 0.01$ ) and bulbs with shape defects ( $P \le 0.05$ ) than in 2018.

IWUE was affected by GS and by IS ( $P \le 0.01$ ; Table 5), with the highest values obtained in 2017, which corresponded to the lowest IWR. For IS, the highest average value ( $P \le 0.05$ ) was obtained with the severe water shortage at the bulb ripening stage (29.7 kg m<sup>-3</sup>; T7) and was also related to the lowest IWA (127 mm for 2017 and 262 mm for 2018). The lowest IWUE value was obtained with the severe water shortage at the vegetative growth stage (24.5 kg m<sup>-3</sup>; T5), as a consequence of the lowest MY obtained with this strategy (5.1 kg m<sup>-2</sup>).

Considering separately the different stages when water restrictions were applied, the MY (kg m<sup>-2</sup>) increased linearly (P  $\leq$  0.01) with increasing IWA (mm), following these equations: Vegetative growth: MY = 0.57 + 0.022\*IWA (r = 0.98; P  $\leq$  0.01); Bulbing: MY = 1.11 + 0.020\*IWA (r = 0.95; P  $\leq$  0.01); and Bulb ripening: MY = 1.03 + 0.022\*IWA (r = 0.97; P  $\leq$  0.01). IWUE decreased linearly with increasing IWA, following the equations corresponding to vegetative growth, bulbing and bulb ripening: IWUE = 27.89 - 0.013\*IWA (r = -0.55; P  $\leq$  0.01); IWUE = 31.79 - 0.026\*IWA (r = -0.71; P  $\leq$  0.01); and IWUE = 32.81 - 0.025\*IWA (r = -0641; P  $\leq$  0.01).

For  $K_y$ , three fitted linear regression equations ( $P \le 0.01$  and  $r \ge 0.91$ ) were obtained, considering together the two GS, with one for each stage of irrigation restriction. The obtained  $K_y$  values were 1.66, 1.75 and 0.75 for the vegetative growth, bulbing and bulb ripening stages, respectively.

#### 3.2.4.5. Onion bulb quality traits

The bulb size (diameter and height) was affected by the GS, IS and their interaction ( $P \le 0.01$ ; Table 6). In general, the bulbs produced in 2017 were shorter and narrower than those produced in 2018. The interaction shows that the shortest bulbs in 2017 corresponded to T4 and T6 while in 2018 these were obtained with T5 (Table 7). The bulb shapes were influenced by the GS ( $P \le 0.01$ ) and the GS-IS interaction ( $P \le 0.05$ ; Table 6) in the sense that the bulbs obtained in 2018 were flatter than those in 2017, being the most elongated bulbs those obtained with T6 in both years and with T4 in 2018 (Table 7).

The dry matter content and SSC were only affected by GS ( $P \le 0.01$ ), corresponding to the higher values in 2018, while the firmness was not affected (P > 0.05) by any factor. In contrast, acidity was affected, in addition to the GS, by IS and by their interaction. The bulbs obtained in 2018 were, in general, more acidic than those obtained in 2017.

**Table 6.** Effects of the growing season and the irrigation strategy on bulb characteristics: size [diameter (D) and height (H)], shape (D/H)]; dry matter content (DMC); firmness; soluble solid content (SSC); acidity and maturity index (MI).

	D	Н	D/H	DMC	Firmness	SSC	Acidity	MI
	(mm)	(mm)		(%)	(N)	(°Brix)	(%)	
Growing season (GS)								
2017	74.4 b	71.1 b	1.05 b	6.2 b	27.4	5.20 b	0.076 b	68.9 b
2018	88.7 a	72.1 a	1.23 a	8.2 a	27.6	7.49 a	0.091 a	83.5 a
LSD	1.1	1.2	0.02	0.52	2.0	0.39	0.004	6.0
Irrigation Strategies (IS)								
T1	83.9 a	73.0 a	1.15	7.2	28.1	6.37	0.094 a	67.4 c
T2	81.1 b	71.9 ab	1.13	7.0	26.4	6.30	0.085 b	74.4 bc
T3	82.2 ab	72.4 ab	1.14	7.9	27.9	6.55	0.089 ab	72.9 bc
T4	82.4 ab	72.6 ab	1.13	6.7	26.4	6.25	0.082 bc	75.5 abc
T5	79.0 c	69.3 c	1.15	7.3	27.5	6.17	0.082 bc	75.0 bc
Т6	80.7 bc	70.4 bc	1.15	7.0	27.0	6.22	0.076 c	81.6 ab
T7	81.6 b	71.5 ab	1.14	7.3	29.2	6.55	0.077 c	86.6 a
LSD	2.1	2.2	0.04	1.0	3.8	0.72	0.007	11.3
ANOVA (df)				% st	ım of square	S		
GS (1)	89.4 **	4.0 ns	88.4**	59.8 **	0.1 ns	81.8 **	44.0 **	32.8 **
IS (6)	3.5 **	22.2 *	0.4 ns	7.6 ns	10.7 ns	1.3 ns	25.5 **	20.1 *
GS*IS (6)	3.3 **	40.2 **	4.2 *	6.6 ns	4.7 ns	1.6 ns	12.2 *	9.6 ns
Residuals (28)	3.8	33.6	7.1	26.0	84.5	15.4	18.3	37.5
Standard deviation	1.8	1.8	0.0	0.8	3.2	0.6	0.0	9.6

df: degrees of freedom (112 for D, H and D/H). SD: standard deviation. Mean values followed by different lower-case letters in each column indicate significant differences at  $P \leq 0.05$  using the LSD

test. \*\* (\*): Indicates significant differences at  $P \le 0.01$  ( $P \le 0.05$ ). ns: Indicates no significant difference.

As a consequence of the respective values of SSC and acidity, the MI was affected by GS ( $P \le 0.01$ ) and by IS ( $P \le 0.01$ ), corresponding the highest values to 2018, and in relation to IS, MI increased with water deficit, particularly when severe water restriction was applied at the bulb ripening stage (T7).

**Table 7.** Growing season - irrigation strategy interaction for bulb characteristics: size [diameter (D) and height (H)], shape (D/H)] and acidity, and gross revenue (GR)

	D (mm)	H (mm)	D/H	Acidity (%)	GR (€ ha <sup>-1</sup> )
2017					
T1	74.7 e	71.9 b	1.037 d	0.080 c	8914 e
T2	74.7 e	72.4 b	1.033 d	0.070 cd	8998 e
Т3	74.6 e	71.7 b	1.043 cd	0.077 cd	8817 e
T4	74.2 e	69.2 cd	1.073 cd	0.080 cd	8718 e
T5	74.2 e	71.6 b	1.037 d	0.090 bc	8669 e
Т6	74.6 e	69.0 cd	1.083 c	0.080 cd	8256 e
T7	74.1 e	71.7 b	1.033 d	0.076 cd	8659 e
2018					
T1	93.1 a	74.2 ab	1.253 a	0.113 a	16882 a
T2	87.6 bc	71.4 bc	1.227 ab	0.077 cd	15443 b
Т3	89.8 b	73.0 b	1.230 ab	0.093 b	16323 ab
T4	90.6 b	76.0 a	1.193 b	0.087 bc	16084 ab
T5	83.8 d	67.1 cd	1.253 a	0.097 b	12732 cd
Т6	86.8 c	71.9 b	1.207 b	0.070 cd	13761 cd
T7	89.1 bc	71.3 bc	1.250 ab	0.077 cd	14772 bc
LSD	2.5	2.5	0.044	0.013	1316

Mean values followed by different lower-case letters in each column indicate significant differences at  $P \le 0.05$  using the LSD test.

#### 3.2.4.6. Crop profitability

The gross revenue and the economic value of water were affected by GS and IS  $(P \le 0.01; \text{ Table 8})$ . The gross revenue was also affected by the GS-IS interaction  $(P \le 0.05)$  and, as observed for many factors, in 2017 there were no differences between the different IS, which were in all cases lower than those obtained in 2018 (Table 7). Applying 50% of the IWR reduced gross revenue in relation to T1 in 2018,

particularly when it was applied at the vegetative growth (T5) and at the bulbing (T6) stages. Regarding the economic value of water, lower values were obtained in 2018 than in 2017, and the highest value was obtained with the severe water restriction applied at the bulbing (T7), while the lowest values corresponded to the severe reduction at vegetative growth (T5).

**Table 8.** Effects of the growing season and the irrigation strategy on the gross revenue and water economic value.

	GR (€ ha <sup>-1</sup> )	WEV (€ m <sup>-3</sup> )
Growing season (GS)		
2017	8719 b	5.91 a
2018	15142 a	5.13 b
LSD	599	0.25
Irrigation Strategies (IS)		
T1	12898 a	5.22 bc
T2	12220 ab	5.37 cb
T3	12570 ab	5.55 bc
T4	12401 ab	5.68 b
T5	10701 c	5.14 c
T6	11008 c	5.43 bc
T7	11716 bc	6.24 a
LSD	1121	0.47
ANOVA (df)	% sum o	of squares
GS (1)	86.8 **	35.5 **
IS (6)	4.9 **	26.8 **
GS*IS (6)	3.3 *	12.9 ns
Residuals (28)	5.0	24.8
Standard deviation	948	0.4

df: degrees of freedom. Mean values followed by different lower-case letters in each column indicate significant differences at  $P \le 0.05$  using the LSD test. \*\* (\*): Indicates significant differences at  $P \le 0.01$  ( $P \le 0.05$ ). ns; Indicates no significant differences.

#### 3.2.5. Discussion

The effective precipitation measured during 2017 was 2.6 times higher than that in 2018, and the ETo during 2017 was 1.7 times lower than that recorded in 2018, as shown in Figure 1. Overall, it can be stated that for the different parameters that were analysed, when the interaction result was significant, it was because the important rainfall registered in 2017 led to no differences between the IS values, contrary to that obtained in 2018, whose differences coincided with the average values, unless otherwise indicated. The IWA during 2017 (167 mm for T1) was approximately 50% lower than that applied during 2018 (331 mm for T1). These volumes are similar to those applied to onions by Martín de Santa Olalla et al. [52]

and Zheng et al. [28]. Doorenbos and Kassam [48] and Pérez-Ortolá and Knox [29] reported that the irrigation water requirements of onions ranged between 350 and 550 mm in the UK, which was the lowest threshold of the same order as the water requirements in 2018.

The VSWC varied between the GS, with higher values in 2017 (on average 92.0% FC) than in 2018 (on average 86.4% FC), which might be related to the higher precipitation levels and to the lower ETc during 2017. The stomatal closure responds earlier to soil water content than to leaf turgor [14] and is different depending on the plant species [53]. The initial plant response to the reduction of water in the soil is stomatal closure, directly affecting the plant water status (RWC and MSI) and reducing the assimilation of CO<sub>2</sub>, as will be discussed later [14,54].

At the beginning of plant growth (establishment stage) both the RWC and MSI were unaffected by the IS, given that all the plants were equally irrigated with water volumes applied that were greater than IWR to ensure adequate plant establishment. These similar values show that all the plants presented a similar water status when the differential irrigation period started. At the end of the vegetative growth stage, both indexes were affected by the GS, IS and their interaction, which was a consequence of the different rainfall amounts and the corresponding VSWC during the two seasons. In general, it can be stated that the lower values for the RWC and MSI in each stage corresponded to the water restriction applied in the respective stage, with lower values observed for the most severe restriction. It is noteworthy that in general, when the MSI value has been reduced in one stage, it is not recovered in the rest of the crop cycle. The RWC and MSI values obtained are in accordance with those reported by Semida et al. [24]. Based on that study, the RWC and MSI obtained for the deficit irrigation strategies in the present experiments could be related to the lower soil moisture and to climate conditions. The leaf water status (and the subsequent plant response) depends on the water deficit in terms of its intensity, duration and the growth stage when it is applied [16]. In accordance with González and González-Vilar [55] an initial reduction in the leaf RWC (100-90%) induces stomatal closure, reducing cellular growth; lower values of RWC (90-80%) induce changes in the tissue composition and changes in the relative rates of photosynthesis and respiration, while a greater decrease in RWC (below 80%) causes changes in metabolism, leading to the cessation of photosynthesis, to an increase of respiration and to the accumulation of abscisic acid. Based on these considerations, the obtained values of the RWC ranging between 74.9 and 83.9%, could be induced by changes in the relative rates of photosynthesis and respiration, until photosynthesis ceased, and could be related to abscisic acid accumulation, leading to negative effects on biomass production. Water restriction had a negative effect on onion plant biomass (fresh and dry weight of the leaves and bulbs) that was, in general, more pronounced when it was applied during vegetative growth than when applied at the bulb ripening stage. A similar trend was observed for plant height and number of leaves per plant, although the differences were not significant. Zheng et al. [28] reported that water restriction during the onion vegetative growth stage had an irreversible effect from which the plant cannot recover and leads to lower plant height and to lower leaf and bulb biomass. These authors also observed a reduction in bulb dry weight under water restriction at the vegetative growth and bulbing stages.

The HI was not affected by the IS, which agrees with the results reported for many other crops, as yield is often directly related to plant biomass [56]. Under moderate water stress, water deficits lead to reduced biomass production due to the reduction in canopy size and, in that case, dry matter partitioning is usually not affected, and the HI is maintained in many crops [10], as occurred in this study. These results imply that the RDI did not alter the partitioning of assimilates between onion plant parts.

The SPAD was not affected by the different IS, indicating that deficit irrigation did not affect the chlorophyll content in the leaves. The SPAD average values obtained during this study are slightly higher than those reported by Leskovar et al. [30]. Since the bulbing ratio is a common indicator of starting bulb formation

[27,38], and considering that there were no differences between IS for the values presented, it can be concluded that plants for all the IS began to form bulbs normally on the same dates, as the plants that were grown with full irrigation.

The yields obtained in the present study under full irrigation in 2018 were similar to those obtained using drip irrigation by Martín de Santa Olalla et al. [33] (until 7.39 kg m<sup>-2</sup>) and Leskovar et al. [30] (until 7.90 kg m<sup>-2</sup>). The important impact of the GS on yield was probably due to two factors: the different climatic conditions registered in each GS [in 2018 there were higher temperatures and higher radiation (2169 and 2317 MJ m<sup>-2</sup> in 2017 and 2018, respectively) and lower rainfall], and to the mildew incidence that took place in 2017 as a consequence of the great rainfall. The mildew incidence, as reported in specialized literature [27,57] leads to an important reduction in yield.

The results of this study indicated that the bulb yield (yield and MY) decreased more when severe water shortage was applied at the vegetative growth (T5) and bulbing (T6) stages than when it was applied at the bulb ripening stage (T7). Similar results were reported by Bekele and Tilahun [58] and Zheng et al. [28], who observed limited effects of deficit irrigation on onion yield when applied at bulb maturity when compared to the effects when the deficits were applied at the crop development and bulb formation stages. Yield reductions were a consequence of both the lower ABW and the higher percentage of small bulbs (although not significant) that were obtained with T5 and T6. This observation agrees with the results reported for field experiments by Martín de Santa Olalla et al. [33] and Zheng et al. [28] in that water shortages applied during the growth and bulbification stages led to higher percentages of small bulbs. Full irrigation led to the highest average bulb weight, decreasing the ABW with the water reduction, in accordance with the results obtained by Kumar et al. [59] and Dirirsa et al. [60].

In addition to genetic characteristics, e.g. the cultivar used, the most important factors for the induction of onion bolting are low temperatures (generally from 5 to 12°C) at certain physiological conditions of the plant (the number of leaves is

generally considered as the best indicator [27]). It is therefore logical that bolting was influenced only by the GS and not by the IS, since the plants of all the IS presented the same physiological conditions (at harvesting there were no significant differences either in height or in the number of leaves of the plants subjected to the different IS).

In addition to genotypes, soil types and agronomic practices, climatic conditions play an important role in IWUE values. The IWUE results are consistent with those reported by Kumar et al. [59] and Patel and Rajput [61], in the sense that the higher IWUE values were obtained with the lower IWA. Tolk and Howell [20] indicated that maximum IWUE usually occurs at an evapotranspiration level that is generally less than the maximum evapotranspiration, thereby suggesting that irrigating to achieve a maximum yield would not correspond to the most efficient use of irrigation water, as occurred in this study. Bekele and Tilahun [58], in a study carried out in Ethiopia without rainfall during the experimental period, stated that all deficit irrigation strategies increased the water use efficiency of onions, from 6% when water stress was applied during vegetative growth to 13% when the IWA was reduced to 75% of the optimum application throughout the growing season. Martín de Santa Olalla et al. [33] obtained the highest IWUE using the following strategy: 80% ET during vegetative growth, 90% ET during bulbing, and 50% ET during bulb ripening.

For the different water restriction stages, the MY increased linearly with the IWA, with high correlation coefficients ( $r \ge 0.95$ ); therefore, reducing the water applied at any stage would decrease the MY relative to full irrigation. Similar positive linear relationships were reported by Zheng et al. [28], indicating that the IWA did not exceed the maximum crop water demands. Kumar et al. [59] presented second-order relationships between yield and IWA, with negative quadratic effects, indicating that the increase in onion yield was not proportional to the increment in IWA because the higher values of IWA exceeded the maximum crop water demands. The negative linear relationships between IWUE and IWA presented lower

correlation coefficients ( $r \ge -0.55$ ) due to the important differences of IWR between years, as a consequence of rainfall.

In this study, the  $K_y$  values were 1.66, 1.75 and 0.75 for the vegetative growth, bulbing and bulb ripening stages, respectively, and these values are consistent with those obtained by Dirirsa et al. [60]. If  $K_y$  is lower than 1, a crop can be considered to be tolerant to water deficits, while if it is greater than 1, this value indicates that the crop response is sensitive to water deficits [22,48]. The lower  $K_y$  seen in the bulb ripening stage indicates that this is a less sensitive period for applying water restriction, suggesting that in the case of deficit irrigation application, the restriction should be applied during the bulb ripening stage.

The RDI led to an important reduction in bulb size (diameter and height), particularly when the severe water stress was applied during the vegetative growth (T5), in agreement with the ABW. Similar reductions in the average bulb weight and size with water restrictions were observed by Leskovar et al. [30], Zheng et al. [28], and Patel and Rajput [61] in India.

The IS did not significantly affect bulb firmness or the SSC, in agreement with reports in the literature [43,62]. The absence of differences between the IS for the bulb SSC could be related to the fact that all bulbs had a similar dry matter content and, therefore, a similar soluble solids dilution.

Bulb acidity values were slightly lower than those reported by Rodríguez et al. [63], with higher values obtained in bulbs subjected to full irrigation. Since the SSC was not affected by the IS, and since for the determination of the MI the acidity appears in the denominator, the trend of MI values is practically the inverse of acidity. Reducing the IWA to 50% of the IWR at the bulbing stage (T6) at the bulb ripening stage (T7) accelerated bulb maturation.

Considering the current climatic conditions in irrigated areas, particularly in dry regions, it is of great importance to increase IWUE and, in turn, the water economic value. Applying moderate (T4) or severe (T7) deficit irrigation at the bulb ripening

stage led to a low reduction in gross revenue relative to full irrigation (4% and 9%, respectively), but these irrigation strategies led to an increase in the water economic value (9% and 20% respectively) relative to full irrigation. The moderate water shortage at the vegetative growth (T2) and bulbing (T3) stages presumed a low reduction in gross revenue (below 5%), but the water savings that they provided were small (below 8%). The greatest reductions in gross revenue were obtained with severe water stress at the vegetative growth (T5) and bulbing stages (T6) (17% and 15%, respectively), which seriously questioned the economic viability of the crop. The average water economic values obtained in this research ranged from  $5.14 \, \text{em}^{-3}$  (T5) to  $6.24 \, \text{em}^{-3}$  (T7), and these values are similar to the ranges of those obtained for other horticultural crops in the area, such as for chufa (*Cyperus esculentus*, L. var. *sativus* Boeck.;  $4.08 \, \text{em}^{-3}$ , [64]) and watermelon ( $6.14 \, \text{em}^{-3}$ , [25]), both in field conditions.

Overall, it can be stated that if water is not a limiting factor, irrigation to full requirements should be applied. Nevertheless, if water is scarce, applying 50% of the IWR during the bulb ripening stage (T7) may lead to important water savings (approximately 22%), while decreasing the MY and, consequently, the gross revenue, by 9%. An intermediate advised IS involves reducing the IWA during the bulb ripening stage to 75% of the IWR (T4), which would slightly reduce the MY (4%), with water savings of approximately 11%.

#### 3.2.6. Conclusions

Field experiments were carried out in Spain under Mediterranean conditions to study the effects of regulated deficit irrigation on the growth, plant water status, yield response, bulb quality, irrigation water use efficiency and crop profitability of the onion 'Hamaemi'. Moderate water shortage (75% of the IWR) at the vegetative growth and bulbing stages presumed a low reduction in gross revenue but with small water savings. Severe deficit irrigation (50% of the IWR) applied at the vegetative growth and bulbing stages negatively affected the biomass production, water status

and marketable yield. Reducing the water applied to 50% of the water requirements during the bulb ripening stage led to important water savings and improved IWUE when compared to full irrigation while reducing, although not drastically, the marketable yield and, therefore, the gross revenue; thus, this strategy can be recommended in cases of severe water shortage conditions. Reducing the irrigation water applied to 75% of the water requirement during the bulb ripening stage resulted in a slight reduction in yield but with similar IWUE to that obtained with full irrigation; therefore, it could be considered as a recommended strategy for onion production under mild water shortage.

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**Chapter 4. Sweet pepper** 

# 4.1. Productive response and irrigation water use efficiency of pepper (*Capsicum annuum* L.) to different deficit irrigation regimes

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#### **4.1.1. Abstract**

Irrigation water is an essential element for food production in the Mediterranean area. Agriculture consumes 70% of the total freshwater, and its shortage is becoming critical in arid and semiarid areas of the world. Irrigation water use efficiency could be improved by adequate irrigation management. For this reason, production and irrigation water use efficiency of pepper in response to three different irrigation strategies (T1, T2 and T3) were studied, applying 100, 75 and 50% of irrigation water requirements, determined as the difference between calculated crop evapotranspiration and effective precipitation. Crop evapotranspiration was determined from reference evapotranspiration, calculated from class A pan evaporation, with a unique crop coefficient adapting the duration of each phase to the growing cycle. The irrigation water applied was 782, 591 and 403 mm in T1, T2 and T3 respectively. The highest marketable yield was observed in T1 (7.72 kg m<sup>-2</sup>) followed by T2 (5.57 kg m<sup>-2</sup>) and finally T3 (1.54 kg m<sup>-2</sup>). Furthermore, T3 had a significantly higher appearance of fruits with blossom end rot (4.10 kg m<sup>-2</sup>), followed by T2 (1.90 kg m<sup>-2</sup>) and T1 (0.94 kg m<sup>-2</sup>). Fruit quality parameters including color indices, firmness, acidity and vitamin C content were not altered by the deficit irrigation strategies, while soluble solids content, polyphenols and carbohydrate content increased with the decrease of irrigation dose. T2 led to improved irrigation water use efficiency (10.04 kg m<sup>-3</sup>) of sweet pepper cultivated in open field. T3 negatively impacted the marketable yield and increase the nonmarketable yield, which in turn leading to a reduction in irrigation water use efficiency (4.21 kg m<sup>-3</sup>). Therefore, irrigating at 75% of water requirements could be an advisable strategy under conditions of water scarcity.

**Keywords**: Evapotranspiration; irrigation doses; sweet pepper; blossom end rot; fruit quality.

#### 4.1.2. Introduction

Irrigation water is an essential element for crop production (Howell, 2001; Steduto et al., 2012). Worldwide, agriculture uses approximately 70% of freshwater; in Spain agriculture consumes around 68% of the total water use (FAO, 2016). During recent years, freshwater shortage has become critical in the arid and semiarid areas of the world, increasing competition for water among agricultural, industrial and urban consumers (Chai et al., 2016). Rapid population growth, incidence of drought caused by climate change, particularly in the Mediterranean area, and diversification of human activities, are factors in predicting that water demand will continue to increase in the foreseeable future (Fereres, 2008). For this reason, and considering irrigation costs, it is necessary to increase the productivity of water use for crop production (Fereres and Soriano, 2007). Irrigation water-use efficiency (IWUE) is a common indicator employed to assess the efficiency of the use of irrigation water in crop production (Tolk and Howell, 2003; Pascual-Seva et al., 2016). At present, there are challenges in maximizing IWUE and increasing crop productivity per unit of water applied. Within this context, the strategy of deficit irrigation implies application of irrigation water at lower levels than optimum crop water requirements, aiming to improve the IWUE (Capra et al., 2008; Chai et al., 2016). The real challenge is to establish deficit irrigation on the basis of maintaining, or even increasing, crop productivity while saving irrigation water and, therefore, increasing the IWUE (Chai et al., 2016). For this reason, deficit irrigation requires precise knowledge of the crop yield response to water applied (Fereres and Soriano, 2007).

Sweet pepper is considered very sensitive to water stress showing large yield reductions (Steduto et al., 2012). It has a very long growth cycle, occurring largely in summer, when evapotranspiration (ET) demands are high and rainfall is scarce, leading to recurrent water stress episodes (González-Dugo et al., 2007). The objective of this study was to evaluate the response of pepper in terms of growth, yield, fruit quality and IWUE under deficit irrigation in open field conditions.

#### 4.1.3. Material and methods

The experiment was carried out in 2016 at the Cajamar Experimental Center in Paiporta, Valencia, Spain (39.4175 N, 0.4184 W). The soil texture was silty loam, pH=7.4, EC 0.39 dS m<sup>-1</sup>, with 1.89% organic matter, 43 mg available phosphorous kg<sup>-1</sup> (Olsen), 340 mg available potassium kg<sup>-1</sup> (ammonium acetate extract). Irrigation water (EC 2.53 dS m<sup>-1</sup> and 77 mg N-NO<sub>3</sub><sup>-</sup> kg<sup>-1</sup>) was pumped from a well. The incorporation of nutrients (200-100-300 kg ha<sup>-1</sup> N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O) was by fertigation following the criteria indicated by Pomares et al. (2007). Sweet pepper seeds of cv. Estrada F1 (Nunhems®) were germinated in polystyrene trays of 209 cells in a peat moss substrate on 21st January in a greenhouse. Plants were transplanted on 15th March, when they reached the four-leaf stage, to open field in a staggered pattern at a spacing of 0.45 m. The row length was 7.2 m, and the distance between the center of the flat raised beds was 2 m, with the raised beds 0.6 m wide at the base and 0.25 m high covered by 0.025 mm thick and 1.0 m wide black polyethylene mulch.

Three irrigation rates (T1, T2 and T3) were evaluated, corresponding to 100%, 75% and 50% of the irrigation water requirement (IWR; mm day<sup>-1</sup>) throughout the growing season. The crop evapotranspiration (ETc; mm) was calculated from the reference evapotranspiration (ETo) determined from a Class A evaporation pan installed in the experimental center, with pan coefficient (K<sub>p</sub>) 0.815 (Doorenbos and Pruitt, 1977) and a single crop coefficient (K<sub>c</sub>), proposed for local conditions by the Instituto Valenciano de Investigaciones Agrarias (IVIA, 2011). The application efficiency (Ef; including percolation and uniformity) was 0.95 (Pomares et al., 2007). The effective precipitation (Pe, mm) was determined from rainfall data using the method of the U.S. Bureau of Reclamation (Stamm, 1967), as presented by Pascual-Seva et al. (2016). The IWR was determined by: IWR = (ETc-Pe)/Ef. Water was supplied by a drip irrigation system with one line per bed with emitters spaced 0.30 m apart and a discharge of 2.2 L h<sup>-1</sup>. From transplanting until establishment, the plants of all treatments were irrigated without restriction, and then the three irrigation

strategies were initiated, with three irrigation events per week until harvesting started. After the beginning of harvesting irrigation was applied daily.

Volumetric soil water content (VSWC) was continuously monitored with ECH<sub>2</sub>O EC-5 capacitance sensors, which were connected to an Em50 data logger, using the ECH<sub>2</sub>O Utility software (Decagon Devices Inc., Pullman, WA, USA). One sensor per treatment was installed horizontally in the middle of a bed next to the irrigation pipe, equidistant between two emitters, at 0.20 m depth for all treatments and additional sensors were placed at 0.30 m depth for T1 and T3 (the two extreme strategies). In order to compare VSWC between treatments, Figure 1 is presented in terms of the rate between the VSWC values to the VSWC at field capacity.

Harvesting started on 23 June and lasted until 23 September. Total fruit yield was separated into marketable and non-marketable yield, following the criteria described by The European Commission (2011). In turn, marketable yield was classified into two categories ('Extra' Class and Class I), and non-marketable yield (Class II) was also classified according to the nature of blemishes, including fruits affected with blossom end rot (BER), sunscald, or showing symptoms of Tomato spotted wilt virus, (TSWV) and fruits that were small or with defects in shape. The IWUE was calculated as the ratio of marketable yield (kg m<sup>-2</sup>) and irrigation water applied (I<sub>applied</sub>, m<sup>3</sup> m<sup>-2</sup>) (Cabello et al., 2009). Yield response to water deficit during the growing season was determined according to Doorenbos and Kassam (1979), using the equation:  $(1-Ya/Ym) = k_v (1-ETa/ETm)$ , where Ya and Ym are the actual and maximum marketable yield (kg m<sup>-2</sup>), respectively; ETa and ETm are the actual and maximum ET (mm), respectively; and k<sub>y</sub> is the yield response factor. ETa and ETm were calculated using soil water balance:  $ET = I_{applied} + Pe$ , considering both the drainage and the variation in the volumetric soil water content negligible. Five representative fruits from each replicate were selected to determine fruit characteristics (length, width and fruit flesh thickness). Fruit color coordinates (L\*, a\* and b\*) were measured using a chroma meter (Minolta CR-300; Konica Minolta Sensing Inc., Tokyo, Japan). Chroma (C\*) was calculated as  $C^*=\sqrt{(a^2+b^2)}$  (Pathare

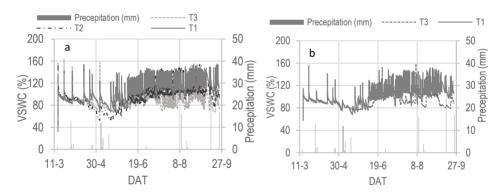
et al., 2013) and Hue angle (H°) was calculated as H° =Arctang(b/a)+180 (McGuire, 1992), and color index (CI) was calculated as CI=(a×100)/(L×b) (Cristina, 2014). Fruit firmness was determined using digital penetrometer with a tip of 8 mm diameter (penefel DFT 14, France). Soluble solids content (SSC, °Brix) was determined with fruit juice using a digital refractometer (Atago®, Pal-1, 0-53%, Japan). Acidity was determined as citric acid (%), by titration with 0.1 M NaOH, using 10 ml of fruits juice. Vitamin C (g ascorbic acid 100 g<sup>-1</sup> fresh fruit) was measured by the volumetric method of 2,6-dichloroindophenol (AOAC International, 2000). Polyphenol content (g gallic acid 100 g<sup>-1</sup> fresh fruit) was determined by the spectrophotometric method of Folin-Ciocalteu with standard curve of gallic acid at 670 nm in UV–vis spectrophotometer (Unicam-Helios  $\alpha$ , USA)(Domene et al., 2014). Total carbohydrates (g total carbohydrates 100 g<sup>-1</sup> fresh fruit) was determined with the spectrophotometric method of phenol-sulfuric acid at 490 nm in UV–vis spectrophotometer (Unicam-Helios  $\alpha$ , USA) (BeMiller, 2014).

Growth parameters were evaluated at the end of plant growth. Plant height and stem diameter were determined in the field on 5 plants from each plot. Leaf chlorophyll content (SPAD) was measured at three points in three fully developed leaves in each plant using a SPAD-502 m (Konica Minolta Sensing Inc., Tokyo, Japan). Aboveground biomass as divided into two parts and analyzed separately: vegetative, including shoots with all their leaves (hereinafter referred to as shoots), and fruit. Each sampled plant part (shoots and fruit) was dried at 65°C in a forcedair oven (Selecta 297, Barcelona, Spain) until reaching a constant weight to obtain dry weights and dry matter content.

This study was performed in a random block design with three replications, each replication consisting of a bed. The results were analyzed by analysis of the variance (ANOVA) using the statistical program Statgraphics Centurion XVI (StatPoint Technologies, 2014). The least significant difference (LSD) at a 0.05-probability level was used as the mean separation test.

## 4.1.4. Results and discussion

The duration of each growth stage based on Allen et al. (1998) was 35, 42, 63 and 53 days corresponding to initial, growth development, mid-season and late season stages, respectively. Kc values were 0.30, 0.95 and 0.80 corresponding to initial, mid-season and late season stages, respectively. The total pan evaporation was 1081 mm and Pe contribution during the growing season was 78 mm. Therefore 2016 can be classified as a dry season. The I<sub>applied</sub> was 782, 591, and 403 mm in T1, T2 and T3, respectively. These values include 37 mm corresponding to the initial irrigation that was applied equally for all treatments, to achieve the correct plant establishment (21 days after transplanting; DAT). In this initial period, VSWC followed a similar trend in all three treatments. Thereafter, differential irrigation was initiated, resulting in the full irrigation treatment with higher VSWC at 0.2 and 0.3 m depth throughout the growing season, while T3 had the lowest VSWC especially after 90 DAT until the end of season, with higher reduction in VSWC at 0.3 m depth (Figure 1).



**Figure 1.** Relative soil water content (VSWC; volumetric soil water content/volumetric soil water content at field capacity) at 0. 20 m depth for T1, T2 and T3 (a) and at 0.30 m depth for T1 and T3 (b), and daily rainfall (vertical bars) during the growing season.

Results of yield, marketable yield components and IWUE are presented in Table 1 and non-marketable yield in Table 2. Water restriction negatively affected yield, T3 resulted in the lowest yield (7.07 kg m<sup>-2</sup>; P<0.05) consequence of the lowest marketable yield (1.54 kg m<sup>-2</sup>; P<0.01). The highest total, 'Extra' Class, and Class I yield were obtained in T1 (7.72, 4.56 and 3.16 kg m<sup>-2</sup>, respectively). The average

fruit weight for marketable fruits was not affected (p<0.05) by the irrigation treatment, which is expectable since small fruits were not considered in the marketable yield. T3 led to the lowest IWUE (4.21 kg  $m^{-3}$ ; p<0.01) due to greater reduction of marketable yield (80%) compared to water savings (51%).

**Table 1.** Effect of irrigation dose on total yield, marketable yield; categories of marketable yield ('Extra' Class and Class I), average fruit weight and irrigation water use efficiency (IWUE).

	Total yield			IWUE		
Treatments	$(kg m^{-2})$		kg m <sup>-2</sup>	g fruit <sup>-1</sup>	$(Kg m^{-3})$	
		'Extra' Class	Class I	Total MY	='	
T1	10.42 a	4.56 a	3.16 a	7.72 a	106.3	10.37 a
T2	9.76 a	3.49 a	2.08 b	5.57 b	100.4	10.04 a
Т3	7.07 b	0.73 b	0.80 c	1.54 c	98.7	4.21 b
LSD	2.26 *	1.29 **	0.47 **	1.72 **	0.13 ns	2.61 **

<sup>\*\* (\*):</sup> Indicates significant differences at P≤0.01 (P≤0.05). ns: Indicates no significant difference.

Non-marketable yield represented 78% of the total yield for T3, while it was 43% in T2, and 26% in T1. T3 led to the highest non-marketable yield (5.53 kg m $^{-2}$ ; p<0.01), mainly because of the high presence of fruits affected by BER (4.10 kg m $^{-2}$ ; p<0.01), accounting for 74% of the non-marketable yield, confirming that water stress increased appearance fruits with BER (Saure, 2001). Nevertheless, this strategy led to the smallest presence of small fruits and fruits with defects in shape (0.85 kg m $^{-2}$ , P≤0.05). The irrigation rate did not affect the incidence of sunburn or TSWV symptoms.

**Table 2.** Effect of irrigation dose on total non-marketable yield (NMY) and their classes including fruit with blossom end rot (BER), sunburn, TSWY symptoms and small and defects shape fruits.

Treatments	Small and defects in	Sunburn	BER	TSWY	Total NMY
	shape fruits (kg m <sup>-2</sup> )	(kg m <sup>-2</sup> )			
T1	1.53 a	0.06	0.94 c	0.27	2.7 с
T2	1.42 a	0.24	1.90 b	0.52	4.19 b
T3	0.85 b	0.07	4.10 a	0.52	5.53 a
LSD	0.54 *	0.18 ns	0.49 **	0.35 ns	1.06 **

<sup>\*\* (\*):</sup> Indicates significant differences at P≤0.01 (P≤0.05). ns: Indicates no significant difference.

Some studies agree with these results, such as Ćosić et al. (2015), who analyzed the effect of three irrigation rates (100, 80, and 70% of ETc) on sweet pepper production during three years, obtained similar results; with higher marketable yield

(8.4 kg m<sup>-2</sup> in 100% ETc), increasing first class fruit yield with increasing irrigation rates. Mardaninejad et al. (2017) stated that severe deficit irrigation (40% and 60% of full water requirements) reduced fruit yield, and increased non-marketable yield, in relation to 100% and 80% of full water requirements. Aladenola and Madramootoo (2014) concluded that IWUE decreased with decreasing irrigation level.

**Table 3.** Effect of irrigation dose on fruit characteristics [length, width, flesh thickness (FT), fresh weight (FW), dry weight (DW), dry matter content (DM) and skin color indexes [Hue angle  $(H^{\circ})$ , Chroma  $(C^{*})$  and color index (CI) of marketable fruits].

Treatments	Length	Width	FT	FW	DW	DM (%)	Н°	C*	CI
	(mm)	(mm)	(mm)	(g fruit <sup>-1</sup> )	(g fruit <sup>-1</sup> )				
T1	21.5 a	44.8	2.9 a	79.9 a	6.1	7.6 b	120.2	38.6	-13.1
T2	19.5 ab	41.2	3.1 a	69.5 ab	5.7	8.2 ab	119.9	36.9	-11.8
T3	18.2 b	40.0	2.5 b	59.6 b	5.7	9.5 a	120.7	34.9	-13.1
LSD	2.48 *	5.5 ns	0.3 **	14.7 *	1.7 ns	1.4 *	2.8 ns	5.3 ns	2.5 ns

<sup>\*\* (\*):</sup> Indicates significant differences at P\u20130.01 (P\u20130.05). ns: Indicates no significant difference.

As for the yield response to water deficits, considering as maximum yield (Ym) the marketable yield obtained under T1, actual yield (Ya) corresponding to T2 and T3 strategies, and ETm and ETa, the corresponding ET to the cited yields, the fitted linear regression is as follows: 1-(Ya/Ym) = 1.47 [1-(ETa/ETm)], which presents a high correlation coefficient (r=0.97) and statistical significance ( $P \le 0.01$ ), and the yield response factor ( $K_y$ ) was 1.47. Marketable yield reduction obtained in T2 was almost proportional and in T3 was drastically increased with reduction of  $I_{applied}$  with  $K_y$  about 1.2 and 1.7 in T2 and T3, respectively. These results confirm that sweet pepper is highly sensitive to water stress (Steduto et al., 2012).

**Table 4.** Effect of irrigation dose on fruit firmness, soluble solid content (SSC), acidity, vitamin C, polyphenols and total carbohydrates content.

Treatments	Fruit firmness (N)	SSC (°Brix)	Acidity (%)	Vit. C (mg AA/100 ml)	Polyphenols (mg GA/100 ml)	Carbohydrates (mg Car/100 ml)
T1	13.0	4.6 b	0.09	0.7	169.3 b	230.1 b
T2	12.0	5.0 ab	0.09	0.8	206.3 a	280.0 a
T3	12.6	5.8 a	0.07	0.7	237.8 a	291.8 a
LSD	1.8 ns	0.8 *	0.02 ns	0.3 ns	33.0 **	40.6 *

<sup>\*\* (\*):</sup> Indicates significant differences at P≤0.01 (P≤0.05). ns: Indicates no significant difference.

Sweet pepper fruit increased ( $P \le 0.05$ ;  $P \le 0.01$ ) their length, flesh thickness and fresh weight with increasing irrigation rate, while the vice versa for dry matter content (Table 3). Irrigation rate did not affect (P≤0.05) fruit width, fruit dry weight or color parameters H°, C\* and CI (Table 3). Similar results were obtained by Ćosić et al. (2015), who found the shortest fruits under 60% ETc, and fruit width not affected by water stress. Fruit quality parameters such as fruit firmness, acidity and vitamin C were not altered (P≤0.05) by the water shortage, while T2 and T3 had a higher polyphenols and carbohydrates contents (P≤0.05, Table 4). Polyphenols are secondary metabolites which contribute to fruit pungency, bitterness, flavor and color (Nagy et al., 2015). SSC increased (P≤0.05) with the decrease of irrigation dose, agreeing with Aladenola and Madramootoo (2014) who stated that highest and lowest SSC were found under 40% ETc and 100% ETc, respectively. Deficit irrigation strategies did not affect (P≤0.05) plant height, stem diameter, leaf chlorophyll content, or shoot fresh and dry weight (Table 5). However, plants irrigated with T3 resulted in smaller fresh and dry fruit weight, as well as total plant weight  $(P \le 0.05; Table 5).$ 

**Table 5.** Effect of irrigation dose on fresh and dry biomass of different parts of the plant, plant height (PH, cm), stem diameter (SD, mm) and leaf chlorophyll index (SPAD).

Treatments	Fresh biomass (g plant <sup>-1</sup> )			Dry b	iomass (g p	lant <sup>-1</sup> )	PH	SD	SPAD
	Shoots	Fruits	Total	Shoots	Fruits	Total	(cm)	(mm)	(-)
T1	1129.3	5266.1a	6395.4a	231.1	400.5 a	631.6 a	123.8	24.2	61.1
T2	1122.8	4612.8a	5735.6a	221.0	374.5 a	595.5 a	123.3	23.7	61.7
T3	1049.5	3400.8b	4450.3b	212.7	269.9 b	482.5 b	123.7	24.3	64.2
LSD	91.2 ns	924.3 **	898.2**	26.3 ns	59.7 **	68.7 **	2.9ns	1.6ns	3.9 ns

<sup>\*\* (\*):</sup> Indicates significant differences at P≤0.01 (P≤0.05). ns: Indicates no significant difference.

#### 4.1.5. Conclusions

The present study analysed the effect of different irrigation rates on the growth, fruit quality, IWUE and yield of sweet pepper cv. Estrada F1. Deficit irrigation at 50% of the nominal crop water requirements resulted in a considerable reduction in total and marketable yield, leading to a reduction in IWUE. This treatment exhibited a larger amount of non-marketable yield, especially caused by BER and produced shorter fruits, with lower flesh thickness, higher dry matter content and higher SSC,

polyphenol and carbohydrate content. Irrigating at 75% of water requirements improved IWUE, with a 28% reduction in marketable yield. Therefore, irrigating at 75% of water requirements could be an advisable strategy under conditions of water scarcity. If water is not a limiting factor, applying 100% of water requirements is advisable.

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4.2. Effects of deficit irrigation on the yield and irrigation water use efficiency of drip-irrigated sweet pepper (Capsicum annuum L.) under Mediterranean conditions

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**Under review in Irrigation Science** 

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#### **4.2.1. Abstract**

Water scarcity is becoming critical worldwide and is seriously affecting agricultural production, especially in arid and semi-arid areas. Therefore, there is increasing interest in improving water productivity in agriculture. This research aims to study the effects of deficit irrigation on the productive response of sweet pepper plants, including plant water status, plant growth, irrigation water use efficiency (IWUE), and fruit quality. Nine deficit irrigation strategies were assayed during two seasons. These irrigation strategies included applying 100%, 75% and 50% of the irrigation water requirement (IWR) during the entire growing period (continued deficit irrigation) or applying 75% and 50% of the IWR during one of the following stages (regulated deficit irrigation): vegetative growth, fruit-setting and harvesting. Pepper plants cultivated under deficit irrigation had reduced fruit biomass and indexes of plant water status. Applying water deficits during the vegetative growth and fruit-setting stages had minimal effects on the marketable yield but with minimal water savings. Irrigating pepper plants with 75% or 50% of the IWR during the entire crop cycle or with 50% of the IWR during harvesting resulted in a high incidence of fruits affected by blossom end rot, which in turn, led to a drastic reduction of the marketable yield in relation to fully irrigated plants (-36%, -55% and -44%, respectively). These strategies also recorded the highest soluble solid and phenolic contents. Reducing the water applied to 75% of the IWR at harvesting led to a yield reduction (-19%) but also provided important water savings (21%). This strategy also vielded acceptable levels of soluble fruit solids and phenolic compounds. Under high IWR-demanding conditions, this strategy could be applied to end the crop cycle at the beginning of September, when most of the marketable yield has already been harvested, providing important water savings and leaving the land available for other crops.

**Keywords:** continued and regulated deficit irrigation, volumetric soil water content, fruit quality traits, harvest index, gross revenue, water economic value.

#### 4.2.2. Introduction

The sweet pepper (*Capsicum annuum* L.) is considered one of the most important vegetable crops worldwide, and it has important economic value. The total land area of pepper cultivation in 2017 was approximately 1.99 million ha, leading to the production of approximately 36 million tons (Faostat, 2018). Worldwide, China is the largest pepper producer, followed by Mexico and Turkey (Faostat, 2018). In Europe, Spain, Italy and Romania are the main producers, and Spain is the second largest exporter of peppers after Mexico (Faostat, 2018).

Drought stress is one of the major limiting factors for vegetable crop production. Water is becoming increasingly scarce worldwide, seriously affecting agricultural production, especially in arid and semi-arid areas (Mancosu et al., 2015; Chai et al., 2016). Globally, agriculture is the largest consumer of freshwater, representing approximately 68% (Aquastat, 2018). During the last decades, global water withdrawal has surpassed population growth by 1.7 times (Aquastat, 2018). By 2050, the world population is expected to be 9 billion people, which would require a 60% increase in agricultural production and a 15% increase in water withdrawal (WWAP, 2016). In the last five decades, the area equipped for irrigation increased worldwide from 196 million ha to approximately 325 million ha (Aquastat, 2018). Forecasts indicate that climate change will affect the agriculture sector, increasing global temperature and evapotranspiration (ET) while reducing precipitation with an altered distribution and pattern, which would consequently increase water demands (Turral et al., 2011; IPCC, 2014; Kahil et al., 2015). The existing drought risks are expected to intensify, particularly in regions where water scarcity is already a concern, as in the Mediterranean region (Iglesias and Garrote, 2015). The Mediterranean climate is characterized by mild winter temperatures and long, hot and dry summers, with precipitation subject to high inter-annual and seasonal variability; therefore, irrigation is essential for crop production (Turner, 2004; Daccache et al., 2014; Galindo et al., 2018).

These indicators point to an increase in food production and irrigation costs, raising competition for water resources among the consumers. To mitigate the effects that climate change will foreseeably entail, researchers are trying to increase water productivity through different approaches (Molden et al., 2010; Levidow et al., 2014; Kang et al., 2017; Galindo et al., 2018).

Irrigation water use efficiency (IWUE) and water use efficiency (WUE) are common indicators to assess the efficiency of irrigation water usage in agriculture (Tolk and Howell, 2003; Pascual-Seva et al., 2016). IWUE improvement is closely related to the reduction of water consumption and loss (ET, runoff and losses in depth) while maintaining crop yield at a certain level (Leskovar et al., 2014; Kang et al., 2017). Several investigators, such as Pereira et al. (2002), Costa et al. (2007), Capra et al. (2008), Geerts and Raes (2009), Chai et al. (2016) and Galindo et al. (2018), have reported that deficit irrigation can improve water productivity. Deficit irrigation (DI) is generally considered to be an irrigation practice whereby crops are irrigated with water amounts below their requirements for optimal plant growth. DI includes continued deficit irrigation (CDI) and regulated deficit irrigation (RDI). The CDI approach is based on imposing the water deficit uniformly over the entire crop cycle, thereby avoiding severe water stress at any particular moment that might affect marketable yield (Iniesta et al., 2009; Galindo et al., 2018). The RDI approach is a stage-based deficit irrigation, consisting of imposing water deficits at specific phenological stages, when crops are less sensitive to water stress (Fereres and Soriano, 2007; Geerts and Raes, 2009; Reddy, 2016; Kang et al., 2017).

As these water reductions may lead to considerable yield reductions (Kuşçu et al., 2014), effective application of this approach requires identification of, the most critical growth stages for each specific crop species and cultivar. Therefore, crop sensitivity to water deficit must be evaluated at different stages to determine the optimal timing and extent of water reduction required to achieve efficient water use while obtaining adequate yield (Chai et al., 2016; Yang et al., 2017). Doorenbos and Kassam (1979) introduced a linear crop-water production function to describe the reduction in yield

when crop is under stress due to a shortage of soil water, being the yield response factor ( $K_y$ ) the factor that describes the reduction in relative yield according to the reduction in the crop evapotranspiration (ETc). Monitoring soil moisture can ensure adequate soil water status, limiting drainage and leading to improved water productivity while minimizing the risk of yield reduction (Fereres and Soriano, 2007; Blanco et al., 2018).

A short period of mild water deficit may affect plant water status (Pérez-Pastor et al., 2014; Chai et al., 2016). Water content and water potential have been used as indicators of leaf water status. The use of water content has been replaced by the relative water content (RWC), which is an index based on the maximum amount of water a tissue can hold (Yamasaki and Dillenburg, 1999). RWC is an index that expresses the absolute amount of water that a plant requires to reach artificial full saturation (González and González-Vilar, 2001). RWC is closely related to cell turgor, which is the process directly driving cell expansion (Jones, 2004), and it is used as a meaningful index for dehydration tolerance (Anjum et al., 2011; Kalariya et al., 2015). Water stress modifies cell membrane structure and composition, which causes leakage of ions (Taiz and Zeiger, 2002). The rate of damage to cell membranes by water stress may be assessed through the cell membrane stability index (MSI), which detects the degree of cell membrane injury induced by water stress (Bajji et al., 2002).

Sweet Italian pepper plants have a very long growth cycle, occurring largely in summer, when ET demands are high and rainfall is scarce, particularly in the Mediterranean climate, where irrigation is needed for any significant summer cropping (Delfine et al., 2002; González-Dugo et al., 2007). Furthermore, the pepper plant is considered very sensitive to water stress, resulting in large yield reductions (Steduto et al., 2012). Earlier reports, such as Fernández et al. (2005) and González-Dugo et al. (2007) in Spain, Dorji et al. (2005) in New Zealand, Sezen et al. (2006) in Turkey and Guang-Cheng et al. (2010) in Southern China, have demonstrated the susceptibility of pepper growth and yield to water shortages. Sezen et al. (2014) reported that CDI decreased the red pepper yield but increased both WUE and IWUE.

Currently, there is an increase in consumer interest for pepper fruit quality, due to their beneficial effects for human health, functional properties and nutritional value, in addition to the sensorial traits of taste and aroma (Howard et al., 2000; Deepa et al., 2007). Fresh pepper fruits are an important source of ascorbic acid (vitamin C) and phenolic compounds (predominantly flavonoids and capsaicinoids), which are well known for their antioxidant activity (Howard et al., 2000; Naczk and Shahidi, 2006; Frary et al., 2008). Many authors have stated that not only water productivity but also fruit quality parameters could be improved by certain levels of deficit irrigation (Chen et al., 2013; Chen et al., 2014; Kuşçu et al., 2014; Yang et al., 2017). Patanè et al. (2011) stated that deficit irrigation improved total soluble solids content (SSC), titratable acidity and vitamin C of tomato fruits, another important solanaceous plant.

Therefore, as mentioned, it is essential to use optimal and innovative irrigation management to maximize both water productivity (Fernández et al., 2005; Mardani et al., 2017) and fruit quality (Yang et al., 2017). These parameters depend to a large extent on the plant material and the environment in which they are grown, so irrigation management should be adapted to each plant material and specific environmental conditions. The objective of this study is to evaluate the vegetative and productive responses of pepper plants, including plant water status, yield, K<sub>y</sub>, IWUE and fruit quality, to CDI and RDI under Mediterranean conditions.

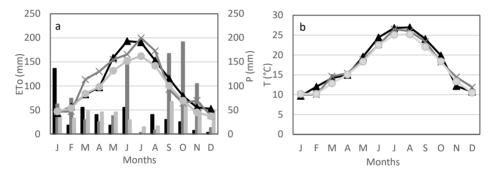
## 4.2.3. Materials and Methods

#### 4.2.3.1. Experimental site description

The field studies were carried out at the Cajamar Experimental Centre in Paiporta, Valencia, Spain (39.4175 N, 0.4184 W) over two consecutive growing seasons (GS; 2017 and 2018). To avoid soil replanting disorders resulting from serial pepper cropping, the experiments were conducted in two subplots within the experimental plot. The soil at the site is deep with a medium texture (silt loam) and is classified as Petrocalcic Calcixerepts, according to the USDA Soil Taxonomy (Soil Survey Staff, 2014). The soil was very slightly alkaline (pH = 7.55) and highly

fertile [organic matter = 1.9%; high available phosphorous (44 mg kg<sup>-1</sup>; Olsen) and potassium (515 mg kg<sup>-1</sup>; ammonium acetate extract) concentrations].

The local climate, according to Papadakis's agro-climatic classification (Verheye, 2009), is subtropical Mediterranean (Su, Me) with hot and dry summers. The annual average rainfall is approximately 450 mm, irregularly distributed throughout the year with the majority occurring in autumn and the beginning of spring. Figure 1 shows the most significant climatological data of the experimental GS, as well as the average values for the 2001-2018 period.



**Figure 1.** Monthly reference evapotranspiration (ETo; a), precipitation (P; a), and average temperature (T; b) during 2017 ( ), 2018 ( ) and average values for the 2001-2018 period ( ).

# 4.2.3.2. Plant material and agronomic details

The sweet Italian pepper 'Estrada F1' (Nunhems®) was used in the experiments. This cultivar was chosen because of its adequate adaptation to the soil and climate conditions in the area, its high productivity under open field cultivation [as evaluated in the Cajamar Experimental Centre (Fundación Cajamar, 2016)] and its great acceptance by consumers (verified in public demonstrations periodically conducted in the Experimental Centre). The fruits, which are adequate for fresh green pepper production, have a triangular longitudinal section 15-30 cm in length with a dark green colour. The plants present an indeterminate growth pattern with intermediate vigour and show intermediate resistance to Tomato Spotted Wilt Virus.

Sowing took place on 27 January 2017 and 12 February 2018, in 104-cell polystyrene trays, in a peat moss-based substrate (70% blonde and 30% dark) recommended for vegetable seedbeds (Pindstrup Mosebrug S.A.E., Sotopalacios, Spain). The trays were maintained in a Venlo-type greenhouse. Thereafter, seedlings were transplanted on 28 March 2017 and 13 April 2018 (when plants reached the four-leaf stage) in an open field in flat raised beds spaced 0.30 m apart with one plant row per bed. The raised beds were 0.6 m wide (the distance from the bed centre-to-centre was 1.5 m), 7.25 m long and 0.15 m high. They were covered by black polyethylene mulch 0.025 mm thick and 1.0 m wide. Plants were horizontally supported by three nylon guide cords parallel to both sides of the plant line as described by Maroto (2002). The incorporation of nutrients (200-100-300 kg ha<sup>-1</sup> N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O) was performed by fertigation, following the criteria indicated by Condés (2017).

### 4.2.3.3. Deficit irrigation strategies and growth stages

The pepper growth period was divided into four stages; (1) initial, from transplanting to plant establishment; (2) vegetative growth, from establishment until early fruit setting; (3) early fruit setting and bearing (hereafter referred as fruit-setting), from setting until starting harvest; and (4) harvesting, which extends until the end of the harvest. All the plants were irrigated without restrictions during the initial stage to ensure correct plant establishment. Afterwards, 9 irrigation strategies (IS) were applied in both GS. These IS differed in the amount of water applied in each irrigation event: T1, T2 and T3 applied 100%, 75% and 50%, respectively, of the irrigation water requirements (IWR) throughout the entire growing season; T4, T5, and T6 reduced the irrigation water applied (IWA) to 75% of the IWR during crop growth stages 2, 3 and 4, respectively; and T7, T8 and T9 reduced the IWA to 50% of the IWR, at the same growth stages.

## 4.2.3.4. Irrigation scheduling

For each irrigation event, the corresponding IWR were determined as:

$$IWR = \frac{ETc - Pe}{Ef}$$

where ETc (mm) is the crop evapotranspiration, Pe is the effective precipitation (mm), determined from rainfall data using the U.S. Bureau of Reclamation method (Stamm, 1967), as presented by Pascual-Seva et al. (2016), and Ef is the irrigation efficiency. This Ef was considered as 0.95, taking into account the distribution uniformity (0.98), the deep percolation ratio (0.97) and that the leaching requirement is negligible, as has been stated in similar experiments carried out in 2016 in the Experimental Centre for the same pepper cultivar (Abdelkhalik et al., in press).

ETc was determined from the reference evapotranspiration (ETo) and the single crop coefficient (Kc), with values of 0.3, 0.95 and 0.8, corresponding to initial, mid-season and late season stages, respectively, which were proposed for local conditions by the IVIA (2011) following the criteria described by Allen et al. (1998) and adapting for the duration of each stage to the growing cycle.

$$ETc = ETo \times Kc$$

ETo was determined according to Allen et al. (1998) as follows:

$$ETo = E_{pan} \times K_p$$

where  $E_{pan}$  (mm day<sup>-1</sup>) is the evaporation from a class A pan installed adjacent to the experimental plot, and  $K_p$  (0.815) is the pan coefficient determined according to Allen et al. (1998).

Plants were irrigated by a drip irrigation system with a single lateral line per bed using a turbulent flow dripline (16 mm; AZUDRIP Compact; Sistema Azud S.A., Murcia, Spain) with emitters (2.2 L h<sup>-1</sup>) spaced 0.30 m apart. The irrigation was managed by an irrigation control programmer (NODE-100 single station controller, Hunter, California, USA). In each IS, the IWA was recorded by a water flow meter (MJ-SDC TYP E, NWM, Czech Republic).

#### 4.2.3.5. Volumetric soil water content

The volumetric soil water content (VSWC; m³ m⁻³) was continuously monitored by ECH₂O EC-5 capacitance sensors connected to an Em50 data logger, using the ECH₂O Utility software (Decagon Devices, Inc., Pullman, WA, USA). One sensor per treatment was placed below the dripline, at a 20-cm depth, equidistant between two adjacent emitters. Furthermore, in the fully irrigated treatment (T1), where the largest amounts of IWA were applied, another sensor was installed at a 35-cm depth to verify that water losses in depth were negligible. The VSWC was measured and stored every 15 min, and its variation was used to determine the in situ field capacity (FC). To compare the VSWC between the IS and GS, their values are presented as the ratio of the VSWC compared with the VSWC at FC (% FC). For each IS, the irrigation event began when the VSWC in T1 dropped to 80% of the FC, thus applying to each IS the corresponding IWA.

#### 4.2.3.6. Data collection and measurements

#### 4.2.3.6.1. Relative water content (RWC) and membrane stability index (MSI)

Both the leaf relative water content (RWC; %) and membrane stability index (MSI; %) were evaluated at the end of each growth stage. The relative water content was determined from fresh leaf discs of 2 cm in diameter, as reported by Barrs (1968), and was calculated using the following equation (Hayat et al., 2007):

RWC (%) = 
$$\frac{(FW - DW)}{(TW - DW)} * 100$$

where FW is the fresh weight (g), TW is the turgor weight (g) and DW is the dry weight (g).

The membrane stability index was determined from samples of fully expanded leaf tissue (0.2 g), as described by Rady (2011), using the following equation:

MSI (%) = 
$$\left(1 - \frac{C_1}{C_2}\right) * 100$$

where  $C_1$  is the electrical conductivity of the solution (samples submerged in distilled water) after 30 min in a water bath at 40°C, and  $C_2$  is the electrical conductivity of the solution after 10 min at 100°C.

# 4.2.3.6.2. Plant growth and harvest index

Growth parameters were analysed at the end of the crop cycle. Plant height, stem diameter and leaf chlorophyll index (SPAD) were determined with three plants from each plot in the field. Plant height was measured with a measuring tape, while the stem diameter was measured by a digital calibre TOP CRAFT (Ovibell GmbH & Co., Mülheim an der Ruhr, Germany). SPAD allows the indirect and non-destructive evaluation of the content of leaf chlorophyll by means of light intensity absorbed by the tissue sample. SPAD was measured at the end of each stage at three points in three fully developed leaves in each plant using a SPAD-502 m (Konica Minolta Sensing, Inc., Tokyo, Japan). The aboveground part of the plants was partitioned into two parts and analysed separately: vegetative, including shoots with all their leaves (hereafter referred to as shoots), and fruits. Each part was weighed with a precision analytical balance (Mettler Toledo AG204, Powai Mumbai, India) and dried at 65°C in a forced-air oven (Selecta 297, Barcelona, Spain) until reaching a constant weight, allowing the measurement of dry weights and fruit dry matter (DM) content. The harvest index (HI) was determined as the ratio of total yield to total aboveground biomass on a dry mass basis (Fernández et al., 2005).

# 4.2.3.6.3. Yield, irrigation water use efficiency and yield response factor (K<sub>y</sub>)

Harvesting of the first GS occurred between 13 June and 16 October 2017 and consisted of 12 passes. Harvesting in 2018 was undertaken from 22 June until 22 October, and required 11 passes. Following the criteria described by European Regulations (Official Journal of the European Union, 2011), yield was partitioned into these categories: «Extra» Class and Class I (together hereafter referred to as marketable yield; MY) and Class II and fruits that due to their defects do not reach this category (jointly hereafter referred to as non-marketable yield). The non-

marketable yield was classified according to the nature of the blemish, including fruits affected with blossom end rot (BER), sunburn and fruits that were small or with defects in shape.

The IWUE was calculated as the ratio of MY (fresh mass; kg m<sup>-2</sup>) to IWA (m<sup>3</sup> m<sup>-2</sup>) as indicated by Cabello et al. (2009). The WUE was calculated as the ratio of MY (kg m<sup>-2</sup>) to actual crop evapotranspiration (ETa; ETa =Pe+IWA; m<sup>3</sup> m<sup>-2</sup>) following that reported by Ko and Piccinni (2009). The yield response to water deficits was determined by the following equation (Doorenbos and Kassam, 1979):

$$\left(1 - \frac{Y_a}{Y_m}\right) = K_y \left(1 - \frac{ET_a}{ET_m}\right)$$

where Ya and Ym are the actual and maximum MY (kg m<sup>-2</sup>), respectively; ETa and ETm are the actual and maximum ET (mm), respectively; and  $K_y$  is the yield response factor. ETa and ETm were calculated as ET = IWA + Pe, considering negligible both the drainage and the variation in the volumetric soil water content.

# 4.2.3.6.4. Fruit quality parameters

Nine representative fruits at similar states of maturation were selected from those harvested from each plot on 31 July 2017 (fifth pass) and on 25 July 2018 (fourth pass) to determine principal fruit quality parameters that included physical, taste and nutrient quality classifications.

# 4.2.3.6.4.1.Physical parameters

Fruit length and width were measured with a measuring tape. The colour indexes [Hue angle (H°), Chroma (C\*) and colour index (CI)] were calculated from CIELAB (CIE 1976 L\*a\*b\*) colour space coordinates, which were calculated from the mean value of four readings, each of which was obtained from each of the cardinal points of the fruit equatorial zone. Fruit colour coordinates (L\*, a\* and b\*) were measured using a chroma meter (Minolta CR-300; Konica Minolta Sensing, Inc., Tokyo, Japan). Hue angle was calculated as presented by McGuire (1992):

$$H^{\circ} = Arctang \left(\frac{b}{a}\right) + 180$$

Chroma was calculated as stated by Pathare et al. (2013):

$$C^* = \sqrt{(a^2 + b^2)}$$

Colour index was calculated described by Cristina (2014):

$$CI = \frac{a * 1000}{L * b}$$

Fruit firmness was measured by a digital penetrometer with an 8-mm diameter tip (Penefel DFT 14, Agro Technologies, Forges les Eaux, France). The flesh thickness was measured with a digital calibre model TOP CRAFT (Ovibell GmbH & Co., Mülheim an der Ruhr, Germany).

# 4.2.3.6.4.2. Taste quality parameters

The 9 fruits used to determine the above-mentioned parameters were liquefied with a domestic blender, filtering the resulting juice. This filtered juice was used to determine the soluble solids content (SSC, °Brix) using a digital refractometer (PAL-1, Atago, Tokyo, Japan). Acidity was determined as citric acid (g citric acid 100 g<sup>-1</sup> FW), as measured by titration with 0.1 M NaOH. Maturity index was calculated as the ratio of SSC (° Brix) and acidity (g citric acid 100 g<sup>-1</sup> FW).

# 4.2.3.6.4.3. Nutrient quality parameters

Ascorbic acid (vitamin C) was determined by the volumetric method of 2,6-dichloroindophenol (AOAC, 2000). Total phenolic content was determined by the spectrophotometric method of Folin-Ciocalteu with a standard curve of gallic acid at 670 nm in UV–vis spectrophotometer (Unicam-Helios  $\alpha$ , USA; Domene et al., 2014).

### 4.2.3.7. Experimental design and statistical analysis

The experiment was performed in a randomized complete block design with three replicates. The results for the different parameters were evaluated by analysis of variance (ANOVA) using Statgraphics Centurion XVII (Statistical Graphics Corporation, 2014). Percentage data were arcsin transformed before analysis. Least significant difference (LSD) at a 0.05-probability level was used as the mean separation test.

#### **4.2.4.** Results

Most of the studied parameters, as shown in Tables 2-5, were affected by GS and IS, ( $P \le 0.05$  or  $P \le 0.01$ ), but in no case by their interaction ( $P \le 0.05$ ). Thus, these factors are discussed separately. In general, only the significantly affected factors ( $P \le 0.05$ ) are shown in the tables.

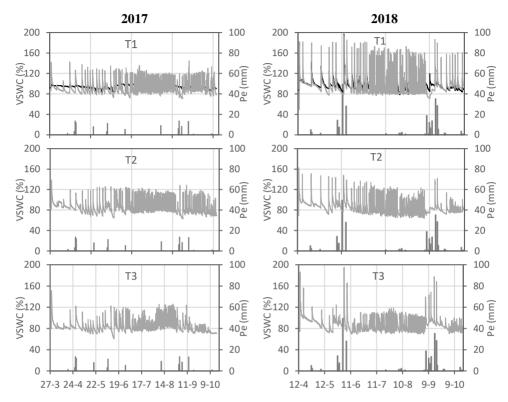
# 4.2.4.1. Growth stages and irrigation water applied

The durations of each growth stage (initial, vegetative growth, fruit-setting and harvesting) are presented in Table 1. The duration of the total crop cycle, including the initial stage, was 202 days in 2017 and 193 days in 2018. The ETo values for 2017 and 2018 were 956 and 905 mm, respectively. However, the Pe registered during 2018 (249 mm) was 2.3 times that in 2017 (109 mm). Therefore, the IWA was lower in 2018 than in 2017, ranging from 274 (T3) to 515 mm (T1) in 2018 and from 389 (T3) to 751 mm (T1) in 2017. These values include 27 and 34 mm in 2017 and 2018, respectively, that correspond to the initial irrigation that was equally applied for all IS to insure adequate plant establishment.

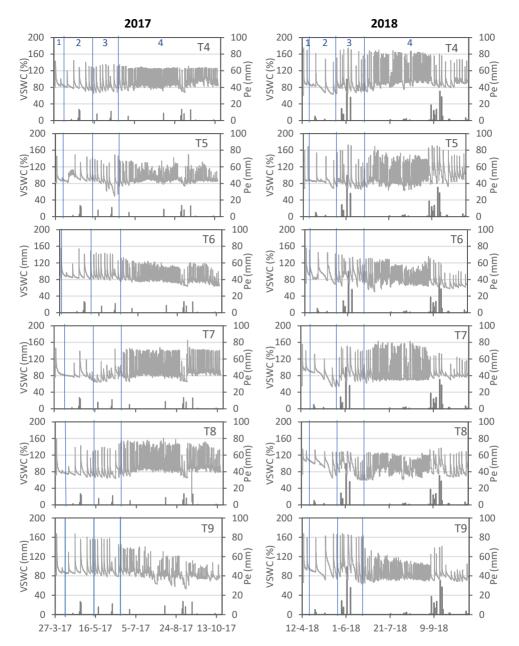
#### 4.2.4.2. Volumetric soil water content

Figures 2 and 3 show the VSWC for the different IS at a 20-cm depth (additionally at 35-cm depths in T1), as well as the Pe during both GS. The average VSWC at 20 cm ranged from 82.7 to 91.0% FC in 2017, and from 82.4 to 94.1% FC in 2018. In general, in both GS, the highest average VSWC values were registered 182

under full irrigation, while the lowest values corresponded to CDI applying 50% of the IWR. VSWC registered at 35 cm deep in both seasons were rather constant, particularly in 2017.



**Figure 2.** Relative soil water content [%; volumetric soil water content/volumetric soil water content at field capacity at a 0.20 m ( —) and 0.35 m ( —) depth] for T1, T2 and T3 irrigation rates and daily rainfall (vertical bars) during each growing season.



**Figure 3.** Relative soil water content [%; volumetric soil water content/volumetric soil water content at field capacity at a 0.20 m] for T4, T5, T6, T7, T8 and T9 irrigation rates and daily rainfall (vertical bars) during each growing season. Crop growth stages: (1) Initial; (2) Vegetative growth; (3) Early fruit setting and bearing; (4) Harvesting.

### 4.2.4.3. Relative water content and membrane stability index

The RWC and the MSI at the end of harvesting were higher ( $P \le 0.01$ ) in 2018 than in 2017 (Table 2), while at the end of the vegetative growth stage there were no differences between years. At the end of the vegetative growth stage, neither RWC nor MSI were affected ( $P \le 0.05$ ) by the IS. At the end of harvesting, the highest values of RWC and MSI corresponded to T1, the lowest to T3, differing or not ( $P \le 0.05$ ) from the other IS; a similar trend was stated at the end of the fruit setting stage.

**Table 2.** Effect of the growing season and irrigation strategy on the leaf relative water content (RWC) and membrane stability index (MSI) at the end of each growth stage: vegetative growth (2), fruit setting (3) and harvesting (4).

		RWC (%)			MSI (%)	
	2	3	4	2	3	4
Growing season (GS)						
2017	81.42	79.05	75.49 b	76.91	74.31 b	74.04 b
2018	80.76	79.81	82.99 a	77.21	75.43 a	76.14 a
LSD	1.16	1.39	0.84	1.14	0.91	0.84
Irrigation strategies (IS)						
T1	82.05	81.47 a	81.50 a	78.10	76.82 a	77.37 a
T2	80.48	77.65 bcd	77.33 b	76.67	73.57 cd	73.35 cd
Т3	79.53	75.55 d	73.57 c	75.72	71.70 d	70.97 e
T4	80.52	80.03 ab	81.10 a	76.18	75.23 abc	77.08 a
T5	81.57	79.62 abc	81. <i>37</i> a	77.90	75.28 abc	76.32 ab
Т6	82.28	81.43 a	80.87 a	78.28	76.15 a	75.12 bc
T7	79.55	80.48 ab	81.20 a	75.68	75.28 abc	76.82 ab
Т8	81.82	77.05 cd	80.98 a	77.85	73.95 bc	76.90 ab
Т9	82.03	81.58 a	75.22 c	77.18	75.82 ab	71.90 de
LSD	2.46	2.94	1.78	2.42	1.94	1.78
ANOVA (df)			% sum of	the squares		
GS (1)	2.5 ns	1.6 ns	58.5 **	0.5 ns	6.4 *	12.8 **
IS (8)	23.9 ns	47.2 **	34.5 **	23.6 ns	43.9 **	61.1 **
GS*IS (8)	6.6 ns	4.2 ns	0.6 ns	5.4 ns	12.6 ns	8.0 ns
Residuals (36)	67.0	47.0	6.4	70.4	37.2	18.1
Standard deviation	2.1	2.5	1.5	2.1	1.7	1.5

df: degrees of freedom. Mean values followed by different lower-case letters in each column indicate significant differences at  $P \le 0.05$  using the LSD test. \*\* (\*): Indicates significant differences at  $P \le 0.01$  ( $P \le 0.05$ ). ns: Indicates no significant difference.

# 4.2.4.4. Plant growth and harvest index

Some pepper plant growth traits were significantly affected ( $P \le 0.01$ ;  $P \le 0.05$ ) by GS and IS (Table 3). Plants grown in 2018 were shorter than in 2017, but they had a wider stem and higher SPAD. Plant height, stem diameter and SPAD were not

affected ( $P \le 0.05$ ) by the IS. Each year the SPAD values were practically constant during the growing season, so only the corresponding values at the end of the crop cycle are presented in Table 3.

**Table 3.** Effect of the growing season and irrigation strategy on plant height (H), stem diameter (D), leaf chlorophyll index (SPAD), shoots dry weight (SDW), fruits dry weight (DW) and harvest index (HI).

	Н	D	SPAD	SDW	FDW	HI
	(cm)	(mm)	(-)	(kg m <sup>-2</sup> )	(kg m <sup>-2</sup> )	(-)
Growing season (GS)						
2017	132.40 a	23.78 b	61.79 b	0.91 b	1.18	0.56 a
2018	129.94 b	27.20 a	66.18 a	1.01 a	1.17	0.54 b
LSD	1.41	0.55	1.84	0.05	0.06	0.01
Irrigation strategy (IS)						
T1	133.67	25.96	64.35	1.06	1.33 a	0.56 ab
T2	131.71	25.25	63.39	0.91	1.13 cd	0.55 ab
T3	129.63	25.64	63.96	0.86	0.92 e	0.51 d
T4	130.58	25.13	64.06	0.99	1.28 ab	0.57 ab
T5	130.71	25.15	63.06	1.01	1.23 abc	0.55 ab
Т6	131.17	25.50	63.33	1.00	1.21 abc	0.54 bc
T7	132.00	25.75	63.48	0.94	1.28 ab	0.58 a
T8	130.04	26.07	64.19	0.94	1.20 bc	0.56 ab
T9	131.00	24.94	64.05	0.94	1.02 de	0.52 cd
LSD	2.99	1.17	3.91	0.12	0.12	0.03
ANOVA (df)			% sum of	the squares		
GS (1)	5.3 **	41.5 **	14.0 **	17.6 **	0.0 ns	15.9 **
IS (8)	4.5 ns	2.0 ns	1.2 ns	22.5 ns	67.8 **	39.7 **
GS*IS (8)	1.7 ns	1.9 ns	4.1 ns	12.1 ns	2.5 ns	4.1 ns
Residuals (144/36z)	88.6	54.7	80.7	47.9	29.6	40.3
Standard deviation	5.3	2.0	6.9	0.1	0.1	0.02

df: degrees of freedom. Mean values followed by different lower-case letters in each column indicate significant differences at  $P \le 0.05$  using the LSD test. \*\* (\*): Indicates significant differences at  $P \le 0.01$  ( $P \le 0.05$ ). ns: Indicates no significant difference. <sup>z</sup>: degrees of freedom for SDW, FDW and HI.

Shoot dry weight was affected ( $P \le 0.01$ ; Table 3) by GS, with higher values in 2018, while it was not affected by IS. Fruit weight [both fresh (Table 4) and dry weight (Table 3)] was not affected by GS. The highest fruit dry weights were obtained in the fully irrigated plants (T1), while the lowest corresponded to the plants that received 50% of the IWR throughout the cycle (T3) and during harvesting (T9). HI was affected ( $P \le 0.01$ ) by GS, with higher ratio in 2017, and it was also affected by the IS, ranging from 0.51 (T3) to 0.58 (T7), with intermediate values for the other strategies, statistically differing, or not, between them.

### 4.2.4.5. Yield, irrigation water use efficiency and yield response factor

Yield was not affected by GS ( $P \le 0.05$ ). Nevertheless, higher ( $P \le 0.01$ ) MY, «Extra class» yield, and lower percentages of the different non-marketable fruit batches (except for BER) were obtained in 2018 than in 2017 (Table 4).

**Table 4.** Effect of the growing season and irrigation strategy on the total yield (Yield), marketable yield (MY; kg m<sup>-2</sup>) and its partitioning into «Extra class» and Class I, non-marketable yield (NMY, % of yield) and its partitioning into fruits presenting with defects of shape (DS), small size, sunburn and blossom end rot (BER), water use efficiency (WUE) and irrigation water use efficiency (IWUE).

	Yield	MY (kg m <sup>-2</sup> )				NMY (%)					IWUE
	(kg m <sup>-2</sup> )	MY	Extra	Class I	NMY	Small	Sunburn	BER	DS	- WUE (kg m <sup>-3</sup> )	(kg m <sup>-3</sup> )
			class		(%)	(%)	(%)	(%)	(%)	(Kg III )	(Kg III )
Growing seaso											
2017	11.89	6.94 b	4.28 b	2.66	42.80 a	8.18 a	2.47 a	20.67	11.43 a	9.59 b	11.39 b
2018	11.98	7.74 a	5.13 a	2.62	36.40 b	4.86 b	1.02 b	20.05	10.12 b	11.77 a	19.29 a
LSD	0.56	0.52	0.39	0.22	2.29	0.62	0.38	2.42	0.97	0.74	1.26
Irrigation strate	egies (IS)										
T1	13.52 a	9.02 a	5.92 a	3.11 a	33.38 d	7.06	1.57	14.36 d	10.35	11.62 a	15.73
T2	10.97 cd	5.78 c	3.56 c	2.22 bc	47.45 b	6.64	1.54	27.83 b	11.14	9.32 b	13.77
Т3	8.81 e	4.02 d	2.40 d	1.61 d	54.69 a	5.88	1.71	36.23 a	10.72	8.34 b	14.35
T4	13.03 a	8.95 a	5.83 a	3.12 a	31.42 d	6.88	1.86	11.40 d	11.01	11.71 a	15.95
T5	12.91 a	8.84 a	5.69 a	3.15 a	31.60 d	6.94	1.57	11.58 d	11.41	11.59 a	15.80
Т6	12.04 bc	7.31 b	4.71 b	2.60 b	39.20 c	6.12	1.83	20.52 c	10.55	11.16 a	16.03
T7	13.43 a	8.80 a	5.62 a	3.17 a	34.56 cd	6.73	2.09	15.80 cd	9.78	11.67 a	15.98
Т8	12.95 ab	8.48 a	5.44 ab	3.03 ab	35.04 cd	6.39	2.19	15.31 d	11.00	11.34 a	15.54
Т9	9.89 de	5.06 c	3.29 c	1.77 cd	49.04 b	6.16	1.38	30.23 b	10.99	9.54 b	15.08
LSD	1.19	0.97	0.83	0.47	4.85	1.31	0.81	5.14	2.06	1.56	2.68
ANOVA (df)					% sum o	f the sq	uares				
GS (1)	0.0 ns	3.8 **	8.5 **	0.1 ns	11.4 **	69.6**	52.5 **	0.1 **	11.9 **	29.6 **	76.8 **
IS (8)	76.3 **	79.7 **	73.1 **	72.6 **	73.6 **	3.9 ns	6.4 ns	79.9 **	5.9 ns	36.8 **	4.1 ns
GS*IS (8)	3.0 ns	2.5 ns	2.7 ns	6.0 ns	2.2 ns	5.2 ns	8.8 ns	5.7 ns	24.8 ns	3.9 ns	1.6 ns
Residuals (36)	20.7	14.0	15.7	21.3	12.7	21.3	32.2	14.3	57.4	29.7	17.4
SD	1.0	0.9	0.7	0.4	4.14	1.11	0.70	4.39	1.76	1.3	2.3

df: degrees of freedom. SD: standard deviation. Mean values followed by different lower-case letters in each column indicate significant differences at  $P \le 0.05$  using the LSD test. \*\*: Indicates significant differences at  $P \le 0.01$ . ns: Indicates no significant difference.

The deficit irrigation negatively affected ( $P \le 0.01$ ) yield components, with IS representing 76%, 80%, 73% and 73% of the data variability (of the total sum of squares) for yield, MY, «Extra» class and Class I, respectively (Table 4). MY corresponding to T2 and T3 was reduced by 36% and 55%, respectively, compared to T1, with slightly greater reductions (40% and 60%, respectively) when «Extra»

class yield was analysed. Water reduction during harvesting (T6 and T9) reduced significantly ( $P \le 0.05$ ) both MY and «Extra» class yield in relation to fully irrigated plants, while applying the water shortage during the first stages did not affect these parameters.

A larger percentage of non-marketable fruits were obtained in 2017 ( $P \le 0.05$ ; Table 4) than in 2018, mainly due to a greater abundance of small fruits and to a lesser extent a higher incidence of sunburnt and deformed fruits. The largest percentage of non-marketable fruits in T3 ( $P \le 0.05$ ) was due to a higher presence of BER in this strategy since the other non-marketable batches were not affected by the IS ( $P \le 0.05$ ).

The highest WUE and IWUE ( $P \le 0.01$ ) values were obtained in 2018, as a consequence of both the higher MY and the lower IWR in 2018 than in 2017. Regarding the IS, the CDI and reduction of water applied to 50% of the IWR during harvesting (T9) led to lower WUE values ( $P \le 0.05$ ) than the other strategies. Although a similar trend could be observed for IWUE, it was not statistically significant ( $P \le 0.05$ ).

Marketable yield (kg m<sup>-2</sup>) increased linearly (P  $\leq$  0.01), with increasing IWA (mm) for CDI and for strategies applying the water reduction at harvesting [(MY: -3.45 + 0.016 IWA; r 0.87; P  $\leq$  0.01), (MY: -1.75 + 0.014 IWA; r 0.79; P  $\leq$  0.01), respectively]. The relationships between MY and IWA for water stress applied at vegetative growth and fruit setting stages were not significant (P  $\leq$  0.05).

Considering both GS together, the  $K_y$  for the CDI was 1.53 while it was 0.80 and 1.32 for the vegetative growth and harvest stages, respectively. All linear regression equations were significant (P  $\leq$  0.01), with correlation coefficients (r) from 0.83 to 0.99.

# 4.2.4.6. Fruit quality traits

# **4.2.4.6.1. Physical parameters**

Fruit diameter and colour indexes (H°, C\* and CI) were unaffected ( $P \le 0.05$ ) by GS, IS or their interaction. The average fruit diameter was 39.3 mm, and the average H°, C\* and CI values were 127.41, 21.40 and -20.51, respectively. Fruit length was only affected ( $P \le 0.01$ ; Table 5) by IS, with shorter fruits obtained with the irrigation water reduction. The shortest fruits were obtained under CDI at 50% of the IWR (T3), followed by CDI at 75% of the IWR (T2) and RDI at 50% of the IWR at harvesting (T9). Flesh thickness was affected ( $P \le 0.01$ ; Table 5) by both GS and IS. Fruits obtained in 2018 presented a thicker flesh than those obtained in 2017. As to IS, the fruits with the thickest ( $P \le 0.05$ ) flesh were those obtained with full irrigation, while the fruits with the thinnest flesh were those corresponding to the most restrictive strategies applied both in CDI (T3) and RDI at harvesting (T9).

**Table 5.** Effect of the growing season and irrigation strategy on fruit traits: length, flesh thickness (FT), average fruit weight (AFW), firmness, dry matter content (DM), acidity, soluble solids content (SSC), maturity index (MI), ascorbic acid (AA) and total phenolic (TPs) contents.

	Length	FT	AFW	Firmness	DM	Acidity	SSC	MI	AA	TPs
	(cm)	(mm)	(g fruit <sup>-1</sup> )	(N)	(%)	(%)	(° Bix)		(mg 100g <sup>-1</sup> )	(mg 100g <sup>-1</sup> )
Growing season	(GS)									
2017	19.73	2.91 b	77.55 b	10.65 b	9.88	0.08 b	5.44 b	67.65	123.4	161.11 a
2018	19.87	3.09 a	86.61 a	12.29 a	9.86	0.11 a	6.67 a	62.10	123.1	116.91 b
LSD	0.37	0.16	5.27	0.45	0.20	0.01	0.38	7.59	6.4	12.94
Irrigation strates	gies (IS)									
T1	20.50 a	3.47 a	94.77 a	11.38	9.80 bc	0.12	5.58 c	50.56	116.7	113.07 d
T2	19.49 b	2.89 bc	77.12 cd	11.55	10.17 ab	0.09	6.34 ab	67.09	116.1	145.48 abc
T3	18.06 c	2.74 c	62.25 e	11.57	10.39 a	0.09	7.14 a	77.35	131.9	163.90 a
T4	20.47 a	3.20 ab	91.82 ab	11.36	9.80 bc	0.09	5.38 c	61.75	121.1	125.97 bcd
T5	20.26 a	3.16 ab	84.43 abc	11.47	9.51 cd	0.09	5.54 c	65.49	128.9	119.61 cd
T6	20.05 ab	3.02 bc	80.90 bcd	11.77	10.04 ab	0.10	6.55 ab	66.14	123.8	148.49 ab
T7	20.28 a	3.08 bc	94.20 a	11.33	9.54 cd	0.10	5.91 bc	61.11	122.2	136.62 abcd
T8	19.81 ab	3.03 bc	82.63 bc	11.13	9.24 d	0.09	5.60 c	63.31	118.5	137.31 abcd
T9	19.31 b	2.84 c	70.62 de	11.69	10.36 a	0.10	6.83 a	71.07	130.0	160.62 a
LSD	0.77	0.34	11.18	0.95	0.43	0.02	0.81	16.10	13.6	27.45
ANOVA (df)					% sum of	f squares				_
GS (1)	0.3 ns	2.6 *	10.4 **	24.9 **	0.2 ns	38.5 **	35.4 **	3.8 ns	4.7 ns	41.3 **
IS (8)	28.6 **	14.1 **	53.8 **	1.3 ns	60.9 **	13.4 ns	33.1 **	23.6 ns	20.6 ns	23.0 **
GS*IS (8)	6.1 ns	7.0 ns	5.2 ns	4.8 ns	1.6 ns	9.6 ns	2.1 ns	10.7 ns	13.8 ns	4.8 ns
Residuals (144)	65.0	76.3	30.7	69.0	37.3	38.5	29.4	61.9	60.8	31.0
SD	1.2	0.5	9.5	1.4	0.4	0.0	0.7	13.7	15.4	23.4

df: degrees of freedom. SD: standard deviation. Mean values followed by different lower-case letters in each column indicate significant differences at  $P \le 0.05$  using the LSD test. \*\* (\*): Indicates significant differences at  $P \le 0.01$  ( $P \le 0.05$ ). ns: Indicates no significant difference.

The average fruit weight for marketable fruits produced in 2018 was higher (P  $\leq$  0.01) than that produced in 2017, and in relation to IS, the results were similar to those of fruit length: the lightest fruits were obtained (P  $\leq$  0.05) under CDI at 50% of the IWR (T3), followed by RDI at 50% of the IWR at harvesting (T9) without differing (P  $\leq$  0.05) between them. As occurred for flesh thickness, fruit firmness was affected by GS (P  $\leq$  0.01), but it was not affected by the IS (P  $\leq$  0.05).

## 4.2.4.6.2. Taste quality traits

Fruit dry matter was only affected by IS ( $P \le 0.01$ ; Table 5), with the highest values obtained for the plants subjected to water stress during harvesting (T3, T9, T2 and T6). Acidity only was affected by the GS ( $P \le 0.01$ ), with higher values obtained in 2018 than in 2017. SSC was affected ( $P \le 0.01$ ) by both GS and IS. In relation to GS, the highest SSC values corresponded to 2018, and in relation to IS, the fruits with the highest SSC were those obtained with the most severe water deficit applied both in CDI (T3) and in RDI at harvesting (T9), followed by those corresponding to moderate water deficit (T2 and T6). MI was not significantly affected ( $P \le 0.05$ ) by any of the analysed factors.

#### 4.2.4.6.3. Nutrient quality traits

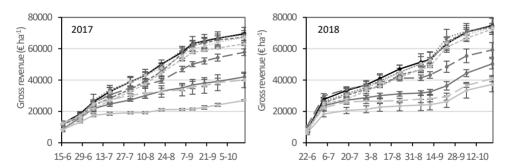
Ascorbic acid content was not affected by the GS or by IS ( $P \le 0.05$ ). However, total phenolic content was affected by both factors ( $P \le 0.01$ ). As for GS, the highest value was obtained in 2017, while in relation to IS, severe water deficit, both in CDI (T3) and RDI at harvesting stage (T9) led to the highest values ( $P \le 0.05$ ). Moderate water deficit (at any stage) or severe water deficit during the first stages led to intermediate results.

# 4.2.4.7. Profitability

Given the importance of crop profitability, the gross revenue and economic value of water has been determined considering the MY, the IWUE and the average pepper fruit price corresponding to the last three years  $(0.80 \, \epsilon \, \text{kg}^{-1}; \text{MAPA}, 2018)$ .

Under the present study conditions, full irrigation (T1) led to the highest MY and therefore to the highest gross revenue (on average 90.2 tons ha<sup>-1</sup> and 71,258 € ha<sup>-1</sup>, respectively), with an economic value per unit of water consumed of 12.43 € m<sup>-3</sup>. Reducing the water applied by 25% of the IWR during the vegetative growth (T4) and fruit-setting (T5) stages led to a reduction of 0.7% and 2.0%, respectively, of the gross revenue in relation to T1, while these reductions increased to 2.4% and 6.4%, respectively, with the severe water restriction (50% IWR; T7 and T8, respectively). The reduction of water applied by 25% of the IWR during harvesting (T6) led to a 21% water savings and a reduction of 19% of the gross revenue (57,749 € ha<sup>-1</sup>) in relation to T1. With CDI strategies, water savings of 25% and 50% were obtained, but the gross revenues were reduced to 45,662 and 31,758 € ha<sup>-1</sup>.

Considering the plant response to the different climatic conditions in the two GS, the gross revenues along the crop cycle are presented separately for each GS in Figure 4. In both GS, CDI and T9 showed lower gross revenue than the other IS since the first harvest pass, increasing the differences between IS throughout this stage.



**Figure 4.** Gross revenue accumulation throughout harvesting during each growing season. Average values; vertical bars represent the standard error. T1: ; T2: ; T3: ; T4: ; T5: ; T5: ; T6: ; T7: ; T7: ; T8: ; T8: ; T9: ; T9: ; T8: ; T9: ; T8: ; T9: ; T

#### 4.2.5. Discussion

The harvest stage is substantially longer than other plant growth stages, averaging 68% of the total crop cycle. During this stage, the highest temperatures and the

highest IWR coincide, as well as the growth of most of the fruits. Therefore, it seems obvious that water restriction applied in this stage would have a greater influence than that applied in the other stages, both in relation to IWA and in the yield and quality of the fruits.

In Figure 1, it can be observed that during the spring and summer months, an increase in temperature and ETo registered for the experimental GS in relation to their average values (2001-2018), corresponding to the general opinion about climate change. The determined ETo values for the crop cycle in 2017 and 2018 (956 and 905 mm, respectively) were rather similar and frequent in the area (on average 914 mm) in the Experimental Centre. Rainfall registered during the crop cycle in 2018 (577 mm) was much higher than that in 2017 (207 mm), with an average value of 280 mm for the crop cycle in the area. Therefore, in 2017 demand for irrigation water was higher than average in the area (higher ETo and lower rainfall), while in 2018, there was an abnormally high rainfall at the end of spring and autumn. The volume of IWA in the full irrigation strategy (T1) in 2017 (724 mm) was similar to that applied to pepper 'Karaisali' in the Mediterranean region of Turkey (743 mm) by Sezen et al. (2019), while that applied in 2018 (481 mm) coincided with that applied to pepper 'Sonora' in Córdoba (Spain; 480 mm) by González-Dugo et al. (2007).

The VSWC registered in 2018 was slightly higher (on average 88.1% FC) than that in 2017 (on average 87.3% FC), which probably related with the higher Pe registered in 2018, particularly that occurring in June, September and October, which exceeded 100 mm per month. For RDI, a decrease in the VSWC was observed during the stages when the water restriction was applied, particularly in the more severe water restriction. Given the long duration of the harvest stage, a slightly downward VSWC trend over time in T6 and T9 led to lower VSWC values than in T1 in that stage. These lower values registered during the water restriction stages were not so clearly observed in the other IS given the shorter duration of the restriction stages.

Although the differences between average values of VSWC corresponding to the different IS were limited, yield was clearly influenced. It has been reported that leaf conductance responds earlier to soil water content than to leaf turgor (Costa et al., 2007), although it differs depending on the plant species (Fahad et al., 2017). Stomatal closure is mediated by hormonal signals (mainly abscisic acid) that travel from dehydrating roots to shoots, increasing the physiologically active abscisic acid concentrations in the leaf apoplast adjacent to guard cells, inducing stomata closure (Costa et al., 2007).

The criterion of initiating each irrigation event when the VSWC dropped to 80% of the field capacity, was already satisfactorily used in preliminary studies (Abdelkhalik et al., in press). Yang et al. (2018) indicated that this irrigation threshold resulted in the highest yield and fruit quality in pepper, when compared with other. The VSWC registered at 35 cm in depth, along the crop cycle, in both seasons indicated that losses in depth were negligible, particularly in 2017. Thus, considering both the irrigation threshold and the irrigation dose applied, it can be stated that the irrigation management was adequate.

At the vegetative growth stage, both RWC and MSI were unaffected ( $P \le 0.05$ ) by GS or by IS, probably because in this stage, the water status difference was small since it immediately followed the establishment stage, in which the amount of water applied was greater than the IWR to ensure adequate plant establishment. With the growth of the plant, the differences in water availability corresponding to the different IS increased, resulting in significant differences at harvesting. At the end of harvesting (and also at the fruit-setting stage for MSI), higher RWC and MSI values were obtained in 2018 (83.0% and 76.1%, respectively) than in 2017 (75.5% and 74.0%, respectively). This result could be related to the differences in VSWC and climatic conditions (temperature, ETo and rainfall), as Figure 1 shows; particularly important was the rainfall that occurred during the fruit-setting and harvesting stages in the 2018 season (181 mm in each stage).

At the end of harvesting (as well as the fruit-setting stage), the highest RWC and MSI values corresponded to T1 (81.5% and 77.4%, respectively), while the lowest corresponded to severe shortages at this stage, both in CDI (T3; 73.6% and 71.0%, respectively) and RDI (T9; 75.2% and 71.9%, respectively). The RWC value for T3 is similar to that reported by López-Serrano et al. (2019; approximately 70%) for water-stressed pepper plants in Spain. Similar results were also found by Okunlola et al. (2017; 69%-95%) in Nigeria and by Camoglu et al. (2018; 60%-80%) in Turkey.

In each analysis performed, the lowest RWC values corresponded to the more severe shortage in the corresponding stage. Difference between the values of the full irrigation strategy (T1) and severe shortages could explain the differences in vegetative growth and yield, given that an initial reduction in leaf RWC induces stomatal closure (González and González-Vilar, 2001). Stomatal closure leads to a reduction of the internal CO<sub>2</sub> availability in leaves, which consequently decreases the rate of photosynthesis and therefore decreases cell division and enlargement, and consequently overall plant growth, reducing the yield (Farooq et al. 2009; Osakabe et al., 2014). In accordance with González and González-Vilar (2001), as a general rule, an initial reduction in leaf RWC (100-90%) induces stomatal closure, reducing cellular growth; lower values of RWC (90-80%) induce changes in tissue composition and modifications in the relative rates of photosynthesis and respiration; a greater decreased RWC (below 80%) commonly implies changes in metabolism, leading to photosynthesis ceasing, respiration increasing and abscisic acid accumulation. On the other hand, under water-stress conditions, Dwivedi et al. (2018) stated that tolerant genotypes of wheat maintained greater RWC (85–90%) compared to susceptible ones (70–75%) due to their greater ability to acquire water from the soil. These differences are consistent with the RWC values obtained in the two extreme IS (T1 and T3) in the present study.

Regarding MSI, in the previously cited study (Dwivedi et al., 2018), their authors stated that, as in RWC, the tolerant wheat genotypes could maintain higher

mean MSI (85%) compared to susceptible ones (75%), and these values are consistent with those obtained in T1 and T3 in the present study.

Given that the sweet Italian pepper is an indeterminate crop, its growth could be affected by water shortage at any moment. Overall, CDI strategies reduced fruit dry weight, which decreased with increasing water stress. These results are similar to those obtained in peppers by Ahmed et al. (2014) and Mardani et al. (2017). In contrast, water shortage at the vegetative growth and fruit-setting stages affected to a lesser extent the fruit dry weight, which is in agreement with that reported by Guang-Cheng et al. (2010). Reducing IWA to 50% of the IWR at harvesting reduced the fruit dry weight to the same extent as the severe CDI. When water restriction was applied only during the vegetative growth stage, it had a reversible effect from which the plant could recover to become of similar height, stem diameter and shoot and fruit dry weight as fully irrigated plants. The SPAD values of the current study (on average 64 for the fully irrigated plants) are slightly higher than those presented by Juan-juan et al. (2012; on average below 60), who showed no significant effects of VSWC on SPAD values.

The HI values obtained in the present study (on average 0.56 for the IS that applied 100% of the IWR) are similar to those obtained by Fernández et al. (2005) for autumn-winter-spring sweet pepper 'Drago' grown in greenhouses in Almeria (Spain; on average 0.53 for plants receiving 100% of the IWR). In soils with high water storage capacity, CDI allows plants to develop slowly and to adapt to water deficits (Fereres and Soriano, 2007). Under CDI with moderate water stress, water deficits lead to reduced biomass production due to the reduction in canopy size and, in turn, in radiation interception. In that case, dry matter partitioning is usually not affected and the HI is maintained, as occurred in T2, but, as the water stress increases in severity, it can affect HI in many crops (Fereres and Soriano, 2007), as occurred in T3. HI for T9 was similar to that obtained in T3 since the same water restriction (50% of the IWR) was applied during harvesting, whose duration corresponded to approximately 68% of the season duration.

The yield (total and marketable) obtained by the fully irrigated plants (T1; on average 13.5 and 9.02 kg m<sup>-2</sup>, respectively) can be considered satisfactory compared to those usually obtained by the growers in the area (4.5 kg MY m<sup>-2</sup>; MAPA, 2018) and to those obtained in greenhouses with "enarenado" soil by Fernández et al. (2005; 9.20 kg m<sup>-2</sup>) in Spain and Ćosić et al. (2015; 8.40 kg m<sup>-2</sup>) in field experiments conducted in Serbia. Variations between seasons in MY traits are not unusual both in the area and in the Cajamar Experimental Centre itself under standard conditions ("under optimum soil water conditions" Allen et al., 1998) (8.9 - 10.5 kg m<sup>-2</sup>; Fundación Cajamar 2017, 2018). The better average results obtained in 2018 in relation to MY, «Extra class» yield, WUE and IWUE, and the lower percentages of the different non-marketable yield batches (except for BER) than in 2017, could possibly be related with the lower seasonal precipitation and slightly higher average summer temperature registered in 2017 (Figure 1).

The CDI strategies led to a drastic reduction of yield and MY (both «Extra» Class and Class I), which decreased as IWA decreased. Pepper fruits of the «Extra» class represent the high-quality yield corresponding to the highest price. Remarkably, «Extra» class fruits represented 65.6%, 61.6% and 59.7% of the corresponding MY for T1, T2 and T3, respectively. Although these values show a negative trend with water deficit, their differences were not significant ( $P \le 0.05$ ). These results agree with those obtained by Camoglu et al. (2018) and Sezen et al. (2019). Ćosić et al. (2015) observed higher first-class fruit yield with full irrigation that decreased with increasing water stress. Applying a water shortage at the vegetative growth (T4 and T7) and fruit development (T5 and T8) stages did not reduce yield and MY parameters in relation to the fully irrigated plants. These results might be attributed to the fact that water shortage at early stages of pepper growth allows plants to develop slowly and to adapt to the water deficits (Fereres and Soriano, 2007). Yang et al. (2017) reported that DI with 33.3% and 66.6% of full irrigation during the vegetative and flowering and fruit-setting stages did not affect the hot pepper yield under greenhouse conditions in northwest China. This result implies that pepper plants have the ability to partially recover from early water deficit effects (Guang-Cheng et al., 2010). However, when water shortage was applied during the harvesting, particularly when IWA was reduced to 50% of the IWR (T9), yield and MY traits were reduced drastically in relation to T1.

Larger percentages of BER and, in turn, non-marketable yield were obtained with the most severe IS during the entire cycle (T3) and at harvesting (T9). These results agree with those of Saure (2001) and Fernández et al. (2005), who stated that water stress increases the incidence of fruits with BER. BER is produced because of the poor translocation of calcium to fruit, and this physiopathy can be accentuated by high temperatures and low relative humidity, under conditions of a certain salinity and, as in the present study, by water deficit (Maroto, 2002; Condés, 2017).

The lower values of WUE obtained with CDI and reduced water application of 50% of the IWR during harvesting indicates that the water savings have not compensated the yield reductions. The result agrees with the results reported for greenhouse experiments by Fernández et al. (2005) in Spain, by Aladenola and Madramootoo (2014) in Canada, and in field experiments by Ćosić et al. (2015) in Serbia, who indicated that the WUE decreased with increasing water shortage during the entire pepper crop growth cycle. Nevertheless, Yang et al. (2017), in an experiment conducted in northwest China, stated that irrigation with 33.3% or 66.6% of the full irrigation at the flowering and fruit-setting stage or at early fruit-bearing and harvesting stage was suitable for improving WUE in hot peppers grown in greenhouses. It is noteworthy that under greenhouse conditions, WUE is equivalent to IWUE since there is no rainfall inside the greenhouse. IWUE was not affected by the IS, due on the one hand to low variability between their values (this factor represented 4.1% of the total sum of squares), which ranged from 13.8% (for T2) to 16.0% (for T6) without a clear trend, while a large variability (76.8% of the total sum of squares) was represented by the GS, whose values were 11.4 kg m<sup>-3</sup> (2017) and 19.3 kg m<sup>-3</sup> (2018).

Linear relationships between MY and IWA for pepper were also reported by Yang et al. (2018) and Gadissa and Chemeda (2009). Other authors (Fernández et al., 2005; Sezen et al., 2019) linearly related MY and IWA. The positive linear relationship between MY and IWA, suggest that IWA did not exceed the maximum crop water demands, as reported by Tolk and Howell (2003) who indicated that curvilinear relationships may be related with a water excess that is used in soil water evaporation.

 $K_y$  in the present study (1.53 for CDI) is consistent with that determined by Gadissa and Chemeda (2009) for peppers grown in Ethiopia under CDI (1.57). Values of  $K_y$  greater than 1 indicate that the crop is sensitive to water deficit, and values lower than 1 indicate that it is tolerant (Doorenbos and Kassam, 1979; Steduto et al., 2012). When considering the different stress stages separately, it can be concluded that the pepper plant is less sensitive to water deficits at the vegetative growth stage ( $K_y = 0.80$ ) than in the later stages ( $K_y = 1.32$  for harvesting), in accordance with the results obtained for yield in this and other studies (Yang et al., 2017). This result implies that, as previously cited, pepper plants have the ability to partially recover from early water deficit effects (Guang-Cheng et al., 2010).

Colour indexes of the fruit skins were not affected by either GS or IS, and they corresponded to the dark green colour characteristic of this cultivar. The length, flesh thickness and average weight of the fruit values obtained with full irrigation are in accordance with those usually obtained with the same cultivar and crop cycle under standard conditions in the Experimental Centre (Fundación Cajamar, 2017, 2018). The CDI led to an important reduction in the values of these parameters, which is in agreement with those reported by Sezen et al. (2014) who observed a reduction in pepper fruit length (and width) under CDI with 75 and 50% of full irrigation. The RDI also led to an important reduction in the average fruit weight when the water stress was applied during harvesting, but not when it was applied during the vegetative growth stage since plants can recover from the stress, as mentioned above. These

results are probably a consequence of partial stomatal closure and alterations in the relative rates of photosynthesis and respiration, as previously cited.

Although marketing standards of the European Regulations (Official Journal of the European Union, 2011) concerning pepper sizing only consider that "elongated sweet peppers should be sufficiently uniform in length", consumers prefer large sizes, so producers usually also classify them by size. These marketing standards do not cover flesh thickness; however, this trait is highly appreciated by consumers and breeders (Maroto, 2002).

Fruit firmness was affected by GS, with the highest values obtained in 2018. Although IS did not significantly affect the fruit firmness ( $P \le 0.05$ ), a similar trend to that of the fruit dry matter content was observed, such that water deficits applied at harvesting led to fruits with higher dry matter content and with greater firmness.

In relation to fruit DM, the IS represented 61% of the data variability, decreasing the GS influence. Reducing the IWA at harvesting or during the entire cycle increased the fruit DM. This increment without reducing its dry weight might improve fruit quality (Dorji et al., 2005; Guang-Cheng et al., 2010). Acidity, which was not affected by the IS, showed values ranging from 0.09% to 0.12%, which are lower than those reported for other cultivars (Domene et al., 2014). The obtained SSC values are in accordance with those reported as reference values for different cultivars by Domene and Segura (2014). Fruits with the highest SSC were those obtained with the CDI (7.14° Brix for T3) and with water shortage at harvesting (6.83 <sup>o</sup>Brix for T9). These results are in accordance with those obtained by Guang-Cheng et al. (2010; 6.2 - 7.6 °Brix) for CDI and by Yang et al. (2017; 7 °Brix) for RDI. Both of them reported an increase in SSC with DI compared to full irrigation, the first one with applying 50% of full irrigation, equivalent to T3 of this experiment, and the second with water stress at the fruit maturation stage with 33.3% or 66.6% of full irrigation. The higher values of SSC obtained in these DI strategies were mainly due to its lower dilution in the fruits, which in turn, was a consequence of the reduction of water uptake

by fruits, not to the accumulation of sugars, in accordance with that stated by Chen et al. (2013) in tomato.

Fruit MI is an important quality criterion for consumer acceptance, being usually a better indicator of acceptability than either SSC or acidity alone (Jayasena and Cameron, 2008). The average MI value was 64.9, which is much higher than those fruits of pepper 'Papri new-E-red' (35.5) produced in greenhouses in Japan (Rahman and Inden, 2012) and of pepper 'Orlando' (California Type; on average 41.4) produced under controlled greenhouse conditions in Spain (Rubio et al., 2010). Although MI was not affected significantly by any of the analysed factors, fruits from full irrigation had a clearly lower value than those from other irrigation strategies, with the highest values obtained in plants exposed to water shortage at harvesting, for both CDI and RDI.

Average ascorbic acid content was on the order of 123 mg 100 g<sup>-1</sup> FW, which is in accordance with values cited in the literature [128 mg 100 g<sup>-1</sup> (Latham, 2001), 131 mg 100 g<sup>-1</sup> (Moreiras, 2013). This content was not affected by IS ( $P \le 0.05$ ), although a tendency to increase with severe water shortage was observed both in CDI (T3) and RDI at harvesting (T9). Keleş and Öncel (2002) found a significant increase of total ascorbic acid in wheat seedlings under salt stress, but they acknowledged a discrepancy with results reported in other plants (such as cotton), probably due to differences in the tolerance of distinct species to salt stress; they concluded that there is no clear relationship between total ascorbic acid content and stress (salt) tolerance.

Severe water deficit, both in CDI or RDI at harvesting, led to fruits with the highest total phenolic compound content. These were followed by fruits exposed to moderate water deficit at harvesting, in the same way as fruit dry matter content and SSC negatively correlated with water availability at this stage. The increase in total phenolic content under drought conditions was also observed by Okunlola et al. (2017) in plant tissues of three pepper species grown in Nigeria, and López-Serrano et al. (2019) in leaves and roots of grafted and non-grafted pepper plants grown in Spain. Keleş and

Öncel (2002) found a significant increase in phenolic compound content ( $\alpha$ -tocopherol) in wheat seedlings under drought and salt stresses.

Environmental stresses, particularly drought stress, stimulate the production of reactive oxygen species that cause oxidative damage (Sharma and Dubey, 2005). Plant cells protect themselves from the damaging effects of reactive oxygen species by a complex antioxidant system comprised of both enzymic and non-enzymic antioxidants, including ascorbate peroxidase and polyphenol oxidase (enzymic), and ascorbic acid and α-tocopherol (non-enzymic) (Farooq et al., 2009). Accumulation of different antioxidants (including ascorbic acid and phenolics) in plant tissues under water stress plays an important role in the alleviation of oxidative damage in the plant itself (Farooq et al., 2009; Galindo et al., 2018; López-Serrano et al., 2019), while this accumulation in the fruits could also alleviate oxidative damage in consumers (Materska and Perucka, 2005).

Currently, several authors have reported that it is necessary to improve irrigation water productivity in agriculture, particularly in arid and semi-arid regions, through increasing the output per unit of water (Howell, 2006). At times, it is even more important to maximize crop water productivity rather than crop yield per unit area (Ruiz-Sánchez et al., 2010), and an adequate DI application requires an evaluation of the economic impact of the yield reduction produced by water stress (Geerts and Raes, 2009).

Important water savings of 25.2% and 50.0% were obtained with CDI strategies, but they led to very important reductions of the gross revenues (35.9% and 55.6%, respectively) to  $45,662 \in \text{ha}^{-1}$  and  $31,758 \in \text{ha}^{-1}$ , seriously questioning the economic viability of the crop. The water economic values for these IS were  $11.07 \in \text{m}^{-3}$  (T2) and  $11.5 \in \text{m}^{-3}$  (T3), lower than the other IS ( $12.6 \in \text{m}^{-3}$  for T1).

Applying RDI during the vegetative growth and fruit-setting stages demonstrated a low reduction in the gross revenue, lower than 2% for moderate water stress (6% for severe restrictions), but the water savings achieved were also

small, particularly for the moderate reduction, which was below 2.5% (4.9% for severe restrictions). The average water economic value for these strategies ranged between  $12.4 \, \in \, \text{m}^{-3}$  (T8) and  $12.8 \, \in \, \text{m}^{-3}$  (T7), like that obtained for fully irrigated plants. These values are much higher than those obtained in the area for watermelon (6.14  $\in \, \text{m}^{-3}$ ; Abdelkhalik et al., 2019) and chufa (*Cyperus esculentus* L. var. *sativus* Boeck.; also known as tigernut) (4.08  $\in \, \text{m}^{-3}$ ; Pascual-Seva et al., 2018) under field conditions.

Given the long duration of the harvesting stage, reducing the water applied to 50% in this stage (T9) led to important water savings (41.5%) but also to a considerable gross revenue loss (43.9%), which makes this IS not recommended for peppers under the studied conditions. When moderate water restrictions were applied during the harvesting (T6), 20.8% of the IWA was saved, while the gross revenue dropped 18.9% in relation to T1.

When the climatic conditions were similar to those in 2017, particularly for rainfall, a consideration would be to end the crop cycle at the beginning of September since it would suppose a gross revenue of  $47070 \in \text{ha}^{-1}$  (81.5% of the MY obtained at the end of the cycle for this IS), a water saving of 23.3% in relation to the entire crop cycle, and a water economic value of  $10.64 \in \text{m}^{-3}$ . Furthermore, this earlier ending of the crop cycle would leave the land available for other crops.

A possible solution to cope with the reduction of yield in some vegetables (as tomato, watermelon and cucumber) because of water stress is the use of grafting technology. Recently, in a study conducted in the same area, López-Serrano et al. (2019) found that water stress severity in pepper plants was alleviated by using a rootstock (code A25) previously selected by Penella et al. (2017). Therefore, it would be interesting to study the response of pepper 'Estrada F1' plants (and other pepper cultivars) when grafted onto drought tolerant rootstocks in response to deficit irrigation strategies.

#### 4.2.6. Conclusions

The present study analysed the effect of deficit irrigation on the plant water status, growth, and productive response of sweet pepper 'Estrada F1' under Mediterranean field conditions. Deficit irrigation negatively affects pepper yield. If water is readily available, full irrigation should be applied. If water restriction is applied during the first stages, plant growth can recover and fruit yield is not reduced, but the water savings are not substantial. Continued deficit irrigation, applying 75% or 50% of the water requirement, or reducing the water applied to 50% of the water requirement at harvesting, leads to a drastic reduction of the marketable yield and, in turn, of the gross revenue, worsening WUE; then, these are not recommended strategies. Applying 75% of the water requirement during harvesting results in a considerable reduction in yield and in the corresponding gross revenue; however, important water savings are obtained in relation to full irrigation. This strategy also led to an improvement of the marketable fruit quality in terms of the soluble solids and polyphenol contents. Under severe limiting conditions, it would probably be feasible to apply 75% of the water requirement during harvesting, ending the crop cycle at the beginning of September, when most of the marketable yield is already harvested, thereby leading to important water savings and leaving the land available for other crops.

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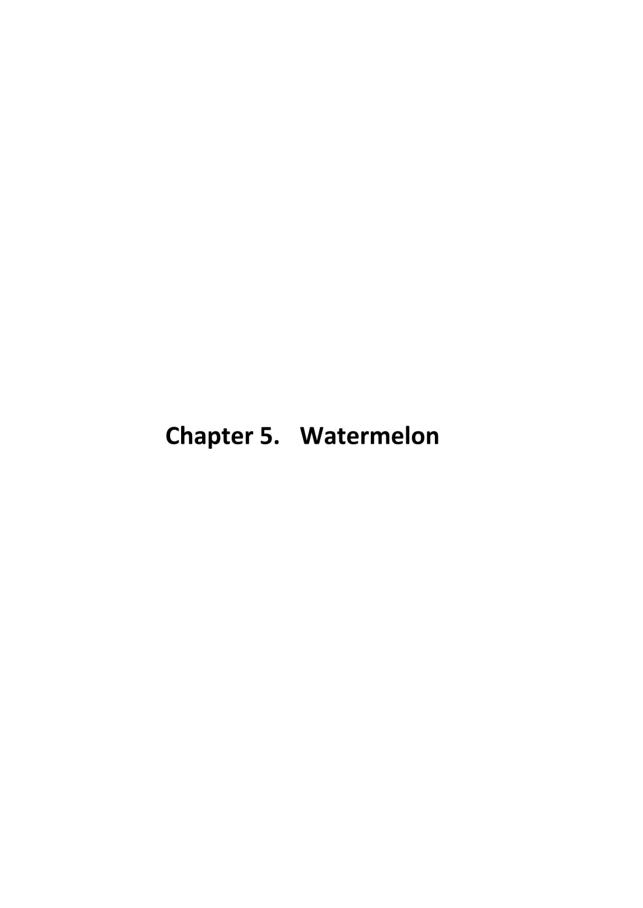
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# 5.1. Yield response of seedless watermelon to different drip irrigation strategies under Mediterranean conditions

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#### 5.1.1. Abstract

Water is an essential resource for food production, as agriculture consumes close to 70% of the total freshwater, and its shortage is becoming critical in arid and semiarid areas of the world. Therefore, it is important to use water more efficiently. The objectives of this project are to determine the productive response and the irrigation water use efficiency of seedless watermelon to three irrigation management strategies over two growing seasons. This was done by applying 100, 75 and 50% of the irrigation water requirements (IWR) the first year, in the second year added six additional treatments, of which three treatments were regulated deficit irrigation with 75% IWR during the vegetative growth, fruit development and fruit ripening stages, and the other three treatments were with 50% IWR during the same stages. The exposure of watermelon plants to severe deficit irrigation resulted in a reduction in dry biomass, total and marketable yield, average fruit weight, fruit number and harvest index, and without improvement of marketable fruit quality. The fruit ripening was the less sensitive stage to water deficits. Relative water content and cell membrane stability index decreased as the water deficit increased. Irrigation water use efficiency decreased to a lesser extend during the fruit ripening stage than when water restriction were applied during different growth stages. If water is readily available, irrigating with 100% of water requirements is recommended, but in the case of water scarcity, applying water shortage during fruit ripening stage would be advisable.

**Keywords:** Evapotranspiration; irrigation water use efficiency; water status; deficit irrigation; soluble solids; fruit size.

# 5.1.2. Introduction

Watermelon [*Citrullus lanatus* (Thun.) Matsum. and Nakai] is an important crop around the world, with a production approximately 117 million Mg from 3.5 million ha (FAO, 2017). Currently, the leading watermelon-producing countries are China, Turkey and Iran. Spain is the main producer of watermelon for the European community, with 969,327 Mg from 17,360 ha (FAO, 2017).

Irrigation water is an essential element for crop production (Howell, 2001; Steduto et al., 2012). Agriculture uses approximately 70% of freshwater; in Spain, agriculture utilizes approximately 68% of total water use (FAO, 2016). During recent years, freshwater shortage is becoming critical in arid and semiarid areas of the world with increasing competition for water across agricultural, industrial and urban consumers (Chai et al., 2016). Rapid population growth, other human activities and the greater incidence of drought, particularly in the Mediterranean area, are increasing the demand for fresh water (Fereres, 2008). This water scarcity and the incremental increase in irrigation costs have led to heightened interest in improving the productivity of water use in crop production (Bessembinder et al., 2005; Fereres and Soriano 2007; Steduto et al., 2012; Reddy, 2016).

Irrigation water-use efficiency (IWUE) is a common indicator employed to assess the efficiency of the use of irrigation water in crop production (Bos, 1980; Tolk and Howell, 2003; Pascual-Seva et al., 2016). At present, there are challenges in maximizing IWUE and increasing crop productivity per unit of water applied. Within this context, the use of deficit irrigation (DI) strategy is a technique of applying irrigation less than the optimum crop water requirements with a result to improve water use efficiency (Pereira et al., 2002; Costa et al., 2007; Capra et al., 2008; Evans and Sadler, 2008; Chai et al., 2016). The real challenge is to establish DI on the basis of maintaining or even increasing crop productivity while saving irrigation water and, therefore, increasing the IWUE (Chai et al., 2016). For this reason, DI requires precise knowledge of the crop yield response to water applied (Fereres and Soriano, 2007). Currently, DI is a common practice throughout the world, especially in dry regions, where it is more important to maximize crop water productivity rather than the harvest per unit land (Ruiz-Sánchez et al., 2010). Regulated deficit irrigation (RDI) is the treatment of water stress during certain crop developmental periods (Fereres and Soriano, 2007).

Water content and water potential have been used as indicators of leaf water status. The use of water content has been replaced by the relative water content (RWC) which

are measurements based on the maximum amount of water a tissue can hold (Yamasaki and Dillenburg, 1999). RWC reflects the metabolic activity in tissues, and it is used as a meaningful index for dehydration tolerance (Anjum et al., 2011; Kalariya et al., 2015). RWC correlates closely with a plant's physiological activities, soil water status (Tanentzap et al., 2015) and is a parameter used for screening the drought tolerance of different genotypes (Tanentzap et al., 2015). On the other hand, the cell membrane stability index (MSI) is also widely used as an indicator of leaf desiccation tolerance (Chai et al, 2010), which detects the degree of cell membrane injury induced by water stress (Bajji et al., 2002).

Watermelon grows in the summer, when evapotranspiration (ET) demands are high and rainfall is scarce, particularly in a Mediterranean-type climate, where irrigation is needed for any significant summer cropping (Turner, 2004). Watermelon is considered to be very sensitive to water stress with larger yield reductions when water use is reduced (Steduto et al., 2012). The timing and extent of water deficit irrigation are important for efficient water use and maximizing yield (Erdem and Nedim Yuksel, 2003; Yang et al., 2017). Currently, there is little available data of DI for seedless watermelon, especially for developed hybrids.

Therefore, it is important to identify the best practices for the water management of watermelon using DI techniques. The objective of this study is to evaluate response of watermelon growth, fruit yield, fruit quality, IWUE, and plant water status under DI in open field conditions.

## **5.1.3.** Materials and methods

# **5.1.3.1.** Experimental site

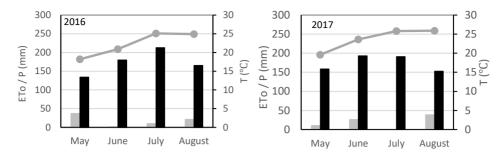
Field experiments were carried out in two plots at the Cajamar Experimental Center in Paiporta, Valencia, Spain (39.4175 N, 0.4184 W), during the 2016 and 2017 growing seasons. The soils are deep, with a coarse texture (Table 1), and are classified as Anthropic Torrifluvents according to the USDA Soil Taxonomy (Soil

Survey Staff 2010). Although the soil of the two plots was apparently similar, soil analyses indicated that the soil in 2017 was sandier than in 2016. In addition, while the soil texture in 2017 was uniform throughout the profile (loam), the soil in 2016 presented a higher percentage of clay (clay loam) at 0.30 m compared to that at a 0.15 m depth. The analyses indicate that the soils have a slightly alkaline pH (on average 7.4), are fertile (1.89% organic matter content; EC 0.39 dS m<sup>-1</sup>), and present high available phosphorous (43 mg kg<sup>-1</sup>; Olsen) and potassium (340 mg kg<sup>-1</sup>; ammonium acetate extract) concentrations. Irrigation water was pumped from a well, with EC 2.53 dS m<sup>-1</sup> and 77 mg kg<sup>-1</sup> N-NO<sub>3-</sub> content.

**Table 1.** Percentages of clay, loam and sand, and soil texture according to the USDA for each irrigation rate (IR: T1, T2 and T3), at a 0.15 and 0.30 m depth in the 2016 and 2017 growing seasons.

	IR	Dept	Clay (%)	Silt (S)	Sand (%)	Texture
2016	T1	0.15	25	51	24	Silt loam
		0.30	27	50.5	22.5	Clay loam
	T2	0.15	25	51	24	Silt loam
		0.30	27	49	24	Clay loam
	T3	0.15	26	50	24	Silt loam
		0.30	27	49	24	Clay loam
2017	T1	0.15	17.5	32.5	50	Loam
		0.30	20	32	48	Loam
	T2	0.15	17.5	32.5	50	Loam
		0.30	20	28	52	Loam
	T3	0.15	17	30.5	52.5	Loam
		0.30	18	30	52	Loam

According to Papadakis's agro-climatic classification (Verheye, 2009), the climate is subtropical Mediterranean (Su, Me) with hot dry summers and an average annual rainfall of approximately 450 mm, irregularly distributed throughout the year, with approximately 40% falling in autumn. Figure 1 shows the most significant climatological data of the growing seasons expressed as average monthly values: temperature (°C), precipitation (mm), and reference evapotranspiration (ET<sub>o</sub>; mm) obtained from a Class A evaporation pan adjacent the experimental plots.



**Figure 1.** Average monthly reference evapotranspiration (ETo; mm; ——), precipitation (P; mm; ——) and temperature (°C; ——) from May to August 2016 and 2017.

# **5.1.3.2.** Plant material and management

Plants of the triploid watermelon cv. *Stellar F1* (Nunhems®) grafted on the hybrid '*Shintoza*' (*Cucurbita maxima* x *Cucurbita moschata*) were transplanted when plants had reached the two-leaf stage in an open field at a spacing of 1.0 m by 3.0 m apart in plastic mulched rows, following traditional practices used in the area, on 19 May 2016 and 15 May 2017. Shortly afterwards, plants were de-topped to force the growth of four tertiary vines per plant, as described by López-Galarza et al. (2004). The row length was 10.0 m, and the width of the raised bed covered by the plastic mulch was approximately 0.60 m.

The cv. *Premium*, also grafted on the hybrid '*Shintoza*', was used as a pollinator with a proportion of 33% to ensure a sufficient pollen amount for the pollination of the triploid cv. The incorporation of nutrients (250-100-250 kg ha<sup>-1</sup> N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O) was performed by fertigation, following the recommendation described by Pomares et al. (2007). Fruit harvest started on 25 July 2016 and lasted until 1 August 2016 and again on 20 July 2017 until 3 August 2017, with three recollections each year.

# **5.1.3.3.** Water requirements and irrigation treatments

From transplanting until establishment (considered as the initial period), the plants of all strategies were irrigated without restrictions. Different irrigation strategies were initiated following this establishment time period. The growth stages are described as follows: (1) initial, from transplanting until establishment; (2) crop development, from

establishment until first fruit setting; (3) fruit growth, from first fruit setting until full fruit size; and (4) fruit ripening, from full fruit size until harvest. These stages correspond to FAO crop growth stages for crop evapotranspiration (ETc) determination (Allen et al., 1998): (1) initial; (2) growth development; (3) mid-season stage; and (4) late-season stage.

<b>Table 2.</b> Irrigation rates (	(R) applied in 2017. Percentages of irrigation water requirements
that were applied in each p	olot during each growth stage.

IR	Initial	Crop development	Fruit growth	Fruit ripening
T1	100%	100%	100%	100%
T2	100%	75%	75%	75%
T3	100%	50%	50%	50%
T4	100%	75%	100%	100%
T5	100%	100%	75%	100%
T6	100%	100%	100%	75%
T7	100%	50%	100%	100%
T8	100%	100%	50%	100%
T9	100%	100%	100%	50%

Two irrigation experiments were completed. The first experiment was conducted in 2016 and 2017 that included three irrigation rates (IR) corresponding to 100% (T1), 75% (T2) and 50% (T3) of the irrigation water requirement (IWR; mm day<sup>-1</sup>) throughout the growing season. The second experiment was carried out in 2017 only, with six additional treatments that included T4, T5 and T6 that corresponded to RDI rates with 75% nominal crop water use at crop growth stages 2, 3 and 4 and T7, T8 and T9 with 50% water use at the same crop stages. The IWR was determined using the following equation:

$$IWR = \frac{ETc - Pe}{Ef}$$

where ETc is the crop evapotranspiration, Ef is the irrigation efficiency including percolation and uniformity) which was considered to be 0.95 (Pomares et al., 2007) and Pe is the effective precipitation (mm), determined from rainfall data using the method of the U.S. Bureau of Reclamation (Stamm, 1967), as presented by Montoro et al. (2011) and Pascual-Seva et al. (2016). The ETc (mm) was calculated from the ETo and a single crop coefficient (K<sub>c</sub>) proposed for local conditions by the Instituto

Valenciano de Investigaciones Agrarias (IVIA, 2011), adapting the duration of each stage to the growing cycle (Table 3).

$$ETc = ETo \times Kc$$

where ETo is the reference evapotranspiration and Kc is the crop coefficient. The ETo was determined according to Allen et al., (1998) as follows:

$$ETo = E_{pan} \times K_p$$

where  $E_{pan}$  (mm day<sup>-1</sup>) is the evaporation from the Class A pan installed in the Experimental Center and  $K_p$  (0.815) is the pan coefficient, determined according to Allen et al. (1998).

**Table 3.** Duration and crop coefficient values (Kc) at different growth stages during 2016 and 2017.

	C	Dates 2016		Dates 2017		Stage duration (d)		
	Growth stages	Initial	Final	Initial	Final	2016	2017	Kc (-)
1	Initial	19 May	29 May	15 May	26 May	11	12	0.2
						7	6	0.32
	C		27			6	6	0.44
2	2 Growth	30 May	27 June	27 May	25 June	5	6	0.56
	development					5	6	0.68
						5	6	0.80
3	Fruit growth	28 June	17 July	26 June	15 July	20	20	0.90
						6	6	0.80
4	Fruit ripening	18 July	1 Aug	16 July	1 Aug	6	6	0.70
						3	5	0.60

The water was supplied by a drip irrigation system with one line, on the soil surface, per bed with emitters spaced 0.30 m apart and a discharge of 2.2 L h<sup>-1</sup>. The amount of water applied for each irrigation event was recorded using totalizing water flow meters connected to the irrigation system. The irrigation events of T1 began when the volumetric soil water content (VSWC) descended to the value of 80% of field capacity, and the other strategies were irrigated at the same time, with the corresponding reductions in irrigation water applied ( $I_{applied}$ ).

#### 5.1.3.4. Volumetric soil water content.

The VSWC  $(m^3 m^{-3})$  was continuously monitored using ECH<sub>2</sub>O EC-5 capacitance sensors connected to an Em50 data logger using the ECH<sub>2</sub>O Utility

software (Decagon Devices Inc., Pullman WA., USA). The sensors were installed one day before transplanting and placed horizontally in the middle of the beds below the irrigation tubing and equidistant between the two emitters, at a 0.15 m depth for all treatments. Additionally, two sensors were installed at a 0.30 m depth for the two extreme strategies, T1 and T3, following the methodology described by González et al. (2009). The VSWC was measured and stored at 15 min intervals. The factory sensor calibration was used directly in the experiments to determine the VSWC. However, in order to compare different irrigation strategies and depths, it was decided to present the VSWC evolution throughout the growing season, as the ratio of the VSWC at each moment compared with VSWC at field capacity (% FC).

# 5.1.3.5. Experimental design and measurements

Each irrigation strategy was replicated three times in a random block design with each replication consisting of a bed (30 m<sup>2</sup>). The external plots were surrounded by similar plots to eliminate border effects.

Three representative plants were sampled from each elemental plot at the end of the growth cycle. Aboveground plants were divided into two parts and analyzed separately: vegetative, including shoots with all their leaves (hereinafter referred to as shoots), and reproductive, including fruits. Each sampled plant part (shoots and fruits) was weighted with a precision analytical balance (Mettler Toledo AG204), dried at 65°C in a forced-air oven (Selecta 297; Barcelona, Spain) until reaching a constant weight to obtain dry weights and dry matter content.

The chlorophyll index (SPAD) allows the indirect and non-destructive evaluation of the content of leaf chlorophyll by light intensity absorbed by the tissue sample. The SPAD was measured at the end of the growth cycle at three points in each of three fully developed leaves in each plant using a SPAD-502 m (Konica Minolta Sensing Inc., Tokyo, Japan).

Total cumulative fruit yield was separated into marketable and non-marketable yield. Marketable yield was classified in accordance with the standard classification,

based on the weight usually used in Spain for this watermelon type, that considers fruits less than 4 kg as small (non-marketable) and those greater than 7 kg as large fruits (marketable). The average fruit weight and number of fruits were determined. The harvest index (HI) was determined as the ratio of marketable yield to total aboveground biomass, both on a dry mass basis (g  $g^{-1}$ ; Turner, 2004).

Three representative fruits per plot were selected to determine the size (height and width) and shape (relation of height/width) of the fruits. Thereafter, fruits were cut to determine rind thickness, and soluble solid content (SSC; ° Brix) was assessed with juice obtained from the central part of the fruit using a digital refractometer (Atago®, Pal-1, 0-53%, Japan). Fruit color coordinates (L\*, a\* and b\*) were taken at the central part of the fruits using a Minolta CR-300 chroma meter (Konica Minolta Sensing Inc., Tokyo, Japan). L\* represents the luminosity, with values ranging from 0 to 100. With a\* and b\* values, the Hue angle (H°) and Chroma (C) were calculated as H° = Arctang (b/a) (McGuire, 1992) and  $C = \sqrt{(a^2+b^2)}$  (Pathare et al., 2013), respectively.

# 5.1.3.6. Irrigation water use efficiency and yield response factor

The IWUE was calculated as the ratio of marketable yield (fresh mass; kg m<sup>-2</sup>) to  $I_{applied}$  (m<sup>3</sup> m<sup>-2</sup>; Cabello et al., 2009).

The yield response to water deficits during the growing season and each growth stage was determined according to Doorenbos and Kassam (1979), using the following equation:

$$\left(1 - \frac{Y_a}{Y_m}\right) = k_y \left(1 - \frac{ET_a}{ET_m}\right)$$

where Ya and Ym are the actual and maximum marketable yield (kg m<sup>-2</sup>), respectively; ETa and ETm are the actual and maximum ET (mm), respectively; and  $k_y$  is the yield response factor. ETa and ETm were calculated using the soil water balance: ET =  $I_{applied}$  + Pe, considering negligible both the drainage and the variation in the volumetric soil water content. Values of Ky greater than 1 indicate that the

crop response is very sensitive to water deficit, while values of Ky lower than 1 mean that the crop is more tolerant to water deficit. When Ky is equal to 1, yield reduction is directly proportional to reduced water use (Doorenbos and Kassam, 1979; Steduto et al., 2012).

# 5.1.3.7. Relative water content and membrane stability index

The relative water content (RWC; %) was determined in fresh leaf discs of 2 cm<sup>2</sup> diameter. The discs were weighed (fresh mass; FM), and immediately floated on double-distilled water in Petri dishes to saturate them with water for 6 h in darkness. The adhering water of the discs was blotted, and turgor mass (TM) was recorded. The dry mass of the discs was noted after dehydrating them at 70°C for 48 h. RWC was calculated using the following formula (Hayat et al., 2008):

RWC (%) = 
$$\frac{\text{FM} - \text{DM}}{\text{TM} - \text{DM}} \times 100$$

The membrane stability index (MSI; %) was determined for 0.2 g samples of fully expanded leaf tissue (Rady, 2011). The leaf sample was placed in a test-tube containing 10 ml of double-distilled water. The content of the test-tube was heated at 40°C in a water bath for 30 min, and the electrical conductivity (C<sub>1</sub>) of the solution was recorded using a multi-parameter analyzer Consort C830 (Consort B2300; Turnhout, Belgium). A second sample was boiled at 100°C for 10 min, and the conductivity was measured (C<sub>2</sub>). The MSI was calculated using the following formula (Rady, 2011):

MSI (%) = 
$$[1-(C_1/C_2)] \times 100$$

Both RWC and MSI were determined by duplicate in each field replication, at the end of each growth stage.

# 5.1.3.8. Statistical analysis

The results of the two experiments were analyzed separately. In the first experiment, T1, T2 and T3 were compared for both years, while in the second

experiment, all IR in 2017 were compared. The results were analyzed using an analysis of variance (ANOVA) using Statgraphics centurion XVII (Statistical Graphics Corporation, 2014). Least significant difference (LSD) at a 0.05-probability level was used as the mean separation test.

#### **5.1.4.** Results

# 5.1.4.1. Sustained deficit irrigation

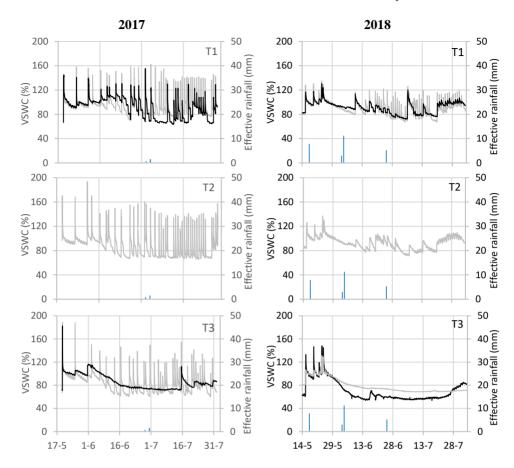
The duration of each growth stage, initial, vegetative development, mid-season and late season, was 11, 28, 20 and 15 days in 2016 and 12, 30, 20 and 17 days in 2017, respectively. The total growth cycle period was 74 days in 2016 and 79 days in 2017. These values, as well as the corresponding Kc values, are presented in Table 3.

The total pan evaporation and consequently ETo during the growing season were lower in 2016 (532 and 433 mm, respectively) than in 2017 (578 and 471 mm, respectively). Values of the monthly precipitation during the two growing seasons were lower than twice the average monthly temperature (°C; data no shown), thus the months included in the experiment are considered dry according to the xerothermic index of Gaussen (Gaussen and Bagnouls, 1952).

During the 2016 growing season T1 received 293 mm while T2 and T3 received 77 and 53%, respectively, of T1. In 2017, T1 received 321 mm while T2 and T3 received 78 and 55%, respectively. These irrigation data indicate that the treatment values of 75 and 50% irrigation rates were accomplished (Table 4). These values include 15 mm in 2016 and 20 mm in 2017 as an initial irrigation across all treatments to ensure good plant establishment.

**Table 4.** Values of irrigation water applied ( $I_{applied}$ ; mm) in the two seasons.

	2016							2017				
	T1	T2	Т3	T1	T2	Т3	T4	T5	T6	T7	T8	Т9
$I_{applied}$	292.7	224.4	155.0	321.1	251.2	177.1	297.1	279.3	275.4	287.6	252.0	244.3



**Figure 2.** Relative soil water content [%; volumetric soil water content/volumetric soil water content at field capacity at a 0.15 m (¬) and 0.30 m (¬) depth] for T1, T2 and T3 management strategies and daily rainfall (vertical bars) during each growing season.

Figure 2 shows the VSWC for T1, T2 and T3 in 2016 and 2017, as well as the Pe. Rain was scarce during the two years, particularly in 2016. Generally, VSWC in the three treatments was relatively higher in 2016 (on average 87.5% FC) than in 2017 (on average 84.7% FC), probably because the soil profile was sandier in 2017 (the sand content was practically two times that in 2016), leading to a higher permeability and less retention of the water supplied on the surface layer. VSWC at a 0.15 m depth was higher under T1 as compared to under T2, which in turn was higher than under T3 (on average 92.5, 89.5 and 76.4 % FC, respectively). T1 had a higher VSWC at a

0.30 m depth (on average 90.9% FC) than that of T3 (on average 82.2% FC), which showed a decreasing trend in their VSWC over time.

Table 5 shows the results of the total yield (in terms of kg m<sup>-2</sup>, fruit number m<sup>-2</sup>, and average fruit weight), marketable yield (indicating the percentage of large fruits), non-marketable yield (differentiating sunburned and small fruit production, which are the only types of culls that were found) and IWUE during the 2016 and 2017 seasons.

**Table 5.** Effect of the growing season and the irrigation rate on the total yield [kg m<sup>-2</sup>, fruit number (No m<sup>-2</sup>) and average fruit weight (kg)], marketable yield [kg m<sup>-2</sup> and large fruits (% on number basis)], fruits affected by sunburn, small fruits and irrigation water use efficiency (IWUE).

		Total yie	ld	Mark	etable yield	Sunburn	Small fruit	IWUE
	kg m <sup>-2</sup>	No m <sup>-2</sup>	kg fruit <sup>-1</sup>	kg m <sup>-2</sup>	Large fruit (%)	kg m <sup>-2</sup>	kg m <sup>-2</sup>	kg m <sup>-3</sup>
Growing season	(GS)							
2016	5.61	1.30	4.32	3.84	7.4	0.36	1.41	16.27
2017	5.79	1.23	4.67	4.57	8.6	0.21	1.01	17.31
LSD	0.65	0.16	0.38	0.88	5.7	0.37	0.64	4.56
Irrigation rate (II	R)							
T1	7.39 a	1.48 a	5.01 a	6.55 a	16.1 a	0.00	0.84 b	21.28 a
T2	5.35 b	1.19 b	4.54 a	4.09 b	5.9 b	0.35	0.92 b	17.23 ab
T3	4.35 c	1.11 b	3.92 b	1.98 c	2.1 b	0.50	1.87 a	11.86 b
LSD	0.82	0.2	0.47	1.08	7	0.45	0.78	5.59
GS*IR								
2016-T1	6.89	1.46	4.73	5.81	12.6	0.00	1.08	19.84
2016-T2	5.50	1.24	4.49	3.95	5.5	0.53	1.02	17.63
2016-T3	4.42	1.19	3.73	1.76	4.2	0.55	2.11	11.34
2017-T1	7.89	1.50	5.28	7.30	19.6	0.00	0.59	22.73
2017-T2	5.20	1.15	4.60	4.23	6.3	0.16	0.81	16.83
2017-T3	4.28	1.04	4.11	2.19	0.0	0.45	1.63	12.38
LSD	1.13	0.28	0.66	1.52	9.9	0.63	1.11	7.91
ANOVA (df)				% Total s	um of the squa	res		
GS (1)	0.4 ns	2.6 ns	9.3 ns	3.2 ns	0.6 ns	4.2 ns	7.5 ns	0.9 ns
IR (2)	81.7 **	55.9 **	60.3 **	83.4 **	57.2 **	31.4 ns	42.4 *	51.5 *
GS-IR (2)	4.2 ns	3.5 ns	2.5 ns	1.8 ns	8.6 ns	4.2 ns	0.8 ns	2.0 ns
Residuals (12)	13.6	38.1	27.9	11.6	33.6	60.2	49.3	45.6
Standard deviation	0.6	0.2	0.4	1.2	5.5	0.4	0.6	4.4

df: degrees of freedom. Mean values followed by different lower-case letters in each column indicate significant differences at  $P \le 0.05$  using the LSD test. \*\* (\*): Indicates significant differences at  $P \le 0.01$  ( $P \le 0.05$ ), ns: Indicates no significant difference.

Water restriction negatively affected ( $P \le 0.01$ ) yield and yield components, but none of the parameters were affected ( $P \le 0.05$ ) by the growing season. The interaction between both factors was not significant ( $P \le 0.05$ ) for any of the analysed parameters. T1 resulted in a higher ( $P \le 0.01$ ) total yield, average fruit weight and total marketable

yield compared to T3, with intermediate values for T2. T1 led to a greater ( $P \le 0.01$ ) proportion of large fruits than T2 and T3. Non-marketable yield represented 55% of the total yield for T3, while it was 11% for T1. Analyzing the different fruits considered as non-marketable, T3 led to a higher ( $P \le 0.01$ ) yield of small fruits compared to that of T2 and T1. Although the sunburned fruit weight was not significantly different among IR, it increased as  $I_{applied}$  decreased.

The IWUE values were high, which corresponds to high-yield crops, and they were affected by IR, with the highest value corresponding to the full irrigation treatment and the lowest to T3. These values are related to the important marketable yield loses of T3 compared to the water saving achieved in relation to T1 (Table 6). Marketable yield (MY) increased linearly with  $I_{applied}$ , following the expression MY = 0.0293  $I_{applied}$  - 2.1171, which presented a correlation coefficient (r) of 0.87 and was significant ( $P \le 0.01$ ). It also increased linearly with the VSWC (% FC), as shows the function MY = 0.2469 VSWC - 17.049 (r = 0.92; P  $\le 0.01$ ).

**Table 6.** Irrigation water savings and marketable yield losses in relation to T1, obtained using the sustained irrigation rates assayed in 2016 and 2017.

	$I_{applied}$ sa	vings (%)	Marketable yield loses (%)		
	2016	2017	2016	2017	
T1	0.00	0.00	0.00	0.00	
T2	23.33	21.77	32.01	42.05	
Т3	47.04	44.85	69.71	70.00	

As for the yield response to water deficits, in both growing seasons, considering as maximum yield (Ym) the marketable yield obtained under T1, actual yield (Ya) corresponding to T2 and T3 strategies, and ETm and ETa corresponding to the cited yields, the fitted linear regression is as follows: 1-(Ya/Ym) = 1. 3 (1-(ETa/ETm), which presents a high correlation coefficient (r=0.99) and statistical significance (P  $\leq 0.01$ ). The yield response factor ( $k_y$ ) was 1.3, being 1.0 for 2016 and 1.6 for 2017.

The fruit size (height and width) and the rind thickness were affected ( $P \le 0.01$ ; Table 7) by the irrigation treatment, with the lowest values corresponding to T3. The

fruits produced in 2017 were wider ( $P \le 0.01$ ) than those produced in 2016, which could be related to the greater average fruit weight obtained in 2017 than in 2016 (Table 5). The rind thickness was affected ( $P \le 0.01$ ) by the interaction of season by IR, in the sense that the rind thickness of the fruits produced under T3 was narrower than that of the fruits under T1 and T2, only in 2016. The fruit shape (height/width ratio) was not affected ( $P \le 0.05$ ) by any of the analyzed factors or interaction.

**Table 7.** Effect of growing season and irrigation rate on different fruit characteristics.

	Height	Width	H/W ratio	Rind thickness (mm)
	(H; cm)	(W; cm)	n/w ratio	Kind unickness (iiiii)
Growing season (GS)				
2016	22.13	20.20 b	1.1	11.52
2017	22.58	21.22 a	1.06	11.96
LSD	0.71	0.49	0.04	0.6
Irrigation rate (IR)				
T1	23.40 a	21.61 a	1.09	12.00 a
T2	22.63 a	20.83 b	1.09	12.48 a
Т3	21.04 b	19.69 c	1.07	10.74 b
LSD	0.87	0.6	0.04	0.73
GS*IR				
2016-T1	23.22	21.06	1.11	12.21 a
2016-T2	22.5	20.33	1.11	12.77 a
2016-T3	20.67	19.21	1.08	9.58 b
2017-T1	23.58	22.17	1.07	11.78 a
2017-T2	22.75	21.33	1.07	12.18 a
2017-T3	21.42	20.17	1.06	11.90 a
LSD	1.34	0.91	0.07	1.12
ANOVA (df)		% Total s	sum of the squares	
GS (1)	2.0 ns	16.5 **	7.1 ns	2.3 ns
IR (2)	38.1 **	39.3 **	1.2 ns	25.7 **
GS-IR (2)	0.5 ns	0.1 ns	0.5 ns	21.3 **
Residuals (12)	59.4	44.2	91.2	50.7
Standard deviation	1.3	0.9	0.1	1.1

df: degrees of freedom. Mean values followed by different lower-case letters in each column indicate significant differences at  $P \le 0.05$  using the LSD test. \*\*: Indicates significant differences at  $P \le 0.01$ . ns: Indicates no significant difference

The fruit quality parameters are presented in Table 8, in terms of fruit dry matter (%), soluble solid content (SSC;  $^{\circ}$  Brix), color parameters L\*, Hue angle and Chroma. Fruit dry matter was only affected by IR with the lowest content under T1, indicating higher water content with the full IR, as expected. IR also affected the SSC in the sense that the lowest value corresponded to T3. There was no difference in color characteristics of Hue and Chroma. L\* was affected ( $P \le 0.01$ ) by both growing

season and IR, with the highest lightness (brightness) values corresponding to 2016 and T3.

**Table 8.** Effect of growing season and deficit irrigation on dry matter content, soluble solid content (SSC) and colour indexes of the fruits: L\*, Hue angle and Chroma.

	Dry matter	SSC	L*	Hue angle	Chroma
	(%)	(°B)			
Growing season (GS)					
2016	8.2	12.68	37.00 a	31.07	37.73
2017	8.2	12.84	34.21 b	30.73	37.67
LSD	0.5	0.27	1.67	1.13	2.47
Irrigation rate (IR)					
T1	7.4 b	12.94 a	34.55 b	31.41	36.63
T2	8.7 a	12.94 a	33.62 b	30.88	39.01
Т3	8.6 a	12.40 b	38.64 a	30.41	37.46
LSD	0.6	0.34	2.05	1.38	3.03
GS*IR					
2016-T1	7.5	12.83	35.00	31.55	38.25
2016-T2	8.7	12.82	36.19	31.40	38.63
2016-T3	8.5	12.38	39.81	30.25	36.31
2017-T1	7.2	13.05	34.09	31.26	35.00
2017-T2	8.7	13.05	31.05	30.37	39.39
2017-T3	8.6	12.42	37.47	30.58	38.62
LSD	0.8	0.52	2.91	1.95	4.64
ANOVA (df)		% Tota	al sum of the	squares	
GS (1)	0.2 ns	2.2 ns	12.3 **	1.1 ns	0.004 ns
IR (2)	72.5 **	21.9 **	30.1 **	6.4 ns	4.8 ns
GS-IR (2)	0.7 ns	0.6 ns	4.9 ns	3.0 ns	6.7 ns
Residuals (12)	26.6	75.3	52.6	89.5	88.5
Standard deviation	0.5	0.5	3.1	1.7	4.5

df: degrees of freedom. Mean values followed by different lower-case letters in each column indicate significant differences at  $P \le 0.05$  using the LSD test. \*\*: Indicates significant differences at  $P \le 0.01$ . ns: Indicates no significant difference.

Table 9 presents the results for leaf chlorophyll content, expressed in SPAD, shoot dry matter (%), shoot and aboveground plant dry biomass and the harvest index (HI), corresponding to T1, T2 and T3 in 2016 and 2017. None of the analyzed parameters were affected ( $P \le 0.05$ ) by the interaction of growing season by irrigation rate. Neither leaf chlorophyll content nor shoot dry matter content were affected ( $P \le 0.05$ ) by growing season or IR. Regarding dry biomass, both shoots and total dry weight were affected ( $P \le 0.01$ ) by IR, with the highest values obtained under the full irrigation

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treatment. T3 had the lowest ( $P \le 0.05$ ) shoot dry biomass. The HI was affected by growing season ( $P \le 0.05$ ) and IR ( $P \le 0.01$ ), with the lowest values obtained in 2016 and T3.

**Table 9.** Effect of deficit irrigation on leaf chlorophyll index (SPAD), shoot dry matter and dry aboveground biomass [vegetative (shoots) and total] and the harvest index (HI).

	SPAD	Shoot dry matter	Aboveground dry biomass		HI
	(-)	(%)	(g m <sup>-</sup>	<sup>2</sup> ) Total	_ (-)
Growing season (GS)			Vegetative	Total	
2016	63.9	21.2	311.8	762.9	0.39 b
2017	62.6	23.3	293.5	698.6	0.50 a
				0,010	
LSD	1.5	3.8	45.42	124.2	0.09
Irrigation rate (IR)					
T1	64.3	21.2	416.9 a	933.4 a	0.51 a
T2	62.9	22.9	274.7 b	687.6 b	0.53 a
T3	62.6	22.6	216.5 с	571.3 b	0.30 b
LSD	1.9	4.7	55.6	152.13	0.11
GS*IR					
2016-T1	65.5	20.7	437.6	949.9	0.43
2016-T2	63.1	21.5	283.3	762.3	0.46
2016-T3	63.3	21.3	214.6	576.4	0.27
2017-T1	63.1	21.6	396.1	916.8	0.58
2017-T2	62.7	24.4	266.1	612.9	0.60
2017-T3	62.0	23.8	218.4	566.2	0.32
LSD	2.7	6.7	78.7	215.16	0.15
ANOVA (df)		% Tota	al sum of the squar	es	
GS (1)	17.7 ns	9.8 ns	1.0 ns	3.0 ns	17.6 *
IR (2)	20.1 ns	5.1 ns	82.8 **	66.1 **	55.9 **
GS-IR (2)	6.1 ns	1.7 ns	1.0 ns	2.69 ns	2.7 ns
Residuals (12)	56.1	83.5	15.2	28.3	23.8
Standard deviation	1.5	3.7	44.2	120.9	0.1

df: degrees of freedom. Mean values followed by different lower-case letters in each column indicate significant differences at  $P \le 0.05$  using the LSD test. \*\* (\*): Indicates significant differences at  $P \le 0.01$  ( $P \le 0.05$ ). ns: Indicates no significant difference.

The RWC and MSI results are presented in Table 10. RWC was affected ( $P \le 0.01$ ) by both growing season and IR, obtaining the highest values in 2016 and T1, which also presented the highest MSI ( $P \le 0.01$ ).

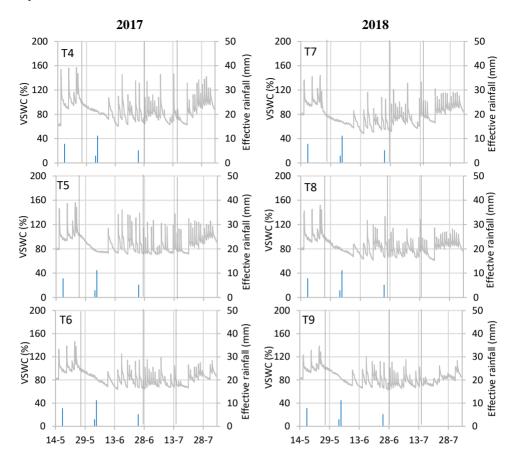
**Table 10.** Effect of growing season and irrigation rate on relative water content (RWC) and membrane stability index (MSI).

	RWC (%)	MSI (%)
Growing season (GS)		
2016	79.7 a	75.8
2017	76.3 b	76.2
LSD	1.4	1.7
Irrigation rate (IR)		
T1	82.9 a	82.9 a
T2	79.2 b	75.8 b
Т3	72.1 c	69.3 c
LSD	1.8	2.1
GS*IR		
2016-T1	83.5	83.1
2016-T2	81.6	75.9
2016-T3	74.4	68.2
2017-T1	82.3	82.6
2017-T2	76.7	75.6
2017-T3	69.9	70.4
LSD	2.5	3.0
ANOVA (df)	% Total sum o	of the squares
GS (1)	12.9 **	0.2 ns
IR (2)	79.3 **	93.0 **
GS-IR (2)	2.7 ns	1.2 ns
Residuals (12)	5.1	5.7
Standard deviation	1.4	1.7

df: degrees of freedom. Mean values followed by different lower-case letters in each column indicate significant differences at  $P \le 0.05$  using the LSD test. \*\*: Indicates significant differences at  $P \le 0.01$ . ns: Indicates no significant difference.

## 5.1.4.2. Regulated deficit irrigation

In the second experiment, there were no considerable differences in VSWC at a 0.15 m depth between the different IRs (Figure 3; on average 83.2% FC) or even during the water restriction stages, as the  $I_{applied}$  in each irrigation event, in every strategy, exceeded the management allowed deficit (corresponding to 20% FC) of the shallower layer of the soil. The  $I_{applied}$  values are presented in Table 4, with the lowest and the highest values corresponding to T3 and T1, respectively, with intermediate values for RDI.



**Figure 3.** Relative soil water content [%; volumetric soil water content/volumetric soil water content at field capacity at a 0.15 m] for T4, T5, T6, T7, T8 and T9 management strategies and daily rainfall (vertical bars) in 2017.

Sustained and regulated deficit irrigation (Table 11) negatively affected ( $P \le 0.01$ ) the yield. The highest value of total yield was recorded ( $P \le 0.05$ ) under T1, and the lowest value was found under T3. Water restriction at 75% IWR during the fruit ripening stage (T6) had a lesser effect on the reduction in fruit yield with respect to full irrigation than when water restriction was applied during the crop development (T4)or fruit growth stages (T5) With the restriction of 50% (T7, T8 and T9) a similar trend was observed, but without statistical differences ( $P \le 0.05$ ).

**Table 11.** Effect of the irrigation rate on the total yield [kg m<sup>-2</sup>, fruit number (No m<sup>-2</sup>) and average fruit weight (kg)], marketable yield [kg m<sup>-2</sup> and large fruits (% on number basis)], fruits affected by sunburn, small fruits and irrigation water use efficiency (IWUE).

	-	Total yield	i	Mark	etable yield	Sunburn	Small fruits	IWUE
•	kg m <sup>-2</sup>	No m <sup>-2</sup>	kg fruit -1	kg m <sup>-2</sup>	Large fruit (%)	kg m <sup>-2</sup>	kg m <sup>-2</sup>	kg m <sup>-3</sup>
Irrigation rates	s (IR)						-	
T1	7.89 a	1.50 a	5.28 a	7.30 a	19.6 a	0.00 c	0.59	22.73 a
T2	5.20 cd	1.15 bcd	4.60 bc	4.23 bc	6.3 bc	0.16 bc	0.81	16.83 ab
T3	4.28 d	1.03 cd	4.11 c	2.19 d	0.0 c	0.45 a	1.60	12.38 b
T4	5.10 cd	1.13 cd	4.53 bc	4.37 bc	2.6 bc	0.00 c	0.73	12.39 b
T5	4.95 cd	1.09 cd	4.55 bc	3.80 bc	8.8 b	0.00 c	1.15	13.62 b
T6	6.38 b	1.43 ab	4.48 bc	4.81 b	7.9 bc	0.00c	1.56	17.48 ab
T7	4.71 cd	0.96 d	4.90 ab	4.08 bc	7.5 bc	0.19 b	0.44	13.41 b
T8	5.11 cd	1.19 bcd	4.35 c	3.45 c	8.9 b	0.07 bc	1.59	13.71 b
T9	5.74 bc	1.28 abc	4.52 bc	4.42 bc	6.4 bc	0.00 c	1.32	18.08 ab
LSD	0.98	0.27	0.55	1.03	8.8	0.17	0.92	6.50
ANOVA (df)		% Total sum of the squares						
IR (8)	82.6 **	63.2 **	57.6 *	87.2 **	59.9 *	76.4 **	49.5 ns	52.4 *
Residuals (18)	17.4	36.8	42.4	12.8	40.1	23.6	50.5	47.6
Standard deviatio	n 0.6	0.2	0.3	0.6	5.1	0.1	0.5	3.8

df: degrees of freedom. Mean values followed by different lower-case letters in each column indicate significant differences at  $P \le 0.05$  using the LSD test. \*\* (\*): Indicates significant differences at  $P \le 0.01$ . ( $P \le 0.05$ ). ns: Indicates no significant difference.

The greatest fruits number  $m^2$  was observed under T1, not differing ( $P \le 0.05$ ) from T6 nor T9. T3 stood out particularly for having the lowest values of commercial yield, no large fruits (0%) and the highest production of sunburned fruits and small fruits (with no significant difference at  $P \le 0.05$ ). Analyzing the different fruits considered as non-marketable, significant differences ( $P \le 0.01$ ) were found in the fruits affected by sunburn; the highest value was obtained with the most restrictive IR (T3), although its importance in the non-marketable yield was low. In contrast, the small fruit yield (fruits less than 4 kg in weight), between 78% and 100% of the non-marketable yield, was not affected ( $P \le 0.05$ ) by the IR, probably due to the high variability of this parameter, with a coefficient of variation (CV; standard deviation as a percentage of the mean value) of 52.7%. The IWUE was negatively affected ( $P \le 0.05$ ) by the sustained and regulated DI, but neither the sustained restriction to 75% (T2) nor RDI when water restriction was applied during the fruit ripening stage (T6 and T9) led to lower values than the full irrigated treatment. The lack of statistical

difference ( $P \le 0.05$ ) among the different DI strategies may be related with the high variability of the IWUE values (CV = 29.3%).

Table 12 presents the  $I_{applied}$  savings and the marketable yield losses obtained using the different IRs. Considering the RDI strategies, the lowest yield losses and the greatest water savings were obtained when the water restriction was applied in the last stage of the crop cycle. The yield increased linearly with  $I_{applied}$ , and the positive linear relationships are presented in Table 13. Obviously, these relations are different depending on the stage in which the water restriction occurred. All the relationships were statistically significant (P  $\leq$  0.01) and showed high correlation coefficients, greater than 0.87. The greatest slope of these relations corresponds to the water restriction in the crop development stage. Other adjustments (i.e. polynomial, exponential, logistic) did not result in significance (P  $\leq$  0.05).

**Table 12.** Irrigation water savings and marketable yield losses in relation to full irrigation, obtained using the irrigation rates assayed in 2017.

	T1	T2	T3	T4	T5	T6	T7	T8	T9
Iapplied savings(%)	0.00	21.77	44.85	7.47	13.02	14.23	10.43	21.52	23.92
Marketable yield losses (%)	0.00	42.05	70.00	40.14	47.95	34.11	44.11	52.74	39.45

**Table 13.** Relationship between yield (Y; kg m<sup>-2</sup>) and irrigation water applied ( $I_{applied}$ ; mm) for the water restrictions applied during the total cultivation cycle (Total), development growth (2), fruit growth (3), and fruit ripening (4) stages.

	Relationship	r
Total	$Y = 0.0354 I_{applied} - 3.0301$	0.96
2	$Y = 0.1012 I_{applied} - 21.755$	0.95
3	$Y = 0.0388 I_{applied} - 3.9957$	0.89
4	$Y = 0.0388 I_{applied} - 3.9957$	0.87

All of them are significant at P≤0.01

As for the yield response to water deficits, for the RDI strategies, there were four fitted linear regression equations: one for the sustained DI and one for each stage of irrigation restriction, considering the yields and ET corresponding to each strategy. All linear regression equations were fitted to the data with adequate correlation coefficients (r from 0.96 to 0.99) and statistical significance ( $P \le 0.05$ ). The yield

response factor (k<sub>y</sub>) was 1.6, 1.4, 1.2 and 0.84 for sustained DI, crop development, fruit growth and fruit maturation, respectively.

**Table 14.** Effect of deficit irrigation on different fruit characteristics.

	Height (H; cm)	Width (W; cm)	H/W ratio	Rind thickness (mm)
Irrigation rate (IR)				
T1	23.58	22.17	1.07	11.78
T2	22.75	21.33	1.07	12.18
Т3	21.42	20.17	1.08	11.90
T4	20.75	19.75	1.05	11.97
T5	23.17	21.58	1.07	12.52
T6	22.58	20.75	1.10	12.50
T7	22.58	20.75	1.07	11.45
T8	22.50	20.33	1.12	12.93
Т9	22.08	20.50	1.10	14.07
LSD	0.71	1.58	0.08	1.98
ANOVA (df)		% Total s	um of the squares	
IR (8)	21.0 ns	24.8 ns	9.1 ns	18.2 ns
Residuals (18)	79.0	75.2	90.9	81.8
Standard deviation	1.7	1.4	0.1	1.7

df: degrees of freedom. Mean values followed by different lower-case letters in each column indicate significant differences at  $P \le 0.05$  using the LSD test. ns: Indicates no significant difference.

None of the analysed fruit characteristics (size, shape and rind thickness) were affected ( $P \le 0.05$ ) by the IR (Table 14), probably due to the observed variability between the fruits under each RDI treatment. Overall, it could be stated that RDI strategies presented intermediate values to the extreme SDI strategies. The water restriction in this experiment did not affect ( $P \le 0.05$ ) the dry matter content, the SSC of fruits, L\* or the Hue angle (Table 15), but it did affect ( $P \le 0.01$ ) the Chroma index. The highest values of Chroma corresponded to T6, and the lowest were obtained under T4 and T7.

**Table 15.** Effect of deficit irrigation on dry matter content, soluble solid content (SSC), colour indexes of the fruits:  $L^*$ , Hue angle and Chroma.

	Dry matter (%)	SSC (°B)	L*	Hue angle	Chroma
Irrigation rate (IR)					
T1	7.2	13.05	34.08	31.26	35.00 ab
T2	8.7	13.05	31.05	30.37	39.38 ab
T3	8.6	12.42	37.47	30.58	38.62 ab
T4	7.5	12.28	35.43	31.00	32.17 c
T5	8.6	12.90	33.62	29.85	38.10 ab
T6	8.7	12.73	33.98	30.10	39.95 a
T7	9.3	12.57	33.38	31.11	30.88 c
T8	8.1	12.92	32.55	30.14	38.37 ab
T9	8.6	12.35	33.10	31.94	38.13 ab
LSD	2.1	0.72	4.54	1.45	4.82
ANOVA (df)		% Total sur	n of the so	quares	
IR (8)	26.2 ns	20.4 ns	18.5 ns	23.6 ns	39.9 **
Residuals (18)	73.8	79.6	81.5	76.4	60.1
Standard deviation	3.7	0.6	3.9	1.2	4.1

df: degrees of freedom. Mean values followed by different lower-case letters in each column indicate significant differences at  $P \le 0.05$  using the LSD test. \*\*: Indicates significant differences at  $P \le 0.01$ , ns: Indicates no significant difference.

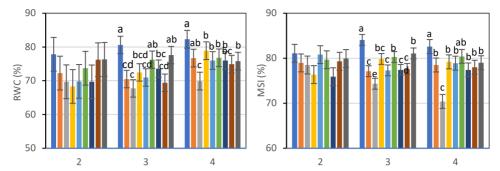
Dry shoot biomass (Table 16) was affected by the IR ( $P \le 0.01$ ), in the sense that the greatest biomass was obtained under the full irrigation treatment, not showing statistical differences ( $P \le 0.05$ ) with T6 nor T9, which, in turn, did not differ ( $P \le 0.05$ ) from the other RDI strategies. The other parameters related to the vegetative part of the plant, such as SPAD, shoot dry matter, total above ground biomass and the HI, were not affected ( $P \le 0.05$ ) by the IR.

**Table 16.** Effect of deficit irrigation on leaf chlorophyll index (SPAD), shoot dry matter and dry aboveground biomass [vegetative (shoots) and total] and the harvest index (HI).

-	SPAD	Shoot dry	Aboveground dry b	iomass (g m <sup>-2</sup> )	HI
	(-)	matter (%)	Vegetative	Total	(-)
Irrigation rate (IR)					
T1	63.1	21.6	396.1 a	916.8	0.58
T2	62.67	24.4	266.1 cd	612.9	0.60
T3	61.97	23.8	218.3 d	566.2	0.32
T4	64.73	21.1	317.8 bc	638.3	0.42
T5	61.63	20.7	311.7 bc	677.7	0.48
T6	61.17	20.4	354.4 ab	749.4	0.56
T7	64.03	22.4	295.6 bcd	660.0	0.57
T8	62.47	22.3	261.7 cd	596.1	0.47
T9	63.87	22.0	333.3 abc	651.7	0.58
LSD	3.66	6.3	77.9	260.4	0.18
ANOVA (df)		%	Total sum of the square	es	
IR (8)	29.3 ns	18.6 ns	64.8 **	38.9 ns	50.7 ns
Residuals (18)	70.7	81.4	35.2	61.1	49.3
Standard deviation	2.13	3.7	45.4	151.8	0.1

df: degrees of freedom. Mean values followed by different lower-case letters in each column indicate significant differences at  $P \le 0.05$  using the LSD test. \*\*: Indicates significant differences at  $P \le 0.01$ . ns: Indicates no significant difference.

Figure 4 presents the evolution of the RWC and MSI indexes through the crop growth periods. Both indexes did not present significant differences ( $P \le 0.05$ ) between IR when irrigation restrictions were applied during growth development (RWC = 77.8%, MSI = 81.1% for T1). There were differences ( $P \le 0.05$ ;  $P \le 0.01$ ) in fruit growth and fruit ripening stages, with the highest values at the fruit ripening stage corresponding to the full irrigation treatment (RWC = 82.3%, MSI = 82.6%) and the lowest under T3 (RWC = 69.9%, MSI = 70.4%).



**Figure 4.** Relative water content (RWC; %) and membrane stability index (MSI; %) for different irrigation rates (■T1 ■T2 ■T3 ■T4 ■T5 ■T6 ■T7 ■T8 ■T9) at the end of the three water restriction stages of growth development (2), fruit growth (3) and fruit ripening (4). Vertical bars represent the LSD for each restriction period.

### 5.1.5. Discussion

The yields obtained in the present study under full irrigation treatment are considered similar to those obtained by López-Galarza et al. (2004) in greenhouse-grown triploid watermelon and those obtained by Özmen et al. (2015) in Turkey.

The notable reductions in both total and marketable yield caused by water restriction are similar to those obtained in seedless watermelon by Bang et al. (2004), Leskovar et al. (2004), and González et al. (2009) and in diploid watermelon by Erdem et al. (2001), Rouphael et al. (2008) and Kuşçu et al. (2015). Rouphael et al. (2008) found that plants grown under full irrigation (100% of  $ET_c$ ) resulted in both higher fruit weight and number than those grown under 75% and 50% of  $ET_c$ . In this study, 100% irrigation had higher fruit weight and fruit numbers compared with reduced irrigation treatments where yield reduction is attributed to the decline in both the number of fruits and fruit size. Moreover, the results agree with those obtained by Bang et al. (2004), in that the marketable yield of large fruits decreased and that of small fruits increased as  $I_{applied}$  decreased.

Water restriction during the fruit ripening stage had a lesser effect on the reduction of fruit yield with respect to full irrigation than compared with water restrictions applied during the crop development or the fruit growth stages. The effect of water restrictions at fruit ripening was minimal because most of the fruits

had reached their final size. Geerts and Raes (2009) presented the main advantage of DI to get the best response is by applying the full water requirement only during the most drought-sensitive stages.

In this research the fruit yield increased linearly with  $I_{applied}$ . Tolk and Howell (2003) reported both linear and curvilinear relationships and stated that nonlinear relationships are explainable if the HI varies with water deficit. In the first experiment, the HI only decreased under T3, and in the second experiment, the HI did not differ between IRs. Therefore, yield-  $I_{applied}$  relationships were lineal when they were analyzed for the water restrictions in both the total cultivation cycle or during separate stages. These positive linear relationships between yield and  $I_{applied}$  agree with the results obtained by Erdem et al. (2001) studying watermelon in Turkey.

IWUE is a key indicator that reveals the optimal water use for plant production. The IWUE obtained in this research for the full irrigation treatment agree with those reported by Kuşçu et al. (2015) and are slightly greater than those presented by Erdem et al. (2005), both obtained using the cv. Crimson sweet in Turkey. In the first experiment, with sustained water restriction, IWUE was affected ( $P \le 0.05$ ) by IR, with the highest IWUE value corresponding to the full irrigation treatment and the lowest to the maximum restriction (T3). Differences were significant due to the important marketable yield losses seen under T3 compared to the water saving achieved, in relation to T1. On the other hand, with RDI, the high coefficient of variation led to a decrease in the level of statistical significance, with similar results shown by Erdem et al. (2005). The lack of statistical significant differences between IRs for some parameters may be consequence of their high values of CV, which might be reduced using larger plots as stated by McCann et al. (2007). Some researchers have stated that IWUE is not affected by IR, such as Erdem et al. (2005). However, other studies have shown that IWUE varies with  $I_{applied}$ , as in the sustained deficit irrigation experiment and in Kirnak et al. (2009), Kirnak and Dogan (2009) and Kuşçu et al.

(2015), which state that IWUE depends on many other factors and particularly on climatic conditions.

All linear regression equations fitted to the data of ET versus yield response confirm the linear relations obtained between yield and  $I_{applied}$  and agree with Erdem and Yuskel (2003) for watermelon in Turkey. The yield response factor obtained for the total growing season coincides with that obtained by Erdem and Nedim Yuksel (2003; 1.27).

Regarding fruit morphological parameters, it is remarkable that fruit dimensions increase with  $I_{applied}$  when extreme rates are considered, as presented by Leskovar et al. (2004); however, there are no differences between RDI treatments, as reported by Özmen et al. (2015). These results were expected, as the analyzed fruits were randomly selected from marketable fruits harvested in their optimal ripening stage, therefore presenting similar characteristics.

Fruit dry matter content was at a minimum (ie the fruits showed the maximum fruit water content) under the full irrigation treatment. This greater water content in the fruits would result in expected lower SSC; however, higher contents were obtained under the full irrigation treatment rather than under the most restrictive treatments. These unexpected results could be related to higher carbohydrate production due to the greater photosynthetic capacity, due to the greater shoot biomass produced under full irrigation. Although SSC depends on many factors, such as genetic variability, cultural practices, etc. (Leskovar et al., 2004), according to different standards for watermelon fruit quality (USDA, 2006; United Nations, 2012), values greater than 10 °Brix are considered to be at a very good sweetness level; thus, the values recorded for all IR in this research are considered as very good quality. The most abundant sugars in the watermelon fruit flesh are initially fructose and glucose (reducing sugars) that decrease at ripening thereby, increasing the sucrose (non-reducing sugar) concentration (Leskovar et al., 2004; López-Galarza et al., 2004).

Although total yield was reduced by 40% in comparison to the full IR, in similar proportion to the aboveground biomass, the greater proportion of non-marketable fruits led to a larger reduction in terms of marketable yield under T3 (70%). For this reason, the HI occurred the most restrictive strategy (T3) presented the lowest HI value. Overall, HI values obtained under T1 (on average 0.51) are somewhat low, and those obtained under T3 are very low, but it must be borne in mind that they have been obtained with respect to total biomass and not only vegetative biomass. These HI values are lower than those reported by Colla et al. (2006) for the cv. *Tex* in Italy and by González et al. (2009) for spring watermelon in Spain, but both determined the HI as the ratio of dry matter partitioned into all fruit (marketable and non-marketable fruits) relative to the total plant biomass, and therefore it led to greater values of HI.

Leaf chlorophyll content was high in relation to the values reported in the literature for watermelon (approximately 42% obtained by Nicolae et al., 2014). It was not affected by water restrictions in any of the experiments.

Under sustained water restriction treatments, a reduction in RWC and MSI was observed, which may be attributed to the negative effect of water shortage on watermelon. Abd El-Mageed et al. (2016) noted a positive relationship between RWC and plant dry biomass in squash plants. This suggests that plants having a greater biomass can maintain a higher water content in leaves, leading to a greater tolerance to drought, as occurred in the present experiment. Our results are also in accordance with those obtained by Rouphael et al. (2008), who observed that the RWC of mini-watermelon cv. *Ingrid* decreased under deficit irrigation treatments of 50% and 75% of ET<sub>c</sub> in comparison to 100% of ETc. Similar results were obtained by Kirnak et al. (2009), Kirnak and Dogan (2009) and Mohammadzade and Soltani (2015).

Regarding the RDI treatments, determinations were made at the end of each restriction stages. At the end of crop development, there were no differences between IR for neither RWC nor and MSI. Treatments that were subjected to a water shortage

in the fruit growth stage showed the lowest RWC values. Regarding MSI, the lowest values were obtained under the treatments that subjected plants to water restrictions during the crop development or fruit growth. The negative evolution of the MSI corresponding to T3 suggests that with the maximum water restriction assayed, the leaves experienced light and permanent cellular membrane damage. These results agree with those reported by Ram et al. (2014) for watermelon seedlings, which indicated that water stress increases membrane permeability causing higher electrolyte leakage into the external medium, resulting in a decrease of MSI values. The RWC and MSI results agree with the greater (except for T1) fruit yield obtained in plants subjected to a water shortage in the fruit ripening stage. Therefore, it can be stated that if water restrictions are required, they should be applied in the fruit ripening stage.

It is important to increase irrigation water productivity throughout the world, especially in dry regions. A pathway to enhance water use efficiency in irrigated agriculture is to increase the output per unit of water (Howell, 2006), being even more important to maximize crop water productivity rather than the harvest per unit area (Ruiz-Sánchez et al., 2010). Nevertheless, considering the IWUE values obtained in 2017 and the average watermelon fruit price (0.27 € kg<sup>-1</sup>; MAPAMA, 2017), in the present study conditions the application of DI in the fruit ripening stage would suppose a decrease in relation to full irrigation in both the gross revenue (19,710, 12,987 and 11,934 € ha<sup>-1</sup> for T1, T6 and T9, respectively) and the economic value per unit of water consumed (6.14, 4.72 and 4.88 € m<sup>-3</sup> for T1, T6 and T9, respectively), which would be greater if the water restriction were carried out in the other stages, seriously questioning the economic viability of the crop. Under limiting conditions, it would probably be interesting to apply the full requirements in a limited area rather than extending the cultivated area (Erdem and Nedim Yuksel, 2003), and to convert to other crops with higher economic value or productivity per unit of water consumed or even to more drought-tolerant crops (Evans and Sadler, 2008).

The herein presented results correspond to the seedless watermelon cv. *Stellar* F1, but it should be noted that the results for seeded cv. *Premium*, used as a pollinator, seem to show a similar trend.

### 5.1.6. Conclusions

The present study analyzed the effect of both sustained and regulated deficit irrigation on the growth and yield of watermelon cv. *Stellar* F1. If water is not a limiting factor, applying 100% of water requirements is advisable. Sustained deficit irrigation at 50% of the nominal crop water requirements led to application of lower water amounts, which resulted in a reduction in total and marketable yield and the average fruit weight, without increasing fruit quality. Irrigating at 75% of water requirements reduced to a lesser extend yield and IWUE than the 50% treatment (compared to full irrigation) and it could be recommended if water is scarce. For regulated deficit irrigation, intermediate results were obtained, highlighting the results obtained for applying water restrictions during the fruit ripening stage, both at 75% and 50% of the water requirements, which lead to acceptable marketable yields and could be recommended. When water is a limiting factor, two options could be recommended, either to apply these regulated deficit irrigation strategies, or to apply the full water requirements in a limited area.

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Chapter 6. General discussion

## 6.1. General discussion

The production of major horticultural crops is located in hot and dry areas, such as Mediterranean (Fereres and Soriano, 2007). In vegetable crops, drought is one of the major constraints, and it seriously affects their production. Furthermore, water shortage is becoming a critical issue in arid and semi-arid areas of the world (Chai et al., 2016; Ghazouani et al., 2019). It is expected that these limitations and risks to food security will be intensified in the Mediterranean area, particularly with the increase in irrigated area, expected climate change and population growth, leading to an increased competition for resources (WWAP, 2015).

Deficit irrigation (DI) have been suggested as a sustainable technique to increase the irrigation water use efficiency with minimal negative effect on yield and that could improve the product quality (Chai et al., 2016; Galindo et al., 2018). The timing and extend of water deficit are key variables in successful DI application. Moreover, crop response varies with the water reductions applied at different development stages, depending on its sensitivity at each growth stage (Galindo et al., 2018; Nadeem et al., 2019). Therefore, some researches have evaluated the effect of continued deficit irrigation (CDI) and regulated deficit irrigation (RDI) on major vegetable crops in the Mediterranean area. This PhD thesis covers different parameters, including plant growth and biomass, plant water status, yield and product quality, and irrigation water use efficiency, in addition crop profitability has been determined, enabling growers to decide of applying DI or not.

The irrigation water requirement (IWR = ETc – Pe) was lower during autumnwinter crops than during spring-summer crops, hence the IWA were higher, resulting (on average for full irrigated plants) 168 mm and 249 mm for cauliflower and onion, respectively, whereas it was 603 mm and 307 for sweet pepper and watermelon, respectively. Monitoring volumetric soil water content (VSWC) to improve irrigation management and starting each irrigation event based on threshold value of VSWC or determining refill limitations has been reported by several investigators, such as Yang et al. (2017) for pepper, watermelon and tomato, Leskovar et al. (2012) and Zheng et al. (2013) for onion and Latif et al. (2016) for cauliflower. Using sensors to monitor soil water content as an irrigation scheduling approach, particularly when using DI to save water, can ensure an adequate soil water status, limiting drainage and leading to improved water productivity while minimizing the risk of yield reduction (Fereres and Soriano, 2007; Blanco et al., 2018). In these studies, the criterion of initiating each irrigation event when the volumetric soil water content (VSWC) dropped to 80% of the field capacity has been proved to be an adequate irrigation management, in the view of the yield obtained under fully irrigated plants. In all crops, soil water sensors recorded the greatest average VSWC (% of field capacity) with full irrigation strategy, while the lowest values were registred with severe water reduction during the entire seasons (50% IWR).

In each analysis performed in each crop, plants grown under the most restricted strategy at the whole season (50% IWR) showed the poorest water status (the lowest RWC and MSI values), while those grown under full irrigation recorded the greatest values.

The yield differences among the growing seasons in all crops (except watermelon) that have been observed in these experiments were due to different climatic conditions. These variations between seasons are not unusual in the area, as stated in other studies performed in the Cajamar Experimental Centre (Fundación Cajamar 2016, 2017, 2018) under "standard" conditions (Allen et al., 1998).

The tested crops have responded differently to water reductions, considering the different climatic conditions between the seasons; the negative effects caused by DI in yield have been minimal in autumn-winter crops, particularly in cauliflower, compared to spring-summer crops. The marketable yield reduction under CDI in autumn-winter crops were (on average) 15% and 18% for cauliflower and onion,

respectively, while this yield decrease was much greater in spring-summer crops (on average) 46% in sweet pepper and 53% in watermelon. A similar trend was observed for crop responses to RDI. On one hand, it is obvious, since irrigation is more important in summer than in autumn-winter, due to the greater ET and the lower rain recorded during the summer months. On the other hand, the different crops show different behavior under water deficits, as stated by Penella et al. (2014) using some pepper genotypes under water stress. This is natural since crops have different susceptibilities to water stress (Fereres and Soriano, 2007), even more some cultivars show different behavior under water deficits (Penella et al., 2014).

For the analyzed crops, full irrigation strategy has resulted in the greatest yield, product average weight and gross revenue. The obtained yield is considered satisfactory compared to those obtained in other experiments conducted with "standar" conditions in the Experimental Centre (Fundación Cajamar, 2016, 2017, 2018). On the other hand, DI with 50% IWR during the entire growing season have caused a drastic reduction of yield, average product weight, and gross revenue.

Crops show different sensitiveness at different stages of their development; on sweet Italian pepper, water shortage at vegetative growth and fruit-setting stages has minimal effect on fruit yield; similar observation was found by Yang et al. (2017). Onion bulb yield has decreased to a greater extend when severe water shortage has been applied at vegetative growth and bulbing stages than when it was applied at bulb repining stage. This agrees with the observation reported by Zheng et al. (2013). In watermelon, water restriction during the fruit ripening stage has had a lower effect on the fruit yield reduction in relation to full irrigation than water restrictions applied during the crop development or the fruit growth stages; similar observations were reported by Kuşçu et al. (2015). In relation to cauliflower, RDI has not affected curd yield.

Overall, yield reduction that has been obtained for all crops under DI is attributed to the decline in the product size and the average weigh, therefore, the effect of water restriction at onion and watermelon ripening was minimal, because most of the

bulbs/fruits had already reached their final size in that stage. Water deficit at early growth stages allows plants to adapt gradually to the water deficits (Fereres and Soriano, 2007; Blum, 2009). Remarkably, continuous severe water shortage during the entire season resulted in large percentages of non-marketable yield in sweet pepper (on average 67%) and a great reduction in watermelon marketable yield (on average 70%). These observations were attributed to the higher presence of BER in sweet pepper and the decline in both watermelon fruit number and size.

When the water is the limiting factor for crop production, it is more important to improve IWUE rather than increasing yield (Geerts and Raes, 2009). In this research, in autumn-winter crops the greatest efficiencies has been recorded under the severe water restriction during the total cultivation cycle (50% IWR), while, in spring-summer crops, neither CDI nor RDI have improved the IWUE, indicating that the water savings have not compensated the yield reductions.

Geerts and Raes (2009) presented that the main advantage of DI to get the best response is by applying the full water requirement only during the most drought-sensitive stages. These studies have estimated the  $K_y$  to determine the most sensitive stages to water reduction in each crop. The obtained  $K_y$  indicate that the most sensitive stages to water stress corresponded to the yield formation (bulbing for onion, fruit setting and bearing in sweet Italian pepper, and fruit growth in watermelon), as stated by Steduto et al. (2012).

Results show that the product quality traits were not affected by neither CDI nor RDI in cauliflower and watermelon, whereas, severe CDI has increased soluble solid content of the onion bulbs, while severe water deficit, both in CDI or RDI at harvesting has led to the highest soluble solid and total phenolic contents in sweet Italian pepper fruits. Rouphael et al. (2012) and Ripoll et al. (2014) noted that, genotypes, climatic conditions, abiotic stresses and agronomic practices are factors that play an important role in determining quality and phytochemicals in vegetable crops.

Among the analyzed crops, sweet Italian pepper led to the greatest gross revenue and water economic value (71258 € ha<sup>-1</sup>and 12.43 € m<sup>-3</sup>, for fully irrigated plants), while that obtained with onion was the lowest one (14719 € ha<sup>-1</sup> and 5.22 € m<sup>-3</sup>). Deficit irrigation can improve the water economic value up to 54% in autumn-winter crops, however, it can reduce the water economic value in spring-summer crops, particularly in watermelon (down to 46%).

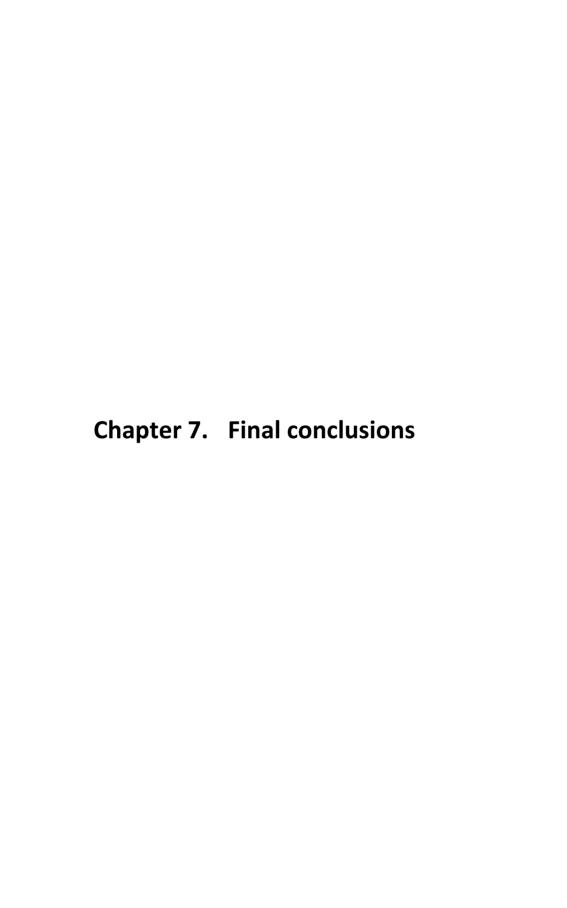
There are some research lines that has not been studied in this PhD thesis, because of time constraints or budget limitations, but it would be interesting to address, as:

- To analyze the grafting effect of high yield genotypes and drought tolerant rootstocks, which could reduce yield losses under severe water stress conditions.
- To evaluate different mulching types in order to reduce the irrigation water requirements.
- To analyze the plant response to external antioxidant application, which may reduce the negative effects of water stress.
- To carry out additional analyses of the product quality traits, evaluating the predominant compounds for each crop.
- To evaluate the root growth and distribution under deficit irrigation.
- To determine further plant water status parameters, such as the leaf water potential, the osmotic potential of leaf sap, and the stomatal conductance, which would help to understand in depth the soil-plant-water relationships.

# 6.2. References

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This PhD thesis evaluates the effects of continued and regulated deficit irrigation on four of the major vegetables grown in open field in the Mediterranean area. As main findings, it can be concluded that:

- Given that seasonal evapotranspiration (ETo) was twice higher for spring-summer crops (sweet Italian pepper and watermelon) than for autumn-winter crops (cauliflower and onion), the irrigation water requirements were higher for the first ones.
- The yield obtained by the full-irrigated plants was satisfactory, which indicates
  that the criterion of starting each irrigation event when the VSWC dropped to 80%
  of the field capacity was an adequate irrigation management.
- The crop response to deficit irrigation depends on the crop cycle, so that, less negative effects of deficit irrigation, particularly for marketable yield, are observed for autumn-winter crops, particularly in cauliflower, in relation to spring-summer crops.
- In cauliflower, continued deficit irrigation applying 75% of the irrigation water requirements, or reducing the irrigation to 50% of the irrigation water requirements during juvenility result in similar curd yield, with important water savings, in relation to full-irrigated plants, improving the irrigation water use efficiency, then these strategies could be recommended.
- In onion, under severe water shortage conditions, it would be advisable to apply continued deficit irrigation with 75% of the irrigation water requirements or reducing to 50% of the requirements during the bulb ripening stage, because they lead to slight decreases in yield and improve the irrigation water use efficiency. In case of moderate water shortage, regulated deficit irrigation reducing to 75% of the irrigation water requirements during the bulb ripening stage, results in a satisfactory bulb yield, increasing the irrigation water use efficiency, although with lower water savings; hence, it could be a recommended strategy for onion production.

- In sweet Italian pepper, applying 75% of the water requirement during harvesting results in a considerable reduction in yield and gross revenue, although, it provided important water savings, and yielded acceptable levels of fruit soluble solids and phenolic compounds. Ending the crop cycle at the beginning of September, when most of the marketable yield has already been harvested, would lead to important water savings. Combining these irrigation and management strategies could be recommended. Exposure of sweet Italian pepper to continued deficit irrigation with 75% or 50% of the irrigation water requirement, or to 50% of the water requirement at harvesting, leads to a high incidence of fruits affected by blossom end rot, which in turn increases the non-marketable yield.
- In watermelon, in case of water scarcity, applying water shortage during fruit ripening stage both at 75% and 50% of the water requirements, leads to an acceptable marketable yield and could be recommended.
- In all crops, continued deficit irrigation at 50% of the irrigation water requirement
  results in a notable reduction of marketable yield, and consequently of gross
  revenue, and also in a poorer plant water status, although in the autumn-winter
  crops it improves the irrigation water use efficiency, not being a recommended
  strategy.
- Cauliflower curd and watermelon fruit quality parameters are not affected by the
  deficit irrigation (neither continued nor regulated). However, deficit irrigation
  improved some quality traits in the other two crops, highlighting the increment of
  the soluble solid and total phenolic contents of sweet Italian pepper.
- Deficit irrigation can improve the water economic value up to 54% in autumnwinter crops, but this value can be negatively affected by the deficit irrigation in spring-summer crops, particularly in watermelon.