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EFFECT OF DEFICIT IRRIGATION ON WATER STATUS, METABOLIC CHARACTERS, FRUITING, YIELD, AND QUALITY OF CLEMENTINE TREES UNDER TUNISIAN SEMI-ARID CONDITIONS

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Abstract

Water scarcity is one of the most serious threats facing humanity, and it is primordial to adopt water-saving strategies to improve irrigation efficiency in agriculture. The current study sought to determine the impact of a partial root-zone drying technique (PRD) used during fruit growth phases II and III (50% of crop water requirement) on water status, yield, and metabolic and technological characteristics of Citrus clementine MA3 during two consecutive seasons (2016-2017 and 2017-2018) under a Mediterranean semi-arid climate. The results of this research revealed that compared to control irrigation, the partial root drying irrigation system decreased the leaf water potential (Ψl) by 35.9% and 12.9%, the leaf water content by 5.2% and 3.2%, and the chlorophyll index by 8.3% and 4.3%, but increased leaf temperature by 2.3% and 3.2%, leaf sugar content by 6.5% and 7.1%, proline content by 8.4% and 18%, and membrane permeability by 6.4% and 7.5%, respectively, in fruit growth stages II and III. The reaction of clementine trees to the application of PRD appeared clearly in the second year by reducing the fruit size by 7.1% and 7.3%, respectively, in phases II and III of fruit growth. PRD did not affect fruit number or yield in the first year but reduced them by 18% and 16% in the second year, respectively. PH was affected by the partial root drying and showed an increase of 0.9% in the second year of the experiment. Fruit weight, TSS, total sugar content, titratable acidity, and maturity index were maintained under partial root drying. Although the non-significant effect of PRD on quality attributes increased slightly, the TSS by 1.7 %, the total sugar content by 1.8 %, the maturity index by 2.5 %, and titratable acidity by 0.6 %. Despite the decrease in chlorophyll index, some water status-related characteristics, and a slight decrease in fruit size and yield, PRD was considered an effective water-saving strategy with no discernible effect on fruit technological attributes.

Key words: Deficit irrigation, Chlorophyll index, Proline content, Leaf sugar content, Citrus, Fruit size, Yield, Technological attributes.

Introduction

Drought is one of the most common global environmental constraints limiting agricultural output. In Mediterranean areas like Tunisia, water resource scarcity and irregular and limited rainfall are the dominant limiting factors in the agricultural system, mainly in summertime. Citrus is a high-water-required fruit crop that is primarily grown in tropical and subtropical climates (Li *et al*., 2017). In Tunisia, the area planted with citrus fruit reaches around 25,000 ha, with an average production of 320,000 T (Anon., 2020).

Citrus are evergreen plants that require water all year round (Ginestar & Castel, 1996; Hutton & Loveys, 2011) to achieve growth and perennity. This species is regarded as one of the most water-stress-sensitive plants (Galindo *et al*., 2018). Citrus water's annual needs depend on growth stages: 10% during induction and floral initiation, 10% during fruit growth stage I (cell division), 40% during fruit growth stage II (cell enlargement), and 10% during fruit ripening (Falivene *et al*., 2006).

Full irrigation is required to avoid plant death and maintain growth ability (Ballester *et al*., 2011). Irrigation is a critical agronomic practice for citrus cultivation success (Singh *et al*., 2004). Water scarcity is a limiting factor for citrus development, particularly in semi-arid areas. To deal with limited water resources, a set of irrigation technologies and/or approaches were adapted as drip irrigation (Abu-Awwad, 2001; Panigrahi *et al*., 2012) and deficit irrigation (DI), which is used to increase water productivity while lowering applied doses.

In citrus orchards, three main DI strategies are used: sustained deficit irrigation (SDI), regulated deficit irrigation (RDI), and partial rootzone drying (PRD) (Dry *et al*., 1996; Panigrahi *et al*., 2014; Ben Messaouda *et al*., 2017; Galindo *et al*., 2018). DI strategies are applied to manage water and improve its use efficiency in a variety of Mediterranean orchards, including olive (*Olea europea* L.) (Romero *et al*., 2006; Ghrab *et al*., 2014; Abboud *et al*., 2021); apple (Ben Messaouda *et al*., 2017); and almond (*Prunus dulcis* Mill) (Egea *et al*., 2009).

RDI causes a decrease in vegetative growth (Ballester *et al*., 2014; Dry *et al*., 2000), but an improvement in fruit quality mainly under severe water-stressed conditions (Ballester *et al*., 2014). During the summer long-term regulated deficit irrigation strategies were successfully implemented on citrus trees, resulting in water savings ranging from 12% to 27% on the "Navelina Orange" tree (Gasque *et al*., 2016). The PRD strategy is a partial drying of the soil in the root zone, which stimulates the synthesis of abscisic acid (ABA), reducing leaf expansion and increasing stomatal conductance. The roots on the watered side, on the other hand, absorb enough water to sustain growth (Liu *et al*., 2006; Zegbe *et al*., 2006).

The PRD reduced shoot growth in several fruit species such as citrus. The reduction of growth in fruiting shoots becomes a limiting factor for flowering and fruit yield. In addition, PRD improved the taste quality of citrus fruit and the retention of sugars (Blanco *et al.*, 1989; Gonzales-Altozano & Castel, 1999; Gonzalez-Altozano & Castel, 2000) in Nules clementine.

Our research aimed to investigate the impact of PRD on plant water status, metabolic parameters, yield, and fruit quality of clementine MA3 in stages II and III of fruit growth.

Material and Methods

Experimental site: The experimental site was located in the region of Zaghouan in the north-eastern of Tunisia (latitude 36°26', longitude 10°05'; altitude 156 m). The climate in this region is semi-arid Mediterranean, with hot, dry summers. Annual rainfall in the study area averaged 422 mm during the first experiment year and 294 mm during the second. During the first year (from July 2016 to June 2017), the average temperature varied between 8.9 and 27.8°C. During the second year (from July 2017 to June 2018), the average temperature ranged between 10.9 and 29°C (Fig. 1). The soil of this assay was loam clay (25% sand, 40% loam, and 35% clay) with 1.99% organic matter.

Plant material: The study was conducted over two years (2016-2018) in a 1.7 ha clementine orchard (*Citrus Clementina*) var. MA3 grafted on sour orange rootstock (*Citrus aurantium* L.). The 7-year-old trees were planted at a 6 m x 3 m spacing.

Irrigation treatments: The irrigation was planned by the crop's water requirements. Indeed, irrigation water supply was scheduled in the field study using the climate module of the "Crop Water model," an agro-climatic model developed by the Food and Agriculture Organization (Anon., 2006). The climate module was used to estimate the crop's evapotranspiration (ETc) using the formula of Penman-Monteith and the method proposed by Allen *et al.*, (1998). Monthly mean data as a minimum and maximum temperature, minimum and maximum relative humidity, wind speed, and sunshine duration. ETc was estimated at different citrus orchard stages of development with less than 50% of coverage, a maximum tree height of 3 m and no cover crops since the cover index (CI) was 30.61% and the maximum height of the trees was 3.15 m. The fertilization, pruning, and pest control practices were used according to local citrus commercial production standards. Since no-till farming has been adopted in this citrus orchard, weed grinding between rows and weed control by herbicides in the rows were used to manage weeds.

A completely randomized design was used with two irrigation treatments (CI and PRD) and four replications per treatment. These two irrigation strategies were installed on 16 tree rows. The experimental unit covered 15 trees. In this experimental essay, two irrigation strategies were applied. CI (Control Irrigation) strategy: irrigation doses and frequencies are managed according to the crop water requirement (100% ETc) to well water the trees. According to the irrigation schedule, water supplies were applied daily. The irrigation system was a double pipe drip irrigation system with 4 drippers per tree (4 l/h auto-regulator drippers). The two irrigation pipes were arranged on the two sides of the tree row (right side and left side). Trees were irrigated at 50% ETc between July and February, corresponding to different phonological growth stages (the second and the final stage of fruit growth, the fruit-maturity period), and at 100% ETc during the rest of the growth period (flowering, fruit set, and fruit drop). This irrigation program reduced irrigation doses by half compared to the CI strategy between July and February.

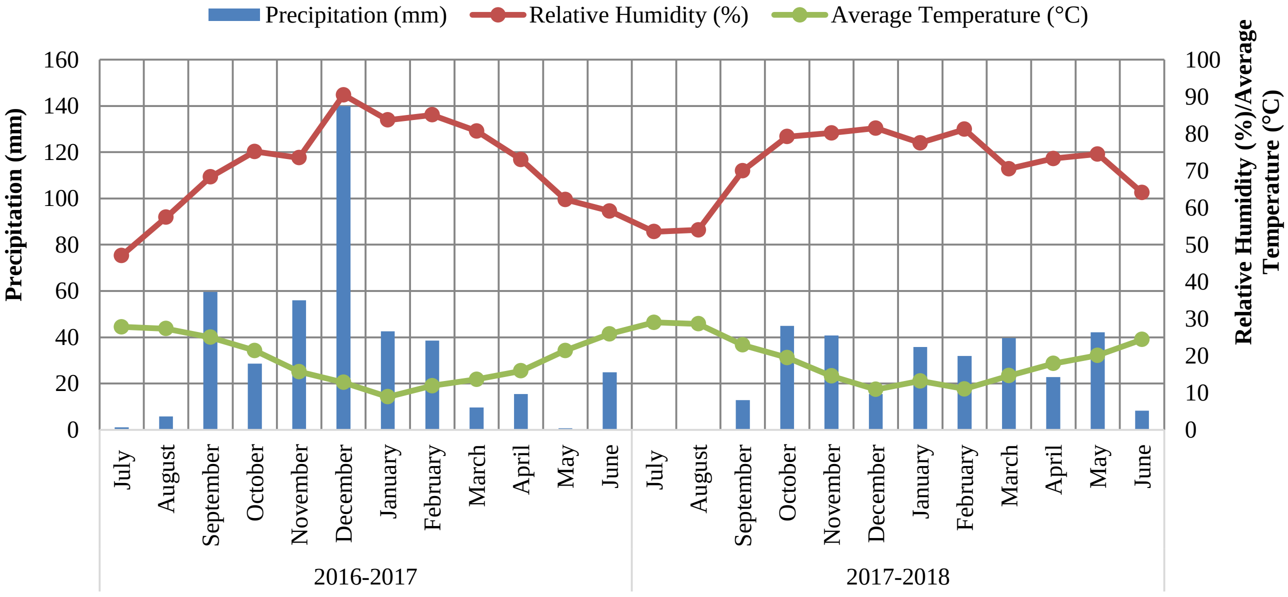

Fig. 1. Variation of Precipitations, Temperature, and Relative Humidity during two consecutive seasons (2016-2017/ 2017-2018).

Measured parameters

Plant water status

Leaf water potential (Ψl): A Scholander pressure chamber was used to measure the midday leaf water potential (Ψl) of 72 samples (PMS Instruments, Model: 600, USA). For two years, a monthly leaf sample was collected from July to December.

Leaf water content (LWC): To investigate the variation of the leaf water content (LWC), a sample of 72 leaves per treatment was measured every 15 days for two years, from June to December. The leaves are weighed immediately in the laboratory to determine their fresh weight (FW) and then dried in an oven at 70°C for 72 hours to determine their dry weight (DW). The leaf water content was determined according to Bowman (1989):

$$LWC\ (\%) = \{(FW - DW) / (FW)\} \times 100$$

Leaf temperature: Leaf temperatures were measured by an infrared thermometer (Lutron Infrared Thermometer: TM-958, Taiwan) every two weeks from July 1 to February 26. For each measurement, 64 leaves per treatment were chosen to carry out leaf temperature measurements between 10 a.m. and 2 p.m.

Metabolic parameters

Soluble leaf sugars: The phenol sulfuric acid method was used to determine the soluble sugars in the leaves (TSS) (Robyt & White, 1990). During the two years of research, a monthly sample of 48 leaves per treatment of the same physiological stage (from vegetative shoots of the year) was collected from July to December. Each leaf sample of 200 mg of fresh plant material was mixed with 5 ml of 80% methanol and heated for 30 minutes in a water bath at 70°C. Following that, 1 ml of the extract was mixed with 1 mL of 5% phenol and 5 mL of concentrated sulfuric acid. After stirring and cooling, the absorbance (Do) was determined using the spectrophotometer (brand: Janeway, model: 6300 Spectrophotometer, country: UK) at a wavelength of 640 nm. Finally, the results were based on a "standard" curve previously established by known glucose solutions (varying between 0.05 and 0.3 mg/ml). The sugar content is expressed in g/g FM (with FM: fresh material).

Chlorophyll content index: To evaluate the effect of partial root drying irrigation on the photosynthetic activity of Clementine, we measured the index of the chlorophyll content, which is expressed as a SPAD index. Indeed, this parameter was measured periodically with a frequency of 15 days during the periods going from 07/01 to 02/26. The SPAD chlorophyllometric index was measured using a chlorophychlorophyll meter (Konica-Minolta, model: SPAD-502, country: Japan) which measures the transmission of two wavelengths (650 and 950 nm) which are absorbed differently by chlorophyll. These measurements were carried out on 120 leaves by treatment at the same physiological stage (from vegetative shoots of the year).

Membrane permeability (%): For the two years of research, the membrane stability index was measured every month from July to December. On each measurement date, 48 leaves of the same physiological stage (from the year's vegetative shoots) were collected. This membrane permeability is determined by measuring the initial electrical conductivity (Lt) of a solution containing 1 fresh leaf cut into small pieces of 1 cm2 immersed in distilled water (after 24 hours of incubation at 25°C) and the final electrical conductivity (L0) of this solution after autoclaving (at 120°C for 20 minutes) using a conductivity meter (brand: Janeway, model: 4510 conductivity meter) (Lutts *et al.*, 1996). The following formula is used to calculate membrane permeability (Pm):

$$PM\ (\%) = (CL / CT) * 100$$

Proline content: Leaf samples were taken at a frequency of 30 days during the period from 07/25 to 12/22 to measure proline content. On each measurement date, 48 leaves of the same physiological stage (from vegetative shoots of the year) were randomly chosen.

In the laboratory, 200 to 400 mg of fresh plant material were weighed using a precision 1/10000 electronic balance (brand: KERN & Sohn GmbH, model: ABS 220-4, country: Germany). Then this plant material was mixed with 5 ml of 40% methanol and heated in a water bath (Memmert) at 80°C for 30 minutes. It should be noted that the heating is done in hermetically sealed tubes to prevent overconcentration of the extract. After cooling, 1 ml of the extract was added to 2 ml of acetic acid (CH_3COOH), 1 ml of ninhydrin solution ($C_6H_6O_4$) (25 mg/ml), and 1 mg/ml of a mixture containing 120 ml of distilled water, 300 ml of acetic acid (CH_3COOH), and 80 ml of orthophosphoric acid (H_3PO_4).

The whole thing was heated in a water bath at 100°C for 30 minutes. The solution gradually turned red. After cooling, 3 ml of toluene was added to this same mixture and stirred vigorously. Two phases were obtained (a red upper phase containing proline and a transparent lower phase without proline). Thus, the upper phase was dehydrated by adding a pinch (spatula) of anhydrous sodium sulfate Na_2SO_4 (to remove the water) and was collected. Finally, the absorbance (Do) of this phase was determined using a spectrophotometer (brand: JANEWAY, mJANEWAY6300 Spectrophotometer, country: UK) at a wavelength of 528 nm. The values obtained were converted into proline levels using a "standard curve" previously established from a series of pure proline solutions of known concentrations (ranging from 0.001 to 0.005 mg/ml) and used to determine the proline content (in µg/g MF with MF: fresh matter) in the foliar samples of the plants.

Yield and technological parameters: At harvest, the yield and number of fruits per tree were determined. A sample of 200 fruits per treatment was taken at random at the time of harvest (Mid-January to February) to measure fruit diameter using a caliper. Then the fruits were weighed and pressed with a scale and a juice machine

(Zumonat). The total soluble solids content of the juice (TSS) was measured with a digital refractometer (Atago) and the pH with a pH meter. The total sugar content was calculated from the following formula given by Moufida & Marzouk (2003):

Total sugar content (g/l) = Brix x 10 x (Juice weight/juice volume)

Titration with 0.1 N NaOH was used to determine the juice's titratable acidity (TA), and the maturity index was expressed as the soluble solids/acidity ratio.

Statistical analysis

In a completely randomized design with three replications, all collected data were analyzed using IBM SPSS 20.0 statistical software. The analysis was carried out using an analysis of variance (ANOVA), and the comparison of treatment means was accomplished using Duncan's Multiple Range Test at 5% and 1% probability levels (Duncan, 1955).

Results

Plant water status

Leaf water potential: The water status of the MA3 clementine tree showed that the water stress applied according to the PRD regime generated more negative leaf water potential (Ψl) than those recorded in the control treatment at all measured growth stages (Fig. 2). The water potentials varied between-2.57 and-1.13 MPa in the trees under the PRD treatment and between-1.65 and-1.04 MPa in the trees under the control treatment (CI).

In addition, the effect of PRD on the leaf water potential (Ψl) of the MA3 clementine was greater during the dry period (between July and September). The decrease in the leaf water potential at midday under water stress varied between 47 and 67% for the two years during this period, while it did not exceed 10% in October.

Leaf water content (LWC): The findings of this study revealed that water stress affects the leaf water content of clementine MA3 (Fig. 3). For the two years of studies from June to January, PRD treatment caused a reduction in leaf water content (LWC). The leaf water content varied during the season between 53 and 63% in leaves under CI and between 51.64 and 62.84% in leaves under PRD. Statistical analysis revealed a significant difference (P 0.01) between different irrigation strategies, especially during the summer and autumn seasons, when the reduction in leaf water content reached 9%.

Leaf temperature: The results of the leaf temperature measurement showed that the water deficit influences the thermal state of the plant. Indeed, in trees subjected to deficit irrigation, the leaf temperature is generally higher than that of the control. The statistical analysis showed that the PRD significantly affects the leaf temperature of the plant, especially for the period between July and November for the two years of study. Furthermore, the PRD treatment increased leaf temperature by 5% and 3% in August and September, respectively, during this period (Fig. 4). Compared to the summer period during the fruit ripening period (from October to February), there is a significant reduction in leaf temperatures and no statistically significant difference between treatments.

Metabolic parameters

Soluble sugar content: According to the findings, PRD-deficient irrigation had a highly significant effect on soluble sugar content (Fig. 5). In fact, during the PRD treatment in July, there was a 14% and 9% increase in sugar accumulation in the leaves, respectively, in 2016 and 2017.

Chlorophyll content index: The chlorophyll content index (SPAD) fluctuated between 51 and 65 in the CI and 45 and 61 in the PRD between July and December (Fig. 6). Irrigation deficiency has an impact on the chlorophyll content index. Indeed, the leaves of trees exposed to water stress had the lowest chlorophyll content indices, particularly during the summer (between July 31 and September 14). The chlorophyll content index was reduced by 10 to 17% in August and September.

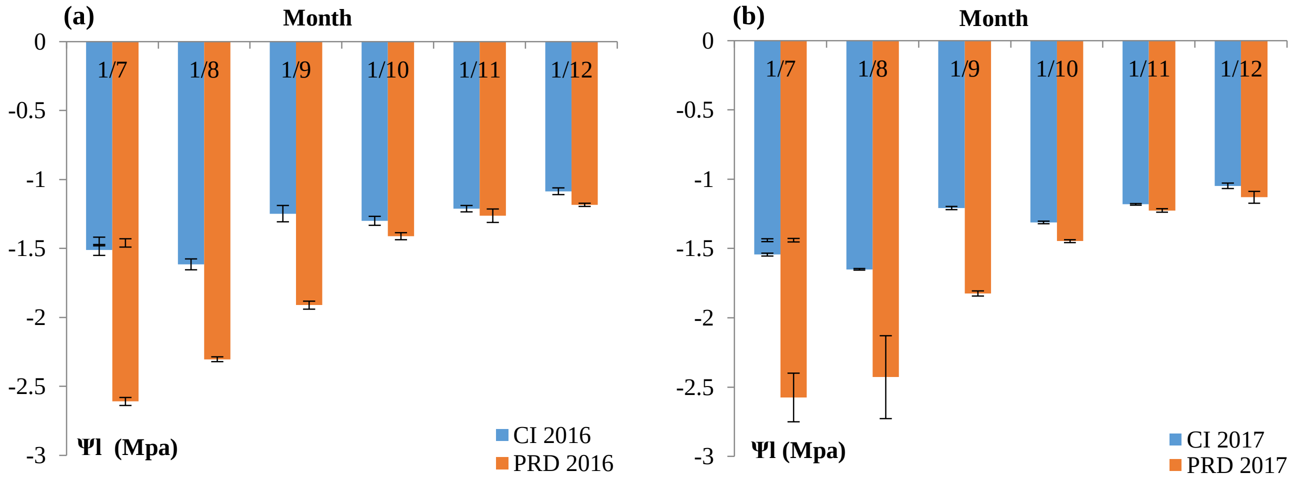

Fig. 2. Variation of leaf water potential (Ψl) under two different irrigation strategies as conventional irrigation (CI) and partial root drying (PRD). (a) 2016-2017; (b) 2017-2018.

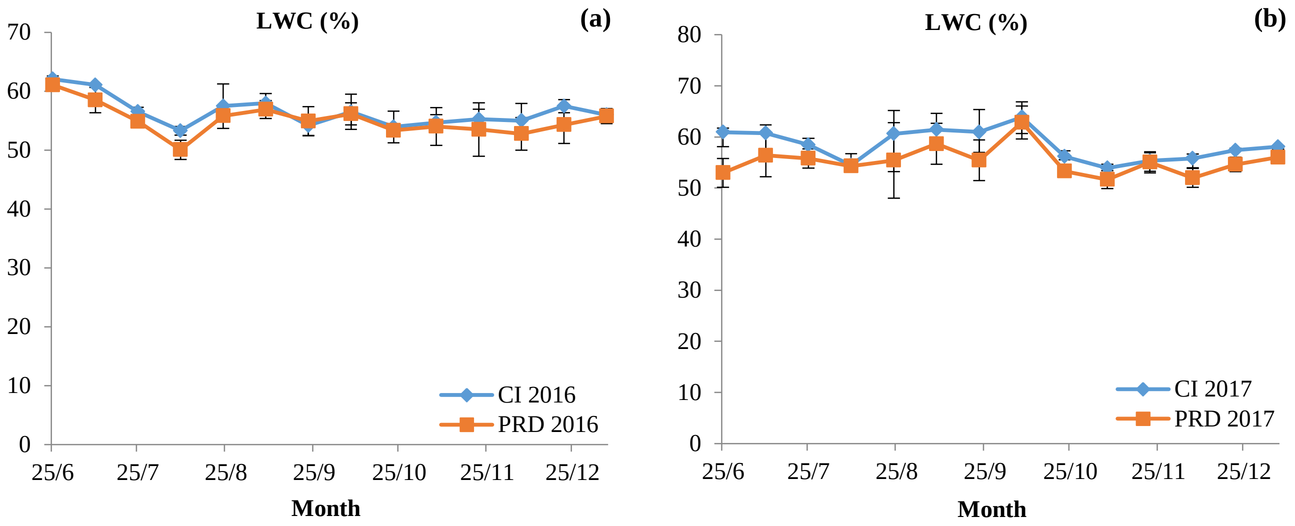

Fig. 3. Effect of irrigation strategies such as conventional irrigation (CI) and partial root drying (PRD) on the evolution of leaf water content (LWC). (a) 2016-2017; (b) 2017-2018.

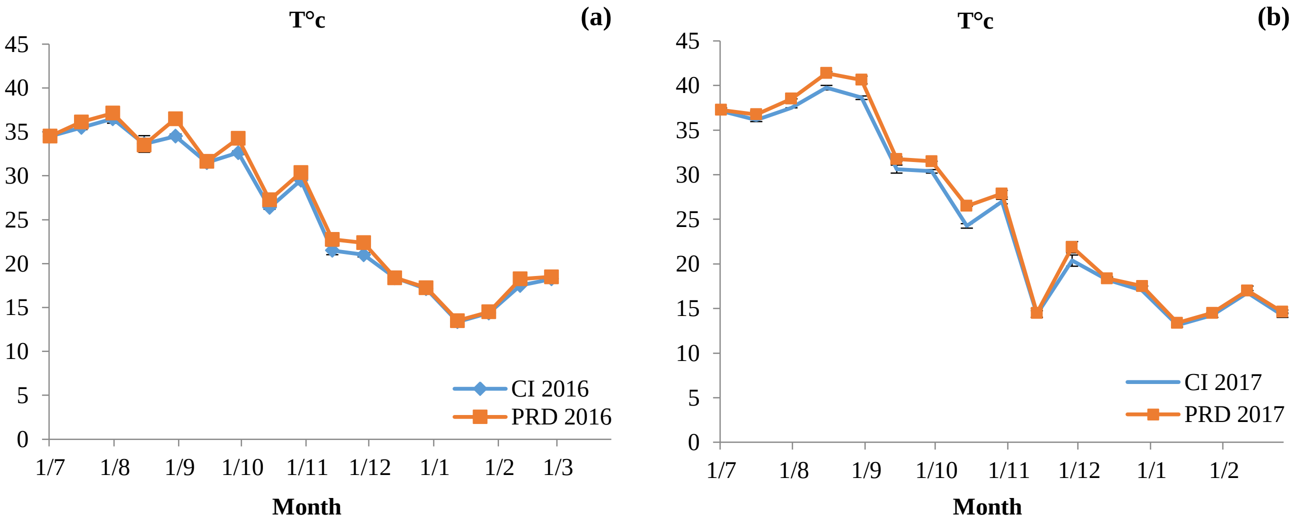

Fig. 4. Leaf temperature variation under two different irrigation strategies as conventional irrigation (CI) and partial root drying (PRD). (a) 2016-2017; (b) 2017-2018.

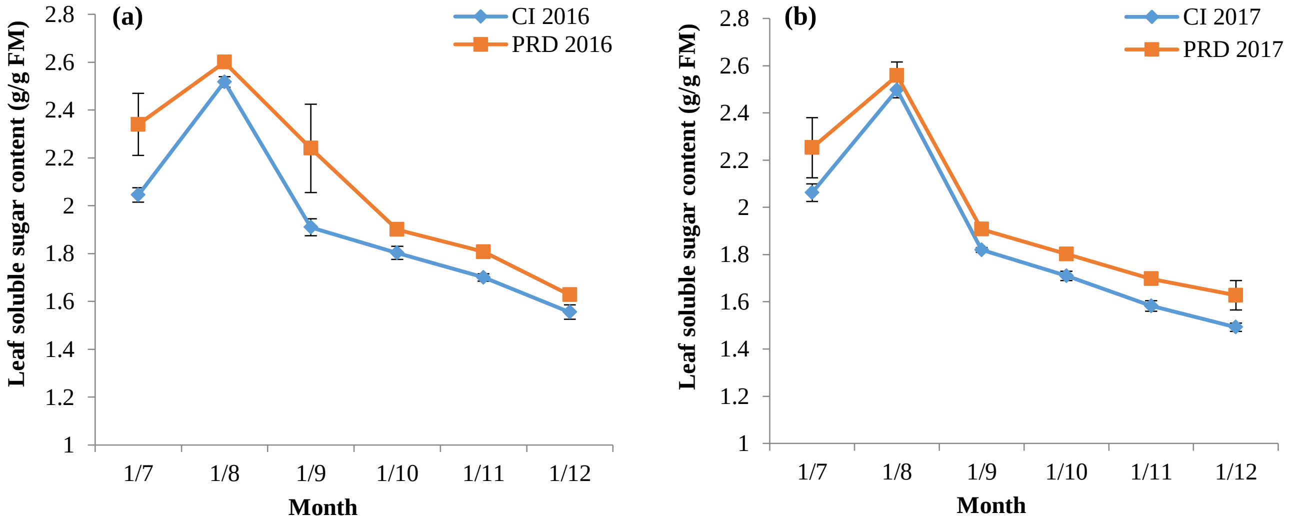

Fig. 5. Effect of irrigation strategies such as conventional irrigation (CI) and partial root drying (PRD) on the evolution of the soluble sugar content of leaves. (a) 2016-2017; (b) 2017-2018.

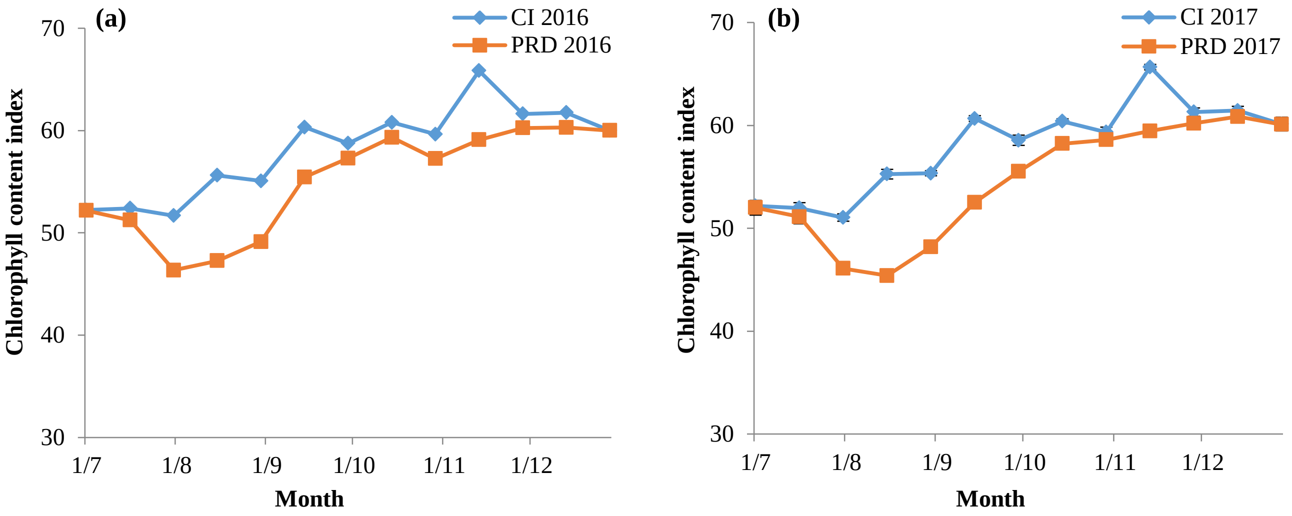

Fig. 6. Variation of the chlorophyll content index (SPAD) as a function of irrigation strategies such as conventional irrigation (CI) and partial root drying (PRD). (a) 2016-2017; (b) 2017-2018.

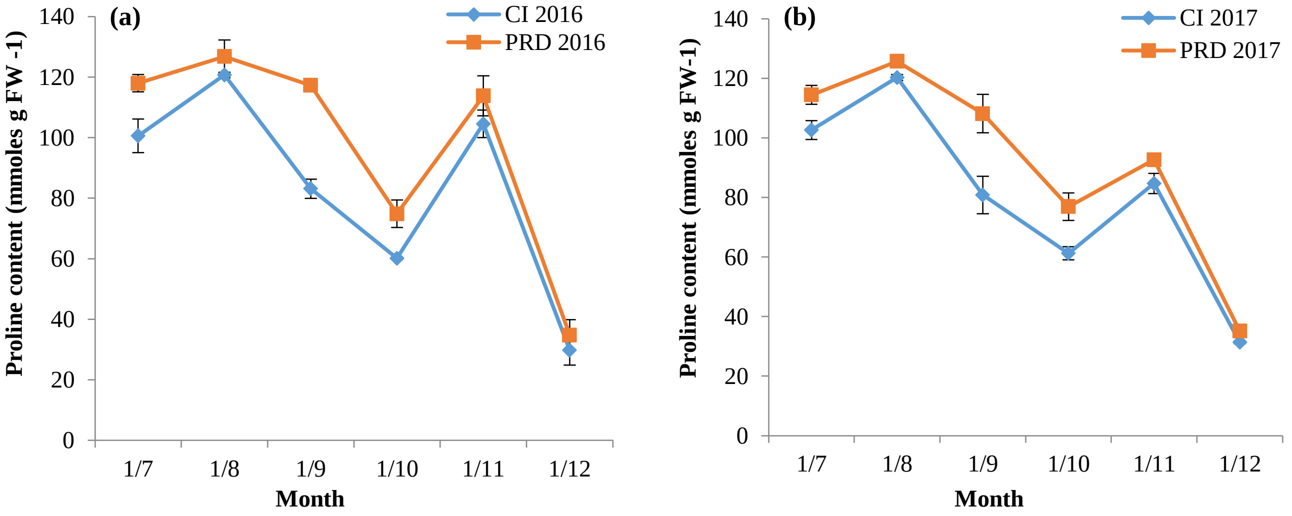

Fig. 7. Proline content in leaves of clementine MA3 trees of irrigation strategies as conventional irrigation (CI) and partial root drying (PRD). (a) 2016-2017; (b) 2017-2018.

Proline content: For the two years of research, there was a highly significant increase in leaf proline concentration in the PRD treatment (Fig. 7). The values of the proline content varied between 31 and 120 in conventional irrigated trees and between 35 and 125 µg/g FW in stressed PRD trees. The water stress caused a significant accumulation of leaf proline content of around 5 to 12% during the summer (July to August) and 34% in September; however, there was a slight variation in October and November.

Membrane permeability: This study showed that the water deficit caused an increase in membrane permeability throughout the period between July and December. Moreover, statistical analysis revealed a highly significant effect on membrane permeability following the application of PRD water treatment. The increase in membrane permeability following the water deficit was approximately 8% at the start of the application of PRD treatment and reached almost 12% two months later (Fig. 8).

Technological parameters

Fruit size: As shown in Figure 9, the clementine fruit growth exhibited a sigmoid growth pattern with two growth phases; phase II (from July to September) and phase III (from September to December). The CI and PRD irrigation strategies showed a similar pattern of fruit diameter in the first year of our study, 2016, with no differences recorded. However, in the second year of the experiment, 2017, clementine fruit diameter of partial root drying decreased by 7.1%, 7.0%, 9.2%, 7.2%, 6.2%, and 6.6%, respectively, from July to December in comparison with the control.

Fruit number, fruit weight, and yield: Our findings revealed that there were no significant differences in fruit yield, fruit number, or fruit weight between conventional and PRD irrigation strategies in 2016–2017. However, the PRD treatment reduced yield and fruit number by 16% and 18%, respectively, in 2017-2018 (Table 1).

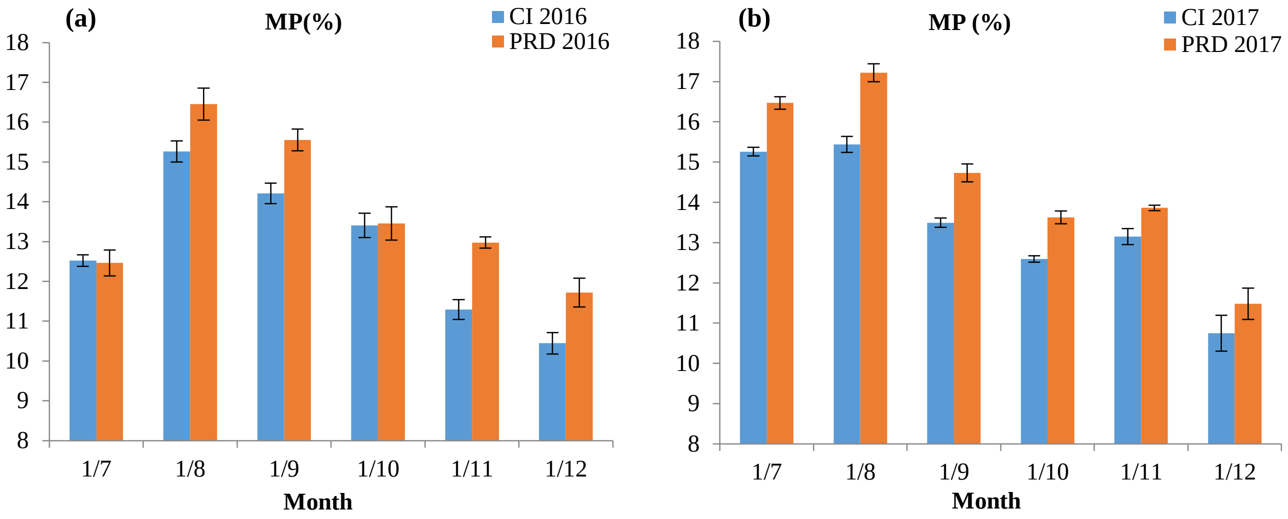

Fig. 8. Variation of membrane permeability as a function of irrigation strategies conventional irrigation (CI) and partial root drying (PRD). (a): 2016-2017; (b): 2017-2018.

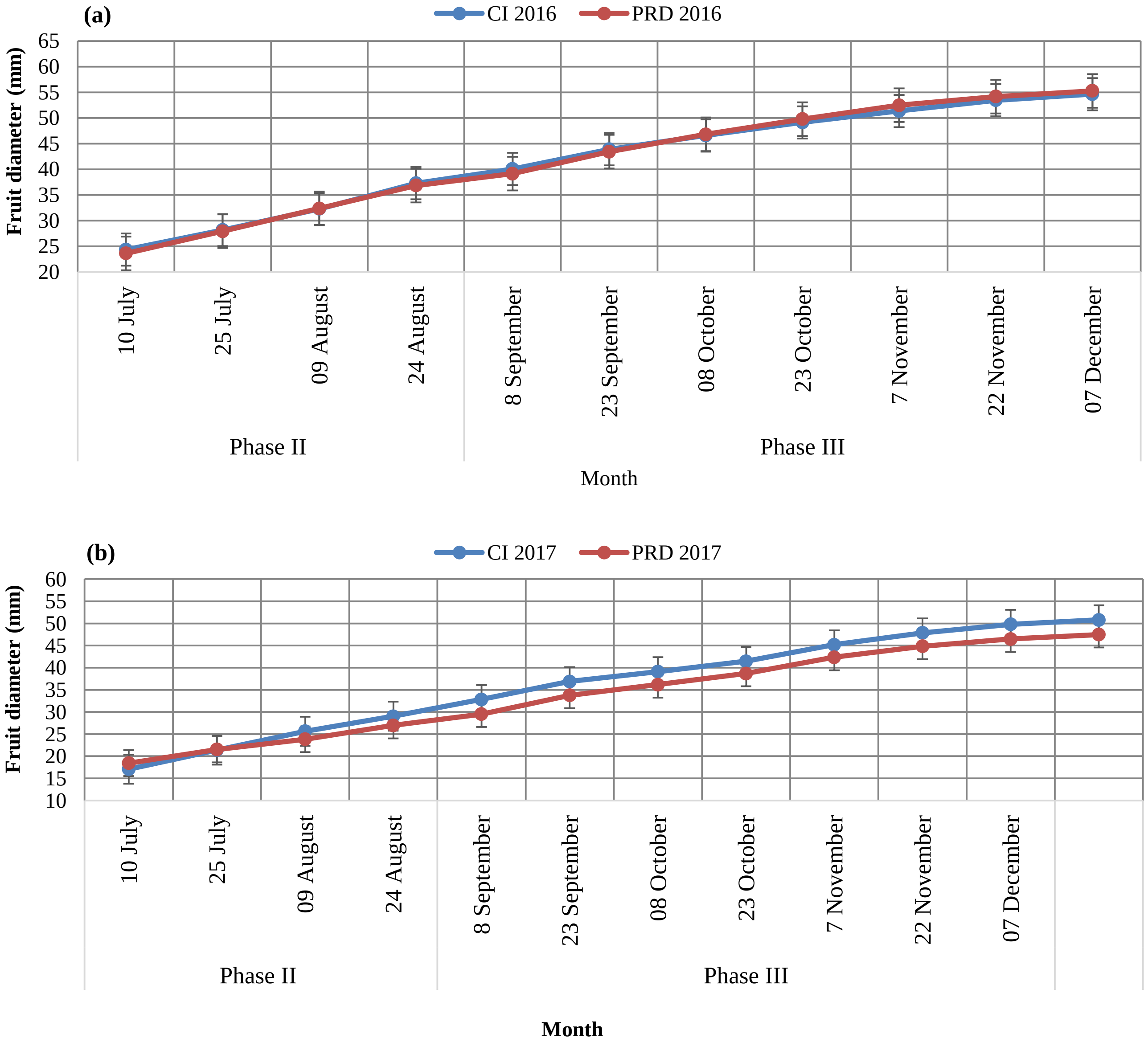

Fig. 9. Evolution of fruit size in Phase II and III as a function of irrigation strategies conventional irrigation (CI) and partial root drying (PRD). (a): 2016-2017; (b): 2017-2018.

Table 1. Fruit number, fruit weight, and fruit yield/tree of 'MA3' clementine under partial root drying (PRD) and conventional irrigation (CI) during 2016 and 2017.

Treatments	2016			2017		
	Fruit yield (kg tree^{-1})	**No. fruits tree^{-1}**	**Fruit weight (g fruit^{-1})**	**Fruit yield (kg tree^{-1})**	**No. fruits tree^{-1}**	**Fruit weight (g fruit^{-1})**
CI	39.1aA	403.6aA	98.3aA	38.3aA	386.0 aA	91.4 aA
PRD	40.5aA	415.6aA	79,4 aA	32.0 bB	339.0 bB	88.4aA
Treatments	ns	ns	ns			
years	*	*	ns			
Treat *Years	ns	ns	ns			

ns: Not significant; *: $p<0.05$; **: $p<0.0$

Table 2. Effect of different irrigation strategies on total soluble solids (TSS), titratable acidity (TA), maturity index (MI), pH, and total sugar content.

	CI		PRD		SL
	2016	**2017**	**2016**	**2017**	
Juice %	61.24	62.08	60.09	59.48	ns
MI	4.31	4.24	4.28	4.27	ns
TA (g l^{-1})	0.86	0.98	0.84	1.02	ns
TSS (°Brix)	10.5	11.6	10.6	11.9	ns
pH	4.31	4.24	4.28	4.27	*
Total sugar content (g/l)	189.90	195.05	195.70	196.25	ns

SL: Significative level, ns: Not significant; *: $p<0.05$; **: $p<0.0$

Fruit quality: There were no significant differences between the control and PRD treatments in terms of juice percentage, maturity index, titratable acidity, total soluble sugar, and total sugar content. Only pH differed significantly between the control and deficit irrigation treatments. The control irrigation treatment had the highest juice percentage in the 2016 and 2017 experiment years (61.24 and 62.08 percent). However, the PRD treatment had the highest TSS (10.6 and 11.9 Brix, respectively), and the highest total sugar content (195.7 and 196.3 g/l, respectively). The maturity index, pH, and titratable acidity were higher under control treatments in the first year of 2016, but they were higher under PRD treatment in the second year of 2017 (Table 2).

Correlation coefficients: The correlation coefficients of different characters of Clementine MA3 are represented in Figure 10. Fruit number was correlated positively with fruit yield (r=0.814**). Leaf water potential at phase III of fruit growth was found to have a significant positive correlation with those measured at phase II of fruit growth (r=0.754**). Membrane permeability at phase II of fruit growth was correlated negatively with fruit weight (r=-0.524*) and leaf water potential II (r=-0.810**). Membrane permeability at phase III of fruit growth was negatively correlated **) with fruit weight (r=-0.627**) but positively with membrane permeability at phase II (r=0.968**). Leaf sugar content at phase II of fruit growth had a significant positive correlation with membrane permeability III (r= 0.574*). Leaf sugar content at phase III of fruit growth was negatively correlated with fruit yield (r=-0.702**) but positively correlated with leaf sugar content at phase II of fruit growth (r=0.815**). Leaf temperature at phase II of fruit growth showed a significant negative relationship with leaf water potential II and III (r=-0.870** and r=-0.546** respectively) but a positive relationship with membrane permeability II and III (r=0.904** and r=0.760** respectively).

Leaf temperature at phase III of fruit growth was negatively correlated with yield (r=-0.565*), fruit number (r=-0.509*) and leaf temperature II (r=-0.627**) but positively correlated with leaf water potential II (r=0.675**), leaf sugar content II and III (r=0.636** and r=0.828** respectively). Leaf water content II was positively correlated with Yield (r=0.626**) and fruit weight (r=0.678**) but negatively correlated with leaf sugar content II and III (r=-0.723** and r=-0.850** respectively) and leaf temperature III (r=-0.643**).

Leaf water content at phase III of fruit growth showed a positive correlation with yield (r=0.631**) and leaf water content II (r=0.796**), but a negative correlation with leaf sugar content II and III (r=-0.616* and r=-0.786** respectively) and leaf temperature III (r=-0.665**). Leaf proline content II had a negative correlation with yield (r=-0.577**), leaf water content II and III (r=-0.564* and r=-0.556* respectively), and a positive correlation with membrane permeability II and III (r=0.590* and r=0.796** respectively), leaf sugar content II and III (r=0.771** and r=0.789** respectively). Leaf proline content at phase III of fruit growth exhibited significant negative association with yield (r=-0.644**), leaf water content II and III (r=-0.814** and r=-0.794** respectively), but positive association with membrane permeability III (r=0.555*), leaf sugar content II and III (r=0.845** and r=0.934** respectively), leaf temperature III (r=0.754**) and leaf proline content II (r=0.810**).

SPAD at phase II of fruit growth showed a positive correlation with yield (r=0.66*) fruit weight (r=0.532*), leaf water content II and III (r=0.574* and r=0.588* respectively), but a negative correlation with membrane permeability II and III (r=-0.813** and r=-0.935** respectively), leaf sugar content II and III (r=-0.652** and r=-0.670** respectively), leaf temperature II (r=-0.614*), and leaf proline content II and III (r=-0.899** and r=0.719** respectively). Finally, SPAD at phase III was positively correlated with yield (r=0.536*), fruit weight (r=0.588*), leaf water content II and III (r=0.593* and r=0.611* respectively) and SPAD II (r=0.946**), but negatively correlated with membrane permeability II and III (r=-0.675** and r=-0.909**), leaf sugar content II and III (r=-0.724** and r=-0.746**), and leaf proline content II and II I (r=-0.930** and r=-0.776** respectively).

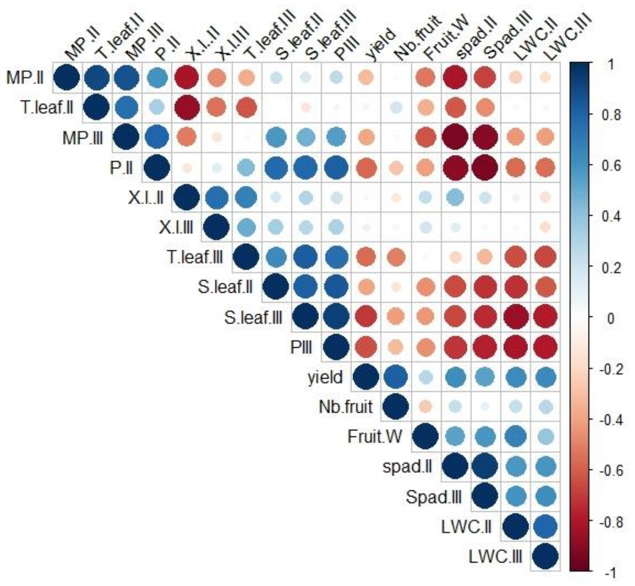

Fig. 10. Correlation coefficient among different characters of Clementine MA3.

Yield: Fruit Yield, Nb. Fruit: Fruit number, Fruit. W: Fruit weight, X.I.II and III: leaf water potential at phase II and III of fruit growth, MP. II and III: Membrane permeability at phase II and III of fruit growth, S. leaf. II and III: Leaf sugar content at phase II and III of fruit growth, T. leaf. II and III: Leaf temperature at phase II and III of fruit growth, LWC. II and III: Leaf water content at phase II and III of fruit growth, P. II and III: Leaf proline content at phase II and III of fruit growth, SPAD. II and III: chlorophyll index at phase II and III of fruit growth. * correlation coefficient significant at (p<0.05), ** correlation coefficient significant at (p<0.01).

Discussion

Plant water status: One of the most common physiological parameters limiting photosynthesis efficiency and biomass productivity in plants is leaf water content (Jin *et al*., 2017). LWC was considered as an important indicator of the water balance of the soil–plant–atmosphere continuum (Zhou *et al*., 2021). In our results, compared to the conventionnel irrigation strategy, the PRD treatment reduced the leaf water content of Clementine trees both in summer and autumn season by 9%.

Stem water potential, leaf water content, and leaf temperature are three of the most commonly used tools for determining plant water status, particularly in water stress conditions.

Leaf water potential was considered a better predictor of plant water status. Our findings revealed that the PRD irrigation regime produced approximately 7.96%–35.80% more negative leaf water potential (l) than the control irrigation regimes.

During the first season of 2016–2017, the PRD decreased the leaf water potential (Ψl) by 35.9% and 13.5%, respectively, in phases II and III of fruit growth. However, it reduced the leaf water potential by 35.8% and 12.3%, respectively, in phases II and III of fruit growth during the second season of 2017-2018 compared to the control. Our findings are consistent with Kirda *et al*., (2007), who discovered that the leaf water potential of PRD mandarin trees is lower than that of control trees.

The maintenance of a high value of leaf water potential is associated with the mechanism of dehydration avoidance (Reddy, 2019), and this was not the case in our study. Under hydric stress, Shahnazari *et al*., (2007) discovered that reduced leaf water potential acts as a hydraulic signal, resulting in reduced leaf area expansion and partial stomatal closure. Shirgure *et al*., (2000) discovered that as soil moisture in the root zone decreased, the leaf water potential of the Kinnow variety of mandarin decreased significantly. During the 2016-2017 growing season, the PRD reduced leaf water content by 3.5% and 1.85%, respectively, in phases II and III of fruit growth. However, during the second season of 2017-2018, it decreased the leaf water potential by 6.9% and 4.5%, respectively, in phases II and III of fruit growth compared to control.

On the other hand, we noted a fluctuation in the leaf water content at the end of August, which continued during the rainy period (September to November). This fluctuation in the value of leaf water content during the fall period could be due to variability in climatic factors, mainly temperature and air humidity. Such variability, which is marked mainly by the alternation of dry and wet periods, is characteristic of autumn in the Mediterranean climate. Then, during the rainy winter period (December to January), the leaf water content continued to improve for the two water treatments (CI and PRD). The results of the correlation study showed that leaf water content was positively associated with yield and negatively with leaf sugar content and leaf temperature, emphasizing that the partial root drying in clementine trees leads to a decrease in leaf water content and thus a decrease in yield, but an increase in leaf sugar content and leaf temperature.

Leaf temperature can be used as an indicator of stomatal opening and it is highly dependent on plant transpiration rate (Sinclair, 1984). The application of a partial root drying irrigation strategy in the Clementine tree caused an increase in leaf temperature of 5% and 3% in August and September, respectively. During the fruit ripening period from October to February, no significant difference in leaf temperature was observed due to the return of the rainy winter with lower temperature degrees.

During the first season of 2016–2017, the PRD increased the leaf temperature by 1.7% and 2.8%, respectively, in phases II and III of fruit growth. However, during the second season of 2017-2018, it increased the leaf temperature by 2.8% and 3.5%, respectively, in phases II and III of fruit growth compared to control. Our results are supported by the findings of Surendar *et al*., (2013), who revealed that throughout the plant growth cycle, the increase in leaf temperature and stomatal diffusive resistance lead to a decrease in transpiration rate, trying to show a negative correlation between leaf temperature and transpiration rate. Our correlation studies revealed that increasing the leaf temperature of clementine trees during stage II of fruit growth increases membrane permeability but decreases leaf water potential. However, the increase in leaf temperature in phase III of fruit growth results in a decrease in yield and fruit number but an increase in leaf sugar content.

According to Sdoodee & Kaewkong (2006), water restriction application caused stomatal closure in the Neck orange variety, resulting in high leaf temperature and confirming the crucial role of the infrared thermometry technique in detecting stomatal closure and thus assessing plant water stress. Not only leaf temperature and spectral emissivity were affected by hydric stress, but also leaf water content, chlorophyll content, and leaf structure (Gerhards *et al*., 2016).

Metabolic parameters: Photosynthesis is the process of converting solar energy into chemical energy. The leaves absorb carbon dioxide and water to produce sugar. During the first season of 2016–2017, the PRD increased the leaf sugar content by 7.7% and 7.9%, respectively, in phases II and III of fruit growth. However, when compared to the control, it increased the leaf sugar content by 5.4% and 6.3%, respectively, in phases II and III of fruit growth during the second season of 2017-2018.

The application of the partial root drying technique in clementine trees showed high soluble sugar content compared to conventional irrigation, explaining the role of moderate hydric stress in the accumulation of sugar in leaves. This increase in the amount of sugar in PRD leaves and cells is a symptom of adaptation to water stress conditions. The application of controlled water stress to Valencia sweet orange trees during stage II of fruit development increased primary osmotic concentrations of fructose and glucose (Barry *et al*., 2004). According to Benirken *et al*., (2013), a difference in rootstock behavior was observed under extreme water deficit conditions. Citrange Carrizo, and *Citrus macrophylla* rootstocks provide the best ability for clementine trees to resist the water stress conditions by increasing the soluble sugar content in their leaves. The findings of our correlation studies showed that an increase in leaf sugar content under water stress conditions leads to an increase in membrane permeability but a decrease in clementine fruit yield.

The leaves of Clementine trees exposed to water stress strategy showed the lowest chlorophyllometric index, particularly during the summer season. During the first season of 2016–2017, the PRD decreased the chlorophyll index by 7.8% and 4%, respectively, in phases II and III of fruit growth. However, during the second season of 2017-2018, it decreased the chlorophyll index by 8.7% and 4.5%, respectively, in phases II and III of fruit growth compared to control. For instance, during August and September, the chlorophyll content index was reduced by 10 to 17%, which may be explained in large part by chloroplast damage caused by active oxygen species or by phytohormones. Our results are similar to those reported by Ghafari *et al*., (2020), who showed that the severe decrease of the chlorophyll content index by 60–85% of the Red Delicious apple variety in partial root drying strategy, particularly at the end of the growing season, was most likely due to a decrease in GA3 and CK hormones. Mafakheri *et al*., (2010) revealed that the implication of drought stress at both vegetative and flowering stages reduced chlorophyll a, chlorophyll b, and total chlorophyll content. The amount of chlorophyll a and b reduced in olive trees was proportional to the degree of drought stress and variety (Arji & Arzani, 2004).

The findings of our correlations revealed that a decrease in chlorophyll index under partial root drying results in a decrease in yield, fruit weight, and leaf water content. However, an increase in membrane permeability in phases II and III of fruit growth, leaf sugar content in phases II and III of fruit growth, leaf temperature in phase II, and leaf proline content in both phases II and III of fruit growth.

Plant proline was considered the most widely distributed compatible solute that accumulated in plants during water stress conditions, playing an important role in plant stress tolerance (Rejeb *et al*., 2012). Our results showed that the water stress caused an increase in proline concentration of 4% to 11.4% in clementine leaves under the PRD strategy, and this proline accumulation varied between 5% and 12% during July and August, and 34% in September. During the first season of 2016–2017, the PRD increased the proline content by 9.6% and 18.5%, respectively, in phases II and III of fruit growth. However, during the second season of 2017-2018, it increased the proline content in phases II and III of fruit growth by 7.2% and 17.5%, respectively, when compared to the control. According to Sarkar *et al*., (2016), water stress increases biochemical components such as total sugar and total soluble protein, increases the accumulation of foliar non-enzymatic antioxidants such as proline, and decreases relative water content and total chlorophyll content in *Citrus reticulata* L. According to our correlation analysis between different parameters, we conclude that an increase in leaf proline content leads to an increase in membrane permeability and leaf sugar content in phases II and III of fruit growth but a decrease in yield and leaf water content in phases II and III of fruit growth.

Between July and December, a lack of water caused an increase in membrane permeability. This increase in membrane permeability following the water deficit was on the order of 8% at the start of the application of the PRD treatment and reached almost 12% two months later. During the first season of 2016–2017, the PRD increased the membrane permeability by 3.9 and 8.1%, respectively, in phases II and III of fruit growth. However, during the second season of 2017-2018, it increased the membrane permeability by 8.8% and 6.9%, respectively, in phases II and III of fruit growth compared to control. Our findings agree with those of Li-Ping *et al*., (2006) in maize plants, who found that severe water stress increased membrane permeability significantly. The obtained correlations in our study showed that there is a strong negative correlation between leaf membrane permeability at phase III of fruit growth with leaf water potential and fruit weight, explaining that the application of partial root drying increased the membrane permeability but decreased the leaf water potential and fruit weight.

Technological parameters: The size of citrus fruits is one of the most important factors in determining their market value. This criterion is strongly influenced by irrigation. The fruit size evolution curve clearly shows a simple sigmoidal shape with three characteristic phases. This is consistent with the findings of various studies on the growth dynamics of citrus fruits (Iglesias *et al*., 2007). The effect of PRD on fruit diameter was significant in the second year of the experiment. Application of PRD in clementine trees reduced fruit size by 7.1 and 7.3% in phases II and III of fruit growth, respectively. These results are in agreement with those of Hutton & Loveys (2011), who indicated a slight reduction in the fruit size of trees irrigated by the PRD irrigation strategy. The reaction of clementine trees to the application of the partial root drying irrigation system appeared clearly in the second year by reducing the fruit number and yield by 18% and 16%, respectively, without effect on fruit weight. When moderate water stress was applied during all three stages of Clementine Nules development, Ballester *et al*., (2011) found no significant effects on fruit weight. This demonstrates that the response to water stress is affected by both the variety and the duration and intensity of the stress. Water stress affects yield differently depending on the phenological stage at which it occurs. The most sensitive phenological stages were blooming and fruit growth (Ginestar & Castel, 1996). The onset, duration, and severity of water deficits during different growth stages will affect yield differently (Kirda, 2002).

Although there were no significant differences in the quality attributes of clementine fruit except for the pH, the application of partial root drying during the 2016 and 2017 experiment years improved the TSS by 0.9% and 2.5%, respectively, and thus the total sugar content by 3% and 0.6%, respectively. The application of PRD increased the pH, maturity index, and titratable acidity by 0.9%, 2.5%, and 0.6%, respectively, during 2017-2018. The increase in TSS and TA is a result of osmoregulation caused by a lack of water, rather than fruit dehydration (Hockema & Etxberria, 2001). Hutton & Loveys (2011) emphasized the importance of partial root drying in increasing the juice quality of Navel orange trees. Similarly, in a study on sweet orange "lane late," Perez-Perez *et al*., (2009) discovered that applying water stress during stage III of fruit growth results in an increase in Brix and acidity titratable, even if the maturity index is unaffected. A similar finding was reported by Barry *et al*., (2004), who discovered that when stress is applied too late in stage III of fruit growth, no increase in sugars and total soluble solids (Brix) occurs, whereas when stress is applied during stage II of fruit growth, which is the period of greatest sugar accumulation, the effect of stress is clearly visible.

Conclusion

Despite a slight decrease in yield and fruit size, as well as physiological and biochemical changes caused by water stress, the use of a PRD irrigation strategy and meeting 50% of the water requirements of Clementine trees during phases II and III of fruit growth had no negative impact on fruit quality. This efficient irrigation method could be used to solve the problem of water scarcity in semi-arid and arid regions.

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