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Research article

Influence of Watering Regimes on Physiological Traits, Growth, Yield, and Capsaicin Content of Chilies

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Abstract: Water stress is a major limiting factor affecting the physiological processes, growth, and productivity of crop plants, including chili. This study evaluated chili plants' responses regarding physiological traits, growth, yield, and capsaicin content under different irrigation levels. The experimental design was a 2x4 factorial in CBD. A pot experiment was conducted on two species of chilies: *Capsicum annuum* 'Super-Hot 2' and 'Huay-Siiton' and *C. frutescent* 'Prik-Kee-Nu-Suan.' The plants were irrigated at four watering regimes such as 100%, 80%, 60%, and 40% of maximum water holding capacity (MWHC) after the anthesis through fruit development. Midday leaf water potential (LWP_{md}) was ranked between -1.30 MPa in 100% MWHC to -2.11 MPa in 40% MWHC indicating the level of water stress. The 40% MWHC caused a drastic decrease in LWP_{md} and reduced the maximum quantum yield of PSII (F_v/F_m) and growth rate in canopy width. Observed leaf greenness was maximized at 60% MWHC. The irrigation regimes also affected the yield of chili, especially the 60% and 40% MWHC, with a reduced number of fruits/plant, fruit fresh and dry weights, and fruit sizes. In the case of 40% MWHC, the dry yield was too low, and it was impossible to analyze the capsaicin content. The reduced irrigation (80% and 60% MWHC) did not significantly induce the capsaicin content or pungency of the studied chili cultivars. Thus the capsaicin yield of studied chili cultivars was reduced considerably by severe reduction of dry fruit yield under restricted water.

Keywords: *Capsicum*; Water Deficit; Water Holding Capacity; Photosynthetic Efficiency, Capsaicinoid

1. Introduction

In Thailand, chili is an important spice used in various traditional dishes and an essential material in the industrial sector, particularly the capsaicinoids that can be extracted and used in the pharmaceutical and cosmetic industries [1-2]. Previous studies have demonstrated that water deficit can alter crop physiological processes, e.g., reducing the stomatal conductance, photosynthetic efficiency, chlorophyll content, and nutrient assimilation [3].

The mentioned changes may lead to a reduction in vegetative growth and a loss of reproductive parts, resulting in a declining yield and yield quality [4]. In chili, water deficit can also alter the pungency level of chili by modifying the capsaicinoid contents [5]. The capsaicinoids are secondary metabolites accumulated in chili fruits, comprising several compounds. The major capsaicin and dihydrocapsaicin account for 90% of total capsaicinoids [6-7]. Studies have found that irrigation regimes below the field capacity (FC) contributed to an increase in secondary metabolites [8-11]. Therefore, irrigation management is of interest as a possible tool for optimizing chili growth and yield and increasing capsaicinoid content. This study aimed to investigate the effects of watering regimes on the physiological traits, growth, yield, and capsaicin content of *Capsicum annuum* and *C. frutescens* under nursery conditions.

2. Materials and Methods

The study was conducted in a nursery from October 2020 to March 2021 at Suranaree University of Technology's farm, Nakhon Ratchasima Province, Thailand. The 3x4 factorial in CRD with 5 replications was used as an experimental design. The studied factors were A) three chili cultivars (*C. annuum* cv. Super-Hot and Huay-Siiton and *C. frutescent* cv. Prik-Kee-Nu-Suan) and B) four watering regimes: 100 (control), 80, 60, and 40% of maximum water holding capacity (MWHC). The chosen chili cultivars are commonly grown in the Northeastern region of Thailand and have been reported to have a high capsaicinoid content and pungency level [12-14].

Seeds of the three cultivars were germinated in peat moss-filled trays under a plastic-covered nursery. The seedlings were regularly irrigated and 30 days after sowing, homogenous seedlings were selected and transplanted into a 5-liter-plastic pot filled with sandy loam soil from Suranaree University of Technology's Farm. Chili plants received the 21-0-0, 15-15-15, and 13-13-21 chemical fertilizers after transplanting, during the vegetative growth stage, and flowering to the fruiting stage, respectively [15]. After transplanting, plants were irrigated to the MWHC, and different irrigation regimes were applied from the flowering stage to the fruit ripening stage of each cultivar. Soil moisture content was regularly observed using the HH2 Moisture Meter (Delta-T Devices Ltd.), and the pots were rewatered when the moisture content dropped below the assigned regimes.

Data collection was started at the onset of anthesis (0 WAA, week after anthesis) for physiological, growth, and development traits. The measurement was retaken at 2, 4, 6, and 8 WAA.

2.1 Physiological traits

Three plants from each treatment were assigned to measure midday leaf water potential (LWP_{md}) to determine the effects of watering regimes on plant water status using a 3005-pressure chamber (Soilmoisture). A fully expanded leaf/plant was cut from the middle of the canopy height of each plant. On the same plants, three fully developed leaves at the middle of the canopy height were marked for the measurement of leaf greenness (SPAD) using the chlorophyll meter SPAD-502plus (Konica Minolta Inc.). A chlorophyll fluorescence meter measured the maximum quantum yield of PSII (Fv/Fm) (Handy PEA, Hansatech Instruments Ltd.). Before the F_v/F_m measurement, marked leaves were clamped with the Dark Leaf Cup (DLC-8) for 20 minutes. These physiological traits were performed before the irrigation, from 11:00 a.m. to 1.00 p.m.

2.2 Plant growth and development

The same plants used for measuring physiological traits were also assigned for measuring plant growth and development: canopy height and width. The values were later used to calculate the relative growth rate (RGR), and the calculation was done according to the following equations:

$$RGR_{height} = (ln\ H_2 - ln\ H_1)/(T_2 - T_1) \quad (1)$$

$$RGR_{width} = (ln\ W_2 - ln\ W_1)/(T_2 - T_1) \quad (2)$$

where H_1 and H_2 were canopy heights at times T_1 and T_2 and W_1 and W_2 were the canopy widths at times T_1 and T_2.

2.3 Yield and capsaicin content

The yield was collected during the 8-14 WAA. The fully ripened fruits were harvested, and the number of fruits harvested was counted. Thirty fruits were randomly sampled from each treatment to measure length and width. All fruits were weighed for fresh fruit weight and dried in a hot-air oven at 70 °C for 48 hours before the dry fruit weight was measured. The dried chili fruits were grounded and stored in a freezer at -20 °C while waiting to measure capsaicin. The capsaicin content was measured using the high-performance liquid chromatography (HPLC) technique [16]. The capsaicin content, the pungency of the samples according to the Scoville organoleptic test, and the capsaicin yield were later calculated [17].

2.4 Statistical analysis

All the traits collected were statistically analyzed using the analysis of variance (ANOVA). The significant difference between treatments was determined using Duncan's New Multiple Range Test (DMRT) at a 95% confidence interval.

3. Results and Discussion

3.1 The physiological response

Chili cultivars had different LWP_{md} and SPAD characters. However, they were similar in the F_v/F_m. With decreasing watering regimes, the LWP_{md} was lowered compared to the control treatment except for the 80% MWHC. On the contrary, it appeared that lowering the water supply to a certain point (60% MWHC) can increase the SPAD of chili. At 100% MWHC, the F_v/F_m was the highest, and it was reduced with the decreased MWHC. The SPAD and F_v/F_m of each studied chili cultivar responded to different watering regimes in similar trends to those observed from the effects of the watering regime (Table 1).

When considering the changes in LWP_{md} between the studied weeks (Figure 1), it was clear that lower watering regimes can cause a reduction in the LWP_{md} of three chili cultivars. However, the degree of decrement has differed. The results indicated that a restricted water supply caused a decrease in LWP_{md} and chili cultivars responded differently to a similarly restricted water supply. Other studies also reported similar results that drought or restricted water supply can cause a reduction in LWP_{md} and differences dropped in LWP_{md} were also observed between studied chili cultivars which can be due to different adaptations to water deficit [18-19].

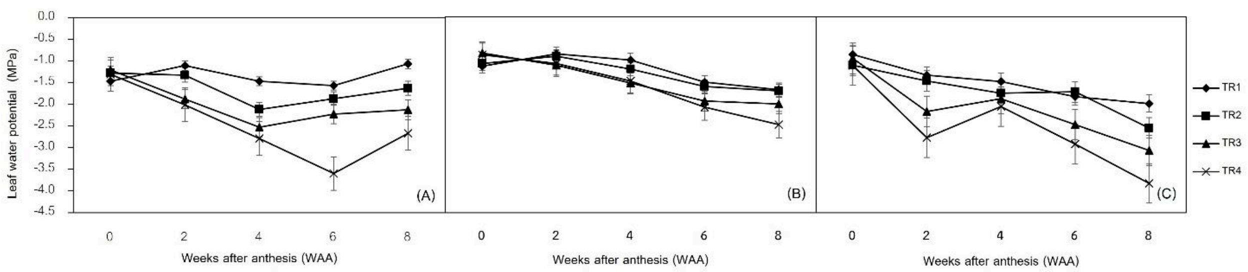

Figure 1. Leaf water potential of *C. annuum* cv. Super-Hot 2 (A) and Hueay-Sii-Ton (B) and *C. frutescens* cv. Kee-Nu-Suen (C) responded to different watering regimes at anthesis (0 WAA) to 8 weeks after anthesis. Means with different letters indicate significant differences by DMRT at a 95% confidence interval.

The SPAD of plant leaves is usually reduced by the effects of water stress [20], and to a certain degree, the result found in this study was contrasted with the previous findings. In this study, the SPAD of water-restricted plants was higher or remained close to the control (Table 2). However, a similar trend was also found in water-stressed Karen KPS chili [21]. Under the water restriction, delayed senescence (or stay-green) may reflect the maintenance of photosynthetic activity and capacity for light harvesting during the re-mobilization of carbon products to the harvested organs of the plant [22-23]. Maintenance of SPAD under drought stress can be considered a positive trait as it indicates the reduction of chlorophyll degradation [24].

Table 1. Effects of chili cultivars, watering regimes, and their interactions on physiological traits and growth rate of *C. annuum* (Super-Hot 2 and Hueay-Sii-Ton) and *C. frutescens* (Kee-Nu-Suen).

Studied factors		LWP_{md} (MPa)	SPAD	F_v/F_m	RGR_{height}	RGR_{width}
Chili cultivars (A)	Super-Hot 2	-1.86 ± 0.09[b1]	56.96 ± 0.33[a]	0.003 ± 0.001[b1]	0.003 ± 0.001	0.759 ± 0.00
	Hueay-Sii-Ton	-1.40 ± 0.06[a]	53.69 ± 0.28[b]	0.00 7 ± 0.001[a]	0.005 ± 0.001	0.759 ± 0.00
	Kee-Nu-Suan	-1.75 ± 0.09[b]	56.91 ± 0.39[a]	0.005 ± 0.001[ab]	0.004 ± 0.001	0.754 ± 0.00
	F-test	**	**	*	ns	ns
Watering regimes (B)	100% MWHC	-1.30 ± 0.05[a]	54.81 ± 0.37[bc]	0.005 ± 0.001	0.006 ± 0.001[a]	0.781 ± 0.00[a]
	80% MWHC	-1.49 ± 0.07[a]	55.85 ± 0.42[b]	0.006 ± 0.001	0.006 ± 0.001[a]	0.758 ± 0.00[b]
	60% MWHC	-1.79 ± 0.10[b]	58.13 ± 0.37[a]	0.006 ± 0.001	0.003 ± 0.001[b]	0.752 ± 0.00[b]
	40% MWHC	-2.11 ± 0.14[c]	54.62 ± 0.40[c]	0.004 ± 0.001	0.001 ± 0.001[b]	0.739 ± 0.00[c]
	F-test	**	**	ns	**	**
AxB	Super-Hot 2 100% MWHC	-1.33 ± 0.08	55.55 ± 0.44[cde]	0.004 ± 0.001	0.005 ± 0.002	0.778 ± 0.003[a]
	Super-Hot 2 80% MWHC	-1.65 ± 0.11	58.79 ± 0.60[ab]	0.004 ± 0.001	0.004 ± 0.002	0.762 ± 0.004[b]
	Super-Hot 2 60% MWHC	-2.00 ± 0.13	60.11 ± 0.65[a]	0.004 ± 0.001	0.002 ± 0.002	0.743 ± 0.006[c]
	Super-Hot 2 40% MWHC	-2.47 ± 0.24	53.38 ± 0.74[f]	0.002 ± 0.001	0.001 ± 0.001	0.754 ± 0.005[bc]
	Hueay-Sii-Ton 100% MWHC	-1.24 ± 0.09	51.39 ± 0.53[g]	0.007 ± 0.002	0.007 ± 0.002	0.787 ± 0.003[a]
	Hueay-Sii-Ton 80% MWHC	-1.30 ± 0.09	54.06 ± 0.56[ef]	0.007 ± 0.001	0.008 ± 0.001	0.752 ± 0.006[bc]
	Hueay-Sii-Ton 60% MWHC	-1.49 ± 0.13	56.13 ± 0.54[cd]	0.007 ± 0.002	0.004 ± 0.003	0.757 ± 0.005[b]
	Hueay-Sii-Ton 40% MWHC	-1.58 ± 0.17	53.18 ± 0.50[fg]	0.005 ± 0.001	0.001 ± 0.002	0.741 ± 0.005[c]
	Kee-Nu-Suan 100% MWHC	-1.34 ± 0.11	57.50 ± 0.77[bc]	0.003 ± 0.001	0.006 ± 0.001	0.778 ± 0.003[a]
	Kee-Nu-Suan 80% MWHC	-1.53 ± 0.12	54.68 ± 0.89[def]	0.007 ± 0.002	0.006 ± 0.002	0.758 ± 0.004[b]
	Kee-Nu-Suan 60% MWHC	-1.88 ± 0.18	58.16 ± 0.69[b]	0.006 ± 0.002	0.003 ± 0.002	0.757 ± 0.003[b]
	Kee-Nu-Suan 40% MWHC	-2.26 ± 0.22	57.30 ± 0.76[bc]	0.004 ± 0.001	0.001 ± 0.002	0.723 ± 0.007[d]
	F-test	ns	**	ns	ns	**

[1] Values are means with SE. The letters indicate significant differences by DMRT at a 95% confidence interval.

Table 2. Leaf greenness of *C. annuum* (Super-Hot 2, Hueay-Sii-Ton) and *C. frutescens* (Kee-Nu-Suen) responded to different watering regimes at anthesis (0 WAA) to 8 weeks after anthesis (8 WAA).

Chili cultivars	Watering regimes (MWHC)	Leaf greenness (SPAD)				
		0 WAA	2 WAA	4 WAA	6 WAA	8 WAA
Super-Hot 2	100%	54.02 ± 0.61[1]	59.29 ± 0.68[b]	56.86 ± 1.03[b]	52.89 ± 1.12[b]	54.69 ± 0.95
	80%	53.40 ± 0.55	66.05 ± 1.10[a]	58.64 ± 1.08[b]	58.29 ± 1.20[a]	57.57 ± 1.43
	60%	52.43 ± 0.53	65.98 ± 1.07[a]	65.15 ± 1.15[a]	59.24 ± 0.85[a]	57.73 ± 1.61
	40%	52.92 ± 0.52	58.57 ± 1.63[b]	50.01 ± 1.62[c]	51.89 ± 1.27[b]	53.49 ± 2.31
F-test		ns	**	**	**	ns
Hueay-Sii-Ton	100%	52.86 ± 0.69[bc]	52.99 ± 0.98[b]	49.94 ± 1.04[b]	50.43 ± 1.16[b]	50.72 ± 1.77[b]
	80%	54.78 ± 1.16[ab]	51.02 ± 1.21[b]	56.20 ± 0.85[a]	57.15 ± 1.35[a]	51.16 ± 1.22[b]
	60%	56.32 ± 1.33[a]	53.37 ± 0.95[ab]	56.17 ± 0.99[a]	58.69 ± 1.44[a]	56.09 ± 1.07[a]
	40%	50.56 ± 0.98[c]	56.56 ± 1.37[a]	49.77 ± 0.51[b]	52.51 ± 0.78[b]	56.50 ± 1.08[a]
F-test		**	**	**	**	**
Kee-Nu-Suen	100%	58.44 ± 0.89	58.72 ± 1.68	58.79 ± 1.92	56.43 ± 2.00[ab]	55.12 ± 1.90[a]
	80%	55.40 ± 1.34	59.76 ± 2.11	57.45 ± 1.98	52.09 ± 1.97[b]	48.72 ± 1.81[b]
	60%	55.46 ± 1.53	56.13 ± 1.44	59.43 ± 1.55	58.96 ± 1.42[a]	60.82 ± 1.62[a]
	40%	56.48 ± 1.16	56.56 ± 1.47	57.09 ± 1.22	61.30 ± 1.74[a]	55.10 ± 2.46[a]
F-test		ns	ns	ns	**	**

[1] Values are means with SE. The letters indicate significant differences by DMRT at a 95% confidence interval.

When considering the changes in F_v/F_m between the studied weeks of the three chili cultivars, it was found that the F_v/F_m had already fluctuated even before the application of watering regimes (Table 3). After applying watering regimes, the F_v/F_m decreased under moderate to severe water deficit. Different watering regimes can cause drought stress on chili and alter the water status inside the plant. In this experiment, despite the apparent changes in LWP_{md}, the F_v/F_m was relatively stable. This contrast can be due to moderate drought's low impact on the F_v/F_m [25-28] and the small decreases can be interpreted as a photoprotection [29].

3.2 Growth and development

Both vegetative growth traits of studied chili plants were low during the experimental period. Chili cultivars differed in the RGR_{height}, in which, Super-Hot 2 had a significantly lower RGR_{height} than Hueay-Sii-Ton. The watering regimes affected the RGR_{width}, the 60 and 40% MWHC resulted in a considerably lower RGR_{width} when compared with the 100 and 80% MWHC. In contrast, the interaction effects were insignificant for the studied factors (Table 1).

It appeared that during the first two weeks after anthesis, chili plants still grew in height and canopy width, and they were affected by reduced water supply. After 2 weeks of anthesis, the growth of the chili plant mostly stopped or declined (Table 4). The mentioned growth pattern can be due to the vegetative growth after anthesis being minimum for chili, and it is not a drought-sensitive period for vegetative development [21]. In addition, the plants may enter the critical phase where the growth rate starts to decrease from abscission and senescence. It can also result from a water supply limitation, which causes a reduction in turgor pressure, wilting, and reduced vegetative growth [30].

Table 3. The maximum quantum yield of PSII (F_v/F_m) of *C. annuum* (Super-Hot 2, Hueay-Sii-Ton) and *C. frutescens* (Kee-Nu-Suen) responded to different watering regimes at anthesis (0 WAA) to 8 weeks after anthesis (8 WAA).

Chili cultivars	Watering regimes (MWHC)	Maximum quantum yield of PSII (F_v/F_m)				
		0 WAA	2 WAA	4 WAA	6 WAA	8 WAA
Super-Hot 2	100%	0.76 ± 0.01[a1]	0.79 ± 0.00[ab]	0.77 ± 0.01	0.80 ± 0.00[a]	0.77 ± 0.01[a]
	80%	0.73 ± 0.01[b]	0.80 ± 0.00[a]	0.77 ± 0.01	0.78 ± 0.01[b]	0.74 ± 0.01[a]
	60%	0.72 ± 0.01[b]	0.78 ± 0.01[b]	0.75 ± 0.01	0.77 ± 0.00[b]	0.69 ± 0.02[b]
	40%	0.78 ± 0.01[a]	0.78 ± 0.00[b]	0.75 ± 0.01	0.77 ± 0.01[b]	0.70 ± 0.02[b]
F-test		**	*	ns	**	**
Hueay-Sii-Ton	100%	0.80 ± 0.00[a]	0.782 ± 0.01	0.790 ± 0.01[a]	0.780 ± 0.00[a]	0.780 ± 0.01[a]
	80%	0.77 ± 0.00[b]	0.769 ± 0.01	0.780 ± 0.01[a]	0.754 ± 0.01[b]	0.686 ± 0.02[b]
	60%	0.77 ± 0.01[b]	0.781 ± 0.01	0.767 ± 0.01[ab]	0.764 ± 0.01[b]	0.707 ± 0.02[b]
	40%	0.72 ± 0.01[c]	0.774 ± 0.01	0.751 ± 0.01[b]	0.750 ± 0.01[b]	0.707 ± 0.02[b]
F-test		**	ns	*	**	**
Kee-Nu-Suen	100%	0.794 ± 0.00[a]	0.771 ± 0.01	0.773 ± 0.01[a]	0.790 ± 0.00[a]	0.76 ± 0.01[a]
	80%	0.770 ± 0.00[b]	0.761 ± 0.01	0.782 ± 0.01[a]	0.766 ± 0.01[a]	0.713 ± 0.01[b]
	60%	0.744 ± 0.01[c]	0.779 ± 0.00	0.763 ± 0.00[a]	0.763 ± 0.01[a]	0.737 ± 0.01[ab]
	40%	0.734 ± 0.01[c]	0.766 ± 0.01	0.737 ± 0.01[b]	0.716 ± 0.02[b]	0.664 ± 0.03[c]
F-test		**	ns	**	**	**

[1] Values are means with SE. The letters indicate significant differences by DMRT at a 95% confidence interval.

3.3 Yield and capsaicin content

The analysis found the effects of cultivars and watering regimes on all studied traits (Table 5). Watering regimes caused a significant reduction in all fruit characteristics and yield. At the same time, the interaction effects indicated that all cultivars had smaller fruit sizes and lower yields when growing under liming water supply. These findings affirm the impact of water supply on crop production [31-32]. The lower watering regimes affected leaf water potential and caused decreases in the number of fruit, fruit weight, and size. If focusing on yield, reducing water supply is not recommended for chili production since it can reduce yield and yield quality by restricting irrigation to 80% of MWHC. The results differed from a previous study suggesting that 80% MWHC can be used to produce *C. frutescens* cv. Karen KPS because it did not affect the yield of studied chili [21]. The contrasted result may be due to the sensitivity of chili cultivars to water stress.

The 40% MWHC yielded a very small yield from the three studied chili cultivars. It was impossible to prepare the dry chili powder to analyze capsaicin content. Thus, Table 6 only showed the capsaicin content, pungency level, and capsaicin yield results from 100, 80, and 60% MWHC treatments. The results show that the capsaicin content and pungency of the three cultivars that grew under different watering regimes were similar. Only capsaicin yield was affected by the combined effects of the chili cultivar and watering regime. Super-Hot 2 was a cultivar with the highest capsaicin yield, followed by Kee-Nu-Suan and Hueay-Sii-Ton. This resulted from different fruit dry weights obtained from chili cultivars, as shown in Table 6. A similar reason can be applied to the effect of watering regimes on capsaicin yield.

The findings were not inconsistent with a previous study which found that restricting water supply to 40% of field capacity resulted in the highest capsaicin yield [7]. In contrast, another study showed no benefit of restricted water supply on the capsaicin content of *C. annuum* [33]. Thus, the restricted watering regime can affect capsaicinoid production in chili cultivars differently. A study suggested that it may depend on the pungency level of the chili cultivars. Those cultivars with low to moderate pungency were more affected by restricted water supply than those with higher pungency [19] in a previous study on *C. frutescens* cv. Karen KPS, the watering regimes at 40% MWHC resulted in the highest capsaicin content and pungency level, while capsaicin yield did not significantly differ [34]. Similarly, a study found that lower water availability can increase capsaicin levels of *C. frutescens* cayenne pepper plants [35].

Table 4. The relative growth rate of *C. annuum* (Super-Hot 2, Hueay-Sii-Ton) and *C. frutescens* (Kee-Nu-Suen) responded to different watering regimes at anthesis (0 WAA) to 8 weeks after anthesis (8 WAA).

Chili cultivars	Watering regimes	RGR_{height} (cm/day)				RGR_{width} (cm/day)			
		Duration (WAA)				Duration (WAA)			
		0-2	2-4	4-6	6-8	0-2	2-4	4-6	6-8
Super-Hot 2	100%	0.016 ± 0.002^{a1}	-0.001 ± 0.002	0.002 ± 0.001	-0.001 ± 0.002	0.016 ± 0.002	-0.006 ± 0.001	0.011 ± 0.001^{a}	-0.001 ± 0.002
Super-Hot 2	80%	0.015 ± 0.002^{a}	0.001 ± 0.001	-0.002 ± 0.001	0.000 ± 0.002	0.015 ± 0.001	-0.006 ± 0.002	0.007 ± 0.002^{a}	0.001 ± 0.004
	60%	0.015 ± 0.003^{a}	-0.002 ± 0.001	0.000 ± 0.001	0.000 ± 0.001	0.014 ± 0.001	-0.007 ± 0.002	0.006 ± 0.001^{a}	-0.004 ± 0.001
	40%	0.008 ± 0.001^{b}	0.000 ± 0.001	0.001 ± 0.001	-0.001 ± 0.001	0.012 ± 0.001	-0.008 ± 0.001	-0.002 ± 0.002^{b}	0.001 ± 0.001
F-test		*	ns	ns	ns	ns	ns	**	ns
Hueay-Sii-Ton	100%	0.025 ± 0.006	0.003 ± 0.001	0.002 ± 0.000	-0.001 ± 0.000	0.017 ± 0.002^{a}	0.010 ± 0.002	0.000 ± 0.001	-0.000 ± 0.001^{a}
	80%	0.020 ± 0.002	0.007 ± 0.001	0.002 ± 0.001	0.001 ± 0.000	0.016 ± 0.002^{a}	0.010 ± 0.002	0.002 ± 0.002	0.002 ± 0.001^{a}
	60%	0.021 ± 0.002	0.008 ± 0.006	0.000 ± 0.005	0.000 ± 0.001	0.010 ± 0.004^{ab}	0.007 ± 0.007	-0.005 ± 0.008	0.003 ± 0.002^{a}
	40%	0.016 ± 0.002	0.004 ± 0.001	0.000 ± 0.001	-0.001 ± 0.001	0.006 ± 0.002^{b}	0.010 ± 0.003	-0.005 ± 0.001	-0.006 ± 0.001^{b}
F-test		ns	ns	ns	ns	*	ns	ns	**
Kee-Nu-Suen	100%	0.012 ± 0.003^{b}	0.001 ± 0.002	0.000 ± 0.001	0.000 ± 0.001	0.016 ± 0.002	0.004 ± 0.002^{ab}	0.004 ± 0.002^{a}	-0.001 ± 0.002
	80%	0.022 ± 0.002^{a}	0.006 ± 0.002	-0.001 ± 0.002	0.000 ± 0.001	0.017 ± 0.003	0.008 ± 0.002^{a}	0.001 ± 0.002^{a}	-0.001 ± 0.002
	60%	0.021 ± 0.003^{a}	0.002 ± 0.002	-0.002 ± 0.001	0.001 ± 0.001	0.019 ± 0.003	0.002 ± 0.001^{ab}	-0.005 ± 0.001^{b}	-0.004 ± 0.002
	40%	0.017 ± 0.002^{ab}	0.001 ± 0.001	-0.002 ± 0.001	0.000 ± 0.001	0.016 ± 0.001	-0.000 ± 0.002^{b}	-0.009 ± 0.002^{b}	-0.001 ± 0.003
F-test		*	ns	ns	ns	ns	*	**	ns

[1] Values are means with SE. The letters indicate significant differences by DMRT at a 95% confidence interval.

Table 5. Changes in fruit characteristics of *C. annuum* (Super-Hot 2 and Hueay-Sii-Ton) and *C. frutescens* (Kee-Nu-Suen) responded to different watering regimes and the interaction effects.

	Studied factors	Number of fruits/plant	Fruit fresh weight (g/plant)	Fruit dry weight (g/plant)	Yield (kg/ha)	Fruit length (cm)	Fruit width (mm)
Chili cultivars (A)	Super-Hot 2	17.00 ± 3.06^{a1}	5.84 ± 3.09^{a}	4.68 ± 0.90^{a}	633.65 ± 123.66^{a}	4.45 ± 0.07^{a}	6.02 ± 0.07^{b}
	Hueay-Sii-Ton	5.07 ± 1.68^{b}	4.05 ± 1.54^{c}	1.45 ± 0.53^{c}	325.61 ± 61.51^{b}	3.52 ± 0.09^{c}	6.49 ± 0.10^{a}
	Kee-Nu-Suan	8.52 ± 2.61^{b}	8.14 ± 2.43^{b}	2.77 ± 0.81^{b}	161.71 ± 97.29^{b}	4.14 ± 0.09^{b}	5.97 ± 0.08^{b}
	F-test	**	**	**	**	**	**
Watering regimes (B)	100% MWHC	22.27 ± 3.38^{a}	20.92 ± 3.54^{a}	6.61 ± 1.00^{a}	836.54 ± 141.49^{a}	4.72 ± 0.08^{a}	6.84 ± 0.06^{a}
	80% MWHC	9.60 ± 2.55^{b}	8.93 ± 2.28^{b}	2.85 ± 0.69^{b}	357.65 ± 91.10^{b}	4.16 ± 0.09^{b}	6.19 ± 0.08^{b}
	60% MWHC	5.80 ± 1.31^{bc}	4.58 ± 1.23^{c}	1.46 ± 0.34^{c}	183.22 ± 49.10^{b}	3.68 ± 0.09^{c}	5.63 ± 0.08^{c}
	40% MWHC	3.11 ± 1.02^{c}	2.93 ± 0.85^{c}	0.94 ± 0.23^{c}	117.22 ± 34.10^{b}	3.54 ± 0.10^{c}	5.72 ± 0.10^{c}
	F-test	**	**	**	**	**	**
AxB	Super-Hot 2 100% MWHC	32.00 ± 3.14	31.53 ± 2.50	9.16 ± 0.68	1,261.01 ± 100.12^{a}	4.94 ± 0.09^{ab}	6.71 ± 0.10^{b}
	Super-Hot 2 80% MWHC	19.33 ± 1.68	17.26 ± 1.14	5.31 ± 0.38	690.48 ± 45.50^{b}	4.66 ± 0.12^{bc}	6.18 ± 0.13^{c}
	Super-Hot 2 60% MWHC	10.33 ± 0.37	9.18 ± 0.36	2.68 ± 0.13	367.31 ± 14.58^{cd}	4.00 ± 0.12^{de}	5.45 ± 0.08^{d}
AxB	Super-Hot 2 40% MWHC	6.33 ± 1.99	5.39 ± 1.36	1.57 ± 0.35	215.81 ± 54.32^{cde}	4.12 ± 0.12^{df}	5.66 ± 0.14^{d}
	Hueay-Sii-Ton 100% MWHC	14.40 ± 1.22	12.60 ± 1.50	4.40 ± 0.50	502.94 ± 59.89^{bc}	4.11 ± 0.12^{de}	7.12 ± 0.13^{a}
	Hueay-Sii-Ton 80% MWHC	3.40 ± 0.76	2.27 ± 0.49	0.80 ± 0.17	91.95 ± 19.47^{e}	3.38 ± 0.14^{f}	6.18 ± 0.16^{c}
	Hueay-Sii-Ton 60% MWHC	1.47 ± 0.35	0.87 ± 0.14	0.37 ± 0.07	34.08 ± 5.70^{e}	3.05 ± 0.18^{f}	6.05 ± 0.21^{c}
	Hueay-Sii-Ton 40% MWHC	1.00 ± 0.50	0.47 ± 0.24	0.23 ± 0.13	17.87 ± 9.47^{e}	3.23 ± 0.20^{f}	6.40 ± 0.20^{bc}
	Kee-Nu-Suan 100% MWHC	20.40 ± 6.97	18.64 ± 6.93	6.27 ± 2.33	745.68 ± 277.07^{b}	5.12 ± 0.22^{a}	6.71 ± 0.23^{b}
	Kee-Nu-Suan 80% MWHC	6.07 ± 1.44	7.26 ± 1.62	2.45 ± 0.55	290.53 ± 64.90^{cde}	4.39 ± 0.12^{cd}	6.20 ± 0.14^{c}
	Kee-Nu-Suan 60% MWHC	5.60 ± 0.42	3.71 ± 0.07	1.34 ± 0.14	148.26 ± 2.79^{de}	3.78 ± 0.14^{e}	5.54 ± 0.10^{d}
	Kee-Nu-Suan 40% MWHC	2.00 ± 0.40	2.95 ± 0.84	1.02 ± 0.18	117.97 ± 33.43^{de}	3.28 ± 0.13^{f}	5.42 ± 0.14^{d}
	F-test	ns	ns	ns	**	**	*

[1] Values are means with SE. The letters indicate significant differences by DMRT at a 95% confidence interval.

Table 6. Changes in capsaicin content, pungency level, and capsaicin yield of *C. annuum* (Super-Hot 2 and Hueay-Sii-Ton) and *C. frutescens* (Kee-Nu-Suen) responded to different watering regimes and the effects of the interaction.

Studied factors		Capsaicin content (ug/g)	Pungency level (SHU)	Capsaicin yield (ug/plant)
Chili cultivar (A)	Super-Hot 2	462.52 ± 13.69[1]	7,588.27 ± 219.00	2,683.70 ± 445.48[a]
	Hueay-Sii-Ton	464.88 ± 0.40	7,312.84 ± 6.41	848.94 ± 299.78[c]
	Kee-Nu-Suan	469.23 ± 0.74	7,545.11 ± 11.78	1,575.49 ± 473.00[b]
	F-test	ns	ns	**
Watering regimes (B)	100%	471.85 ± 4.45	7,423.11 ± 71.21	3,072.83 ± 470.08[a]
	80%	454.63 ± 7.56	7,476.76 ± 120.96	1,341.13 ± 317.79[b]
	60%	469.15 ± 11.35	7,546.36 ± 181.57	694.17 ± 164.82[b]
	F-test	ns	ns	**
AxB	Super-Hot 2 100%	463.03 ± 14.14	7,447.20 ± 226.19	4,268.72 ± 393.65
	Super-Hot 2 80%	470.28 ± 24.58	7,563.20 ± 393.26	2,492.31 ± 58.72
	Super-Hot 2 60%	482.23 ± 36.74	7,754.40 ± 587.89	1,290.06 ± 32.10
	Hueay-Sii-Ton 100%	454.91 ± 0.65	7,317.20 ± 10.39	2,014.07 ± 228.95
	Hueay-Sii-Ton 80%	454.46 ± 0.41	7,310.00 ± 6.58	374.55 ± 76.50
	Hueay-Sii-Ton 60%	454.54 ± 1.13	7,311.33 ± 18.08	158.20 ± 34.10
	Kee-Nu-Suan 100%	466.63 ± 1.17	7,504.93 ± 18.74	2,935.69 ± 1,082.83
	Kee-Nu-Suan 80%	469.90 ± 0.28	7,557.07 ± 4.43	1,156.53 ± 259.90
	Kee-Nu-Suan 60%	470.92 ± 0.22	7,573.33 ± 3.45	634.24 ± 63.50
	F-test	ns	ns	ns

[1] Values are means with SE. The letters indicate significant differences by DMRT at a 95% confidence interval.

4. Conclusions

Restricted water supply affects the physiological traits of the chili plant. It can reduce midday leaf water potential and maximum quantum yield of PSII. The leaf greenness can be enhanced under irrigation of 60% MWHC. The restricted water supply during anthesis to fruit ripening did not affect the relative growth rate in plant height, but the 60% and 40% MWHC can reduce the growth rate of canopy width. The reduction of water supply (80%, 60%, and 40% MWHC) also decreased the yield and fruit size of the three chili cultivars. It cannot significantly induce the capsaicin content or pungency of the studied chili cultivars. Thus, a severe reduction of dry fruit yield significantly reduced the capsaicin yield. Therefore, a reduced water supply during the anthesis through fruit development should be avoided for the three chili cultivars. In addition, the restricted water supply may not be a suitable management for the high capsaicinoid production in chili. However, further study is required since the effects found in pot-grown plants may differ from field-grown plants.

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