

Impact of Drought Stress on the Accumulation of Capsaicinoids in Capsicum Cultivars with Different Initial Capsaicinoid Levels

Paongpetch Phimchan and Suchila Techawongstien¹

Department of Plant Science and Agricultural Resources, Faculty of Agriculture, Khon Kaen University, Khon Kaen 40002, Thailand

Saksit Chanthai

Department of Chemistry, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand

Paul W. Bosland

Department of Plant and Environmental Sciences, New Mexico State University, NM 88003

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Abstract. Capsaicinoids are the alkaloids in hot pepper that cause the sensation of heat when eaten and are affected by a genetic and environment interaction. Drought stress is well recognized as an environmental condition that influences capsaicinoid accumulation. This investigation identified the responses of capsaicinoid accumulation in hot pepper cultivars under drought stress condition. A total of nine cultivars with a different initial pungency level, i.e., low, medium, and high, was subjected to gradual drought stress during the flowering stage. Plants in this drought stress group were supplied with reduced water applications of 25%, 50%, and 75% by volume at 10, 20, and 30 days after flowering (DAF), respectively. Leaf water potential and relative water content were recorded to measure the level of drought stress. The results indicated that all cultivars were subjected to drought stress because of their decrease in leaf water potential and changes in physiological characteristics, e.g., growth and yield performance. In addition, leaf area and shoot-to-root ratio were good criteria for identifying hot pepper cultivars under drought stress because their responses were correlated with the stress level and yield components. Yield performances of the high pungency group did not decrease under drought stress, whereas those of the low pungency group did decrease. In conclusion, capsaicinoid levels increased for all cultivars studied when subjected to drought stress, except for the cultivars in the high pungency group. A yield response under drought stress for the medium pungency group varied and was not found to be associated with drought stress.

Hot peppers (*Capsicum* spp.) are one of the most important vegetables and spices in the world. It has been domesticated for more than 6000 years (Perry et al., 2007). Pungency or the sensation of heat when eaten is caused by capsaicinoids, a unique alkaloid restricted to *Capsicum* species (Suzuki and Iwai, 1984). There is increased use of the capsaicinoids in

pharmacy products, sprays for riot control, self-defense, and the pesticide industries (Shur, 2002). Capsaicinoid accumulation is related to a fruit's age, size, and stage of development (Estrada et al., 1997) and is also regulated by a genotype and an environment interaction (Zewdie and Bosland, 2000).

Hot peppers vary widely in pungency level. Some of the hottest cultivars are found in *C. chinense* that contains 'Habanero', 'Red Savina', and 'Bhut Jolokia' with pungency levels up to more than 1 million Scoville heat units (SHUs) (Bosland and Baral, 2007). The pungency levels of *C. annuum* cultivars are lower than *C. chinense*, but they are the most cultivated *Capsicum* species worldwide. Environmental factors such as temperature, light, water stress, and soil nutrient have been reported to affect the capsaicinoid contents (Murakami et al., 2006; Quagliotti, 1971; Sung et al., 2005).

World production of hot pepper is mostly in tropical countries, e.g., India, Indonesia, Myanmar, Bangladesh, Pakistan, and Thailand

(FAOSTAT, 2009). In Thailand, hot peppers are normally grown under rain-fed and open-field conditions. In most years the hot pepper plant is subjected to gradual drought stress for 30 to 45 d during flowering to fruiting stage, which is a critical stage for yield development (Jaimez et al., 2000). Capsaicinoid content is increased under drought stress (Estrada et al., 1999) with some of the increase resulting from varietal differences (Sung et al., 2005). In addition, the stress level and physiological responses related to growth, yield, and pungency level were good criteria for evaluating hot pepper cultivars with high capsaicinoid levels under drought stress (Sung et al., 2005). Therefore, the objective of this experiment was to study the yield and capsaicinoid responses under drought stress of hot pepper cultivars with different initial capsaicinoid levels.

Materials and Methods

A total of nine hot pepper cultivars with varying growth habit, leaf and fruit characteristics, and pungency levels were selected (Table 1). A low pungent group, less than 50,000 SHUs, consisted of three cultivars, Keenoo-Sakonnakorn (*C. chinense*, a mutant cultivar, which was selected for non-pungency, perennial growth habit, big leaf size, and small fruit size), Num Keaw Tong 80, and Yuyi (both *C. annuum*, annual growth habit, big leaf size, and big fruit size). A medium pungent group (50,000 to 100,000 SHUs) consisted of four cultivars, C 04872 and Takanotsume (*C. annuum*, annual growth habit, big leaf size, and big fruit size), Keenoo-Pama, and Huay-Siiton (*C. annuum*, perennial growth habit, small leaf size, and small fruit size). A high pungent group (greater than 100,000 SHUs) consisted of two cultivars, i.e., Perennial (*C. annuum*) and BGH 1719 (*C. chinense*), both with perennial growth habit, small leaf size, and small fruit size.

The experiment was conducted under a plastic net house at the experimental farm of Khon Kaen University, Khon Kaen Province, Thailand (lat. 16°28' N, long. 102°48' E, 200 m MASL) during May to Oct. 2009. Plants were grown in 2-L plastic pots filled with a mixed media consisting of rice husk, charcoal rice husk from the rice mill industry, filter cake (organic waste materials from sugar mill industry), and cow manure in a ratio of 2:1:1:0.5 (v:v), respectively. Fertilizers were applied in 3-d intervals with the same concentration between control and drought-stressed plants throughout the experiment (adapted from Patricia, 1999). Environmental conditions, i.e., air temperature, light intensity, relative humidity (RH), and rainfall, were recorded. The monthly average maximum air temperature was 43 °C and the minimum was 26 °C (Fig. 1A), and monthly average light intensity was quite high (207 to 231 $\mu\text{mole}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) (Fig. 1B) during the time between transplanting and flowering stage, and it was low (58 to 62 $\mu\text{mole}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) during fruit set and the ripening stage. The monthly average RH was quite high, averaging $\approx 80\%$ throughout the experiment

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Table 1. List of nine hot pepper cultivars and species, pungency category, characteristics, and origin.

Pedigree	Capsicum species	Pungency category ^z	Leaf characteristics	Fruit characteristics	Growth habit ^y	Source
Keenoo-Sakonnakorn	<i>chinense</i>	Low	Big leaf, deltoid shape	Short, slim, elongated, pointed end	Perennial	KKU/Thailand
Num KeawTong 80	<i>annuum</i>	Low	Big leaf, ovate shape	Cayenne, elongated, pointed end	Annual	KKU/Thailand
Yuyi	<i>annuum</i>	Low	Big leaf, ovate shape	Bell, campanulate, sunken end	Annual	Japan
C 04872	<i>annuum</i>	Medium	Big leaf, ovate shape	Cayenne, elongated, blunt end	Annual	AVRDC/Taiwan
Keenoo-pama	<i>annuum</i>	Medium	Small leaf, lanceolate shape	Short, slim, elongated, pointed end	Perennial	Myanmar
Huay-Siiton	<i>annuum</i>	Medium	Small leaf, lanceolate shape	Thai chilli, elongated, pointed end	Perennial	KKU/Thailand
Takanotsume	<i>annuum</i>	Medium	Big leaf, ovate shape	Cluster, elongated, pointed end	Annual	Japan
BGH 1719	<i>chinense</i>	High	Small leaf, deltoid shape	Short, slim, elongated, pointed end	Perennial	USDA/Brazil
Perennial	<i>annuum</i>	High	Small leaf, deltoid shape	Short, slim, campanulate, sunken end	Perennial	USDA/Mexico

^zHigh = greater than 100,000 SHUs; medium = 50,000 to 100,000 SHUs; low = less than 50,000 SHUs.

^yAll cultivars are normally grown and harvest within one season as annual type (plant growth habit of these cultivars is typically normally perennial type, but actually grown and harvest as annual type).

SHUs = Scoville heat units.

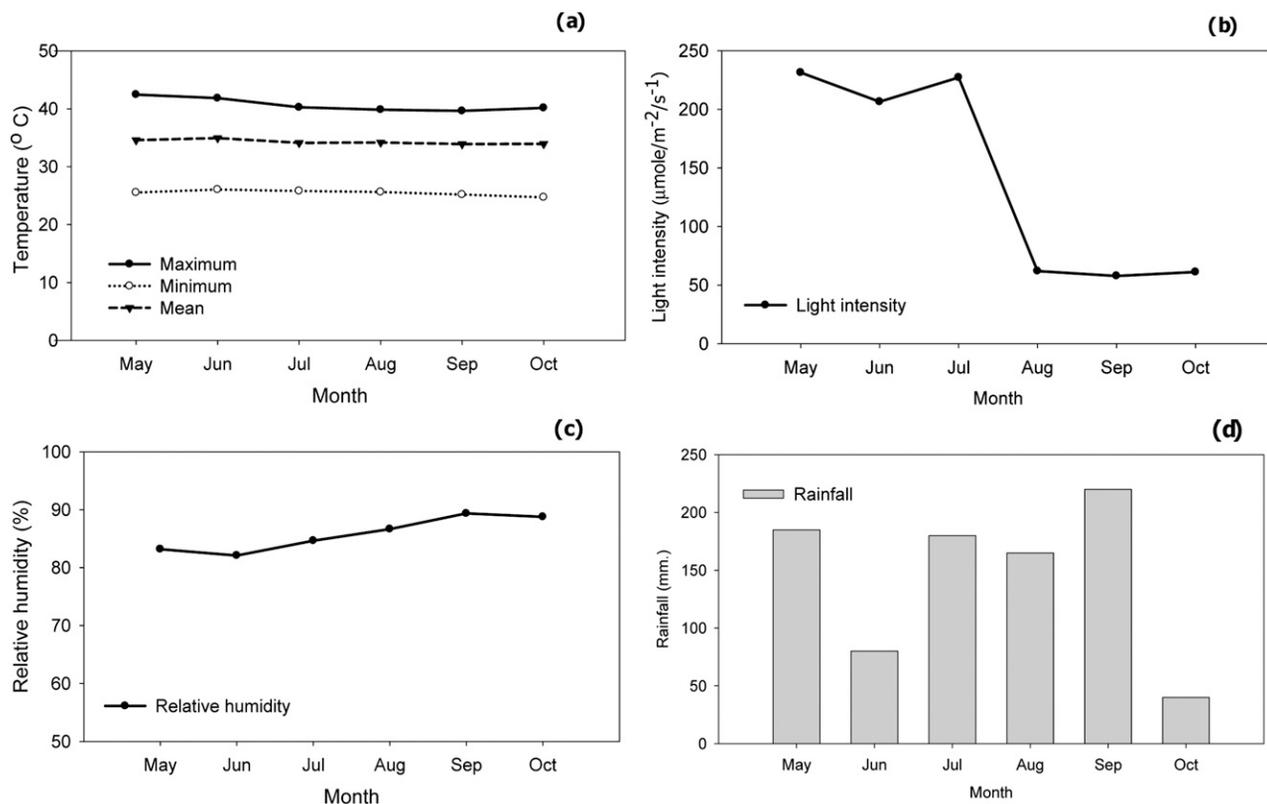


Fig. 1. (A–C) Monthly mean air temperature, relative humidity and light intensity under a plastic net house and (D) monthly mean rainfall under field condition at the experimental farm at Khon Kaen University during the rainy season (May to Oct. 2009).

(Fig. 1C). In addition, the minimum monthly rainfall was 32 mm in October and maximum was 208 mm in September (Fig. 1D).

Plant growth and development were different among cultivars; sowing date of each cultivar was different to synchronize the flowering date for all cultivars. The seeds were sown during 10 Apr. to 30 Apr. 2009. The treatments were arranged in a randomized complete block design with four replications. Plants of each cultivar were divided into two groups: non-drought (control) and drought stress treatments. They were watered daily with different amounts of water. The first group was watered at field capacity for each cultivar throughout the experiment and assigned as the control treatment. The amount of water applied in the control treatment was different based on the difference in field capacity of each cultivar. Plants in the second group

had a gradual decrease in the amount of water supplied after flowering, which was applied during the period from 15 June to 30 Aug. 2009. The amount of water applied in the drought stress treatment was 100%, 75%, 50%, and 25% of the water volume that was supplied to the control plants for each cultivar at 1 to 10, 11 to 20, 21 to 30, and 31 to 40 DAF, respectively (Techawongstien et al., 1992). When severe wilting persisted overnight, the plants in the drought stress treatment were re-watered at the rate of the control plants. The mean values of leaf water potential, relative water content, plant height, stem diameter, leaf area, and specific leaf area were recorded at 10-d intervals for 50 DAF. Midday leaf water potential was measured by using a pressure chamber model DIK-700 (Daiki Rika Kogyo Co. Ltd., Japan), and relative water content was recorded following Kramer (1980).

For leaf area, the third fully expanded mature leaf from the apical main stem was measured by using a leaf area meter (LICOR-3100; LICOR Inc., Lincoln, NE). Specific leaf area was calculated as the ratio of leaf area to leaf dry weight ($\text{cm}^2/\text{g}^{-1}$). Weekly harvests of ripe mature fruits were done for 6 weeks to record the fresh weight. For dry fruit yield, fruits were oven-dried at 80 °C until constant weight. At the second harvest, average fruit weight and fruit size were recorded and their capsaicinoid levels were analyzed, whereas the shoot-to-root ratio was recorded at the final harvest.

Capsaicinoid was extracted from the dried fruit and quantified with high-performance liquid chromatography (HPLC) using a modification of the “short run” protocol (Collins et al., 1995). For each determination, 1 g of hot pepper powder was extracted by shaking

in 10 mL of acetonitrile at 80 °C for 4 h. The extract was filtered with a syringe through 0.45 µL of polyamide and 10 µL of the filtered extract was injected for HPLC analysis using a Shimadzu-Model, 10AT-VP series (Shimadzu Company, Japan). The mobile phases were methanol: deionized water at a ratio of 80:20 at a flow rate of 1.5 mL·min⁻¹ with the ODS C-18 column. The wavelength of detector was set at 284 nm. The external standards for capsaicin and dihydrocapsaicin (Fluka #37274; Fluka Chemie, Buchs, Switzerland) were prepared in 0, 50, 100, 500, and 1,000 ppm solutions. Capsaicin and dihydrocapsaicin were converted to SHUs following Collins et al. (1995). Capsaicinoid content was calculated from the summation of capsaicin and dihydrocapsaicin. A “capsaicinoid yield” was calculated by multiplying the capsaicinoid content per plant by the fruit dry weight per plant.

Statistical analyses were conducted following Gomez and Gomez (1976). Analysis of variance in a randomized complete block design with a *t* test to compare the significant difference between treatments within a cultivar was accomplished. The percentage ratio of drought stressed to control [$100 \times (\text{drought/control})$] for every trait was separated by least significant difference test at $P \leq 0.05$ for comparison between cultivars (Techawongstien et al., 1992).

Results

Under the control conditions, leaf water potentials were similar (–0.2 to –0.3 MPa) throughout the experiment (Fig. 2; Table 2). However, in the drought-stressed plants, the leaf water potentials of all cultivars were significantly lower than those of the control plants at 31 to 40 DAF. Significant differences

of leaf water potential between the drought-stressed and control plants were observed within all cultivars. In addition, relative water content of the drought-stressed plants in all cultivars was significantly lower than those of the control plants (Fig. 3; Table 2). The significant differences between the control and the drought-stressed plants were observed earlier at 21 to 30 DAF until 31 to 40 DAF in all cultivars studied, except for the cultivar Keenoo-pama that had a significant difference only at 31 to 40 DAF (Fig. 3). However, the distinction between leaf water potential of the control and drought-stressed plants in ‘Huay-Siiton’, ‘Keenoo-Pama’, and ‘Perennial’ was less than that in the other cultivars (Fig. 2; Table 2).

Leaf area in the drought-stressed plants was significantly lower than that of the control plants in all of the low pungent cultivars,

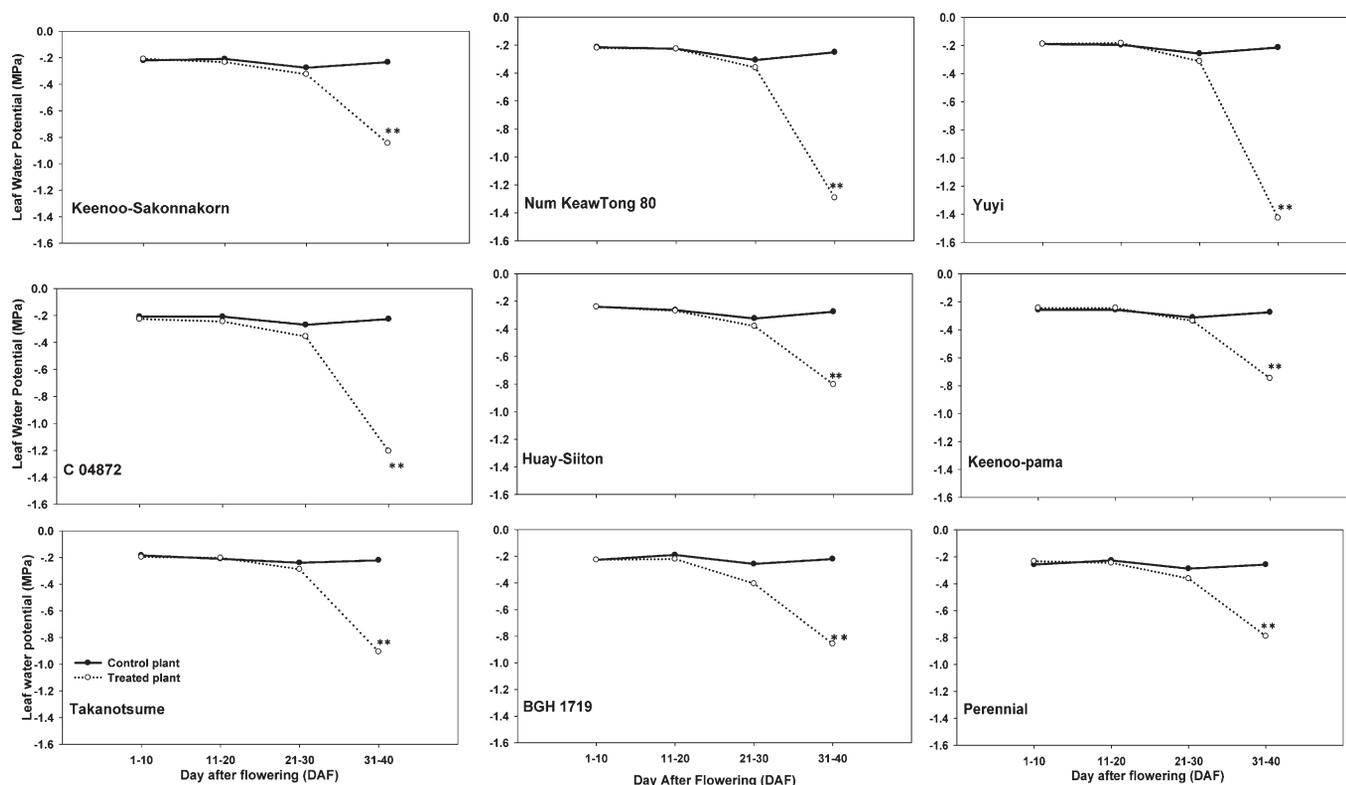


Fig. 2. The values of leaf water potential response at 10-d intervals of the control (—●—) and drought-stressed plants (.....○.....) in nine hot pepper cultivars. **Significant difference between control and drought treatments within cultivar at $P \leq 0.01$ level by *t* test.

Table 2. Leaf water potential (LWP), relative water content (RWC), leaf area (LA), and specific leaf area (SLA) between the control plant (C) and drought-stressed plant (T) of nine hot pepper cultivars at 31 to 40 d after flowering.

Pedigree	LWP (MPa)			RWC (%)			LA* (cm ²)			SLA (cm ² ·g ⁻¹)		
	C	T ^z	%T/C ^y	C	T	%T/C	C	T	%T/C	C	T	%T/C
Keenoo-Sakonnakorn	–0.2	–0.9**	450	90.3	68.6**	75.9	31.7	26.3*	83.0	364.5	311.6*	85.5
Num Keaw Tong 80	–0.3	–1.3**	433	90.0	66.2**	73.5	30.6	25.2*	82.4	321.1	241.9**	75.3
Yuyi	–0.2	–1.4**	700	92.7	65.8**	70.9	37.6	30.0*	79.7	354.5	287.8*	81.2
C 04872	–0.2	–1.2**	600	92.7	64.4**	69.3	27.2	24.3	89.2	339.9	275.2**	80.9
Huay-Siiton	–0.3	–0.8**	267	88.5	67.2**	75.9	12.2	10.4	85.5	373.1	351.1	94.1
Keenoo-Pama	–0.3	–0.8**	267	92.2	74.0**	80.1	10.5	10.4	99.5	377.9	355.7	94.1
Takanotsume	–0.2	–0.9**	450	90.3	63.7**	70.5	23.3	19.2*	82.5	301.4	274.0	90.9
BGH 1719	–0.2	–0.9**	450	91.7	66.4**	72.3	14.4	12.9	89.8	339.4	293.4*	86.4
Perennial	–0.3	–0.8**	267	90.8	70.6**	77.7	15.1	14.1	93.6	364.1	318.9*	87.6

*, **Significant difference between control and drought stressed plants within cultivar at $P \leq 0.05$ and 0.01 *P* levels by *t* test, respectively.

^yPercentage ratio of drought stressed to control [$100 \times (\text{drought-stressed/control})$].

*Leaf area was measured from the third fully expanded leaf of the apical main stem.

whereas there were not significant differences in the high pungent cultivars (Table 3). However, leaf area of the medium pungent cultivars varied because leaf area of the three cultivars in this group was not significantly lower than their control plants. The leaf area of cultivar Takanotsume was significantly decreased under drought stress. In addition, specific leaf area of the drought-stressed plants was significantly lower than those of the control plants in both low and high pungent cultivars, whereas performance was inconsistent in medium pungent cultivars. Specific leaf area of cultivar C04872 was significantly decreased compared with their control plants. Nevertheless, specific leaf area of the other three cultivars in the medium pungent group was not significantly decreased under drought stress. It was interesting to note that leaf area and specific leaf area of cultivars Keenoo-pama and Huay-Siiton were not significantly decreased under drought stress.

Under drought stress conditions, plant height was not significantly shorter than the control plants in any of the cultivars, except for the cultivar Takanotsume (Table 3). In addition, the stem diameter of two cultivars of the low pungent group (Yuyi and Keenoo-Sakonnakorn), one cultivar of the medium pungent group (Keenoo-pama), and one cultivar of the high pungent group (BGH 1719) was significantly lower than that of their control plants, whereas the others were not. Shoot-to-root ratios in the drought-stressed plants of all low pungent cultivars were significantly decreased, whereas they were

not for the high pungent cultivars. The responses of the shoot-to-root ratios between the drought-stressed and control plants were varied in the medium pungent cultivars. The shoot-to-root ratios of cultivars C04872 and Takanotsume were significantly decreased but were not significantly decreased for cultivars Keenoo-Pama and Huay-Siiton (Table 3).

Fruit length and fruit width were not significantly different between the drought-stressed plants and the control plants (Table 4). The dry fruit yield in the drought-stressed plants of all low pungent cultivars were significantly lower as compared with those of the control plants, i.e., 'Yuyi' (51.7%), 'Keenoo-Sakonnakorn' (74.2%), and 'Num Keaw Tong' 80 (48.8%). The decreases of dry fruit yield in the water-stressed plants of medium pungent cultivars were inconsistent, in which cultivars C04872

(54.2%) and Takanotsume (68.3%) were significantly lower than those of the controls, but cultivars Keenoo-Pama (82.1%) and Huay-Siiton (81.4%) were not. In addition, fruit number per plant in the drought-stressed plants of low and medium pungent cultivars were significantly lower than those of the control plants, except for cultivar Keenoo-Pama (90.4%). However, this decrease was not observed in the high pungent cultivars (Table 4).

Capsaicin, dihydrocapsaicin, and total capsaicinoid contents in the drought-stressed plants of low and medium pungent cultivars significantly differed from the controls (Table 5). Capsaicinoid contents were decreased in the high pungent cultivars under drought stress, but this decrease was not significantly different from the control plants. The total capsaicinoid contents in the drought-stressed plants

Table 3. Plant height, stem diameter, and shoot-to-root ratio between the control plants (C) and drought-stressed plants (T) of nine hot pepper cultivars at second harvest.

Pedigree	Plant ht (cm)			Stem diam (cm)			Shoot-to-root ratio		
	C	T ^z	%T/C ^y	C	T	%T/C	C	T	%T/C
Keenoo-Sakonnakorn	151.9	136.8	90.1	2.0	1.7*	82.8	5.2	3.7**	71.5
Num Keaw Tong 80	167.9	161.3	96.1	1.7	1.5	87.8	5.2	3.1**	59.8
Yuyi	121.3	120.1	99.0	1.6	1.3*	83.5	5.1	3.8**	74.9
C 04872	155.5	139.5	89.7	1.7	1.5	86.0	5.0	3.2**	63.7
Huay-Siiton	146.5	133.3	91.0	1.7	1.6	88.5	5.2	4.8	92.8
Keenoo-Pama	128.9	138.6	107.5	1.4	1.2*	85.9	5.1	4.9	95.0
Takanotsume	125.3	103.8*	82.8	1.2	1.1	92.5	5.0	3.6**	71.6
BGH 1719	120.1	111.9	92.8	1.7	1.1**	67.0	5.0	4.6	91.4
Perennial	144.8	140.3	96.8	1.4	1.3	89.5	5.1	4.8	94.7

^z*, **Significant difference between control and drought stressed plants within cultivar at $P \leq 0.05$ and 0.01 P levels by t test, respectively.

^yPercentage ratio of drought stressed to control [$100 \times (\text{drought-stressed}/\text{control})$].

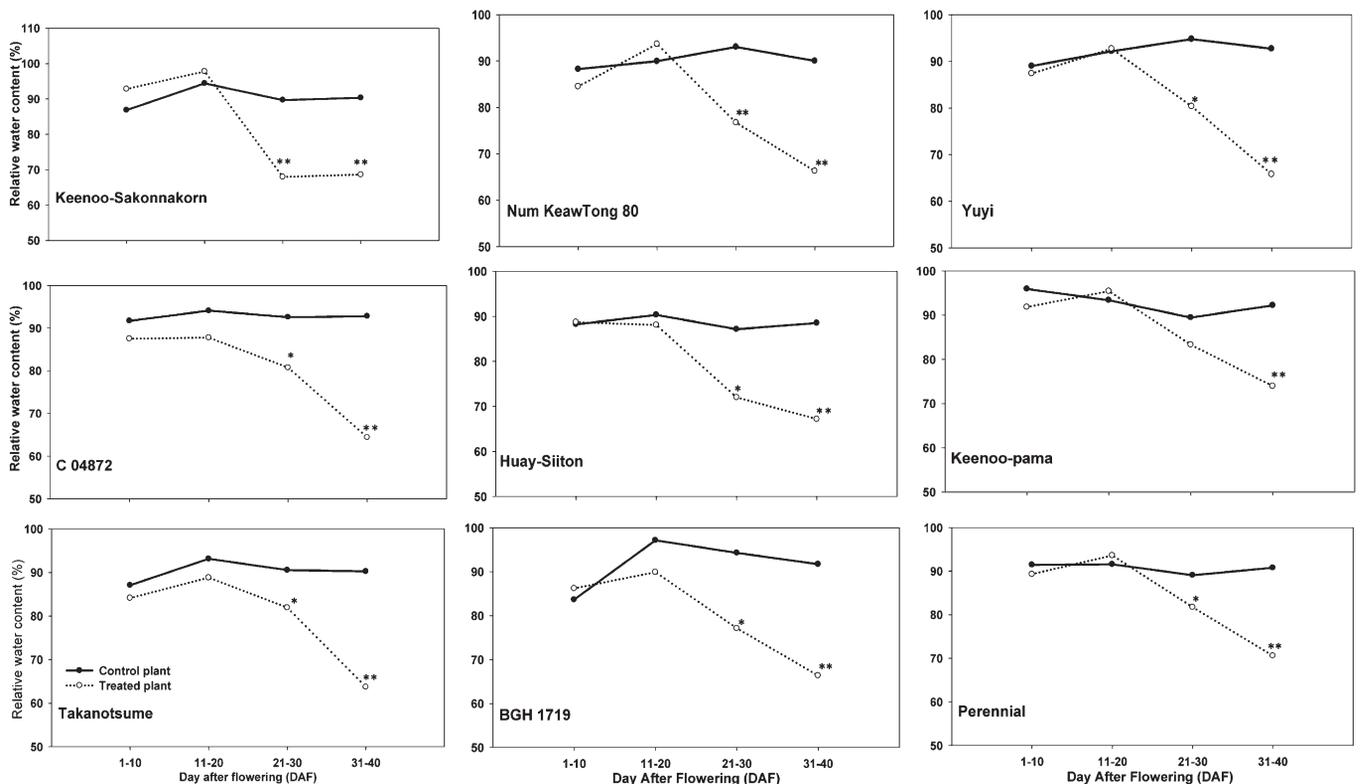


Fig. 3. The values of relative water content response at 10-d intervals of the control (—●—) and drought-stressed plants (---○---) in nine hot pepper cultivars. **Significant difference between control and drought-stressed plants within cultivar at $P \leq 0.01$ level by t test.

Table 4. Yield and fruit quality between the control plants (C) and drought-stressed plants (T) of nine hot pepper cultivars.

Pedigree	Dry fruit yield (g/plant)			No. of fruit/plant			Fruit length (cm)			Fruit width (cm)		
	C	T ^z	%T/C ^y	C	T	%T/C	C	T	%T/C	C	T	%T/C
Keenoo-Sakonnakorn	69.9	51.7**	74.2	166.8	124.8*	74.8	3.2	3.2	98.8	0.38	0.37	97.4
Num Keaw Tong 80	120.8	59.0**	48.8	32.3	18.3**	56.6	7.3	7.1	97.1	1.54	1.52	98.7
Yuyi	128.0	66.3**	51.7	71.8	42.3**	58.9	5.6	5.5	97.7	3.74	3.73	99.7
C 04872	161.1	87.3**	54.2	44.5	21.3**	47.8	7.8	7.5	96.9	1.72	1.65	95.9
Huay-Siiton	128.1	104.2	81.4	259.0	194.5*	75.1	4.3	4.2	96.7	0.58	0.57	98.3
Keenoo-Pama	119.6	98.3	82.1	308.0	278.5	90.4	3.6	3.6	98.3	0.51	0.50	98.0
Takanotsume	41.4	28.4**	68.3	88.3	46.0**	52.1	8.4	6.2**	74.0	2.21	2.20	99.5
BGH 1719	96.4	80.4	83.5	281.0	233.0	82.9	2.8	2.4*	88.1	0.68	0.67	98.5
Perennial	89.1	71.0	79.8	264.0	241.5	91.5	2.8	2.8	98.6	0.55	0.53	96.4

** , **Significant difference between control and drought stressed plants within cultivar at $P \leq 0.05$ and 0.01 P levels by t test, respectively.

^yPercentage ratio of drought stressed to control [$100 \times (\text{drought-stressed}/\text{control})$].

Table 5. Capsaicin (Cap), dihydrocapsaicin (Di), capsaicinoid (CAPS), and capsaicinoid yield (CAPS yield) between the control plants (C) and drought-stressed plants (T) of nine hot pepper cultivars.

Pedigree	Cap (SHU ^s)			Di (SHU)			CAPS (SHU)			CAPS yield (mg/plant)		
	C	T ^z	%T/C ^y	C	T	%T/C	C	T	%T/C	C	T	%T/C
Keenoo-Sakonnakorn	ND	1,404**	—	411	1,500**	379.1	411	2,904**	721.9	—	9**	—
Num Keaw Tong 80	10373	24,300**	235.1	4,423	36,450**	855.0	14,796	55,350**	378.4	120	218**	181.7
Yuyi	17998	27,000**	150.1	12,723	25,050**	197.4	30,721	52,200**	170.2	263	231*	87.8
C 04872	33680	37,106	110.3	16,492	25,650**	155.7	50,172	62,700**	125.0	539	365**	67.7
Huay-Siiton	32773	36,906	112.6	15,809	18,000*	113.9	48,582	54,900*	113.0	415	382*	92.0
Keenoo-Pama	41732	49,950*	119.8	21,347	24,000	112.5	63,079	74,100*	117.5	504	486	96.4
Takanotsume	34503	44,850*	130.0	22,888	36,150**	158.0	57,391	81,000**	141.2	159	154	96.9
BGH 1719	78913	77,616	98.4	32,773	31,086	94.9	11,1686	108,702	97.3	718	583**	81.2
Perennial	78239	71,661	91.6	50,723	47,629	93.9	12,8961	119,289	92.5	766	565**	73.8

** , **Significant difference between control and drought stressed plants within cultivar at $P \leq 0.05$ and 0.01 P levels by t test, respectively.

^yPercentage ratio of drought stressed to control [$100 \times (\text{drought-stressed}/\text{control})$].

^sSHU is the pungency unit (Scoville heat unit).

ND = not detected.

of the low pungent cultivars ranged from 2,850 to 55,350 SHUs, whereas those in the medium pungent cultivars ranged from 54,900 to 81,000 SHUs and in the high pungent cultivars ranged from 108,702 to 119,289 SHUs. It is interesting to note that the relative increase of capsaicinoids in the drought-stressed plants of low pungent cultivars (170% to 721%) was obviously higher than those of the medium pungent cultivars (113% to 141%). However, capsaicinoid contents of the drought-stressed plants in the high pungent cultivars were slightly decreased (3% to 8%) as compared with the other two groups. Nevertheless, the capsaicinoid yield, that was calculated from capsaicinoid amounts multiplied by dry fruit yield, was decreased under drought stress for all cultivars, except 'Keenoo-Sakonnakorn' and 'Num Keaw Tong 80'.

Discussion

Under drought stress, a plant's physiological characteristics are affected such as photosynthesis and transpiration (Cornic and Massacci, 1996), which in turn affect plant growth and yield (Aloni et al., 1991; Dorji et al., 2005). In our experiment, all the drought-stressed plants expressed a decrease in leaf water potential implying a difference in plant water status (González-Dugo et al., 2007). The leaf area, which is an indicator of photosynthetic rates (Ismail et al., 2002), shoot-to-root ratio, and dry fruit yield were significantly decreased as a result of drought stress. Kramer (1980) state that good criteria to evaluate different responses among plant cultivars to water

stress are ones that correlate with the stress level, their growth, and yield components. From our results, leaf area and shoot-to-root ratio responses were a good criterion for hot pepper responses under drought stress, because the responses were correlated with the water status and fruit yield performances. Plant height and stem diameter showed a varied response with stress level and yield performance; therefore, they would not be a good criterion for identifying cultivar responses under drought stress. The results reported here show that decreases in leaf water potential were different among the cultivars. It was interesting to note that cultivars with small leaf, small fruit, and perennial habit, e.g., 'Keenoo-pama', 'Huay-Siiton', and 'Perennial', had better water retention. In addition, all of the mentioned cultivars are medium and high pungent cultivars. The cultivar Keenoo-Pama maintained normal water status longer than the other cultivars. 'Yuyi', 'C04872', and 'Takanotsume', with a big leaf, big fruit, and annual habit, showed poorer water retention. These observations might be attributed to their smaller leaf area, resulting in less transpiration during drought stress and lower decreases in shoot-to-root ratio. Although the decrease in shoot-to-root ratio between the control and drought-stressed plants within the four cultivars, BGH 1719, Keenoo-Pama, Huay-Siiton, and Perennial, were similar, water retention in 'BGH1719' was slightly lower than the other three cultivars. This might be because cultivar BGH 1719 is *C. chinense*, which normally has a bigger leaf

size on the lower part of stem than the upper part and resulted in higher transpiration.

Drought stress had no significant effect on fruit size, except for 'Takanotsume' and 'BGH 1719'. Drought stress did affect fruit number and subsequently fruit yield. The fruit yield of the high pungent cultivars did not decrease under drought stress, which corresponds with the leaf area and shoot-to-root ratio responses. As one may expect, a decrease in fruit yield performance of the low pungent cultivars under drought stress was associated with a large decrease in leaf area and shoot-to-root ratio. However, among the medium pungent cultivars, no trends could be determined for all the responses under drought stress. Although all the cultivars in the medium pungent group are *C. annuum*, their growth habit and fruit type are different from the low and high pungent groups, which may explain the inconsistent responses. Cultivars Keenoo-pama and Huay-Siiton are semiperennial types and categorized as "chili" in Thailand as a result of their fruit type and pungency level, whereas cultivars C 04872 and Takanotsume are annual types and are categorized as "long cayenne" and "cluster chili," respectively. Therefore, genetic differences among the medium pungency cultivars could explain their expression as physiological and drought responses (Ismail and Davies, 1997). Based on their less susceptibility to reductions in leaf area, shoot-to-root ratio, and fruit yield, 'Huay-Siiton' and 'Keenoo-Pama' might be categorized as drought-tolerant as compared with those of the other cultivars in this group.

Capsaicinoids start accumulating early fruit development and reach a maximum at 30 to 50 DAF depending on the cultivar (Estrada et al., 2000). Hence, all cultivars studied were affected by drought stress because they were subjected to the stress since flowering stage and lasted until 40 to 55 DAF, depending on the cultivars. Previous studies have reported that capsaicinoid content increased under drought stress (Estrada et al., 1999). However, in our study, the significant increases in capsaicinoids were observed only for the low and medium pungent cultivars but not for the high pungent cultivars. These results might be explained by the fact that a genotype and genotype–environment interaction affected capsaicinoid content (Zewdie and Bosland, 2000), in which the genotype effect was larger than the environmental effect (Gurung et al., 2011b; Zewdie and Bosland, 2000). In addition, Gurung et al. (2012) found high stability of cultivars with high pungency, whereas the lower pungent cultivars were very sensitive to environment. From our results, it is notable that the fruits of the high pungent cultivars are smaller than the other cultivars used, and this might indicate that the capsaicinoids in the small fruit of the high pungent cultivars are less affected by drought stress than those of the big fruit with low and medium pungency. A similar finding was reported by Gurung et al. (2001a) in which the environment has less effect on plant growth, yield, and capsaicinoid contents for small fruit cultivars. Moreover, when considering differences between leaf sizes, we found that all high pungent cultivars and some of medium pungent cultivars (‘Huay Siiton’ and ‘Keenoo-Pama’) have smaller leaf sizes than the other cultivars and resulted in less responses to drought stress for physiological, growth, yield, and including capsaicinoid contents. In addition, it is notable that a very significant trend in the low pungent group was that the dihydrocapsaicin content was higher than the capsaicin content particularly for ‘Keenoo-Sakonnakorn’ and ‘Num Keaw Tong 80’. This might be because the low pungent cultivars (Keenoo-Sakonnakorn and Num Keaw Tong 80) gave the low ratio of capsaicin to dihydrocapsaicin and might have resulted in the dihydrocapsaicin in the drought-stressed plants being higher than capsaicin in these cultivars. This result is similar to Gurung et al. (2012) in that the low ratio of capsaicin to dihydrocapsaicin was observed in the low pungent cultivars, and dihydrocapsaicin in these cultivars was affected by environment stress more than capsaicin.

The high pungent cultivars had good water retention and showed minimum effect of drought stress on yield and capsaicinoid contents. Drought stress effects were high in the low and medium pungent cultivars. An explanation may be attributed to the different responses in capsaicinoid biosynthesis among the different cultivars, which will be studied in the future.

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