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Valcárcel-Germes, M.; Lahoz, I.; Campillo, C.; Martí-Renau, R.; Leiva-Brondo, M.; Rosello, S.; Cebolla Cornejo, J. (2020). Controlled deficit irrigation as a water-saving strategy for processing tomato. Scientia horticulturae (Online). 261:1-8. https://doi.org/10.1016/j.scienta.2019.108972



The final publication is available at https://doi.org/10.1016/j.scienta.2019.108972

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Additional Information

1 CONTROLLED DEFICIT IRRIGATION AS A WATER-SAVING STRATEGY FOR 2 PROCESSING TOMATO

3

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18 Abstract

19 In a complex scenario characterized by climate change and increasing population demand, it is necessary to adopt production strategies involving a lower use of water. For this purpose, 20 21 controlled deficit irrigation, CDI, strategies with an initial full irrigation covering 100% crop 22 evapotranspiration (ET_c) followed by mild (50%ET_c) and moderate (75%ET_c) irrigation 23 restrictions after the fruit set stage were evaluated. The study was performed during two years in 24 two growing areas of processing tomato in Spain, using conventional and high-lycopene cultivars. 25 With moderate levels of CDI an average saving of water of 26.1% was achieved, but productivity was reduced on average by 10.7%. It should be considered though, that the effect was dependent 26 27 on the environment and genotype, and high-yield cultivars seemed to be more sensitive to the 28 negative effects of moderate CDI on productivity. In contrast, mild CDI levels did not affect 29 productivity and still entailed a water saving of 13%. Moderate CDI levels increased soluble 30 solids content, but this increase was not due to a higher accumulation of taste-related compounds, 31 such as sugars and acids. Indeed, irrigation dose had no significant effect on the levels of fructose, 32 glucose, citric and glutamic acid nor on derived variables related to tomato acceptability and CDI 33 decreased the levels of malic acid. Considering previous results, mild CDI strategies would be 34 preferred to continuous deficit irrigation strategies as the former contribute to reduce the use of 35 water, an increasingly scarce resource, avoiding negative impacts on productivity, a key 36 parameter to promote farmer adoption of CDI strategies.

37

38 Keywords: Solanum lycopersicum L.; commercial yield, sugar, acid, lycopene, irrigation

40 Highlights

- 41 Mild controlled deficit irrigation, CDI, (100-75%ET_c) does not affect yield
- 42 High yield cultivars are more sensitive to deficit irrigation strategies
- 43 Increased soluble solids with 100-50%ET_c CDI levels are not due to sugar accumulation
- 44 CDI entails a water saving of 13% with 100-75% ET_c and 26% with 100-50% ET_c

46 **1 Introduction**

Tomato (*Solanum lycopersicum* L.) is one of the most important horticultural crops worldwide,
with a global production that exceeded 177 million tons in 2016 (http://www.fao.org/faostat). Its
consumption is present in countries all around the world, with high levels of intake that exceed
35.8 kg per person per year in North America and 34.8 kg per person per year in southern Europe

51 (http://www.fao.org/faostat).

52 As an irrigated crop, tomato performance depends on the availability of water to obtain high 53 vields. Therefore, tomato production is jeopardized in warm areas already affected by water 54 shortages, such as the Mediterranean basin. Models predicting the effects of climate change 55 anticipate a 10-30% decrease in runoff in Southern Europe by 2050 with a clear drying of the area 56 (Milly et al. 2005). In this context, the main consequences expected in water resources for 57 agricultural production include i.a. an increased evapotranspiration in response to increased 58 temperatures and water shortages, particularly during spring and summer, and raised water 59 requirements for irrigation (Iglesias and Garrote 2015). But climate change is not the only factor 60 affecting water availability, as rising water demands due to population growth and industry 61 outweigh greenhouse warming in defining the state of global water systems in 2025 (Vörösmarty 62 et al. 2000).

In this scenario, it is necessary to develop alternatives to reduce water usage in tomato production anticipating the negative effects of climate change. Several alternatives have been proposed, including the removal of irrigation following plant establishment. However, an early water shortage has a marked impact on marketable yield, due to fruit losses higher than 40% (Patanè et al. 2011).

Another approximation is to apply deficit irrigation (DI) which consists of applying lower water 68 69 volumes than the crop requirements. In this case, the use of water is also considerably reduced, 70 while the effects on yield are less dramatic. For example, a moderate deficit irrigation strategy 71 covering 75% of crop evapotranspiration (ET_c) under Spanish conditions entailed a mean 72 reduction in water use of 28.2% and a moderate impact on yield, with a mean decrease of 16.4%, 73 with a soluble solids increase of 8.4% (Lahoz et al. 2016). Under certain conditions and varieties, 74 the production of aroma volatiles related to flavor also increased. Despite the possible benefits in 75 quality, the truth is that the drop in commercial yield, being moderate, is still too high to represent 76 an alternative for farmers. Under other environmental conditions, the effect of DI on yield may be milder. For example, in Italian conditions, a higher level of DI with 50% ET_c represented a 77 78 water saving of 46.2% and improved soluble solids content, titratable acidity, and vitamin C 79 content, while it did not significantly affect the marketable yield (Patanè et al. 2011).

80 Partial root-zone drying (PRD) can also lead to a substantial improvement in water use efficiency, 81 but it seems that similar results can be achieved by closely monitored deficit irrigation, without the complexity and additional cost of PRD (Sadras 2009). Controlled deficit irrigation (CDI) 82 83 represents an interesting alternative for areas where DI evidenced a negative effect on yield, as it 84 seeks to optimize the use of water stress only in non-critical periods of the development of the 85 plant. Tomato requires a constant and sufficient water supply during the vegetative growth stage (Nuruddin et al. 2003; Patanè and Cosentino 2010), but also during the reproductive stage 86 87 involving flowering and fruit set (Waister and Hudson 1970). For that reason, CDI (eg. 100-x% 88 ET_c) maintains a full supply of water to cover 100% ET_c and irrigation is only reduced after fruit set, covering a variable proportion of crop evapotranspiration (x%). 89

90 CDI represents another advantage. Recent studies showed that CDI would also have a positive 91 effect on the functional quality of tomato, as it favored the accumulation of L-ascorbic acid 92 (vitamin C) and certain phenolic compounds such as chlorogenic and ferulic acids, and rutin while it had a limit effect on carotenoids (Martí et al. 2018). Nevertheless, the development of products 93 94 with the highest functional value depended on the right combination of climatic conditions, mild 95 levels of CDI and certain genotypes (especially high-lycopene cultivars). Increasing the levels of bioactive compounds in tomato would entail an added value, satisfying the demand of quality 96 97 markets. Indeed, the functional value of tomato has gained importance, as this species represents 98 a main dietary source of bioactive compounds including carotenoids, vitamin C and polyphenols 99 (Chun et al. 2005; Garcia-Closas et al. 2004). These compounds have been related not only with the prevention of certain types of cancer (Martí et al. 2016; Rao and Rao 2007) but also 100 101 cardiovascular diseases and Alzheimer (Martí et al. 2019) and consumers are increasingly aware 102 of the role of food in the prevention of diseases (Siró et al. 2008).

103 In Italian conditions, as for Patanè et al. (2011), a CDI of 100-50% ET_c offered a similar 104 performance compared to DI covering 50% ET_c, with no significant impact on marketable yield and an increase in TSS. It would be interesting to know if in other environmental conditions CDI 105 106 can also reduce the impact on yield. A more in depth study on the impact on the accumulation of 107 sugars and acids would also complete the analysis of the impact of DI strategies on quality. This 108 is, in fact, the objective of the present work, to analyze the effect of mild (100-75% ET_c) and moderate (100-50% ET_c) CDI on the agronomic performance and the accumulation of taste-109 110 related compounds in processing tomato of conventional high yield cultivars and high lycopene 111 varieties targeted to quality markets. In order to obtain reliable results, the study was performed 112 during two years, in two of the main processing tomato growing areas of Spain: Extremadura and 113 Navarra. The results obtained would enable comparison with previous work dealing with DI, and identify effective strategies to reduce the use of an increasingly scarce input such as water while 114 115 offering the maximum functional and organoleptic quality to the consumer.

116

117 2 Materials and methods

118 2.1 Experimental design and plant material

119 The experiment was conducted during two consecutive years and in two Spanish growing areas (Extremadura and Navarra) to evaluate CDI on four processing tomato cultivars: two cultivars 120 121 with good agronomical performance 'H-9036' and 'H-9661' (Heinz Seeds) widely used by local farmers, and two mid to high lycopene cultivars 'H-9997' (Heinz Seeds) and 'ISI-24424' 122 (Diamond seeds S.L.; Isi Sementi S.P.A.). Three irrigation regimes were evaluated. A control 123 treatment satisfying the crop evapotranspiration (ET_c) of the plant during the entire growth cycle 124 125 (100-100% ET_c), and two CDI strategies. Both of them comprised irrigation covering 100% ET_c (phase 1) until fruit set and the start of the growing phase. Then a mild (25%) or moderate (50%) 126 127 reduction of irrigation (phase 2) was applied in order to cover a 75% and 50% of ET_c respectively (Table 1). For each treatment, three field sections of 100 plants each for cultivation (4 cv. x 25 128 129 plants) were employed. The statistical design was a split plot with three replications where the 130 main factor was the irrigation rate (100-50%, 100-75% and 100-100% ET_c) and the second factor 131 was cultivar.

		Nava	arra	Extremadura								
	2012			2013			2012			2013		
Irrigation (%ETc)	100-100	100-75	100-50	100-100	100-75	100-50	100-100	100-75	100-50	100-100	100-75	100-50
Planting	50.6	50.7	51.8	45.8	45.2	44.9	5.3	5.3	5.3	4.2	4.4	5.1
Planting to fruit set	180.0	178.2	175.6	168.3	169.9	168.9	201.1	202.2	200.8	468.8	468.6	467.9
Fruit set to harvest	266.6	196.1	123.9	261.3	194.7	129.7	457.2	355.7	256.6	255.0	192.2	129.5
Total	497.2	425.0	351.3	475.3	409.8	343.5	663.6	563.2	462.7	728.0	665.2	602.5
Water savings		14.5%	29.3%		13.8%	27.7%		15.1%	30.3%		8.6%	17.2%

Table 1. Irrigation doses applied to the plants (L m⁻²) at different development stages.

137 2.2 Growing conditions

138 The trials were conducted in the two Spanish main production areas of processing tomato:

139 Extremadura (research center Finca 'La Orden-Valdesquera', Badajoz, lat. 38⁰ 53' 26'' N, long.

140 6⁰ 40' 00'' W, 186 m. a. s. l.) and Navarra (INTIA Experimental Farm, Cadreita, lat. 42⁰ 12' 34''

141 N, long. 1⁰ 43' 1'' W, 267 m. a. s. l.). Integrated pest management with the typical fertilization

doses and phytosanitary treatments of each cultivation site was applied.

143 Planting was carried out when adequate climatic conditions for a spring-summer cycle were

present (Fig. 1). For Extremadura, it was performed on April 24th in 2012 and May 2nd in 2013.

145 In Navarra, planting was performed on May 10th in 2012 and May 23rd in 2013. A spacing of 1.50

146 m x 0.2 m (3.33 plants m⁻²) was used in Extremadura and a spacing of 1.60 m x 0.35 m (3.57

147 plants m^{-2}) in Navarra. Plants were grown in the open air under 15 μ m black polyethylene plastic.

148 2.3 Irrigation scheduling

The scheduling of the irrigation was carried out considering the water requirements of the plant. 149 The Penman-Monteith (Allen et al. 1998) method was used to calculate the ET_c. Evaporative 150 151 coefficient was not considered because the soil was cover with plastic. A flow meter was installed 152 in the field sections destined for each of the different treatments to measure the real volume of 153 water applied (Table 1). Weekly monitoring of the phenology of each variety, starting with the first fruit set, was made in order to establish the end of the period of fruit set. Irrigation should 154 155 ensure the survival of plants. In both growing areas, maximum temperature and relative humidity 156 were registered using an HMP45C probe (Vaisala, Helsinki, Finland). In Extremadura, solar 157 irradiance was recorded using a CMP3 pyranometer (Kipp&Zonen, Delft, the Netherlands). A 110/S pyranometer (Skye, Powys, United Kingdom) was used for this purpose in Navarra. A 158 detailed description of the climatic conditions was reported by Martí et al. (2018). 159

160 *2.4 Sampling*

A single collection of fruits was conducted for each cv. and irrigation treatment at the optimum
moment for harvest following commercial practices in the area (i.e. a minimum of 85% fruits in
the red-ripe stage and no more than 5% of over-ripened fruits). Harvest was performed by hand.
The specific dates of harvest were August 21st in 2012 and August 20th in 2013 for Extremadura,
and August 29th in 2012 and September 26th in 2013 for Navarra.

166 2.5 Agronomic and basic quality indicators

167 Commercial yield, total yield and mean fruit weight were determined for each of the 25 plants of the replicate. Two commercial red ripe fruits of each of the 25 plants were blended to obtain a 168 169 homogenized sample to determine TSS, pH, dry matter and color. TSS were evaluated using a digital refractometer (ATAGO PR-1, Tokyo, Japan), with a precision of 0.1^oBrix (results 170 expressed as ⁰Brix at 20⁰C). The Hunter 'a' and 'b' color parameters were obtained using a digital 171 172 colorimeter (CR 300, Minolta, Japan) and the Hunter a/b ratio was calculated. The soluble solids 173 yield (t ha⁻¹) was estimated from the product of soluble solid content by commercial yield in 174 accordance with Patanè et al. (2011). Aliquots of the sample were frozen at -80°C until further 175 analysis.

177 2.6 Chemicals and reagents

Analytical grade standards of malic, citric and glutamic acids, sucrose, fructose, and glucose were
 purchased from Sigma-Aldrich (Steinheim, Germany). 2,6-pyridine dicarboxylic acid (PDC)

- 180 hexadimethrine bromide (HDM) and sodium dodecyl sulfate (SDS) were also supplied by Sigma-
- 181 Aldrich. Sodium hydroxide (NaOH) was purchased from Panreac (Castellar del Vallés, Spain). A
- 182 Milli-Q water system (Millipore, Molsheim, France) was used to obtain ultrapure water.

183 2.7 Analysis of organic acids and sugars

184 Defrosted samples were centrifuged at 12,000 rpm (10,483g) for 5 min at 4° C to separate the 185 upper phase, which was diluted 1:20 with ultrapure water. The resulting solution was filtered 186 using a 0.22 µm-Nylon centrifuge tube filter (Costar Spin-X, Corning, NY, USA) before its 187 analysis by capillary electrophoresis (CE).

Ouantification of organic acids and sugars was performed following the procedure described by 188 189 Cebolla-Cornejo et al. (2012) using a 7100 CE system (Agilent Technologies, Waldbronn, Germany) with diode array detection and temperature control of sample compartment. Prior to its 190 191 first utilization, uncoated fused silica capillaries (67 cm total length, 60 cm effective length, 375 192 µm od, 50 µm id) from Polymicro Technologies (Phoenix, AZ, USA) were rinsed with NaOH 1M at 50°C (5 min), NaOH 0.1M (5 min), water (10 min) and running buffer at 25°C (30 min). 193 194 Between runs, the capillary was flushed with SDS 58 mM (2 min) and running buffer (5 min). 195 Running buffer consisted of a solution 20 mM 2,6-pyridinedicarboxylic acid (PDC) with 0.1% 196 (w:v) hexadimethrine bromide (HDM) adjusted to pH 12.1. A hydrodynamic injection at 3400 Pa for 10 s was used. Separation was performed using a voltage of -25 kV at 20° C. Indirect detection 197 198 of target compounds was recorded at 214 nm.

199 2.8 Statistical analysis

200 All statistical analyses were performed using the SPSS 22.0 software (NYSE: IBM, Armonk, NY, 201 USA). The Pillai trace test was employed to calculate the *p*-value in MANOVA analysis. 202 Individual ANOVAs and Tukey B multiple range tests (p < 0.05) were calculated for the 203 individual variables. Graphical MANOVA Biplot representations were performed using the 204 freeware licensed by Prof. Vicente-Villardon (2015). The confidence intervals ($\alpha = 0.05$) were 205 represented by the projection of Bonferroni circles on each variable for the identification of 206 differences statistically significant between groups. In the MANOVA Biplot, dashed lines 207 represent non-significant effects.

208 3 Results and discussion

209 3.1. Environmental conditions

210 Extremadura, with a lower latitude, usually records higher temperature and radiation than 211 Navarra. This general trend was observed during both years. In 2012, though, the differences 212 between both locations were limited, but still, Extremadura had higher maximum temperatures and accumulated radiation (Fig. 1), while in 2013 the accumulated radiation was higher in 213 214 Navarra. In this latter year, the climatic conditions were not optimal for tomato production. In 215 Extremadura, a considerably lower accumulated radiation was registered compared to 2012, while 216 the differences in maximum temperature were not so pronounced. In Navarra during this second 217 year, the bad weather during May forced to delay transplant. Lower maximum temperatures and 218 accumulated radiation during the whole cycle resulted in a delayed growth of plants and lower 219 water requirements. As a result, the growth cycle was extended (Fig. 1). Bad weather was also

registered at the end of the cycle, accordingly, the gradient of accumulated radiation decreased

and maximum temperatures dropped. These conditions favored an outbreak of *Alternaria solani* that affected especially the cultivar 'ISI-24424'.

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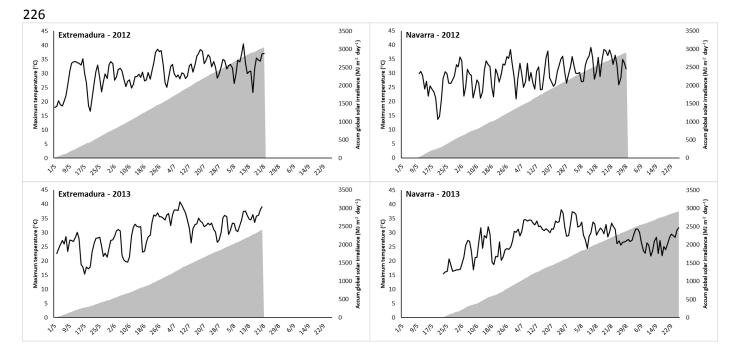


Fig. 1. Maximum temperatures (black continuous line) and accumulated global solar irradiance (grey area) for Extremadura and Navarra during the growth cycle (day/month) in 2012 and 2013.

230 *3.2. Agronomic performance and basic quality indicators*

231 Water is one of the key factors affecting tomato fruit growth and production (Wang et al. 2011). 232 Usually, an increase in the water supply results in increased yield but reduced fruit quality, due 233 to the higher water content of the fruit (Dorais et al. 2001). This is true up to certain limits, as increasing irrigation doses over the recommended 100% ET_c would have no significant effect on 234 235 the agronomical performance, while it would bring about a dilution effect reducing soluble solids 236 and lycopene content (Lahoz et al. 2016). Therefore, a smart irrigation schedule and strategy 237 obtains a good equilibrium between yield and quality, being more beneficial for farmers and more 238 sustainable for the environment.

239 However, water is an increasingly scarce resource. In a complex scenario characterized by climate 240 change and increasing populations, the optimistic future depends on whether agriculture is able 241 to manage and consume water in a sustainable way (Iglesias and Garrote 2015). In this context, 242 the development of technological advances and their transference to farmers via educational programs will be crucial. Among existing alternatives, deficit irrigation strategies acquire an 243 244 important role, as they limit the use of water providing an adaptation to scenarios with lower 245 water availability. However, the success of these strategies relies on their adoption by farmers. 246 Currently, strategies that significantly affect yield, even at low rates, have a negative prognostic 247 in terms of farmer adoption.

- 248 In our work, the year, site of cultivation, irrigation dose, and genotype significantly affected the 249 performance of tomato crop, as revealed by the MANOVA analysis (Pillai trace, p < 0.01). The 250 uncontrolled environment (year and site of cultivation) had a marked effect on the agronomical behavior of the crop. Individual ANOVAs revealed that climatic differences for the two 251 252 cultivation years significantly affected commercial yield, the percentage of commercial fruits, 253 soluble solids yield, pH, dry matter, and color (Table 2). In general, higher values corresponded 254 to the year 2012, except for the pH which was higher in 2013. In addition, the site of cultivation 255 had a significant effect on all agronomical and basic quality parameters except for dry matter and color (Table 2). The conditions of Navarra were more favorable for all significant variables with 256 257 the only exception of soluble solids, as a higher value for the latter was obtained in Extremadura.
- The irrigation dose had a lesser effect on the agronomical performance compared to the uncontrolled environmental conditions (Table 2). Nevertheless, there was a significant reduction in commercial yield (10.9%) and average fruit weight (9.2%) when CDI 100-50%ET_c was applied, compared to the control dose (100-100%ET_c). A milder CDI level (100-75% ET_c) had no significant impact on commercial yield. The percentage of commercial fruits showed the opposite behavior, with a higher proportion of commercial fruits with a CDI of 100-50%ET_c compared with CDI 100-75%ET_c and the control 100-100%ET_c.

The most restrictive CDI level $(100-50\% \text{ET}_c)$ significantly increased the soluble solids content and dry matter %, while no significant differences were obtained between 100-75% ET_c and the control (Table 2). The pH followed the opposite trend, being slightly higher in the control (100-100% ET_c), while the no differences were observed between the two CDI regimes (Table 2). On the other hand, neither soluble solid yield nor the color of the fruit were affected by irrigation dose, but a significant genotype x irrigation dose interaction was observed for both parameters (Table 2).

		Commercial yield (t ha ⁻¹)	Commercial fruits (%)	Average fruit weight (g)	Soluble solids (°Brix)	Soluble solids yield (t ha ⁻¹)	pН	Dry matter (%)	Hunter colour a/b	Malic acid (g kg-1)	Citric acid (g kg-1)	Glutamic acid (g kg-1)	Fructose (g kg-1)	Glucose (g kg-1)
Year (Y)	p value	***	***	ns	ns	***	***	*	***	***	ns	**	***	***
	2012	157.88	89.07	80.23	4.61	7.25	4.16	4.88	2.50	0.80	3.87	1.55	11.80	11.86
	2013	118.18	86.25	79.60	4.52	5.29	4.31	4.79	2.05	1.13	3.80	1.37	14.76	13.70
Site of cultivation (S)	p value	***	***	***	*	***	***	ns	ns	***	***	***	ns	ns
	Extremadura	123.07	85.60	77.29	4.62	5.65	4.14	4.84	2.26	1.02	3.44	1.10	13.16	13.04
	Navarra	153.00	89.72	82.54	4.51	6.90	4.33	4.83	2.28	0.92	4.23	1.81	13.40	12.51
Irrigation	p value	***	***	***	***	ns	**	***	ns	*	ns	ns	ns	ns
	100-	145.41b	86.35a	83.49c	4.41a	6.37	4.25b	4.77a	2.28	1.01b	3.74	1.41	13.29	12.53
dose ¹	100%ET _c													
(I)	100-75%ETc	139.14b	86.31a	80.40b	4.47a	6.23	4.23a	4.79a	2.28	0.93a	3.78	1.49	13.22	12.54
	100-50%ETc	129.54a	90.31b	75.85a	4.80b	6.22	4.22a	4.95b	2.26	0.96ab	3.98	1.47	13.32	12.27
Genotype (G)	p value	***	***	***	ns	***	***	***	***	***	***	**	**	**
	'H-9661'	134.83b	89.62b	74.98a	4.51	6.06b	4.22ab	4.84b	2.20b	0.92a	4.11bc	1.57b	12.27a	12.22a
	'H-9997'	134.30b	89.48b	73.52a	4.61	6.20b	4.24b	4.97c	2.35c	0.96a	4.22c	1.31a	13.00ab	12.53a
	'H-9036'	166.40c	88.44b	73.42a	4.57	7.55c	4.21a	4.83b	2.14a	0.89a	3.78b	1.54b	14.22b	13.87b
	'ISI-24424'	116.59a	83.10a	97.72b	4.56	5.27a	4.28c	4.70a	2.40c	1.11b	3.23a	1.40ab	13.62b	12.49a
YxS	p value	*	ns	***	***	ns	***	**	***	ns	ns	**	**	**
YxG	p value	ns	***	***	ns	**	***	*	***	*	ns	**	ns	ns
Yxl	p value	ns	ns	**	*	ns	ns	***	*	ns	ns	ns	ns	ns
SxG	p value	**	ns	**	**	***	***	**	***	ns	ns	ns	*	ns
SxI	p value	ns	ns	**	ns	ns	***	*	ns	ns	ns	ns	ns	ns
Gxl	p value	**	ns	ns	*	*	ns	***	***	ns	ns	ns	ns	ns

Table 2. Effect of the year, site of cultivation, irrigation dose and genotype on agronomic performance and quality parameters. The statistical relevance is273provided (ns, not significant; * p < 0.05; ** p < 0.01; *** p < 0.001). ¹Different letters indicate significant differences at p < 0.05 (Tukey B test).

276 CDI treatments led to an important decrease in the amount of water used for irrigation during 277 phase 2, i.e. following the first fruit set (Table 1). In Navarra water savings for the whole cycle were similar both years. With the 100-75% ET_c regime, 14.5% and 13.8% of water were saved in 278 279 2012 and 2013 respectively. With the 100-50% ET_c regime, water savings doubled, reaching 280 levels of 29.3% and 27.7% respectively. On the other hand, in Extremadura water savings were 281 much lower in 2013 due to the poor climatic conditions. In 2012 savings reached 15.1% and 282 30.3% for the 100-75% and 100-50% regimes respectively, while in 2013 these values almost 283 halved (8.6% and 17.2% respectively).

284 The genotype had a significant effect on all the agronomic and basic quality parameters, with the exception of soluble solids content (Table 2). The highest commercial yield corresponded to the 285 286 cultivar 'H-9036'. Its high productivity (23.4% higher than the next cultivar) lead to the highest 287 soluble solids yield. But, this cultivar showed the lowest Hunter a/b ratio and thus, a less intense red pigmentation. The lowest commercial yield, the percentage of commercial fruits, and soluble 288 289 solids yield were found in the high lycopene cultivar 'ISI-24424'. However, 'ISI-24424' was 290 characterized by the highest average weight per fruit and water content. Both high lycopene cultivars 'ISI-24424' and 'H-9997' registered the highest Hunter a/b ratio related to an intense 291 292 red pigmentation. 'H-9997' also had the highest percentage of dry matter. The differences in pH 293 among cultivars, being significant, were limited. 'ISI-24424' reached the highest pH values and 294 'H-9036' the lowest.

295 Most of the studied traits showed important environmental interactions (Table 2). Therefore, a 296 detailed analysis was performed for each location and year using MANOVA biplots. In 297 Extremadura, the effects of CDI were much limited than in Navarra, as fewer variables were 298 significantly affected by DI (solid lines) and confidence circles were larger in the MANOVA 299 biplot (Fig. 2). Indeed, in Extremadura, in 2012, the differences between the control and 100-300 75%ET_c were limited for most parameters while 100-50%ET_c clearly increased soluble solids 301 content (Fig. 2). On the other hand, in 2013 the differences between CDI strategies and the control 302 were negligible. In fact, as stated above, the differences in water savings were limited. For 303 Navarra, differences were already found in the agronomical performance between the control and 304 the milder CDI treatment (100-75% ET_c). Nevertheless, in 2013, with the worst climatic 305 conditions, the differences between CDI 100-75% ET_c and the control were much lower. In both 306 years, CDI 100-50% had clear negative effects on agronomical performance.

307 In order to provide an idea of the genotypic interactions with irrigation treatments and years, 308 relative commercial yields were calculated comparing the values obtained with CDI treatments 309 and the control (Fig. 3). This analysis revealed that the effect of CDI on yield was highly 310 dependent on the genotype and environmental conditions. In fact, in Extremadura, CDI treatments 311 only had a significant effect on yield for cultivar "H-9036", while in Navarra the effects of CDI 312 were detected at some point for most of the cultivars. In general, the conventional cultivar 'H-313 9036', with maximum productivity (Table 2), was highly sensitive to CDI treatments. It was also 314 the cultivar that experienced a higher reduction in yield when a high $(100-50\% \text{ET}_{c})$ and mild 315 (100-75% ET_c) CDI treatments were compared. In contrast, the high lycopene cultivar 'ISI-316 24424', characterized by its low productivity, registered no impact of CDI on yield, even with the 317 most restrictive dose in most environmental conditions. Considering the loss of production for 318 each year and cultivar, at the higher CDI level (Fig. 3) a mean reduction of 10.3% was obtained 319 in Navarra, and 7.3% in Extremadura.

Previous studies regarding DI during two years in Navarra showed that continuous restricted
 irrigation from transplant to harvest using 75% ET_c requirements, despite saving 28.2% of water,

led to a general loss of 16.4% of commercial production. Nevertheless, significant interactions
were detected and the impact depended on the cultivar and the specific environmental conditions
of each year (Lahoz et al. 2016). The mean loss of productivity may seem low, but it is still too
important for DI to be considered as a production alternative for farmers.



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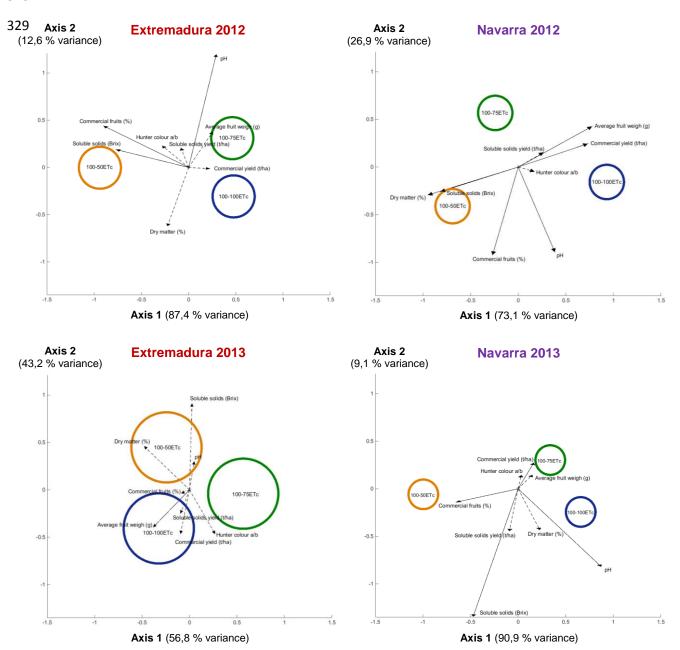


Fig. 2 MANOVA biplot representing the impact of mild 100-75% ET_{c} and moderate 100-50% ET_{c} controlled deficit irrigation applied after first fruit set, CDI, strategies over the control (100-100% ET_{c}) on agronomic performance and basic quality parameters. Discontinuous lines indicate a non-significant effect (ANOVA, p=0.05). Circles represent univariate confidence limits.

🗆 CDI 100-75% ETc 🛛 🛛 CDI 100-50% ETc

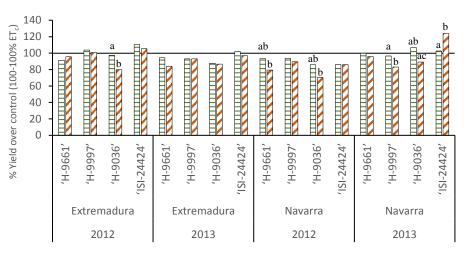


Fig. 3 Relative commercial yield of CDI treatments compared to the control receiving 100% ET_{c} (horizontal thick line) during the whole cycle. For each cultivar, no letters: non significant effect of water dose (ANOVA, p=0.05); a: no significant differences with 100% ET_{c} control; b or c: significant differences with other treatments (Tukey, p=0.05).

In the present work, using similar materials and areas of production, CDI, with water restrictions applied only after fruit set, has proved to be a better strategy to reduce water usage in tomato production and minimize the impact on yield. Mild CDI levels, 100-75% ET_c resulted in a mean reduction of water usage of 13.0%, and in the specific conditions of Navarra a mean reduction of 14.2%. At the same time, this water saving was obtained with no significant impact on commercial yield. A higher CDI level would double water savings (mean 26.1% reduction in water use), but it would imply a mean reduction in yield of 10.9%. Interestingly, the effect of CDI was strongly dependent on the cultivar considered. Highly productive materials seem to be more sensitive to water shortages than cultivars with lower production levels and consequently, CDI strategies are more suitable for quality-based markets, rather than quantity-based markets.

It is true that DI and CDI were not contrasted in the same environmental conditions, but both studies included four cultivars and two years of cultivation, thus the results are quite robust. That would mean that a CDI strategy with a higher water restriction level (100-50% ET_c) following fruit set would probably lead to approximately the same water saving than applying a milder water reduction during the whole cycle (75% ET_c) and the effects on productivity would be more limited. Anyway, a CDI level of 100-50% ET_c would still be too harsh for farmer adoption, at least in Spanish conditions, and a milder reduction would represent the best deficit irrigation strategy to contribute to reduce water use and still represent a real alternative for farmers.

In other conditions, the impact of DI and CDI strategies seem to be lower. For example, in Italian
conditions, Favati et al. (2009) found that continuous DI with moderate restrictions (50% ET_c)
led to a clear decrease of production (37.6% and 46.2% in a two year study), while a CDI strategy
(100-50% ET_c) had no significant effect one year and slightly reduced yield, a 7% during the

379 other year. Also in Italy, Patanè et al. (2011) found an even lower impact of DI strategies, as

continuous restriction down to 50% ET_c did not significantly affect yield. Similarly, under Indian conditions, a continuous DI of 80% ET did not have a negative impact on commercial yield, but further restrictions (60% ET) did have a negative effect, reducing yield by 24.8% (Nangare et al. 2016). In the same conditions, a CDI of 60% ET during the fruiting stage had a limited impact in yield (5.4%).

385 In their review on DI strategies, Geerts and Raes (2009) concluded that DI was successful in 386 increasing water productivity for various crops without causing severe yield reductions if a certain minimum amount of seasonal moisture is guaranteed. Nevertheless, the authors stated that DI 387 388 strategies are targeted to maximize water productivity and to stabilize, rather than maximize, 389 yield. This conclusion is strongly supported by the present and previous works. Effectively, DI 390 strategies based on mild restrictions optimize the use of water with a slight impact on yield and 391 could be an option for certain areas. On the other hand, mild CDI strategies entail a lower 392 reduction in water use but at the same time have no impact on yield, being a better option for 393 quantity based agricultural systems.

394

395 *3.3. Effect on sugar and acid accumulation*

396 Apart from reducing the use of water, DI has been proposed as an alternative to offer high quality 397 products. An approach that has also been pursued in other studies promotes the use of saline water 398 (De Pascale et al. 2001). A moderate level of continuous DI (50%) proved to increase dry matter 399 and soluble solids in Italian conditions, an effect that could also be achieved applying water 400 restriction during the last phases of the cycle following a CDI approach (Favati et al. 2009). In 401 that study, the mean increase with CDI, 8.1%, was considerably lower than using a DI strategy, 402 13.5%, and highly dependent on environmental conditions. In Spanish conditions, a mild level of 403 DI also led to a modest mean increase in the soluble solids content of 8% (Lahoz et al. 2016). The 404 present study confirmed that moderate CDI levels (100-50% ET_c) increase dry matter (3.8%) and 405 soluble solids content (8%), thus reaching the same improvement as with mild (75%) DI.

406 Other studies have reported a larger effect. In this sense, Nangare et al. (2016) found that DI with 407 80% ET_c increased soluble solids content by 50%, though further restrictions (60% ET_c) had a 408 similar effect (60% increase). This effect could also be achieved with a CDI of 60% ET_c (56% 409 increase). Not always has continuous DI been related to these effects. For example, Pernice et al. 410 (2010) found that a reduction of 50% of water usage compared to normal irrigation had no effect 411 on dry matter and soluble solids content neither in processing nor fresh tomatoes. 412 Notwithstanding, other authors have found more marked differences. Ripoll et al. (2016) found 413 that a restriction of water usage (40%) specifically during the maturation stage would lead to an 414 increase of 17.6% of dry matter, an effect not achieved if the restriction was applied in earlier 415 stages. Nevertheless, in that case, soluble solids content was not affected.

416 In our work, MANOVA test confirmed that the site and year of cultivation, irrigation dose, and 417 genotype all had a significant effect on the accumulation of sugars and organic acids (Pillai trace, 418 p < 0.01).

Individual ANOVAs confirmed a significant effect of the year on the concentration of all
compounds with the only exception of citric acid (Table 2). The year 2012 was more favorable
for the accumulation of glutamic acid, while the year 2013 showed higher contents of sugars,

422 malic acid.

423 The site of cultivation had a significant effect on the contents of organic acids (Table 2). The 424 higher contents in citric and glutamic acid found in Navarra, and the lack of effect of the site of 425 cultivation on sugar accumulation, would imply lower sugar to acid ratios in this location. The 426 conditions of Extremadura were more favorable for the accumulation of malic acid.

427 The effect of irrigation dose was limited since it was only significant for the accumulation of 428 malic acid, with higher contents in the control dose, 100-100%ET_c (Table 2). The highest effect 429 on sugar and acid accumulation, in fact, depended on the genotype. The conventional cultivar 'H-430 9036' showed the highest levels of sugars and glutamic acid. The high lycopene cultivar 'ISI-431 24424' was outstanding for sugar accumulation and showed the highest malic acid contents and 432 the lowest citric acid contents. The high lycopene cultivar 'H-9997' showed the highest citric acid 433 content. The conventional cultivar 'H-9661' had high glutamic acid content and low sugar 434 accumulation, which imply low sugar to acid ratios.

435 Significant year x site of cultivation, year x genotype, and site of cultivation x genotype
436 interactions were detected. There were no interactions with irrigation dose, which confirmed that
437 the low impact of CDI on sugar and acid accumulation was independent of the genotype or
438 environment considered (Table 2).

Altogether, these results show that even achieving a modest increase in soluble solids, this
increase would not be due to a higher accumulation of sugars and acids, as the effect of irrigation
dose on the accumulation of fructose, glucose and citric acid was not significant, and the contents
of malic acid were only slightly reduced. Therefore, CDI even at moderate levels, would not
promote the accumulation of taste-related compounds in processing tomato.

444 As reviewed by Ripoll et al. (2014) water deficit entails an increase in sugar content, especially 445 when deficit affects the last stage of fruit ripening. For organic acids, the effects of water deficit 446 are less clear as some studies correlate with higher contents and other with the opposite. In fact, 447 moderate water deficit has been suggested as a useful tool to improve fruit quality, mainly by 448 metabolic effects, especially for sugars and acids related to taste, as no positive impact is expected regarding carotenoid and ascorbic acid contents (Ripoll et al. 2016). In this latter study contents 449 450 of fructose and glucose tended to increase, while citric and malic acid tended to decrease, though 451 the response was higher in less tasty cultivars than in tasty ones. It was also suggested that 452 different cultivars would show a different rate at which they develop adaptive responses to water 453 deficit. In the present study, the sugar content was not significantly increased independently of the sugar accumulation capacity of the cultivars, as important differences were found for fructose 454 455 and glucose contents among cultivars in the control treatment. It seems then, that the CDI levels 456 applied here would not be as intense as to provoke changes in sugar and acid accumulation or that the cultivars included in the study would have a slow adaptive response. 457

458 With respect to other quality parameters, Zegbe-Domínguez et al. (2003) found that DI or partial 459 rootzone drying led to maintenance or increase of the redness of fruit. Favati et al. (2009) stated that the redness of the fruit only increased with moderate DI regimes (50% ET_c). Lahoz et al. 460 (2016) also found a small increase with milder DI treatments (75% ET_c), but the effect on 461 462 lycopene accumulation was not significant. In the present work, the color of tomatoes was not 463 affected by CDI, not even with the higher level. The evaluation of CDI on functional value already had confirmed that CDI would not have a significant effect on the accumulation of carotenoids 464 465 (Martí et al. 2018). However, they also confirmed a positive effect on the accumulation of the 466 hydroxycinnamic acids chlorogenic and ferulic acids, the flavonoid rutin and L-ascorbic acid. 467 That means, that despite not influencing the accumulation of taste-related compounds, CDI would 468 represent an added value increasing the levels of hydroxycinnamic acids and flavonoids related 469 with the prevention of cancer (Martí et al. 2016) and other degenerative diseases (Martí et al. 470 2019). Nonetheless, this positive effect on the functional value of tomato would be highly 471 dependent on the genotype and environmental conditions, with Navarra conditions and mild CDI 472 level (100-50% ET_c) being more favorable (Martí et al. 2018).

473

474 4 Conclusions

475 CDI strategies applying the same level of water restrictions after fruit set seem to have a lower 476 impact on yield than DI strategies based on a continuous water restriction. In the present work, 477 moderate CDI levels (100-50 ET_c) led to considerable water savings but had a significant negative 478 impact on yield. Nevertheless, this effect was highly dependent on the environment and cultivar 479 considered. In Navarra, with environmental conditions leading to maximum productivity, the 480 effects of moderate levels of CDI were noted, while in conditions such as those of Extremadura 481 the effect was be limited. In both environments, moderate levels of CDI had a higher impact on 482 high-yielding cultivars. This fact could represent a considerable handicap restraining farmer adoption of the strategy, as quantity still seems to be more valued than quality. On the other hand, 483 484 CDI would have a slight positive impact on soluble solids content. Nevertheless, irrigation dose 485 had no significant impact on the accumulation of fructose, glucose, citric and glutamic acids and 486 reduced the levels of malic acid. Accordingly, the positive impact on soluble solids would not necessarily result in better organoleptic quality. Milder CDI levels (100-75 ET_c) had no impact 487 488 on the accumulation of sugars and acids, nor on soluble solids, but more importantly, it had no 489 impact on commercial yield. At the same time, considering previous results mild levels of CDI 490 would increase the functional quality of the production increasing the levels of polyphenols and 491 L-ascorbic acid. Consequently, this would represent the ideal deficit irrigation strategy to combine 492 sustainability both from the environmental and the farmer point of view. Thus, it would be easy 493 to be assimilated by farmers, contributing to save an increasingly scarce resource, water, and to 494 contribute to enhanced health benefits of tomato intake on consumers.

495

496 Acknowledgments This research was funded by Instituto Nacional de Investigación y Tecnología
497 Agraria y Alimentaria with the project RTA2011-00062 and GRU 15112 and AGROS financed
498 by Gobierno de Extremadura. These projects were co-funded by the Fondo Europeo de Desarrollo
499 Regional (FEDER, EU).

500 **Conflicts of Interest** The authors declare that they have no conflict of interest.

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