INFLUENCE OF IRRIGATION REGIMES ON PHYSIOLOGICAL TRAITS, YIELD, AND CAPSAICINOIDS PRODUCTION IN CHILI

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อิทธิพลของการให้น้ำต่อลักษณะทางสรีรวิทยา ผลผลิต และการสร้างสารแคปไซซินอยด์ในพริก

พิมพ์วิภา อารมณ์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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Suranaree University of Technology has approved this thesis submitted in partial fulfillment of the requirements for a Master's Degree.

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พริก/สภาวะเครียดจากการขาดน้ำ/ศักย์ของน้ำในใบพืช/ความสามารถในการอุ้มน้ำ/ประสิทธิภาพการสังเคราะห์แสง/แคปไซซิน

น้ำเป็นปัจจัยหลักซึ่งส่งผลต่อกระบวนการทางสรีรวิทยา การเจริญเติบโต ผลผลิต และองค์ประกอบของไฟโตนิวเทรียนท์ในพริก การให้น้ำอย่างเหมาะสมสามารถส่งเสริมการเจริญเติบโตและทำให้พริกให้ผลผลิตได้ตามศักยภาพของพันธุ์ การศึกษาในพืชหลายชนิดพบว่าความเครียดจากการขาดน้ำสามารถกระตุ้นการสร้างและสะสมสารทุติยภูมิในพืชได้ อย่างไรก็ตาม การตอบสนองนี้มีความจำเพาะเจาะจง หากต้องการนำเอาการจัดการน้ำมาใช้เพื่อการผลิตพริกให้มีปริมาณแคปไซซินอยด์สูงจึงจำเป็นต้องมีการศึกษาการตอบสนองของพันธุ์พริกที่สนใจ การศึกษานี้มีวัตถุประสงค์เพื่อตรวจสอบผลของการให้น้ำต่อลักษณะทางสรีรวิทยา การเจริญเติบโต ผลผลิต และปริมาณแคปไซซินของพริก *Capsicum annuum* และ *C. frutescens* ภายใต้โรงเรือน และเพื่อประเมินผลของการให้น้ำต่อลักษณะทางการเกษตร และปริมาณแคปไซซินอยด์ของพริก *C. annuum* และ *C. frutescens* ในสภาพแปลง โดยการศึกษาแบ่งออกเป็นสองการทดลอง

การทดลองที่ 1 เป็นการทดลองในกระถางซึ่งปลูกภายใต้โรงเรือน วางแผนการทดลองแบบสุ่มสมบูรณ์ โดยจัดตำรับการทดลองแบบ 3x4 แฟคทอเรียล ดำเนินการทดลองโดยใช้พริก 3 สายพันธุ์ ได้แก่ ซุปเปอร์ฮอต 2 และห้วยสีทน (*C. annuum*) และ ขี้หนูสวน (*C. frutescent*) โดยต้นพริกได้รับน้ำที่แตกต่างกันสี่ระดับ คือ 100, 80, 60 และ 40% ของความสามารถในการอุ้มน้ำสูงสุดของดินปลูก (maximum water holding capacity, MWHC) ในระยะหลังจากดอกบานจนถึงการพัฒนาของผล ผลการศึกษาพบว่าพริกทั้งพันธุ์ 3 มีศักย์ของน้ำในใบ (LWP_{md}) ความเขียวใบ (SPAD) อัตราการเจริญเติบโตสัมพัทธ์ทางด้านความสูง (RGR_{height}) ดัชนีพื้นที่ใบ (LAI) จำนวนผล/ต้น น้ำหนักผลสดและแห้ง ขนาดผล ดัชนีการเก็บเกี่ยว (HI) รวมถึงผลผลิตแคปไซซินที่แตกต่างกันอย่างมีนัยสำคัญทางสถิติ ในขณะที่ระดับน้ำที่ลดลงยับยั้งกระบวนการทางสรีรวิทยา การเจริญเติบโต ผลผลิต และปริมาณแคปไซซินในพริกที่ทำการศึกษา โดยการให้น้ำที่ 40% MWHC ทำให้ LWP_{md} และประสิทธิภาพการสังเคราะห์แสงสูงสุด (F_v/F_m) ลดลงอย่างมากเมื่อเปรียบเทียบกับการให้น้ำที่ระดับอื่น ๆ ทั้งยังจำกัดอัตราการเจริญเติบโตสัมพัทธ์ทางด้านความกว้างทรงพุ่ม (RGR_{width}) และ LAI และทำให้ผลผลิตและผลผลิตแคปไซซินลดลง ในทางตรงกันข้าม การให้น้ำที่ 60% MWHC สามารถทำให้พริกมี SPAD สูงที่สุด การให้น้ำที่ 60 และ 40% MWHC ทำให้จำนวนผล/ต้น น้ำหนักผลสดและแห้ง ตลอดจนขนาดผลลดลง สำหรับการให้น้ำที่ 40% MWHC ให้ผลผลิตแห้งในระดับต่ำมาก และไม่สามารถวิเคราะห์ปริมาณแคปไซซินได้ ขณะที่การให้น้ำที่ 80 และ 60% MWHC ไม่สามารถกระตุ้นปริมาณแคปไซซิน

หรือความเผ็ดของพริกที่ทำการศึกษาได้อย่างมีนัยสำคัญทางสถิติ ด้วยเหตุนี้ผลผลิตแคปไซซินของพริกที่ได้รับน้ำในปริมาณจำกัดจึงลดลงอย่างมากจากการลดลงของผลผลิตผลแห้ง เมื่อพิจารณาการลดลงของผลผลิตแคปไซซินเมื่อได้รับความเครียดจากการขาดน้ำพบว่า พริกพันธุ์ห้วยสีทนมีเปอร์เซ็นต์การลดลงของผลผลิตแคปไซซินสูงกว่าพริกอีก 2 พันธุ์ที่ทำการศึกษา จึงเลือกพริกพันธุ์ซุปเปอร์ฮอต 2 และ ขี้หนูสวน รวมถึงเลือกการให้น้ำที่ระดับ 60% MWHC สำหรับการศึกษาในการทดลองที่ 2

การทดลองที่ 2 เป็นการศึกษาภายใต้สภาพแปลง วางแผนการทดลองแบบ 2x2 split-plot (3 ซ้ำ) โดยใช้พริก 2 พันธุ์ ได้แก่ ซุปเปอร์ฮอต 2 และขี้หนูสวน และให้น้ำ 2 ระดับ คือ 100 และ 60% MWHC ในระยะหลังจากดอกบานจนถึงการพัฒนาของผล ผลการทดลองพบว่า พริกขี้หนูสวนมี RGR_{height} และ RGR_{width} จำนวนผล/ต้น น้ำหนักผลแห้ง HI ปริมาณแคปไซซิน ไดไฮโดรแคปไซซิน และความเผ็ด สูงกว่าพริกซุปเปอร์ฮอต 2 ทั้งยังสามารถให้ผลผลิตแคปไซซินอยด์สูงกว่าพริกซุปเปอร์ฮอต 2 ถึง 1.5 เท่า สำหรับการให้น้ำระหว่างหลังดอกบานถึงผลพริกสุก ไม่ส่งผลต่อ RGR_{height} และ RGR_{width} LAI และ HI การให้น้ำที่ 60% MWHC ทำให้ขนาดผลของพริกทั้งสองพันธุ์ลดลง หากสามารถส่งเสริมน้ำหนักผลแห้ง ปริมาณแคปไซซินอยด์ ระดับความเผ็ด และผลผลิตแคปไซซินอยด์ของพริกทั้งสองพันธุ์ที่ทำการศึกษาได้อย่างมีนัยสำคัญทางสถิติ

จากผลการศึกษาสามารถสรุปได้ว่าการจำกัดการให้น้ำในระยะหลังจากดอกบานจนถึงการพัฒนาของผลไม่เหมาะสมสำหรับการนำมาใช้เพื่อการกระตุ้นการสร้างและสะสมแคปไซซินในพริกทั้งสามพันธุ์ซึ่งปลูกในกระถางภายใต้โรงเรือน เนื่องจากการขาดน้ำจำกัดกระบวนการทางสรีรวิทยา การเจริญเติบโต และการให้ผลผลิตของพริก โดยไม่สามารถส่งเสริมการสร้างและสะสมแคปไซซินในผลพริกได้ ในขณะที่การจำกัดการให้น้ำที่ระดับ 60% MWHC ในสภาพแปลงปลูกเป็นวิธีการที่มีศักยภาพในการส่งเสริมการสร้างและสะสมแคปไซซินอยด์ในพริก ทั้งนี้ จำเป็นต้องคัดเลือกพันธุ์พริกที่สามารถให้ผลผลิตแคปไซซินอยด์สูงภายใต้สภาวะเครียดจากการขาดน้ำมาใช้ร่วมกับการจำกัดการให้น้ำ

สาขาวิชาเทคโนโลยีการผลิตพืช
ปีการศึกษา 2565

ลายมือชื่อนักศึกษา พิมพ์ชนิดา อารมณ์
ลายมือชื่ออาจารย์ที่ปรึกษา

PIMVIPA AROM : INFLUENCE OF IRRIGATION REGIMES ON PHYSIOLOGICAL TRAITS, YIELD, AND CAPSAICINOIDS PRODUCTION IN CHILI. THESIS ADVISOR : DR.WANPLOY JINAGOOL, 104 PP.

Capsicum/Drought stress/Leaf water potential/Water holding capacity/Photosynthetic efficiency/Capsaicin

Water supply is a primary factor affecting the physiological process, growth, yield, and phytonutrient composition in chili. Optimum watering can improve crop growth and allow yield production according to the potential of cultivar. In several studies, drought stress can stimulate the production and accumulation of secondary metabolites in plants, however, this response is specific. To use water management for high capsaicinoid production in chili, it is necessary to study the responses of the interested chili cultivars. This study aimed to investigate the effects of watering regimes on the physiological traits, growth, yield, and capsaicin content of *C. annuum* and *C. frutescens* under greenhouse conditions and to evaluate the effects of watering regimes on agronomic traits and capsaicinoid content of *C. annuum* and *C. frutescens* under field conditions. The study was divided into two experiments.

Experiment 1 was a pot experiment grown under greenhouse conditions. The experimental design was a 3x4 factorial in CRD, conducted on three chili cultivars: Super-Hot 2 and Huay-Siiton (*C. annuum*) and Kee-Nu-Suan (*C. frutescent*). The chili plants were irrigated with four watering regimes: 100, 80, 60, and 40% of maximum water holding capacity (MWHC) after the anthesis through fruit development. It was found that leaf water potential (LWP_{md}), leaf greenness (SPAD), relative growth rate in plant height (RGR_{height}), leaf area index (LAI), fruits/plant, fruit fresh and dry weights, fruit sizes, harvest index (HI), and capsaicin yield of three chili cultivars were significantly different. The study also found that reduced watering regimes can inhibit physiological process, growth, yield, and capsaicin content in studied chilies. The watering regimes at 40% MWHC drastic decreased LWP_{md} and the maximum quantum yield of PSII (F_v/F_m) compared with other watering regimes. It also limited relative growth rate in canopy width (RGR_{width}) and LAI, and reduced yield and capsaicin yield. On the contrary, the 60% MWHC gave the highest SPAD. The watering regimes at the 60 and 40% MWHC decreased fruit/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 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 greatly reduced by severe reduction of fruit dry yield under restricted water supply. Considering the reduction of capsaicin yield under restricted watering regimes, Hueay-Sii-Ton had higher percentage of capsaicin yield reduction than the other two studies cultivars. Therefore, Super-Hot 2 and Kee-Nu-Suen, and 60% MWHC watering regime were selected for the study in experimental 2.

Experiment 2 studied in field conditions with 2x2 split-plot design and three replications. Super-Hot 2 and Kee-Nu-Suan were irrigated at two watering regimes: 100, and 60% of MWHC after the anthesis through fruit development. Results of the experiment revealed that Kee-Nu-Suan had higher RGR_{height}, RGR_{width}, fruits/plant, fruit dry weights, HI, capsaicin contents, dihydrocapsaicin, and pungency levels than the Super-Hot 2. It also gave 1.5-fold higher of capsaicinoids yield than the Super-Hot 2. The irrigation regimes during anthesis to fruit ripening did not affect RGR_{height} and RGR_{width} LAI, and HI. The 60% MWHC decreased fruit size in both chili cultivars, but significantly promoted dry fruit weight, capsaicinoid contents, pungency level, and capsaicinoid yield of both studied chili cultivars. Fruit dry weight was, however, increased under irrigation of 60% MWHC. In terms of capsaicinoids, restricted water can significantly induce the capsaicinoid content, pungency level, and capsaicinoid yield of the studied chili cultivars.

From the results, it can be concluded that restricted water supply after the anthesis through fruit development is not suitable for stimulating production and accumulation of capsaicin for all three chili cultivars grown in pots under the greenhouse conditions. Water deficit limited physiological processes, growth, and yield of chili without being able to promote the production and accumulation of capsaicin in chili fruit. Watering regime at 60% MWHC in field conditions is a potential method to promote the production and accumulation of capsaicinoids in chili. Nevertheless, it is necessary to selected chili cultivar that can provide high capsaicinoids yield under water stress to use with water restriction management.

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LIST OF ABBREVIATIONS

AAT	=	after anthesis
ACC	=	aminocyclopropanecarboxylate
AMT	=	aminotransferase
AWC	=	available water capacity
AWHC	=	available water holding capacity
Ca	=	calcium
Ca3H	=	cinnamic acid-3-hydroxylase
Ca4H	=	cinnamic acid 4-hydroxylase
CAGR	=	compound Annual Growth Rate
$C_{18}H_{27}NO_3$	=	capsaicin
$C_{18}H_{29}NO$	=	dihydrocapsaicin
CoMT	=	caffeic acid O-methyl transferase
CO_2	=	carbon dioxide
CRD	=	complete randomized design
CS	=	capsaicinoids synthase
DAA	=	day after anthesis
DAF	=	days after flowering
DAT	=	days after transplanting
DM	=	dry mass
EC	=	electrical conductivity
ETR	=	electron transport rate
F_0	=	minimal level of fluorescence
FAO	=	food and agriculture organization

LIST OF ABBREVIATIONS (Continued)

FC	=	field capacity
Fe	=	Iron
F_m	=	maximum possible yield of fluorescence
F_v	=	variable fluorescence
HI	=	harvest index
HPLC	=	high-Performance Liquid Chromatograph
K	=	potassium
K_2O	=	potassium oxide
LAI	=	leaf area index
LWP	=	leaf water potential
LWP_{md}	=	midday leaf water potential
Mg	=	magnesium
MWHC	=	maximum water holding capacity
N	=	nitrogen
OM	=	organic matter
P	=	phosphorus
PAL	=	phenylalanine ammonia lyase
P_2O_5	=	phosphorus pentoxide
pH	=	positive potential of the hydrogen ions
PSII	=	photosystem II
PWP	=	permanent wilting point
RCBD	=	randomized complete block design
S	=	sulfur

LIST OF ABBREVIATIONS (Continued)

SHU	=	scoville heat units
WAA	=	weeks after anthesis
WHC	=	water holding capacities

CHAPTER I
INTRODUCTION

1.1 Background of the selected topic

Chili (*Capsicum* spp.) is a member of Solanaceae, it is considered the world's economically important crop. FAOSTAT (2020) reported that, in 2019, the important fresh chili producers were China, Mexico, Turkey, Indonesia, and Spain. The global production was around 38,027,164 tons while the area harvested was 12,443,287.5 rai. In Thailand, chili production is distributed throughout the country, but the Northeastern region is a major production area accounting for 68% of the total country's plantation. Nakhon Ratchasima, Chaiyaphum, and Ubon Ratchathani are the provinces where chili production is concentrated (Pangjan *et al.*, 2017). Bird's eye chili, green chili, and bell chili are the cultivars widely grown in Thailand, presently, the most cultivated chili cultivar in the Northeastern region is bird's eye chili.

Chili is used in several sectors, the majority is used in the food production sector as fresh ingredients and primary products for processed food such as chili powder, chili sauce, dried chili, and chili paste. Currently, the domestic and external demands for chili are increasing because of the rising demand for capsaicinoids in the pharmaceutical and cosmetic industries (Gurung *et al.*, 2012). Capsaicinoids, the sources of heat in chili, consist of two main substances: capsaicin, and dihydrocapsaicin. Both substances are accountable for 91% of the total volume of capsaicinoids found in chili (Kosuge and Furuta, 1970). The chili with high capsaicinoid content or pungency, approximately 0.6-3.9% capsaicin content or 80,000 to 500,000 Scoville heat units (SHU) is desired for the previously mentioned industries (Prasad *et. al*, 2020). The fresh chili fruits of high-pungent cultivars can be sold at a rather high price compared with normal cultivars, for example, a kilogram of chili with a pungency level of 800,000 SHU can be sold to a company at a guaranteed price of 200 baht/kg while the price of chili in a normal market is 20 - 50 baht/kg (Thairath, 2017, 2020). In Thailand, several products containing capsaicin are commercialized, these products normally used imported capsaicin because normal chili production still yielded rather low capsaicin contents. Thus, the production of highly pungent chili is of interest.

The level of capsaicinoids depends on several factors including the cultivars and environmental factors such as plant nutrients and watering levels (Zewdie and Bosland, 2001; Phimchan *et al.*, 2012; Jeeatid *et al.*, 2017). The high-pungent chili cultivars are, for sure, of interest for industrial uses but the cultivation of these cultivars can be limited by their vulnerability to insects and diseases, as well as their sensitivity to environmental factors; these resulting in a high production cost (Wisitpanich *et al.*, 2011). The breeding program for high-pungent cultivars has been carried out in Thailand and cultivars such as Yodson Khem 80 and Akanee Pirote were introduced (Techawongstien, 2014). Nevertheless, the cultivars are yet to be commercially produced. Super-Hot 2 and Huay-Sii-ton from *C. annuum* and Kee-Nu-Suan from *C. frutescens* are chili cultivars that are wildly grown in the country because of their high demand in the market. They have the potential to produce a high yield per area with medium pungent levels of around 35,000 to 100,000 SHUs (Bureau of Food Safety Extension and Support, 2019; NSTDA, 2019). Therefore, they are chosen for this study.

Environmental management is another possible strategy to produce high-pungent chili products. The study by Phimchan *et al*. (2012) found that when subjecting different chili cultivars to drought stress, capsaicinoid level was induced in the cultivars classified as low and medium pungent groups. While the pungency of high-pungent chili cultivars did not respond to given drought stress. This finding suggested different responses in capsaicinoid biosynthesis among the different chili cultivars. The results of a study by Jeeatida *et al*., (2017) indicated that a suitable level of drought stress can increase the capsaicinoids biosynthesis of hot pepper (*C. chinense*). Thus, this study is interested in water management to control the biosynthesis and the accumulation of capsaicinoids in commonly grown chili cultivars.

Nevertheless, it is necessary to keep in mind that drought stress is one of the limiting factors for the plant, it can affect the physiological processes by decreasing leaf water potential, metabolism activities, disturbed growth, and development of plant cells, and decreased photosynthesis, and assimilation activities, thus affecting plant growth (Gomes-Laranjo *et al*., 2006; Hamad *et al*., 2004; Ou and Zou, 2012; Panella *et al*., 2014; Sam-Amoah *et al*., 2013), and leading to a massive loss in crop yield and quality. It seems that the water management to induce high capsaicinoid contents in chili production maybe contrasts with the normal practices to maintain high yield. Therefore, it is necessary to clearly understand chili plant responses to different irrigation levels before it can be introduced to the growers for high capsaicinoid chili production.

1.2 Research objectives

1.2.1 To investigate the effects of watering regimes on the physiological traits, growth, yield, and capsaicin content of *C. annuum* and *C. frutescens* under greenhouse conditions.

1.2.2 To evaluate the effects of watering regimes on agronomic traits and capsaicinoid content of *C. annuum* and *C. frutescens* under field conditions.

1.3 Research hypotheses

1.3.1 Different watering regimes can affect the processes of plants' physiology, growth, yield, biosynthesis, and accumulation of capsaicinoids.

1.3.2 Water stress at a suitable level can stimulate capsaicinoids biosynthesis and accumulation in chili.

1.4 Scope and limitation of the study

This study focused on the evaluation of physiological responses, vegetative growth, yield, and yield characteristics, as well as the capsaicinoids production of 3 chili cultivars from 2 chili species: *C. annuum* (Super-Hot 2 and Huay-Siiton) and *C. frutescens* (Kee-Nu-Suan) responded to different watering regimes. The experiment was divided into 2 parts: **Experiment 1** which was conducted under nursery conditions and **Experiment 2** which was done under field conditions at Suranaree University of Technology, Nakhon Ratchasima. For the first experiment, all 3 cultivars were evaluated with 4 different watering levels: 100% of maximum water holding capacity (MWHC) as a control, 80, 60, and 40% MWHC. In experiment 2, only a cultivar from each species and 2 watering regimes (100% MWHC as a control and a chosen watering regime) were studied.

CHAPTER II
LITERATURE REVIEWS

2.1 Chili: origin, distribution, and species

Chili (*Capsicum* spp.) is native to tropical and temperate Americas and distributed from Mexico to Brazil, Paraguay, and Central Argentina (Carrizo-García *et al*., 2016). It is, nowadays, spread throughout the globe via the Portuguese traders during the colonial era (Figure 2.1 A). In many regions, it became an important cash crop and greatly contributed to the region's economy.

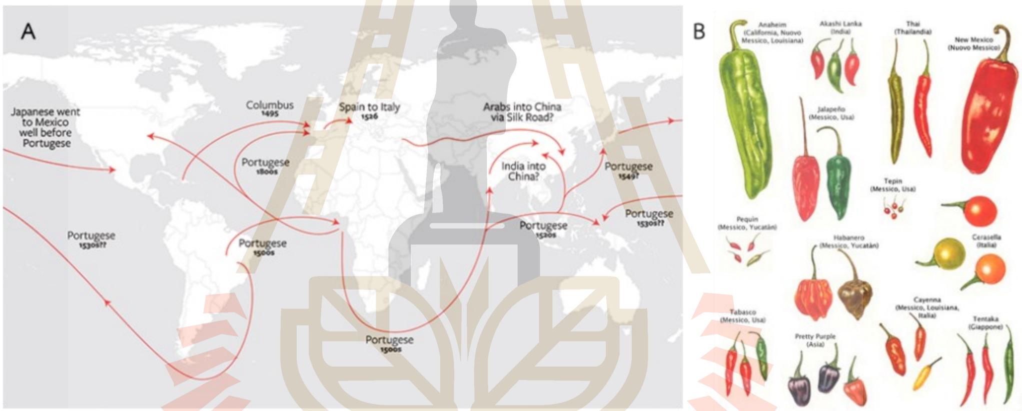

Figure 2.1 The global spreading of chili was attributed to the Portuguese during the colonial era (A, Legal Nomads, 2020) and the most known and consumed chili varieties. Source: Made in Yle (2015).

The genus *Capsicum* contains five domesticated and commercialized species: *C. annuum*, *C. frutescens*, *C. chinensis*, *C. pubescens*, and *C. baccatum* (Pickersgill, 1997) as shown in Figure 2.1 B. The *C. annuum* includes many common varieties such as bell peppers, wax, cayenne, jalapeños, chiltepin, and all forms of New Mexico chile. The *C. frutescens* includes malagueta, tabasco, Thai chili, piri piri, and Malawian Kambuzi while *C. chinensis* are the hottest chili such as the naga, habanero, Datil, and Scotch bonnet. *C. pubescens* includes the South American rocoto peppers, and *C. baccatum* is the South American aji peppers (Bhatt *et al*., 2021). In addition, there are semi-domesticated species and wild pepper species with approximately 35 species (Carrizo-Garcia *et al.,* 2013) that are commercially used. These mentioned chilies are members of the nightshade family, Solanaceae. Some important characteristics of these species are shown in Table 2.1 Karen chili is one among these semi-domesticated species, it was previously grown by Karen people in the border area of Thai and Myanmar. The domestication and commercialization of Karen chili later occurred because it has a good aroma and spiciness which is suitable for Thai cuisine (Kongtoom *et al*., 2019; Hadthamard *et al*., 2021).

Table 2.1 Characteristics and commercial cultivars of five domesticated and commercialized *Capsicum*.[1]

Species	Characteristics	Area of origin	Annual/ perennial	Pungency	Commercial cultivars
C. annuum	Milky white large corolla, single flower at each node, presence of calyx teeth, yellow and smooth seeds, annual, medium to large size fruits	Central and south America regions	Annual	Non-pungent and pungent	Sweet pepper, bell pepper, Cayenne, Jalapeños, Chinda, and, Huay-Sii-ton
C. baccatum	Cream to white colored corolla with yellow to green spots, one or more flowers at each node and smooth seeds.	Argentina, Bolivia Paraguay, Peru	Perennial	Non-pungent and pungent	Aji lemon drop, bishop’s crown, and Brazilian Starfish
C. chinensis	Dull white corolla, two or more flowers at each node, devoid of calyx teeth, constriction between the base of calyx and pedicel, yellow and smooth seeds.	Central America, Colombia, Ecuador, south-eastern Brail, Veneuela	Perennial	Highly pungent	Naga, Habanero, Ghost pepper, and Scotch Bonnet.
C. frutescens	Greenish white corolla, two or more flowers at each node, devoid of calyx teeth, no constriction between base of calyx and pedicel, yellow and smooth seeds, small size fruits.	Central America, central-eastern Brail, Colombia, Ecuador, Venezuela	Perennial	Highly pungent	Prik-Kee-Nu-Suan, Karen, and Tobasco pepper
C. pubescens	Deep purple to faintly violet corolla, one or more flowers in each node, presence of calyx with small teeth, black to brown and rough seeds.	Argentina, Bolivia, central America, Ecuador, Peru	Perennial	Non-pungent and pungent	Rocoto, Manzano, and Locoto

[1]Adapted from: Greenleaf, 1986; Tripodi and Kumar, 2019; Bhatt *et al*., 2005

2.2 Production and uses of chili

2.2.1 Chili production and its contribution to the economy

After its spread and domestication, chili became a cash crop for many countries around the globe. Asia is a main production site of chili, FAO (2020) estimated that between 2015 – 2019, 72.3 and 68.3% of dry and fresh chili were produced in this region while Africa, America, Europe, and Oceania contributed at a lower production share (Figure 2.2 A and B).

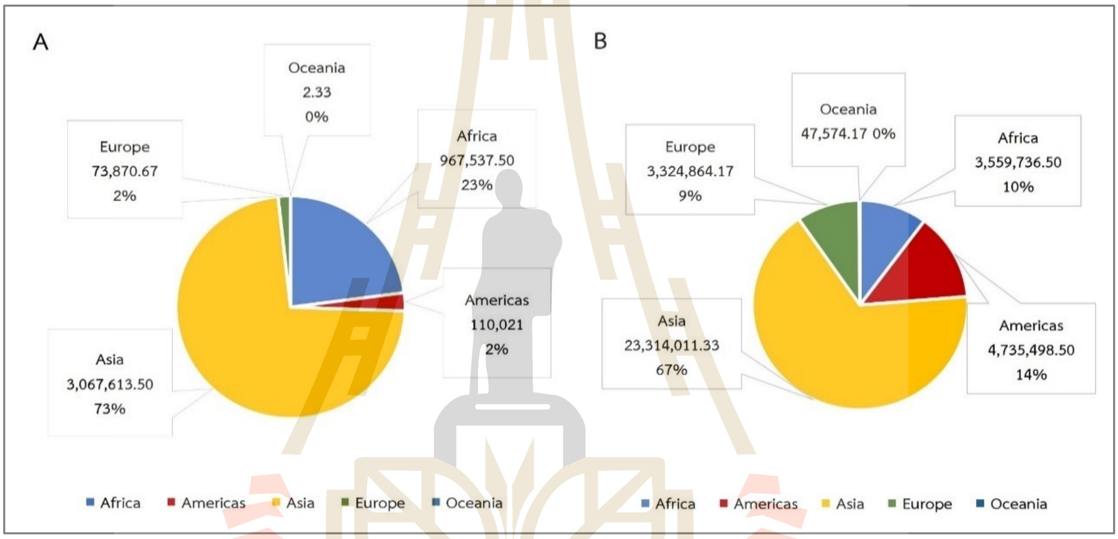

Figure 2.2 Percentage of production share (2015 – 2020) of dry (A) and fresh (B) chili products. Source: FAO (2022).

It can be seen from Figure 2.3 that from the years 2010 to 2018, global chili production continuously increased. In 2018, the production reached the highest volume of 3.9 million tons which increased by approximately 0.5 times compared with the production in 2010. However, the production volume was slightly reduced to approximately 3.5 million tons in 2019 due to the production of dry chilies in Asian countries having decreased slightly or remained stable. In addition, the COVID-19 outbreak directly and negatively impacted the dry chilies market globally. The figure also showed the market share of the top 10 countries for dry chili production and found that between those past 10 years, India was the leading chili-producing country followed by Thailand, China, Ethiopia, and Ivory Coast, respectively (Mordor-Intellingence, 2022).

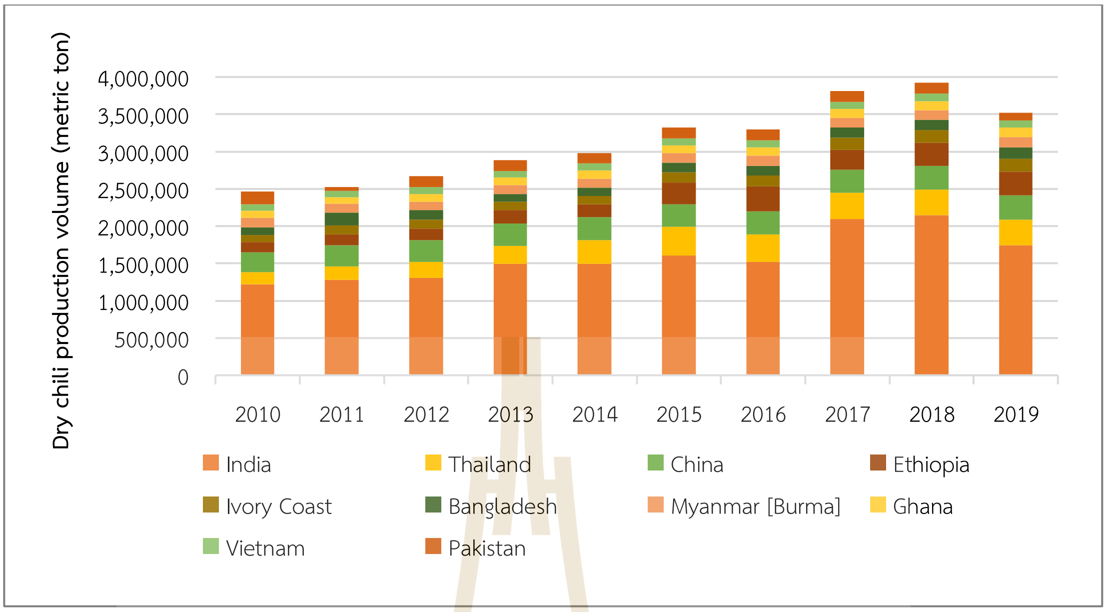

Figure 2.3 Dry chili production volumes and market shares 2010-2019. Source: TRIDGE (2020).

In Thailand, *C. annuum*, and *C. frutescens* are commonly grown, they are industrial crops that greatly contributed to the Thai economy. In 2021, 135,051 rai of Thailand area was used for chili cultivation which produced 252,960 tons of chili yield. The value of the production was approximately 2,100 million baht in that year and the most exported chili products of Thailand were fresh chili and frozen chili with a total value of 2,186 million baht, chili sauce 4,397 million baht. The important chili production areas in Thailand are spread throughout the country, the northeastern region contributes around 40% of the country's chili growing areas (50,800 rai) which produced a yield of 114,690.11 tons, followed by the northern, central, and southern regions (DOA, 2022). Si Sa Ket, Ubon Ratchathani, and Nong Khai are considered the main chili production areas in the northeastern region.

2.2.2 Production practices of chili and markets in Thailand

Chili production in Thailand belongs to small-scale to medium-scale growers with an average cultivation area of 8.30 rai/household. The labor used for chili cultivation is usually family members and, in each household, the average labor force is around 4 people (Jirawadee and Piansak, 2011). Chili production is a labor-intensive activity, therefore if the household cannot provide a sufficient labor force, chili growers may have to spend more on hiring labor for their production activities (Asravor *et al.*, 2015). DOA (2021) suggests that the cost of chili production was approximately 15,900 bath/rai including seed, land preparation, manure and fertilizers, pesticides, plastic mulch, and labor for harvest. Out of that value, 31.44% was a labor cost. This show that labor cost in Thailand is rather high and at a time may be very difficult to find.

Most chili growers use traditional cultivation methods, for example, the application of chemical fertilizers without information concerning their soil fertility and the intensive use of pesticides (DOA, 2015). The irrigation for chili plants depends on the nature of the water source in the cultivation area. In general, it can be divided into 2 groups: the irrigated area in which growers have water resources and equip the cultivation area with an irrigation system, and the rainfed area which depends on the rainfall. The irrigated area includes the production in Chaiyaphum and Loei provinces where chili is grown after the rice production season and chili cultivation in the plains along the Mekong River in Nong Khai province. In the rainfed area, chili plants are usually grown in the paddy field after rice harvesting. In the latter case, the production is relying on moisture residue in the paddy field or rainfall (Phompanjai *et al*., 2015)

Yield from Thai chili production is delivered to the customers via 3 levels of the market: local market, wholesale market, and international market. The local market depends on the infrastructure for the local transportation and marketability of growers. While the wholesale markets are the place where fruits and vegetables are gathered and the shopkeepers will distribute these products to different smaller markets (Ekaphong *et al*., 2006). The products from a wholesale market can also be exported to international markets, but they will have to meet a rather high standard for food safety depending on the importer's requirement. These high-quality products can be sold at a higher price but also heavily relies on international demand. The selling price of chili can be largely different, it may depend on the variations of demand or supply sides. Figure 2.4 shows chili wholesale prices in Thailand by variety. In some seasons, the domestic chili yield was insufficient, or the price was high due to the high production cost and the average yield was relatively low. In general, we can observe the fluctuation of chili prices between seasons. The chili production in the northeastern region can be classified into 3 groups; chili cultivation during the dry season, in the upland area, and the production in the paddy field after the rice was harvested from October to May (Phompanjai *et al*., 2015). From these cultivation periods, the fruits can be harvested in November, and there will be a period in which products may be scarce in May-June of each year for both green and red chilies. The prices of green chili and red chili are high from November – December (20-25 baht/kg) and from December – mid-January, (40-50 baht/kg), respectively (Rakbankerd, 2017). The price of chili is depending on supply and demand, at the beginning and the end of the season, chili supply is yet to meet the market's demand, and thus the selling price can be higher than during the middle of the season when the chili fruit is released to the market in a large quantity (Ekaphong *et al*., 2006).

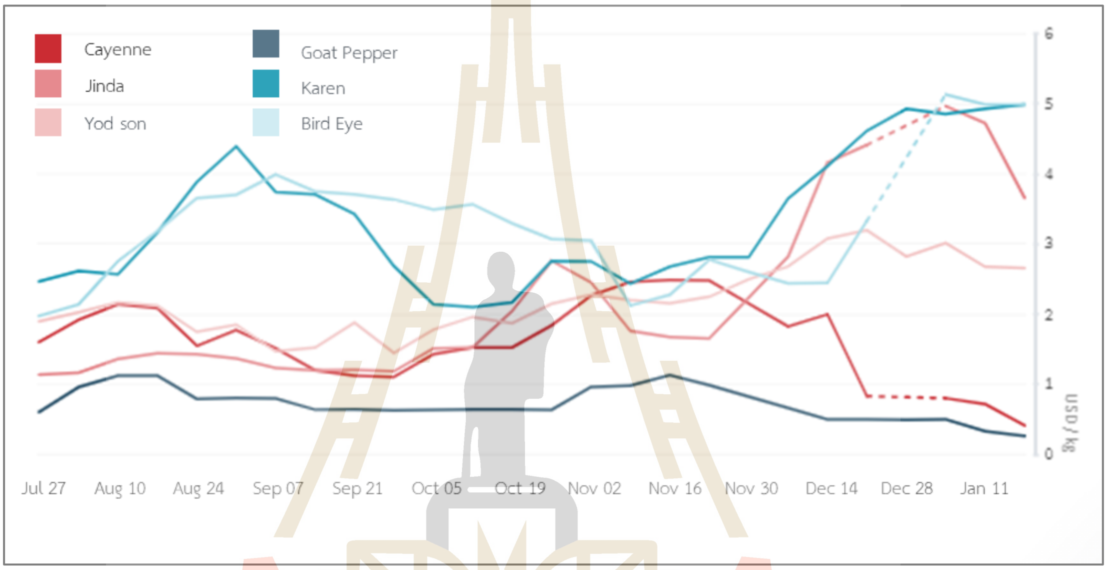

Figure 2.4 Chili wholesale prices in Thailand by different varieties between 2020 July, 27 – 2021 January, 11. Source: TRIDGE (2020).

Chili's selling price is also determined by the middlemen, in general, the growers do not have the power to bargain for the price. A lack of incorporation between chili growers may be the cause of this problem (Sudangnoi and Phakdee, 2011). Besides, the price of chili is also affected by the number of imported chilies from foreign countries. These chilies usually have a lower price and are very competitive with local chilies. The import can result in an oversupply of chili in domestic markets and cause a reduction in selling price.

In Nakhon Ratchasima, the area of interest for this research, around 4,400 rai was occupied with chili which gave a yield of 2,454 tons in 2018 (DOA, 2018). The *C. annuum*, especially large fruit bird's eye chili such as Chinda, Haurue, Huay-Sii-ton, and Yord-Soen, is the most popular chili species among the growers in this area (Phakuthai, 2010). Chili growers in Nakhon Ratchasima usually sell their fresh or dried products to retailers and wholesalers in the local markets, as well as the provincial-level markets (Prasarthinphimai *et al*., 2018). The price of these products largely depends on the types of chilies and the season when they are released to the market. These resulted in price fluctuations throughout the year. In recent years, areas of chili cultivation are decreasing, especially for the large fruit bird's eye chili, which has the highest planting area proportion in the northeast region. In 2017, the area was 64,794 rai and reduced to 31,815 rai in 2018 while the yield remained constant from 2017 to 2020 (Table 2.2).

Table 2.2 Production of large fruit bird's eye chili in the northeast region in 2017-2020[(1)].

Year	Cultivated area (rai)	Production (ton)	Average yield (kg/rai)	Average price (baht/kg)
2020	31,815.20	36,314.28	1,340.47	40.62
2019	50,823.49	45,351.15	1,055.71	52.82
2018	63,556.38	71,014.70	1,087.10	30.23
2017	64,794.88	61,061.75	865.38	34.36

[(1)]Source: DOA, (2020).

2.2.3 Uses of chili

Fresh and dry chili fruits are primarily used for consumption, some are directly used as cooking ingredients while some are processed into various products such as curry paste, chili sauce, and chili paste before they were used to add spiciness to the dish. The demand for chili in the food processing industry is increasing due to the increasing consumption of spicy food and the rising popularity of international cuisines. The changing taste of consumers and the introduction of new flavors in the market have driven the growth of the chili sauce market since it is an affordable and easy method to experiment with food (Growth Market Reports, 2019). In 2019, the global chili sauce market had a value of 4,232.7 million USD and is projected to reach 6,824.7 million USD by 2027, expanding at a CAGR (Compound Annual Growth Rate) of 6.5% during the forecast period, from 2020 – 2027 (Growth Market Reports, 2020). Thailand is benefiting from this growth as shown in Figure 2.5 the export value of chili sauce was continuously increased since 2016. In 2020, Thailand earned approximately 3,100 million baht from chili sauce exportation while frozen chili, chili powder, and dry chili were Thailand's other exported chili goods (Ministry of Commerce, 2019).

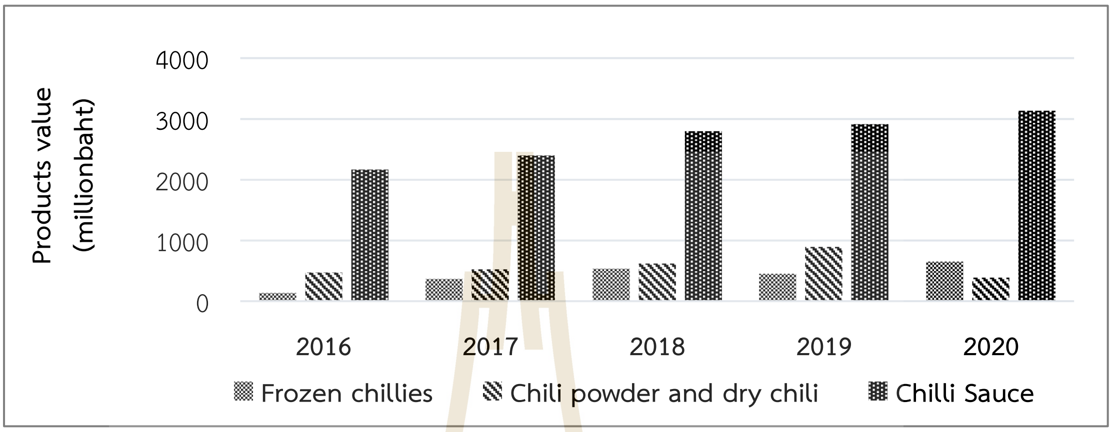

Figure 2.5 Export value of chili products in Thailand 2016-2020. Source: Ministry of Commerce (2020).

Chili is rich in nutrients and various substances, for example, vitamins A, vitamin C, carotenoids, capsanthin, capsorubin, beta-carotene, cryptoxanthin, lutein, phytofluene, xanthophyll, steroids, capsicoside, and capsaicinoids can be found in chili fruits. The important substances that reflect the unique characteristics of chili are carotene and capsaicinoids (Govindarajan, 1986) which are phenolic alkaloids specific to the genus *Capsicum* (Giacalone, 2015). Capsaicinoids are not only given chili fruits spiciness but also have many other important pharmaceutical effects from their antioxidative property. They have an analgesic effect, can reduce infection and inflammation, and stimulate the digestive system (Thapankaew, 2011). These beneficial properties of capsaicinoids stimulate their demand in pharmaceutical sectors. In Thailand, the pharmaceutical industry has also become more active. In the forecast for the 2021 – 2022 pharmaceutical industry trend, it is expected that Thailand's domestic drug sales will accelerate to an average of 4.5-5.0% in line with the increasing demand for drugs. The increment is a result of the increasing illness, the better access to treatment channels of the Thai population, the increasing number of foreign patients in 2021-2022, the increasing health-conscious flow of Thai people, as well as being a high-tech industry group supported by the government (Food Peak Asia, 2020). This is an opportunity to develop Thai manufacturing potential to reduce dependence on imported drugs including the development of capsaicinoid-based pharmaceutical products. Bangkok Lab & Cosmetic Co., Ltd., is the company that has researched and developed the extraction of capsaicinoids from chili produced in Thailand for use in the manufacture of pharmaceutical and cosmetic products, especially in the analgesia product group.

Chili is also used as an additive in the pet and livestock feed production sector. The demand is driven by the concern of the owners about using chemical drugs or substances on their beloved pets as well as the use of antibiotics in animal feeds according to the European Union Register of Feed Additives Regulation (EC) No. 1831/2003 (Cardozo *et al*., 2005). The use of antibiotics in animal supplements is prohibited because of the concern for the potentially harmful effects of extensive use of low-level antibiotics in feeds due to the development of resistant strains of organisms in host animals that might compromise animal as well as human health (Stallones *et al*., 1980). This allowed natural extracts to play a bigger role in the animal feed additive industry. Table 2.3, outlines some commonly used plant extracts and their functions and it is shown that chili is used in the feed additive as a substitute for antibiotics to improve gut health, well-being, and livestock productivity (Chisor, 2016).

Table 2.3 Examples of some often-used plants and functions in livestock[(1)].

Aromatic species		Pungent species		Herbs	
Plant	Function	Plant	Function	Plant	Function
Nutmeg	Digestion stimulant, anti-diarrhoeic	Capsicum	Digestion stimulant	Rosemary	Digestion stimulant, antiseptic, antioxidant
Cinnamon	Appetite and digestion stimulant, antiseptic	Pepper	Digestion stimulant	Thyme	Digestion stimulant, antiseptic, antioxidant
Cloves	Appetite and digestion stimulant, antiseptic	Horseradish	Appetite stimulant	Sage	Digestion stimulant, antiseptic, carminative

[(1)]Source: Chisor (2016).

Table 2.3 Examples of some often-used plants and functions in livestock[1] (continues).

Aromatic species		Pungent species		Herbs	
Plant	Function	Plant	Function	Plant	Function
Cardamom	Appetite and digestion stimulant	Mustard	Digestion stimulant	Laurel	Appetite and digestion stimulant, antiseptic
Coriander	Digestion stimulant	Ginger	Gastric stimulant	Mint	Appetite and digestion stimulant, antiseptic
Cumin	Digestive, carminative, galactagogue	Garlic	Digestion stimulant, antiseptic		
Anise	Digestion stimulant, galactagogue				
Celery	Appetite and digestion stimulant				
Parsley	Appetite and digestion stimulant, antiseptic				
Fenugreek	Appetite stimulant				

[1]Source: Chisor (2016).

In addition, chili is also used as an insecticide. According to the report, capsaicin has broad-spectrum insecticidal activity against many species of insects, eg, stored product beetles (*Sitophilus zeamais* and *Tribolium castaneum*), rice grain insects (*Sitotroga cerealella*), *Alfalfa weevil*, *Myzus persicae*, *Bemisia tabaci*, and *Plutella xylostella* (Al-Doghairi *et al*., 2003; Ho *et al*., 1997; Jia, 2006; Jin *et al*., 2008; Liu and Lin, 2003; Liu *et al*., 2008; Prakash and Rao, 2006; Zhao *et al*., 2012). The study by Claros-Cuadrado *et al*. (2019) indicated that both capsaicinoids and glucosinolates have a biocidal effect on *A. cytisorum*, and act within a fairly short time. The mortality of aphids at 87–97% can be archived by the application of 5% capsaicinoids, 50% glucosinolates, or a mixture of 5% capsaicinoids and 45% glucosinolates. The insecticidal activity of natural capsaicinoids was weaker than that of the corresponding chemical pesticides, but increasing the concentration of capsaicinoids and the number of sprayings can effectively control the tested agricultural insects (Li *et al*., 2019). Although the use of chemical pesticides was highly efficient, it can lead to health problems for users and consumers as well as environmental issues (Aktar *et al*., 2009). On the other hand, several pieces of evidence show that the use of plant extracts with insecticidal properties is one of the main ecologically friendly and economically feasible alternatives to synthetic pesticides (Gupta and Dikshit, 2010; Mazid *et al*., 2011). Currently, there are commercialized insecticides from capsaicinoids such as the product from General Hydroponics (insect repellant/insecticide), and Neptune's Harvest (insect repellent).

Despite the production of large quantities of chili in Thailand, it is still necessary to import capsaicinoids from foreign countries as pure extracts to use in industrial sectors. The reason for this situation is that suitable chili for capsaicinoid extraction in the industrial sectors needs to have a high level of capsaicinoid content. For chili growers, not only high-pungent cultivars should be used, but they also need cultivars that can give high productivity.

2.3 Factors affecting chili production

The success of chili production depends on several factors. The plant generally requires suitable environmental factors, for example, light, temperature, plant nutrients, and water for normal growth and development. In addition, the requirement of these factors varies according to cultivars, varieties, and species. Therefore, growers need to understand their requirements to manage production factors accordingly to ensure adequate yield quantity and high-quality products for the market. Chili production is not an exception, the following section is dedicated to factors influencing chili production and quality.

2.3.1 Chili species and seed quality

As previously mentioned, there are various species and varieties of chilies, each suitable for a specific purpose. Some chilies can be grown only in tropical areas while others may be grown in subtropical areas. Some have a very high pungency level such as *C. chinense* with a pungent level of up to 1,000,000 SHU and some of the *C. frutescens* can have a very low pungency level that can be consumed as a fresh vegetable (Kraikruan *et al.*, 2015). Some varieties are tolerant to biotic and abiotic stresses and there are those which are vulnerable. These characteristics are controlled by the interaction between genetics and the environment in which they grow. For instance, the evaluation of Sha and Madhavan (2016) on certain growth and yield of 12 *C. annuum* genotypes found that there was a superior genotype in terms of growth, reproduction, and yield compared with others. The reasons for these differences may be due to the high accumulation of photosynthates (Erwin *et al*., 2019) and the specific morphology of the genotype. Therefore, growers need to choose the right species and varieties for their production purposes and their production environments.

2.3.2 Temperature and light

The optimum climate conditions for the growth of chili are a constantly warm and humid environment with a temperature of 20-30 °C and an annual rainfall of 850–1,200 mm. In such conditions, chili can be grown throughout the year. Figure 2.6 shows that the Northeast region of Thailand, in general, has suitable temperatures and rainfall for chili production. However, with climate change, this situation might change. Increasing temperature and irregular rainfall are expected to occur more often in the future according to the climate model scenario, they may cause a reduction in yield quality and pest and disease outbreaks (Thammawong, 2010).

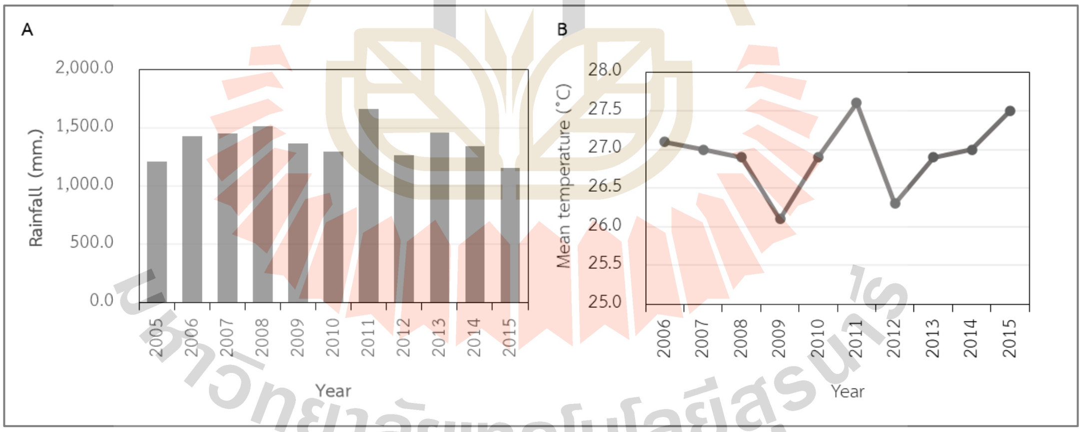

Figure 2.6 Average rainfall (A) and mean temperature (B) in the Northeast region of Thailand between 2005-2015. Source: National statistical office (2015).

Climate change can affect chili production through an increase in pollination failures, floral abortion, reduced fruit size and quality under higher temperatures, increased incidence of physiological disorders (sunscald and blossom end rot), increased risk of soil-borne diseases (leaf blight and fruit rot) (Khaitov and Umurzokov, 2019). The experiment conducted by Lee *et al*. (2018) revealed that chili exposed to extremely high temperatures and increased CO_2 concentration shortened the ripening period and reduced the fruit yield. Furthermore, negative effects were also observed on morphogenesis, photosynthesis, and fruit characteristics. The results are seen in reducing crop yield if adaptable cultivars are absent since extreme heat can negatively affect plant reproductive development (Kafizadeh *et al*., 2008).

2.3.3 Soil properties and plant nutrients

Chili can be grown in almost all soil types, but sandy loam is the most favorable one because it contains organic matter which facilitates water drainage. It is slightly acidic with a range of 6.0 – 6.8 (Thammawong, 2010) under suitable soil moisture. In the northeastern region, chili production is experiencing several soil problems because most of the soil in this area is sandy soil. This type of soil, in general, rarely has sufficient nutrients for crops to reach their potential yield because of its low organic matter content. It is also affecting soil physical properties such as structure, bulk density, and water-holding capacity, chemical properties such as nutrient availability, cation exchange capacity, and allelopathy, and biological properties such as nitrogen mineralization bacteria, dinitrogen fixation, mycorrhizal fungi, and microbial biomass (Fageria, 2012). With low organic matter content, plant nutrients are also easily leached into the underground water. Nowadays, growers use chemical fertilizers at a higher rate, especially N (nitrogen), P (phosphorus), and K (potassium), which, if used more than necessary, could have a negative effect. In fertilization, growers must know the effective use of fertilizers. However, the use of fertilizers must be considered in conjunction with several factors, such as plant species, soil fertility, transplanting process, and climate (Suksawas, 2000). In the study of Phadung *et al*. (2018), the suitable ratio of N-P-K for *C. annuum* L. was 4:1:5. The average amounts of N, P_2O_5, and K_2O required through the developmental stages were 3.25, 0.56, and 4.04 kg/rai, respectively. While the N, P_2O_5, and K_2O removed by yield were 3.49, 1.19, and 4.28 kg/ton, respectively. However, Altaf *et al.* (2019) found that in *C. annuum* L., the highest yield (248.31 kg/rai) was recorded with the application of organic manures (farmyard manures) 1.3 tons/rai with 100:50:50 of (N: P: K) when compared with the control (161.42 kg/rai). Also, organic manures coupled with NPK fertilizer increased the uptake of nutrients (N, P, K, Ca, S, and Fe) as compared to the control. These show the influence of fertilizers on plant growth and yield. Chomthaisong (2008) recommends chili growers in the northeastern provinces apply the following fertilizer formula (NPK)

15-20-20, 13-13,21, and 12-12-17. The recommendation is based on the low K content in sandy soil which is the main soil type found in this production area (Chomthaisong, 2008). Moreover, the continuous production of chili in the same place for a long time can disturb the balance of plant nutrients in the soil and affect the growth and productivity of chili (Wonprasaid and Tira-umphon, 2014).

2.3.4 Water

The effect of water on plants emerges from its physiological importance, an essential factor for successful plant growth, involving photosynthesis and several other biochemical processes such as the synthesis of energetic composites and new tissue (Chavarria and Santos, 2012). Therefore, the growth and production of the plant are depending on the water supply. Normally, the water state of a plant is controlled by relative rates of loss and absorption. Moreover, it depends on the ability to adjust and keep an adequate water status. For chili, rainfed production requires an annual rainfall of 850–1,200 mm which can be found in humid regions (Thammawong, 2010).

The water potential in soil affects water reservoirs and their availability for plants, hence it has a large impact on plant growth and production. Furthermore, the soil water content exerts a great influence on some physical and chemical properties, such as the level of available nutrients, the aeration state of the soil, oxygen content, which interferes with root breathing, and microbial activity Maria (Gavrilescu, 2021). Water potential is directly dependent on soil physical characteristics, varies with time and space, and depends on soil water balance. Figure 2.7 shows the soil moisture balance which is determined by the input (rain and/or irrigation) and output of the soil (drainage, evaporation, and root absorption) (Allen *et al*., 1998). According to the physiological aspect, the water content in soil is associated with three terms: the field capacity (FC), the permanent wilting point (PWP), and the available water content (AWC) (Zotarelli *et al*., 2010).

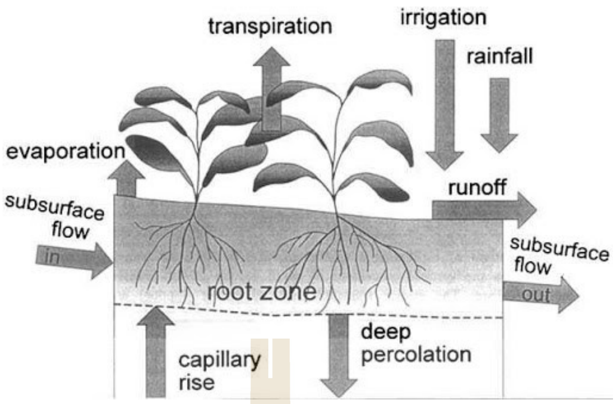

Figure 2.7 The perspective of the soil water balance of the root zone at the field levels. Source: Allen *et al*. (1998).

The FC corresponds to the maximum water content that a given soil can retain by capillarity, after saturation and gravity drainage, and it is conventionally estimated as the water content when the matrix potential is –0.03 MPa (–0.3 Bar). While the PWP can be defined as the amount of water per unit weight (or volume) of soil that is so tightly retained by the soil matrix that roots are unable to absorb causing the wilting of plants and estimated as the water content when the matrix potential is –1.5 MPa (–15 bar, Chavarria and Santos, 2012). The water content in the soil at FC and PWP is essential for calculating the AWC for the plants. The AWC is calculated considering the soil volume explored by roots and the percentage of water content determined as the difference between FC and PWP. Due to this interval of water availability, one may assume that water could be absorbed by the roots with the same facility in the range between FC and PWP (Chavarria and Santos, 2012). The relationship between soil and water in different soil textures is shown in Figure 2.8.

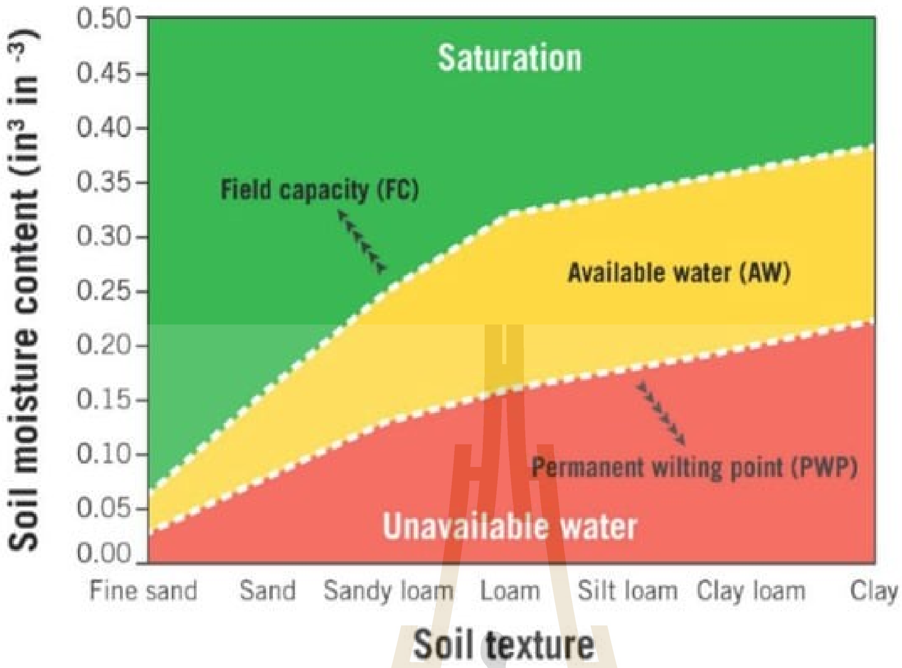

Figure 2.8 The relationship between soil saturation, field capacity, soil available water, permanent wilting point, soil unavailable water, and soil texture. Source: da Silva *et al*. (2019).

Effect of water deficit

Water shortage is primarily sensed by the roots, inducing a signal cascade to the shoots via the xylem causing physiological and morphological changes. Several genes are regulated up or down with osmotic stress; the majority of these responsive genes can be driven by either an ABA-dependent or ABA-independent pathway. Some studies suggest that ethylene shuts down leaf growth very fast after the plant senses limited water availability. Ethylene accumulation can antagonize the control of gas exchange and leaf growth upon drought and ABA accumulation (Salazar *et al*., 2015).

Soil water deficit directly affects the water potential of plants and relative water content decrease by restricting water uptake from the soil. For instance, the study by Wijewardana *et al*., (2019) found that exposure to severe soil moisture stress resulted in decreasing midday leaf water potential (LWP_{md}) by 45% as compared to the control in soybean. Similarly, in chili under the control conditions (watered at FC), leaf water potentials were maintained at –0.2 to –0.3 MPa in all chili cultivars studied, while the 25% FC watering regime resulted in lower leaf water potentials, around –0.8 to –1.4 MPa (Phimchan *et al*., 2012). In the northeastern region of Thailand, hot and dry weather combined with the limitations of the irrigation system and problems with the soil in the area can cause water shortages during some growth stages of chili and result in lower yield compared with the variety's potential (Wonprasaid and Tira-umphon, 2014). Most of the agricultural areas in the northeastern regions are outside the irrigation zone, thus growers have to rely only on rainfall. Water deficit (drought) is the main limiting factor for plant growth and productivity. Physiologically, water deficit decreased metabolism activities, disturbed growth, and development of plant cells, decreased photosynthesis, transpiration, stomatal conductivity, nutrients translocation, and assimilation activities in the plant (Gomes-Laranjo *et al*., 2006; Hamad *et al*., 2004; Ou and Zou, 2012; Panella *et al*., 2014; Sam-Amoah *et al*., 2013), and ultimately leading to a massive loss in crop yield and quality.

It has been established that drought stress is a very important limiting factor in the initial phase of plant growth and establishment. It affects both elongation and expansion growth (Anjum *et al*., 2003; Bhatt and Srinivasa-Rao, 2005; Kusaka *et al*., 2005; Shao *et al*., 2008). The reduction in plant height is associated with a decline in cell enlargement and more leaf senescence in *Abelmoschus esculentus* under water stress (Bhatt and Srinivasa-Rao, 2005). The development of optimal leaf area is important to photosynthesis and dry matter yield. Water deficit stress mostly reduced leaf growth and in turn the leaf areas in many species of plants such as *Populus* (Wullschleger *et al*., 2005), and many other species (Farooq *et al*., 2009). A common adverse effect of water stress on crop plants is the reduction in fresh and dry biomass production (Farooq *et al*., 2009). Similarly, in chili, water deficit also affects plant growth and morphology. In the vegetative stage, the stress developed during the water stress period markedly suppressed the vegetative growth and the plant became stunted. The leaf, stem, and root dry weight subjected to water stress treatment at the vegetative stage is lower than that of the flowering and fruiting stage (Khan *et al*., 2008). The study of Pérez-Gutiérrez *et al*. (2017) on the effects of drought stress on vegetative growth of five habanero pepper genotypes (*C. chinense*) found large differences in plant height and stem diameter at different watering regimes. Plant height and stem diameter were lower under reduced irrigation regimes. Ahmed *et al*. (2014) also showed that *C. annuum* plants subjected to different soil water-holding capacities (WHC) can grow differently. Under 55% WHC, the chili height decreased by around 57% compared to the control plant. In addition, leaf area and stem diameter also decrease when plants are subjected to a lower percentage of WHC (Jeeatid *et al*., 2018).

The reproductive stage is the most critical stage for drought stress during crop growth because it strongly impacts yield and seed quality. Drought stress negatively affects flower pollination by decreasing the amount of viable pollen grain, increasing the unattractiveness of flowers to pollinators, and decreasing the amount of nectar produced by flowers. Consequently, the crop seed set is lowered. Moreover, drought stress affects crop yield by reducing grain yield and all yield components (Alqudah *et al*., 2011). The study by Rosmaina *et al*., (2018) revealed that water deficit at the vegetative stage can affect the growth and production of chili indicated by a severe decrease in the yield (71.0%), the number of fruits per plant (69.2%), and fruit set (68.9%). The decline in growth significantly occurred starting from 50% FC when compared with the control (100% FC). Whereas the severe water deficit (25% FC) can induce nearly 5% higher flower abortion when compared with the 100% FC. Despite a low reduction in the percentage of flower abortion, the impact of water deficit was greater on the percentage of fruit setting and fruit fresh weight which were reduced by 38.0% and 71.4%, respectively. A study by Khan *et al*. (2008) suggested that water deficit at different growth stages can affect the chili yield differently. When plants were exposed to water deficit at the vegetative stage, there was a decrease in fruit number, while exposed chili plants to water deficit at the matured stage can decrease the fruit weight but the degree of yield reduction will not be severe. Similarly, the study by Ichwan *et al*. (2017) found that water deficit at the vegetative stage can affect the growth of eight *C. annuum* varieties. It can reduce the number of branches and shoot dry weight, as well as the yield by decreasing the fruit number and fruit weight. The reduction of fruit numbers was between 16.83% - 46.91% at 50% FC. While the fruit weight greatly decreased by approximately 18.50% - 45.99% at 50% FC. Moreover, the study by Yang *et al*. (2017) found that the largest yield reduction (13-20%) was recorded when a water deficit occurred during the middle stage (flowering and fruit enlargement) in *C. annuum* L. in an arid environment. Additionally, Akhami *et al*. (2019) found that increased ethylene production has caused accelerated senescence as an adaptive measure to decrease the water demand to the whole-plant level.

In terms of physiological responses, they are linked to a recognition of stress by the root system, turgor changes, and water potential. Consequently, stomatal conductance, internal CO_2 concentration, and photosynthetic activity can be reduced (Chavarria and Santos, 2012). Apart from the influence of soil moisture status, leaf water potential (LWP) is also influenced by climatic conditions (Gil, 1995; Turner and Begg, 1981), a drop in the LWP in the middle hours of the day can be observed under any given irrigation levels. The study of Moreno *et al*. (2003) found that LWP and stomatal conductance slightly differed between irrigation levels, but the growth was more affected by severe water deficit. Several studies also observed a reduction of stomatal conductance arising from a diminished leaf water potential in chili plants subjected to severe water deficit (Janoudi *et al*., 1993; Lsmail and Davies, 1997; Srinivasa Rao and Bhatt, 1988) while the mild water stress is not severe enough to inhibit stomatal conductance and photosynthesis capacity (Hsiao, 2000). The study of Okunlola *et al*. (2017) found that different drought stress levels can differently affect the total chlorophyll content of chili. Moderate and severe stresses during the vegetative stage can greatly reduce the total chlorophyll content of *C. annuum*, *C. chinense*, and *C. frutescense* studied. However, these drought stresses did not affect the total chlorophyll content during the flowering and fruiting stages. This result shows the higher impact of drought stress at the vegetative stage than at the fruiting and flowering stages of chili species. Pérez-Gutiérrez *et al*. (2017) found that water stress can reduce the photosynthesis efficiency (F_v/F_m) of different genotypes of Habanero, particularly when the irrigation given to plants was reduced from 40 to 20% of available water capacity (AWC).

Despite the mentioned negative impacts, some studies also reported positive effects on product quality, such as activating the biosynthesis of secondary metabolites (Gonalez-Chavira *et al*., 2018; Jeeatid *et al*., 2018; Kopta *et al*., 2020; Sangwan *et al*., 2001). Gonalez-Chavira *et al*. (2018) demonstrated that drought stress can stimulate the metabolism of phytochemicals with health-promoting properties. Jeeatid *et al*. (2018) reported that appropriate drought stress could increase capsaicinoid contents in hot pepper while a recent study by Kopta *et al*. (2020) showed that drought stress can increase the pungency and ascorbic acid contents in mature chili fruits by booting the activity of antioxidant enzymes in leaves and fruits. The increase in ethylene production in hot pepper fruits under severe (9-day interval irrigation) and moderate (7-day interval irrigation) stress conditions could be attributed to the metabolic reaction between ACC oxidase with its substrate (ACC). This phenomenon is caused by decreased compartmentation due to the deterioration of membrane integrity. Additionally, moderate and severe stress conditions can increase the accumulation of capsaicin in chili fruits harvested at 45 days after anthesis (DAA). This effect of drought stress is highly correlated with the ethylene produced (Haris *et al*., 2020).

Plant adaptations to water deficit

Plant species are responses differently to drought stress. Some plant species could endure a higher level of water deficit and/ or survive a long period of drought stress than others (Klos *et al*., 2009; McDowell *et al*., 2008; Mueller *et al*., 2005). These differences are the results of defense mechanisms against the water deficit adopted by the species. In nature, plants have evolved and responded to acclimatization and survive drought stress with an array of morphological, physiological, and biochemical adaptations (Bohnert *et al*., 1995). Drought tolerance could be defined as the plant's ability to preserve vegetative growth and crop yield under drought conditions. However, plant adaptations for drought stress could be divided into three strategies which involve: drought escape, drought avoidance, and drought tolerance.

Drought escape is the ability of a plant to complete its life cycle before the onset of drought. Thereby, plants do not experience drought stress, as they can modulate their vegetative and reproductive growth according to water availability (Jones *et al*., 1981). In these conditions, plants develop rapidly and reduce the vegetative growth period. Also, early flowering and producing a few flowers and seeds is an important mechanism plant use to adapt to drought. Therefore, a short life cycle is considered the mechanism to escape from climatic stresses (Abobatta, 2019).

Drought avoidance is the response that plant avoids tissue dehydration, mainly from the reduction of water loss. The processes can be done by closing stomata, decreasing transpirational area through leaf growth inhibition and leaf shedding, reducing light absorbance by leaf rolling, increasing trichome layer density, changing the angle of leaves, and also increasing water use efficiency (Chaves *et al*., 2003). At the same time, the root system plays a vital role in the drought-avoiding mechanism and the root system characters change (become deeper and thicker) to adsorb water from extra depths can contribute to producing yield under drought conditions (Abobatta, 2019).

Drought tolerance is defined as the ability to grow, flower, and display economic yield under a sub-optimal water supply. Drought tolerance involves the ability of the plant to keep its metabolism functioning under low water potential (Jinagool, 2015) by enduring low tissue water content through adaptive traits. Drought-tolerant plants initiate defense mechanisms against water deficit and plants use different mechanisms of drought tolerance at different levels of aridity (Abobatta, 2019). Examples of these mechanisms include morphological mechanisms such as escape from drought, drought avoidance, and phenotypic flexibility. Also, there are physiological mechanisms to avoid the negative effects of drought on plant growth such as osmotic adjustment, antioxidant systems, and resistance to xylem cavitation (Chen and Murata, 2002; Cochard *et al*., 2007; Kranner *et al*., 2002).

2.3.5 Pests, diseases, and weeds

The three factors are called reducing factors for crop yield. Chili is vulnerable to several diseases including root knots caused by nematodes (*Meloidogyne incognita*) and anthracnose (*Colletotrichum* species) cause serious losses of fruits in pre- and post-harvest stages (Mishra *et al*., 2018; Ridzuan *et al*., 2018). Phytophthora blight caused by *Phytophthora capsica* is one of the most destructive pathogens of chili (Quirin *et al*., 2005). Wilt caused by *Fusarium oxysporum*, root rot caused by *Sclerotium rolfsii*, and leaf curl caused by the virus, are also important diseases in chili. Several important pests in chili production are shown in Figure 2.9 (Parisi *et al*., 2020). Broad mite damages the outer cells of leaves. Leaves become distorted, bronze-colored, stiff, and rolled is observed (Figure 2.9 D). The tobacco whitefly, *Bemisia tabaci* (*Hemiptera: Aleyrodidae*), (Figure 2.9 B) has being a vector capable of transmitting efficiently plant viruses (Morales, 2007). Thrips are a major pest of chili plants, they attack the buds, young leaves, and flowers of chili plants (Kalshoven, 1981).

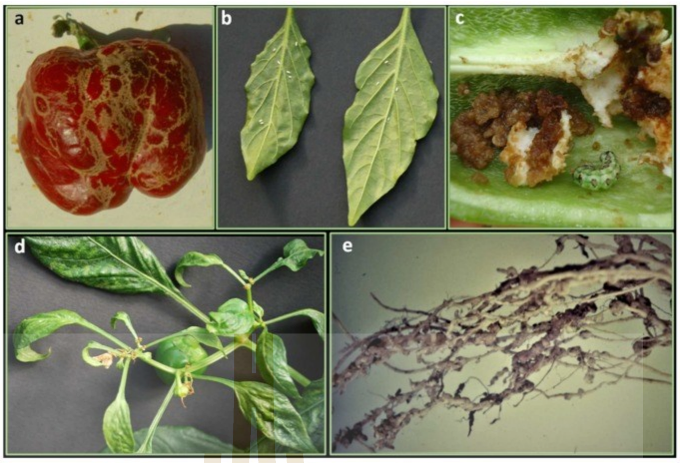

Figure 2.9 Important pests in chili production (a) stripes on fruits caused by thrips feeding;(b) adult stages of whiteflies; (c) damages on fruit caused by cotton bollworm larvae; (d) distorted leaves and damages on inflorescences caused by broad mite feeding; (e) galls or "knots" on pepper roots caused by nematode feeding. Source: Parisi *et al*. (2020).

The outbreak of diseases and insects can occur due to the continuous production of chili in the same place for a long time, moreover, climate change is another factor driving the spread of pests and diseases. Rising temperature and CO_2 levels, as well as the changes in moisture, can affect the population size, survival rate, growth rate, and geographical distribution of pests and diseases (Doody, 2020) As a result, growers require pesticides and insecticides to prevent chili yield loss (Pangjan *et al*., 2015).

Weeds are another major problem for crop production. They compete with crops for light, moisture, and plant nutrients. In most cases, weeds can grow faster than commercial crops and they can easily overshadow the commercial crop during the beginning of the growing season. Weeds can then, reduce the amount of photosynthetic active radiation and CO_2 absorption of the crop plants. With fast-spreading root systems, weeds are very effective in absorbing water and nutrients from the soil, thus depleting soil moisture and fertility. Therefore, they can affect crop growth, yield, and yield quality.

The cost of chili production is relatively high, especially when considering the chemical fertilizers, herbicides, pesticides, as well as fungicides required in the production (Sornin and Athipanyakul, 2014).

2.4 Capsaicinoids

2.4.1 Types and properties of capsaicinoids

Capsaicinoids are alkaloid substances that contributed to the unique characteristic of chili, the spiciness. Capsaicin, dihydrocapsaicin, nordihydrocapsaicin, homocapsaicin, and homodihydrocapsaicin are members of capsaicinoids found in chili fruit but the proportion of each capsaicinoid can differ (Conforti *et al*., 2007; Koleva-Gudeva *et al*., 2013), Table 2.4 shows the percentage of capsaicinoids in red chili.

Table 2.4 The percentage of capsaicinoids in red chili[1].

Capsaicinoids	Proportion found in the fruit (%)
Capsaicin	69
Dihydrocapsaicin	22
Nordihydrocapsaicin	7
Homocapsaicin	1
Homodihydrocapsaicin	1

[1]Source: Conforti *et al*., 2007; Koleva-Gudeva *et al*., 2013.

Capsaicin (8-methyl-N-vanillyl-6-nonenamide, $C_{18}H_{27}NO_3$, (Figure 2.10 A) can be found in the placenta and septa of chili, which is equal to 2.5 % capsaicin, while in seeds, pericarp the capsaicinoids can be found at approximately 0.7% and 0.03 % capsaicin, respectively (Hundal and Dhall, 2005). This substance is a white crystalline powder, odorless, and has a weak acid property. Its molecular weight is 305.4 g/mol, the boiling point is between 210 - 220 ˚C, and the melting point is 64.5 ˚C (Prapannaphasin, 2001). Capsaicin is accountable for 70% of total capsaicinoids (Table 2.4). Therefore, capsaicin is the main compound used in industries and pharmaceutical sectors.

Dihydrocapsaicin ($C_{18}H_{29}NO$) is a substance that has the second-highest proportion in chili fruit. The chemical name is N-(4-Hydroxy-3-methoxybenzyl)-8-methylnonanamide, it has the chemical structural formula as shown in (Figure 2.10 B) with a molecular weight of 307.44 g/mol. The structural formula of capsaicin and dihydrocapsaicin are similar, with the same amount of 18 carbon atoms. The difference between these two capsaicinoids is the bond between the carbon atom and the oxygen atom at the final position; capsaicin has double bonds, but dihydrocapsaicin has a single bond. In nature, capsaicin is transformed into dihydrocapsaicin by the hydrogenation reaction.

Homocapsaicin is a substance that is found in a small proportion of capsaicinoid groups. It has the structural formula as shown in Figure 2.10 (C) while nordihydrocapsaicin is a substance that has a high volume, followed by dihydrocapsaicin and the chemical structural formula as shown in Figure 2.10 (D). From the chemical structure formula, nordihydrocapsaicin is a substance in the capsaicinoids group that has the least amount of carbon atoms (17 carbon atoms). Homodihydrocapsaicin is a substance that is found in a small proportion of capsaicinoid groups similar to homocapsaicin, its formula is shown in Figure 2.10 (E).

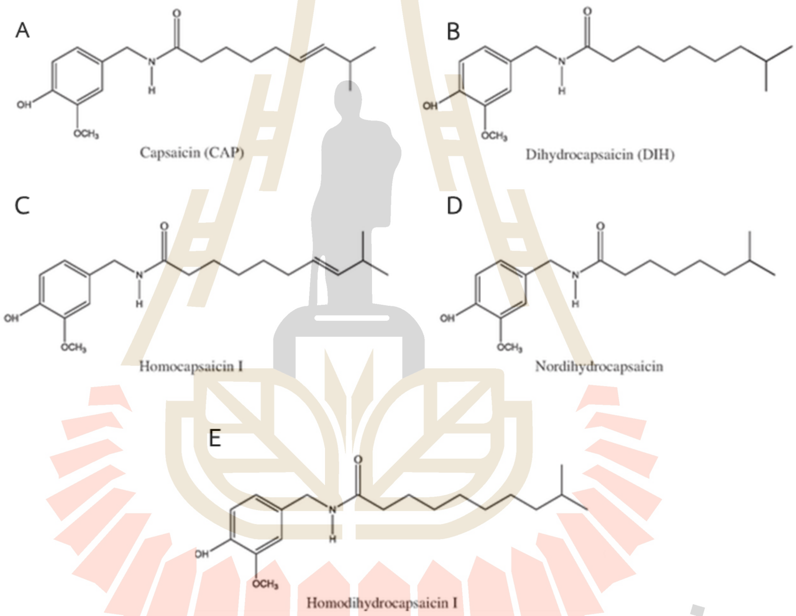

Figure 2.10 Structures of major capsaicinoids identified in *Capsicum* species. Source: Vera-Guzmán *et al*. (2017).

2.4.2 Biosynthesis and accumulation of capsaicinoids in chili

Capsaicinoids were synthesized through two biosynthetic pathways: the phenylpropanoid and the branched-chain fatty acid pathways. Phenylalanine is the precursor of the phenylpropanoid pathway while valine or leucine are precursors of the branched-chain fatty acid pathway. Enzyme products such as phenylalanine ammonia lyase (PAL), cinnamic acid 4-hydroxylase (Ca4H), cinnamic acid-3-hydroxylase (Ca3H), caffeic acid O-methyl transferase (CoMT), aminotransferase (AMT), and capsaicinoids synthase (CS) are catalysts in these pathways. Figure 2.11 (A) shows the capsaicinoid biosynthesis pathway model in plant cells which can be divided into 8 steps. In the first step, chorismate is produced from the shikimate pathway, it is used to synthesize Phenylalanine in the plastid (step 2). In the cytoplasm, associated with the endoplasmic reticulum, Phenylalanine is converted to feruloyl-CoA by phenylpropanoid metabolism (step 3) and is likely converted to vanillylamine by an unknown enzyme in an unknown compartment (step 4). Pyruvate is the precursor to Val (step 5), which is exported to the mitochondria and catabolized to isobutyryl-CoA (step 6). Isobutyryl-CoA returns to the plastid, where it is elongated to 8-methylnonenoic acid by the fatty acid synthase (step 7). Export from the plastid is concomitant with the formation of the CoA thioester. The location and mechanism of the final condensation and export of capsaicin out of the cell (step 8) are debated. Other capsaicinoids are formed through variations in steps 5 to 7, and ester forms of the molecule are likely formed by variations in steps 3 and 4 (Figure 2.11 B). These enzymes combine intermediates from the phenylpropanoid pathway with the fatty acid metabolism reaction in the synthesis of capsaicinoids. Considering the different structural substances of capsaicinoids, the similarity is in the part of vanillylamide, caused by the breakdown reaction phenylpropanoid. The difference is due to the variance in the carbon chain caused by the breakdown of fatty acid metabolism, which directly affects the types of capsaicinoid created (Pasorn *et al.*, 2012).

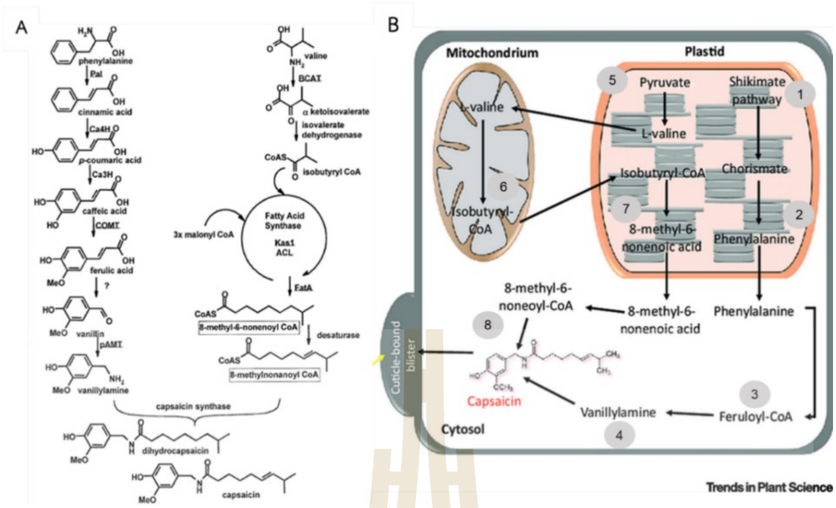

Figure 2.11 Capsaicinoid biosynthetic pathway (A) source: Blum *et al*. (2004), and schematic representations of the subcellular locations of the main steps involved in capsaicinoid biosynthesis (B) source: Naves *et al*. (2019).

Capsaicinoids accumulate in different parts of chili fruit; the placenta, pericarp, and seed are the main parts where capsaicinoids accumulated. Estrada *et al*. (2000) measured the capsaicinoids in *C. annuum* L. var. *annuum* cv. Padron. They detected the maximal levels of free phenolics during the early stages of development (Figure 2.12 (A)) with a different pattern of capsaicinoid accumulation. Phenolic compounds and lignin were presented in a similar pattern in the early accumulation and gradually decrease while the capsaicinoid accumulation as the free phenolics, gradually increase 28 to 42 days after flowering (Figure 2.12 (B)), Estrada *et al*., 2000). Such a decrease in lignin content in the Padron chili coincided with the softening process that the fruit undergoes. Softening is a process that entails a major chemical restructuring of the cell walls that comprise the body of the fruit (Gross *et al*., 1986), and as capsaicin synthesis *in vivo* coincides with fruit softening, it has been proposed that such softening may provide a huge supply of substrates for capsaicin synthesis. Therefore, capsaicinoids start to accumulate at an early fruit development stage and gradually increase until it reaches a maximum value around 30 to 50 days after flowering, depending on the cultivar. In *C. frutescens*, accumulation continues to increase after the length of the fruit reaches a maximum value (Estrada *et al*., 2000; Hall *et al*., 1987; Sukrasno and Yeoman, 1993), but in other *Capsicum* species, capsaicinoid content somewhat dropped in the ripening stage (Iwai *et al*., 1979; Salgado-Garciglia and Ochoa-Alejo, 1990).

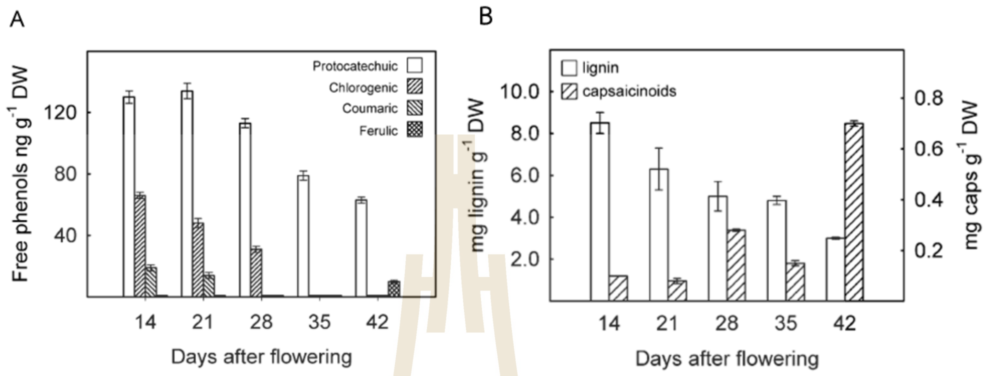

Figure 2.12 Evolution of free phenolics during the ripening of chili fruit and changes in capsaicinoid and lignin content during the ripening of chili fruit (B). Values are means ± SD. Source: Estrada *et al*. (2000).

The study by Pandhair and Sharma (2008) found that in ripened chili fruits (49 days after flowering, DAF), capsaicin accumulation in the placenta can be up to 63.96 mg /g of fruit dry weight. While in pericarp and seed, capsaicinoids can be found up to 7.12 mg/g and 5.06 mg/g, respectively in *C. annuum* L. The capsaicin content of the placenta was found to be about ten-fold higher compared with chili fruits followed by pericarp and seeds (Figure 2.13).

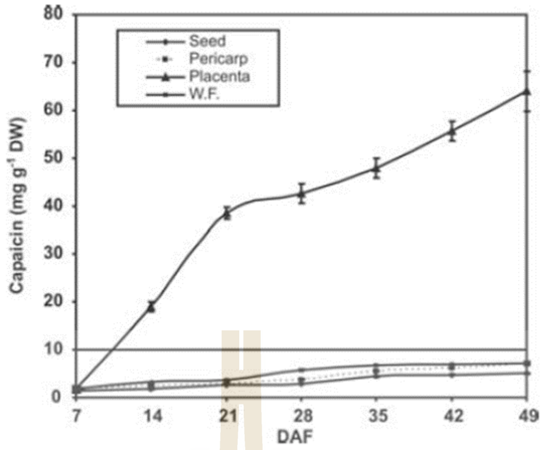

Figure 2.13 Capsaicin accumulation (mg/g dry weight) in different parts of fruits at various physiological stages of growth. Vertical bars indicate ±SE. Source: Pandhair and Sharma (2008).

The decline of capsaicinoid content in chili fruit can occur due to chemical decomposition by photooxidative reactions (Iwai *et al*., 1979) or the action of some enzymes such as peroxidase (Contreras-Padilla and Yahia, 1998; Estrada *et al*., 2000). The developmental changes in capsaicinoid content to peroxidase activity have been studied in some chili fruits. Contreras-Padilla and Yahia (1998) described the evolution of capsaicinoids during the development of the fruit in different hot chili widely used in Mexico and observed that the peroxidase activity increased at the time when the concentration of capsaicinoids started to decrease after the end of ripening (80 days). This might indicate that this enzyme is involved in developmentally regulated capsaicin degradation.

2.4.3 Pungency and capsaicinoid contents

The pungent of chili is measured in Scoville Heat Units (SHU) and it can be used to classify chilies into 5 groups: non-pungent (0-700 SHU), low pungent (700-3,000 SHU), medium pungent (3,000-25,000), high pungent (25,000-70,000 SHU) and highest pungent > 80,000 SHU (Weiss, 2002). The pungent level of chili is directly related to the amount of capsaicinoid contained in the chili fruit (Scoville, 1912); 1 ppm of capsaicin is equal to 16 SHU. The pungent level and capsaicinoid contents in chili depend on many factors: the species of chili, genotypes, or cultivars, node position, the development stage of the fruit, the size and age of chili fruit (Sukrasno and Yeoman, 1993; Zewdie and Bosland, 2000), as well as environmental factors during the cultivation, crop management, nitrogen and potassium contents, water availability, sunlight, temperature, and postharvest management (Ornelas-Paz *et al*., 2010; Uarrota *et al*., 2021; Wilson *et al*., 1995). The following section describes the important factors that can alter the capsaicinoid content and pungency of chili.

Chili species and cultivars

The production of capsaicinoids is inherited as a dominant character and is controlled by the Pun1 locus (Blum *et al*., 2002), whereas under pun1 / pun1 recessive conditions, they are not produced by chilies. Cazáres-Sánchez *et al*. (2005) reported in Habaneros chilies values of 60,901 Scoville units (SHU), while in sweet chilies only 1,519 SHU. The degree of pungent is also regulated by the environment and the genotype-environment interaction (Gurung *et al*., 2011; Jeeatid *et al*., 2017), which generates a high variation in the level of pungency and capsaicinoids between and within the genotypes (Zewdie and Bosland, 2000). Variations of capsaicinoid content in chili fruits depend on the genotypes and production environments (Gurung *et al*., 2011). Some examples are Bhut Jolokia has a pungent level of 800,000 – 1,200,000 SHU, which can provide capsaicinoids yield of 1,300 mg/plant, while Akanee Pirote has a pungent level of 400,000 – 600,000 SHU and can provide higher capsaicinoids yield at 3,835 mg/plant. If calculating capsaicinoid yield per rai (1,600 m^2) from the number of chili plants in the area using 1.2 × 0.8 m planting spacing (Sitathani, 2013) these two varieties can produce 42.61 kg/rai and 290.46 kg/rai of dry fruit yield, respectively. From the mentioned yield, Bhut Jolokia and Akanee Pirote can produce 2.88 kg/rai and 8.52 kg/rai of capsaicinoid yield, respectively (Jeeatid *et. al*., 2021).

Despite the increasing demand for capsaicinoid extracts, the cultivation of chili with the highest pungent in Thailand is still limited, especially for *C. chinensis.* Growing the *C. chinensis* cv. Habanero and Bhut Jolokia in Thailand is rather challenging because the climate in Thailand is unfavorable for the species and the susceptibility of the species to diseases and pests (Kraikruan *et al*., 2015).

Stages of fruit ripening

The stages of development and maturation of chili fruit are other factors determining capsaicinoid content. The stages of development are extended to the size and age of the fruit. Research by Iwai *et al*., (1979) found that capsaicinoids were first discovered around 20 days after flowering (DAF) in both the placenta and pericarp and gradually increased to a maximum at about 40-50 days after flowering, then they decreased. Contreras-Padilla and Yahia (1998) found that the peak of capsaicinoid accumulation in *C. chinense* fruits was between 45 and 50 DAA and maximum peroxidase activity at 60 DAA followed by a drastic decrease in capsaicinoids afterward. The most likely cause of capsaicinoid loss at the start of fruit senescence is peroxidase activity.

Environmental factors

Environmental factors are complex and have a great influence on the response of plants. They are related to plants at the genetic level in terms of plant responses to the variation of environment, these responses are including the synthesis and accumulation of capsaicinoids in chili. The following are the important environmental factors that can greatly affect the capsaicinoid content of chili: light, temperature, water, and plant nutrients.

1) Light stimulates the synthesis of capsaicinoids in unripe fruit. It has a positive influence on the expression of the capsaicin synthase gene. A study by Jeeatid *et al*. (2016) tested chili cultivars of *C. chinense* with different pungent levels in response to changes in light intensity. They found that Akanee Pirote under 50% of shading during the flowering stage gave a maximum capsaicinoid content of 4,820 mg/plant which was 2-fold higher than unshaded plants (full light intensity). Whereas Bhut Jolokia gave the highest capsaicinoids under 70% shading. These findings indicate that shading levels at a specific growth stage can induce capsaicinoid content in chili cultivars that are in a highly pungent group. On the other hand, the study by Uarrota *et al*., (2021) found that high light intensity and heat treatments may reduce capsaicinoid content in fruits probably due to the loss of activity of capsaicin synthase (CS) and phenylalanine ammonia lyase (PAL).

2) Ambient temperature can influence the capsaicinoid content of chili, however, the influences can be positive or negative depending on the species and cultivars. Therefore, it cannot yet exclusively conclude the effect of temperature on the capsaicinoid content of chili. In some cultivars, a higher temperature can positively affect the accumulation of capsaicinoids. A study by Otha (1960) found that chili plants that grew under 30 °C of day and night temperatures can produce higher capsaicinoids when compared with those that grew under a temperature of 21 to 24 °C. The study

also found that higher night temperatures, in particular, can greatly influence the synthesis and accumulation of capsaicinoids. The accumulation of capsaicinoids in other cultivars was negatively affected by an increasing temperature. Gurung (2011) found that under low temperatures *C. annuum and C. chinense* could produce and accumulate higher capsaicinoids when compared with the plants cultivated at a higher temperature. The study by Van-Soest (1987) reported high ambient temperature increased lignifications of the plant cell wall and promoted rapid metabolic activities occurred, which can result in a decrease in other metabolism pathways. Capsaicinoids are also products of metabolic pathways, then the competition between capsaicinoids and other more important compounds may result in a reduction of capsaicinoid biosynthesis at higher temperatures.

3) Plant nutrients can influence the biosynthesis and accumulation of capsaicinoids in positive and negative ways, similar to the effects of temperature. Some plant nutrients, especially nitrogen, can stimulate capsaicinoids. Nitrogen can directly affect capsaicinoid accumulation because the biosynthesis of these compounds is related to three amino acids: phenylalanine, valine, and leucine. It can also provide the essential amino groups for the formation of vanillylamine. Johnson and Decoteau (1996) found that nitrogen rate affected the pungency of *C. annuum* L. 'Jalapeno' fruits when nitrogen treatment began at transplanting. The observed capsaicinoid contents and pungency level in response to nitrogen fertility rate suggested that the optimum nitrogen fertilization for high pungency fruits was between 7.5 to 22.5 mM. While 1 mM nitrogen reduced capsaicin levels in fruit compared to other nitrogen rates. Furthermore, observation in *C. chinense* Jacq. 'Habanero' pepper found that nitrogen fertilization significantly increased plant growth and fruit as well as maintaining high capsaicin levels. The optimum response was produced with 15 mM urea as the nitrogen source while plants under fertilization stress (control) had high capsaicin content (Medina *et al*., 2008). However, Johnson and Decoteau (1996), observed no effect of K on pungency in *C. annuum* fruit, suggesting that K does not notably interfere in capsaicin metabolization.

4) Water levels have been reported to affect capsaicinoid biosynthesis and accumulation in chili. Several studies have shown that the influence of water affected capsaicinoid biosynthesis and accumulation. Estrada *et al*. (1999) investigated the level of pungent in *C. annuum* (Padron pepper) under different watering regimes through the microprocessor-controlled irrigation system. The experimental treatments were

control treatment that was watered 13 minutes/day, 20 minutes/day (T1), and 5 minutes/day (T2). The study evaluated changes in capsaicinoid content at different maturation stages of fruit and found that capsaicinoid content consists of capsaicin and dihydrocapsaicin content there is a similar level of increase in each treatment. The results showed that in all three treatments, capsaicinoid can be detected starting at 14 days after flowering (DAF), and the maximum capsaicinoid content was found in 20 minutes/day irrigation (T1). However, there was an interesting change in T2, with the level of capsaicinoids increasing continuously, showed a large increase on the 21 DAF, and remained at high levels on the 35 DAF. While T1 capsaicinoid content increased at 21 DAF and gradually reduced at 28 and 35 DAF with lower capsaicinoid content compared with T2. This study shows that the comparison of the 3 treatments provides a different amount of water in a large and low volume that can positively affect capsaicinoid content at all stages of fruit development. Ruiz-Lau *et al*. (2011) studied *C. chinense* Jacq. (Habanero pepper) under the water deficit regimes. They compared the control and stress treatment groups: watered at first anthesis (26 d after transplanting), 1 liter every 7 days (T1), and 1 liter every 9 days (T2). They found that fruit fresh weight in the stress treatment groups at 45 DAA was not significantly different compared with the control. Both water stress treatments led to a significant increase in the concentration of capsaicin (an increase of 16 mg/g dry weight compared with the control) and dihydrocapsaicin (19 mg/g dry weight increase compared with the control) in the placenta at 45 DAA. The treatments also affected the capsaicin and dihydrocapsaicin in seeds and pericarp at 25 and 45 DAA, in which capsaicinoids were found to be increased. These results suggest that the water stress level and the age of the fruits have the effects on the increment of capsaicin and dihydrocapsaicin contents as well as the yield, such as the fruit fresh weight in Habanero pepper. Another study by Jeeatid *et al*. (2017) studied the influence of water stresses on capsaicinoid production in hot chili (*C. chinense* Jacq.) cultivars with different pungency levels. They found that water stress can influence capsaicinoid content, pungent level of chili, and yield. The response depends on chili cultivars and the levels of water stress that the plants received. For example, every third-day watering can increase capsaicinoids content in Akanee Pirote to 4,653 mg/plant, compared with normal watering which can produce 3,835 mg capsaicinoid/plant, while in Bhut Jolokia and BGH 1719, capsaicinoids were decreased compared with the control. Only 1,300 and 417 mg of capsaicinoid/plant were received, respectively. Haris *et al*. (2020) studied the growth, yield quality, and capsaicin concentration of *C. annuum* under drought conditions. They found that drought stress affected capsaicinoid accumulation. Fruits treated under moderate (7-day interval irrigation) and severe (9-day interval irrigation) stress had the highest concentration of capsaicin and dihydrocapsaicin when harvested at 36 and 45 days after anthesis. Capsaicin concentration at 36 after anthesis under moderate (7-day interval irrigation) and severe (9-day interval irrigation) stresses was 260 µg/g and 270 µg/g, respectively when compared with the control that gave 90 µg/g capsaicin. Rathnayaka *et al*. (2021) studied the relationship between water supply, sugar, and capsaicinoid contents in chili fruits (*C. annuum* L.), the chili plants were treated with three water supply treatments: excess (260 mL), standard (130 mL), and drought (50 mL) per application. They found that two chili varieties 'Botankosho' and 'Sapporo Oonaga Nanban' had higher capsaicinoid content under drought treatment at 40 and 50 DAF. While capsaicinoid content was lower in plants treated with excess water and standard water supply at all DAF compared with drought treatment. Moreover, the water stress levels also decreased the fruit number and fruit dry yield. Although water deficit may cause a reduction in yield, it only slightly affects placental tissue formation which is an important plant tissue for the synthesis and storage of capsaicinoids (Sung *et al*., 2005). As a result, the capsaicinoids that are synthesized may remain high when applying water stress treatment.

According to the information demonstrated above, it shows that chili production was important to the economic sector of Thailand. It is used in many sectors, whether it is a direct consumption or an important raw material in various industries. Chili becomes more important to high-value industries, especially in the pharmaceutical sector. Thus, one may expect an increasing demand for capsaicinoid extracts in the coming future. This highlights the importance of a study involving the production of chili with high capsaicinoid yield. As mentioned above, many factors can affect the synthesis and accumulation of capsaicinoids in chili, and water management is one of the factors that can effectively stimulate capsaicinoids synthesis and accumulation. Applying water stress at a suitable level and at an appropriate time may lead to the stimulation of capsaicinoids biosynthesis and accumulation in chili. This precise water management can also benefit chili production, especially in the northeastern region where water is limited. At present, studies on the effect of water stress to stimulate the production and accumulation of capsaicinoids have been widely studied. However, due to the different responses of chili cultivars to water stress, studies have not been well-covered. Furthermore, the information obtained from the various studies had yet to investigate the physiological responses that may involve capsaicinoid production of the chili. Therefore, an investigation for suitable watering regimes that can result in optimal yield and high capsaicinoid content is of interest.

CHAPTER III
RESEARCH METHODOLOGY

The experiment in this study was divided into two parts: the effects of water regimes on physiological traits, growth, yield, and capsaicin content in pot-grown chili and the effects of water regimes on agronomic traits and capsaicin content of chili under field conditions. Therefore, the information in chapter 3 and chapter 4 is separated into two parts as well.

3.1 The effects of water regimes on physiological traits, growth, yield, and capsaicin content in pot-grown chili

3.1.1 Experimental design

In this study, 3 x 4 factorial in complete randomized design (CRD) was used. The experiment included three chili varieties (factor A): Super-Hot 2, Huay-Sii-ton, and Kee-Nu-Suan, and 4 different watering regimes (factor B): 100% of maximum water holding capacity (MWHC) or control, 80%, 60%, and 40% MWHC. The plants were assigned to 12 treatment combinations as follows:

1) Super-Hot 2 with 100% MWHC watering (control)
2) Super-Hot 2 with 80% MWHC watering
3) Super-Hot 2 with 60% MWHC watering
4) Super-Hot 2 with 40% MWHC watering
5) Huay-Sii-ton with 100% MWHC watering (control)
6) Huay-Sii-ton with80% MWHC watering
7) Huay-Sii-ton with 60% MWHC watering
8) Huay-Sii-ton with 40% MWHC watering
9) Kee-Nu-Suan with 100% MWHC watering (control)
10) Kee-Nu-Suan with 80% MWHC watering
11) Kee-Nu-Suan with 60% MWHC watering
12) Kee-Nu-Suan with 40% MWHC watering

Each treatment had 3 replications and contained 5 plants in a replication.

3.1.2 Plant materials

Three chili cultivars used in this study were Super-Hot 2 and Huay-Sii-ton from *C. annuum* with a pungent level of around 35,000 to 70,000 SHUs (NSTDA, 2019) and Kee-Nu-Suan from *C. frutescens* with the pungent level around 100,000 to 250,000 SHUs (Bureau of Food Safety Extension and Support, 2019). These cultivars are in market demand and wildly grown in the country. Super-Hot 2 and Huay-Sii-ton are large fruit Kee-Nu that have a large production area in the northeastern region, both have the potential to produce high yield per area, especially Super-Hot 2 can produce a yield of around 1.2 - 2 ton/rai (Si Sa Ket Horticultural Research Centre, 2017). Kee-Nu-Suan is small fruit Kee-Nu, It is produced in many areas across the country and has a very spicy taste that is popular with consumers.

The seeds were soaked in warm water (about 50°C) for 30 minutes, they were germinated in plastic germination trays filled with peat moss. The germination trays were placed under a plastic-covered nursery at Suranaree University of Technology's farm in Nakhon Ratchasima Province, Thailand. They were regularly watered while sufficient sunlight throughout the day and well-ventilated conditions were ensured during the whole experiment. On 15-20 days after sowing, a solution of chemical fertilizer (15:15:15) at a concentration of 100 g/ 20 L of water was given to the seedlings. At 30 days after sowing, homogenous seedlings were selected and transplanted into 10-inch plastic pots, a seedling per pot. Each pot was filled with a similar amount of sandy loam soil from the planting field at Suranaree University of Technology's farm where the second experiment took place. Pots were kept under an open greenhouse with a plastic-covered roof and irrigated to the MWHC (the determination was indicated in 3.1.3). The chili plants were fertilized as the recommendation of Sitathani (2013) while pests and diseases were managed as recommended by the GAP regulation (National Bureau of Agricultural Commodity and Food Standards, 2005).

3.1.3 Soil properties, water holding capacity, and watering regimes

Soil properties were obtained from the Suranaree University of Technology's farm and indicated in Table 3.1.

Table 3.1 Properties of the sandy loam soil used in the experiment.

Soil sample	Sandy loam soil	Value interpretation
EC (ds m^{-1})	0.09	None-saline
pH	7.97	Slightly alkaline
OM (%)	1.13	Slightly low
Available P (mg kg^{-1})	36.78	High
Exchangeable K (mg kg^{-1})	111.42	High
Exchangeable Ca (mg kg^{-1})	198.80	Very low
Exchangeable Mg (mg kg^{-1})	73.30	Low

The MWHC was determined through the calculation started from the estimation of the FC and PWP of the soil, the soil at the root zone depth (40-50 cm) was collected from the assigned field. The soil was dried in a hot air oven at 105˚C for 24 hours. After that, the soil sample was transferred into the crucibles until they were ¾ filled. The soil was packed by tapping the crucibles, this process was repeated until the crucibles were almost full. The soil moisture contents at FC and the PWP were determined using a pressure plate apparatus (Model 505, 20 bar compressors, Soilmoisture) at suction pressures of 0.33 and 15 bars, respectively. Then, the crucibles were placed in a basin and distilled water was added to a depth of about 3 cm. They stood in the water-filled basin for 24 hours, and at the end of this period, the soil inside the crucibles was saturated with water. After wiping the outside dry, the crucibles were weighed and the weight of the crucibles plus the saturated soil was recorded. The crucibles and their contents were then transferred to the hot air oven and air-dried at a temperature of 105 ˚C for 24 hours. After drying the crucibles and their dried soil were weighed again and the dry weight was recorded (Mbah, 2012). The soil moisture content by mass can be calculated as follows:

$$\text{Water weight} = (\text{crucible weight} + \text{wet sample}) - (\text{crucible weight} + \text{dry sample}) \quad (3.1)$$

$$\text{Dry soil weight} = (\text{weight of crucible} + \text{dry sample}) - \text{weight of crucible} \quad (3.2)$$

$$\text{Soil moisture (\%)} = (\text{water weight/dry soil weight}) \times 100 \quad (3.3)$$

The 100% MWHC was calculated as follows:

$$\text{MWHC} = \text{\% soil moisture at FC} - \text{\% soil moisture at PWP} \quad (3.4)$$

After that, the soil moisture contents at 80, 60, and 40% MWHC were calculated. The FC, PWP, and soil moisture contents of the studied treatments are shown in Table 3.2.

Table 3.2 Water content at different watering regimes of the sandy loam soil used in the experiment.

Soil moisture contents	Moisture contents (%)
Field capacity	12.12
Permanent wilting point	6.57
100% MWHC[(1)]	12.12
80% MWHC	11.01
60% MWHC	9.90
40% MWHC	8.79

[(1)] Maximum water holding capacity

Watering regimes were applied to the plants at the flowering stage and maintained these levels until fruit development (November 2021 – February 2022). Soil moisture content in pots was daily observed using the HH2 Moisture Meter (Delta-T Devices Ltd.) and changes in soil moisture were used to calculate the volume of water that needed to be given to the plants for controlling soil moisture content in the pots. The pots were reirrigated when the moisture content dropped below the assigned regimes.

3.1.4 Environmental conditions

Air temperature and relative humidity (RH) under greenhouse conditions at Suranaree University of Technology's farm are indicated in Figure 3.1. From September 2021 to February 2022, the average air temperature was contained between 25 to 39 °C, while RH ranged between 30 to 88%. Early in the experiment in September, the air temperature was still not high, but there are gradually increased high in January. While RH was high in the early stage of the experiment and gradually decreased.

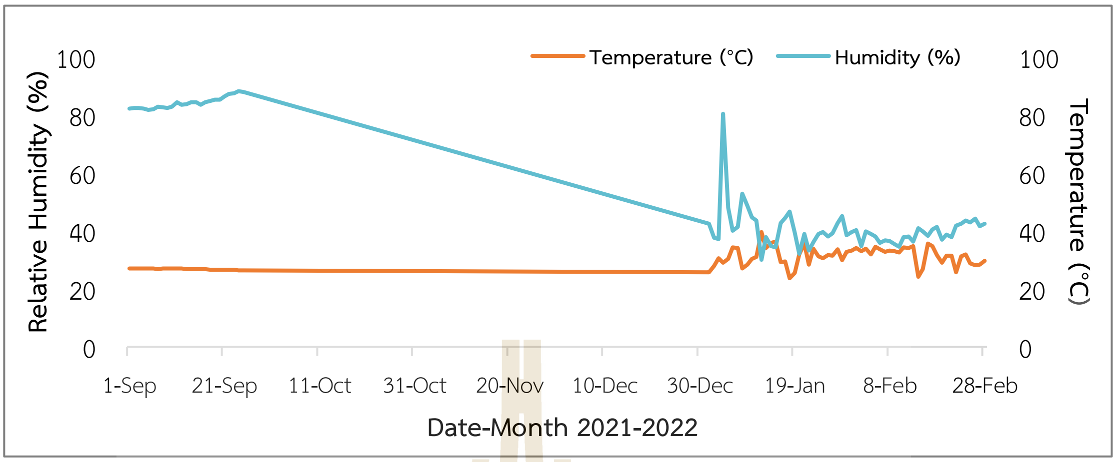

Figure 3.1 Air temperature and relative humidity (RH) under greenhouse from September 2021 to February 2022. Source: Pairintra (2022).

3.1.4 Data collection

The chili plants were subjected to the following measurement:

Physiological traits

1) Leaf water potential (LWP_{md}) was determined using a pressure chamber model 3005 (Soilmoisture, Japan) on a fully expanded leaf. Three plants were randomly selected from each treatment and a leaf from each plant was cut from the middle of the canopy height. The measurement was performed before the watering from 11:00 a.m. to 1.00 p.m. at 0 (at the anthesis), 2, 4, 6, and 8 weeks after the anthesis (WAA).

2) Leaf greenness (SPAD) was measured using the SPAD-502 plus chlorophyll meter (Konica Minolta, Japan). Three plants from each treatment that were not used for the measurement of LWP_{md} were randomly selected for the measurement, they were tagged and continuously used for the measure of SPAD and maximum quantum yield of PSII throughout the experiment. On each plant, 3 leaves at the middle of the canopy height were selected. The SPAD was the average value from 3 points on a leaf. This trait was measured on the same day as the LWP_{md} measurement.

3) The maximum quantum yield of PSII (F_v/F_m) is the measurement of chlorophyll fluorescence yield, shortly before and during a pulse of saturating light. It was measured using a chlorophyll fluorescence meter (Handy PEA, Hansatech

Instruments Ltd., UK) on the same set of leaves that were used to measure leaf greenness. The measurement was done on the same day as the measurement of the previous traits, but it was performed only between 9.00 a.m. – 2.00 p.m. The leaves were clamped with the Dark Leaf Cup (DLC-8) for 15-20 minutes to allow the movement of electrons through the PSII to complete. Then using the red-light beam from the device to excite the chlorophyll; the minimal level of fluorescence (F_0), and reaction centers are opened. The saturating pulse of light also gives information on the maximum possible yield of fluorescence (F_m) and F_v (variable fluorescence) can be calculated from the difference between F_m and F_0. Finally, the maximum quantum yield of PSII can be calculated by dividing F_v by F_m. It represents the ratio of photons used in the photosynthesis process relative to the number of photons all the leaves absorb (Walz-Gmbtl, 1999).

Growth and development

1) The growth in plant height and canopy width were randomly measured on 5 plants/treatment. The plants were measured on the same day as the measurement of physiological traits. The height was measured from the soil surface to the last node of the shoot while the measurement of canopy width was measured 3 times across the canopy and the average value was recorded.

2) Leaf area index (LAI) was measured using the LI-3100C leaf area meter (LI-COR) at 12 WAA. Three plants/treatment was randomly selected, their leaves were separated and measured for leaf area and LAI was calculated as followed:

$$\text{LAI} = \text{leaf area } (m^2)/\text{ground area } (m^2) \quad (3.5)$$

in this experiment, the ground area was 0.25 m^2 (planting area/plant).

3) Plant dry mass (DM) was evaluated at 12 WAA that finally in the fruits harvest of three chili cultivars. A plant/replication was randomly harvested, cleaned, and dried at 70°C until the dry weight stabilized. The dry weights of the vegetative part and reproductive part were then measured; they were used to calculate the harvest index (HI), which is the ratio between the dry weight of the yield to the dry weight of the whole plant was calculated as followed:

$$\text{HI} = \text{Economic yield / Biological yield} \times 100 \quad (3.6)$$

Yield and capsaicin content

Flowers were daily tagged and marked for the date of anthesis. It was used to track fruits' age since the capsaicinoid contents can vary according to the fruit age (Vázquez-Espinosa *et al*., 2020). The study suggested that capsaicinoids started to accumulate from the early stages of fruit development and continue to increase their content during ripening until a maximum concentration is reached, which is usually after 40–60 days postanthesis (DPA). In this experiment, the ripened chili fruits at 40-60 DAA were harvested to use in the assessment of capsaicin content.

1) Chili fruits were harvested at the ripening stage. The number of fruits per plant was counted, then, the pedicel and calyx were separated from the fruit and the yield fresh weight was recorded. At each harvest time, 10 chili fruits/treatment were randomly sampled and recorded for fruit size (length and width), fruit fresh weight, and dry weight (oven-dried at 60 ˚C for 2 – 5 days until the dry weight stabilized). These dried chili fruits were grounded and kept in closed containers at -20 ˚C while waiting for the analysis of capsaicin content.

2) Capsaicin and pungency, the previously grounded chili powder was used for the extraction of capsaicin using the modified method of Collins *et. al.* (1995) with the HPLC (Chin *et. Al.,* 2011). For capsaicinoid extraction, 2 g of ground chili powder was mixed with 20 ml of acetonitrile and incubated at 80 °C for four hours. The extract solution was filtered and 10 µl was injected into an Agilent 1260 Infinity-Model HPLC series (Agilent Technologies, United States) for analysis. The mobile phase was methanol and deionized water at a ratio of 80:20 at a flow rate of 0.6 ml min^{-1} with the SB-C18 column, column size 3.5 µm, 4.6 x 250 mm. The detector's wavelength was set at 280 nm. The standard capsaicin (≥95% capsaicin; Sigma-Aldrich, Burlington, United States) was prepared for different solution concentrations of 31.125, 62.50, 125, 250, and 500 ppm. The capsaicin data was converted to Scoville Heat Units (SHU), as described by Collins *et al*. (1995). The capsaicinoid yield was calculated using the following formula:

$$\text{Capsaicinoid yield} = (\text{capsaicinoid (mg)} \times \text{fruit dry weight})/\text{sample weight} \quad (3.7)$$

3.1.5 Data analysis

The analyses of variance (ANOVA) were performed and means were compared using Duncan's new multiple range test (DMRT) at a 95% confidence level.

3.2 The effects of water regimes on agronomic traits and capsaicin content of chili under field conditions

3.2.1 Experimental design

The experimental design was a 2 x 2 split-plot design of three replications. Two watering regimes (100 or control and 60% MWHC) were the main plot and two chili cultivars (Super-Hot 2 and Kee-Nu-Suan) were the subplot. In total, there were 4 treatment combinations as follows:

1) Super-Hot 2 at 100% MWHC watering (control)
2) Super-Hot 2 at 60% MWHC watering
3) Kee-Nu-Suan at 100% MWHC watering (control)
4) Kee-Nu-Suan at 60% MWHC watering

Each treatment had 3 replications and there were 120 plants in a replication.

3.2.2 Plant materials

In this experiment, Super-Hot 2 and Kee-Nu-Suen, chili cultivars from *C. annuum* and *C. frutescens* previously studied in experiment 3.1 were selected for evaluation under field conditions. The reasons for this selection were the ability of the two cultivars to maintain yield under restricted watering regimes (80 and 60% MWHC) which finally resulted in a lower reduction of capsaicin yield in Super-Hot 2 and Kee-Nu-Suen than Hueay-Sii-Ton.

The seeds of two chosen cultivars were soaked in warm water (50°C) for 30 minutes then germinated in the germination trays filled with peat moss, 1 seed/hole, covered the seeds slightly. The germination trays were placed under a plastic-covered nursery at Suranaree University of Technology's farm. They were regularly watered to keep the constant moisture of the germination. Sufficient sunlight throughout the day and well-ventilated conditions were ensured through seed germination and seedling growth. During the 15-20 days after sowing, chemical fertilizer (15:15:15) at a concentration of 100 g/ 20 L of water was given to the seedlings.

While waiting for the germination, the assigned field at Suranaree University of Technology's farm was plowed and dried under the sun for 7-14 days to kill pathogenic organisms and weeds, after that, the soil was thoroughly tilled. The 1-m-wide and 10-m-long raised beddings were made with an interval distance of 1 m. Installed the drip irrigation system on the soil surface using drip tape with a flow rate of 2 liters/hour, and 0.5 m distance between drip holes, on a raised bedding, placed 2 drip tape lines. Covered the bedding with plastic to prevent weeds and before transplanting, the beddings were prepared by drilling holes in the plastic cover in double rows of planting; the between row and between plant spacing were 0.5 m and 0.5 m, respectively.

When seedlings were 30 days old, homogenous seedlings were selected and transplanted to the field. In each treatment, there were 120 plants/chili cultivar. The seedlings were irrigated to 100% MWHC, and watering regime treatments was applied after the anthesis through fruit development. Fertilization was provided according to soil analysis values (Thongket, 2016) and pests and diseases were managed following the GAP regulation (National Bureau of Agricultural Commodity and Food Standards, 2005).

3.2.3 Soil water holding capacity and watering regimes

The field plot used in this experiment was a similar plot in which the soil was previously used to fill the pots in experiment 1. Therefore, its properties were similar as shown in Table 5. As previously indicated that 60 and 100% MWHC were chosen for this experiment. The reasons behind these choices of watering regimes were that 80% MWHC had a soil moisture content close to 100% MWHC shown in Table 6, which may not show the difference when compared under field conditions. However, the reduction of the water content in the field condition can be slow when compared to the condition of the pots in the greenhouse, due to several factors such as the amount of soil available to the roots that different, depth of soil wetting, and evapotranspiration. Thus, the pot's condition can be different from the field condition due to differences in soil water status (Ray *et al*, 1998; Wang *et al*, 2001). A study by Liu *et al.* (2021) reported that drought stress levels can be divided into four water stress levels; non-stress (75–80% of the MWHC), mild drought stress (55–60% of the MWHC), moderate drought stress (40–45% of the MWHC), and severe drought stress (20–25% of the MWHC). This report demonstrates that, if using a water regime at 80% MWHC, it was not stressful under field conditions, while 60% MWHC can induce drought stress.

Since the soil was similar, we used the FC and PWP that were previously calculated (Table 3.2) to determine the available water holding capacity and soil water holding capacity as shown in Table 3.3.

Table 3.3 Soil moisture contents and soil water holding capacity for watering regimes calculation.

Soil moisture contents	Moisture contents (%)
Field capacity (FC)	12.12
Permanent wilting point (PWP)	6.57
Available water holding capacity (AWHC)	5.87
Soil water holding capacity (SWHC) for 1 cm (mm)	0.587

Then, the crop water requirement was calculated according to the following equation:

$$\text{ETc (mm/day)} = \text{ET}_p \times \text{Kc} \tag{3.8}$$

when ETc was crop evapotranspiration, ETp was potential evaporation of plant, and Kc was crop coefficient.

Afterward, soil water holding capacity (SWHC), SWHC for root depth, SWHC for the crop at each irrigation time (mm), and watering frequency (day) were calculated as followed:

$$\text{SWHC for root depth (mm)} = \text{SWHC for 1 cm} \times \text{Root depth (cm)} \tag{3.9}$$

$$\text{SWHC for crop in each time (mm)} = (\text{SWHC for root depth} \times \text{allow crop use water (\%)}) \times 100 \tag{3.10}$$

The calculated crop water requirements of chili plants during the experimental months were shown in Table 3.4.

Table 3.4 Crop water requirements and soil water holding capacity for watering regimes calculation.

Crop water requirements	2022					
	April	May	June	July	August	September
Day of month	30	31	30	31	31	30
Etp (mm/day)	5.15	4.00	3.96	3.57	3.63	3.44
Kc (crop coefficient)	0.67	0.67	0.67	0.67	0.67	0.67
Etc (mm/day)	3.45	2.68	2.65	2.39	2.43	2.30
Etc (mm/month)	103.52	83.08	79.60	74.15	75.40	69.14
Drip flow rate(l/h)	1.6	1.6	1.6	1.6	1.6	1.6
Flow rate (mm/h)	16.0	16.0	16.0	16.0	16.0	16.0
SWHC[1] for 1 cm (mm)	0.587	0.587	0.587	0.587	0.587	0.587
SWHC for root depth (mm)	5.87	11.74	17.61	17.61	17.61	17.61

(1) Soil water holding capacity

From the information on crop requirements in Table 3.4, we calculate the amount of water and frequency of watering for the 100 and 60% MWHC using the following equation:

$$\text{Watering frequency (day)} = \text{SWHC for crop in each time (mm)/ETc} \qquad (3.11)$$

The details of watering regimes were shown in Table 3.5.

Table 3.5 Watering schedule for the assigned regimes used in the experiment.

Watering regimes		2022					
		April	May	June	July	August	September
100% MWHC[2]	SWHC for crop (mm)	3.45	2.68	2.65	2.39	2.43	2.30
	Water frequency (day)	1	1	1	1	1	1
60% MWHC	SWHC for crop (mm)	2.35	4.70	7.04	7.04	7.04	7.04
	Water frequency (day)	1	2	3	3	3	3

(1) Soil water holding capacity

(2) Maximum water holding capacity

Watering regimes were applied to the plants at the flowering stage until fruit development, which started from May–August 2022. The watering regimes were managed in combination with total rainfall (mm/day) to maintain soil moisture content at the assigned regimes.

3.2.4 Environmental conditions

The total rainfall, air temperature, and relative humidity (RH) were obtained from Northeastern Meteorological Center (Lower Part) in March-August 2022 indicated in Figure 3.2. The range of minimum air temperature was between 16 to 26°C while the maximum air temperature was between 22 to 38 °C. The RH was between 56 to 91%. Throughout the experiment, air temperature and RH were rather stable and consistent, while the total rainfall began to increase in August.

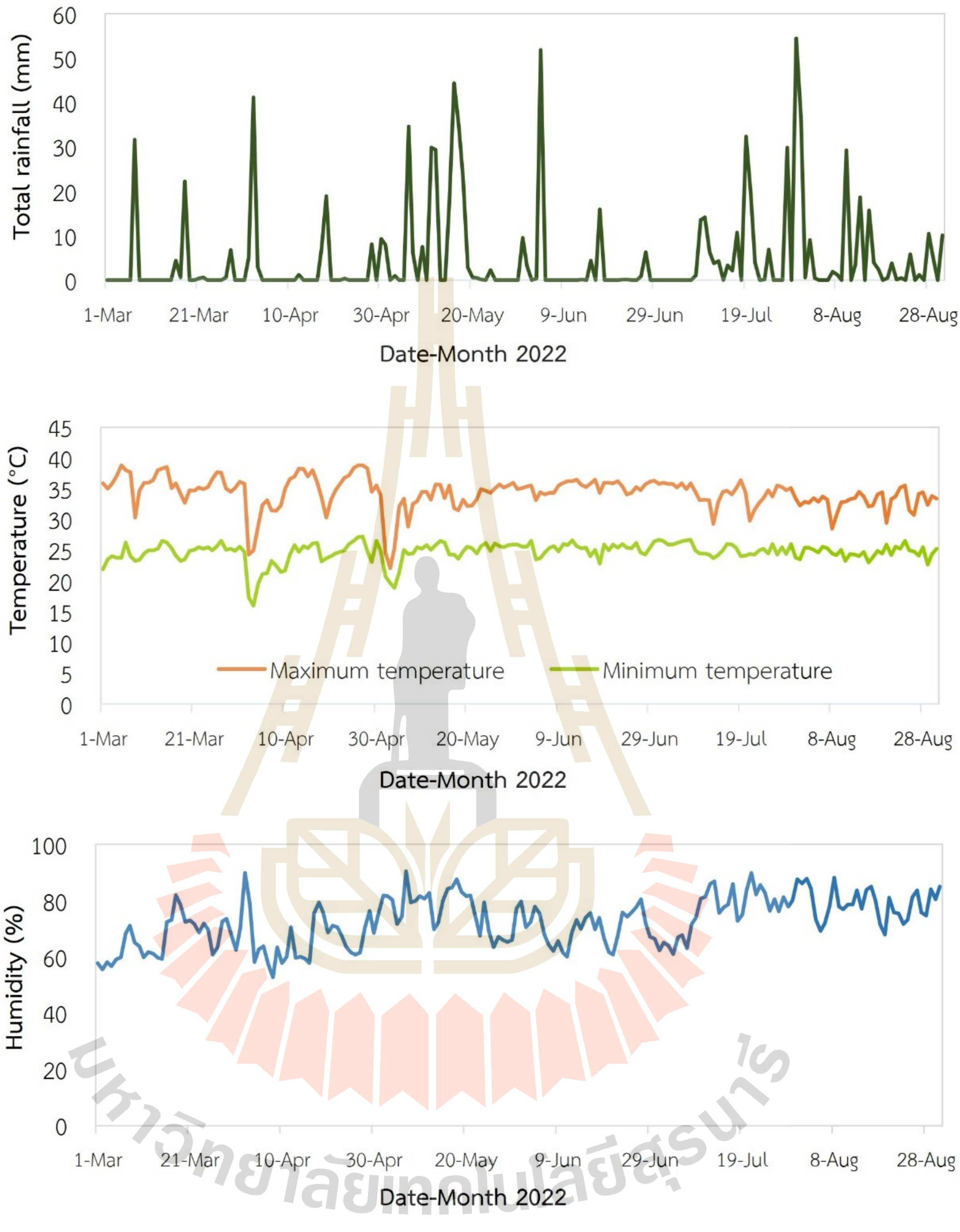

Figure 3.2 Rainfall, air temperature, and relative humidity (RH) in March-August 2022.

3.2.4 Data collection

Growth

1) Plant height and canopy width were randomly measured from each treatment on 5 plants per replication. They were measured at 2, 4, 6, and 8 WAA on the last day of no watering. Plant height was measured from the soil surface to the last node on top of the apical bud while the measurement of canopy width was measured 3 times across the canopy. In addition, the leaf area index (LAI) was randomly measured at five random points in the plots, the measurement was done from 10:00 a.m. to 1.00 p.m. using Sunscan (Delta-T devices, UK).

Agronomic traits and capsaicinoid content

The harvesting procedure started with flower tagging to ensure the same age of chili fruits at the harvest, it was done on ten plants/replication/watering regime for each chili cultivar.

1) Agronomic traits, consisting of the number of fruits/plant, fruit fresh and dry weight, fruit length and width, and total yield were measured. The ripened chili fruits (fully red color fruits) were harvested. The harvest time of studied chili cultivars has differed: Super-Hot 2 was at 50 DAA while Kee-Nu-Suan was at 45 DAA. The harvesting was regularly done for a month; the number of fruits per plant was counted, then, the pedicel and calyx were removed from the fruit and yield fresh weight was recorded. At each harvesting time, 10 chili fruits/treatments were randomly selected and recorded for fruit size (length and width), fresh weight, and dry weight (dried at 60°C for 2-5 days in a hot-air oven).

2) Capsaicinoid contents were measured from studied chili cultivars. Chili fruits of Super-Hot 2 and Kee-Nu-Suan were harvested at 50 and 40 DAA, respectively. Pedicel and calyx were removed from the fruit, and then whole chili fruits were dried in a hot-air oven at 60°C for 2-5 days until the dry weight stabilized. The dried chili fruits were grounded, and stored in a closed container at -20°C while waiting for the study of capsaicinoid contents. Capsaicin and dihydrocapsaicin contents were analyzed using the HPLC technique by adopting the methods described by Collins *et al*. (1995) and Chin *et al*. (2011). The capsaicin and dihydrocapsaicin data were converted to Scoville Heat Units (SHU), as described by Collins *et al*. (1995), and the capsaicinoid yield was calculated using the following formula:

Capsaicinoid yield = (capsaicinoid (mg/g) + dihydrocapsaicin (mg/g)) × fruit dry weight)/sample weight (3.12)

3) Harvest index (HI) was measured at 6 and 8 WAA by randomly harvesting three plants/replication. The whole plants were collected, cleaned, and dried at 70 °C for 48 h. Afterward, when the dry weight was stabilized, the dry weights of yield and whole plant were measured. The HI was calculated as the ratio between the dry weight of the yield to the dry weight of the whole plant.

3.2.5 Data analysis

The analyses of variance (ANOVA) were performed and means were compared using Duncan's new multiple range test (DMRT) at a 95% confidence level.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 The effects of water regimes on physiological traits, growth, yield, and capsaicin content of chili under greenhouse conditions

The responses of three chili cultivars to the watering regimes were divided into three parts: physiological responses, growth and development, and yield and capsaicinoid contents.

4.1.1 Physiological traits

During the application of watering regimes, changes in LWP_{md} were observed. The analysis shows significant differences in LWP_{md} when compared between chili cultivars and between watering regimes but without a significant difference between the interaction effects of the two studied factors (Table 4.1). Among the three studied chili cultivars, Hueay-Sii-Ton was the chili with the highest LWP_{md} which was significantly higher than Kee-Nu-Suan and Super-Hot 2. The applied watering regimes can influence the LWP_{md} of chili plants. With decreasing watering regimes, the LWP_{md} was lowered when compared with the control treatment except for the 80% MWHC.

Table 4.1 Effects of chili cultivars, watering regimes, and their interaction on physiological traits of *C. annuum* (Super-Hot 2 and Hueay-Sii-Ton) and *C. frutescens* (Kee-Nu-Suen) responded to different watering regimes (% maximum water holding capacity; MWHC) and the interactions.

Studied factor		LWP_{md} (Mpa) (2)	SPAD	F_v/F_m
Chili cultivar (A)	Super-Hot 2	-1.86±0.09^{b}	56.96±0.33^{a}	0.759±0.00
	Hueay-Sii-Ton	-1.40±0.06^{a}	53.69±0.28^{b}	0.759±0.00
	Kee-Nu-Suan	-1.75±0.09^{b}	56.91±0.39^{a}	0.754±0.00
	F-test	**	**	ns
Watering regimes (B)	100% MWHC (1)	-1.30±0.05^{a}	54.81±0.37^{bc}	0.781±0.00^{a}
	80% MWHC	-1.49±0.07^{a}	55.85±0.42^{b}	0.758±0.00^{b}
	60% MWHC	-1.79±0.10^{b}	58.13±0.37^{a}	0.752±0.00^{b}
	40% MWHC	-2.11±0.14^{c}	54.62±0.40^{c}	0.739±0.00^{c}
	F-test	**	**	**
AxB	Super-Hot 2 100% MWHC	-1.33±0.08	55.55±0.44^{cde}	0.778±0.003^{a}
	Super-Hot 2 80% MWHC	-1.65±0.11	58.79±0.60^{ab}	0.762±0.004^{b}
	Super-Hot 2 60% MWHC	-2.00±0.13	60.11±0.65^{a}	0.743±0.006^{c}
	Super-Hot 2 40% MWHC	-2.47±0.24	53.38±0.74^{f}	0.754±0.005^{bc}
	Hueay-Sii-Ton 100% MWHC	-1.24±0.09	51.39±0.53^{g}	0.787±0.003^{a}
	Hueay-Sii-Ton 80% MWHC	-1.30±0.09	54.06±0.56^{ef}	0.752±0.006^{bc}
	Hueay-Sii-Ton 60% MWHC	-1.49±0.13	56.13±0.54^{cd}	0.757±0.005^{b}
	Hueay-Sii-Ton 40% MWHC	-1.58±0.17	53.18±0.50^{fg}	0.741±0.005^{c}
	Kee-Nu-Suan 100% MWHC	-1.34±0.11	57.50±0.77^{bc}	0.778±0.003^{a}
	Kee-Nu-Suan 80% MWHC	-1.53±0.12	54.68±0.89^{def}	0.758±0.004^{b}
	Kee-Nu-Suan 60% MWHC	-1.88±0.18	58.16±0.69^{b}	0.757±0.003^{b}
	Kee-Nu-Suan 40% MWHC	-2.26±0.22	57.30±0.76^{bc}	0.723±0.007^{d}
	F-test	ns	**	**

(1) Maximum water holding capacity

(2) Values in columns are means with SE, the different letters indicate significant differences by DMRT (**, $p < 0.01$; * $p < 0.05$; ns, $p > 0.05$).

When considering the changes in LWP_{md} between the studied weeks (Figure 4.1), it was clear that lower watering regimes can cause a reduction in LWP_{md} of three chili cultivars. However, the degree of decrement has differed. The 40% MWHC caused a steep reduction in LWP_{md} of Super-Hot 2 starting from 2 WAA and at 6 WAA, the LWP_{md} reached the lowest value of -3.60 Mpa, but it was increased to -2.67 Mpa at 8 WAA. The LWP_{md} of Kee-Nu-Suan also followed a similar trend but a slight fluctuation along the observation was observed. In the cases of Hueay-Sii-Ton, LWP_{md} gradually decreased until 6 WAA and stabled in the last week except for the 40% MWHC which the LWP_{md} continued to decrease to the lowest point of -2.47 Mpa. The results indicated that a restricted water supply caused a reduction of LWP_{md} and chili cultivars differently responded 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 in morphological, physiological, and biochemical adaptations to water deficit in different chili cultivars (Phimchan *et al*., 2012; Sato *et al*., 2004). In our experiment, water deficit treatments expressed a decrease in LWP_{md} implying a difference in plant water status (Gonza´lez-Dugo *et al*., 2007). In addition, the study of Liu *et al*. (2021) reports that plant leaf water loss became increasingly severe with prolonged drought stress duration and increased stress intensity in *Phedimus aizoon* L. showed a correlation to LWP_{md} trend as shown in Figure 4.1. Moreover, the temperature in the greenhouse of this experiment was extremely high and RH was rather low. These conditions increased the evaporation which can increase the stress intensity, when combined with limiting water supply, they caused a severe decrement in LWP_{md}. Therefore, the combined effect of high-temperature and drought stress on crops could be more severe than the individual stress impact, and when considering the growth stage, the reproductive stages of crops are more vulnerable to drought, high temperature, and combined stress than the vegetative stages (Choukri *et al*., 2020; Sehgal *et al*., 2017).

Watering regimes also affected the leaf greenness of studied chili cultivars as shown in Table 4.1. Hueay-Sii-Ton was a cultivar with a significantly lower leaf greenness compared with the other two cultivars. It appears that lowering the water supply to a certain point can increase the leaf greenness of chili. The watering regime at 60% MWHC gave the highest leaf greenness, it was significantly higher than the control (100% MWHC) and the 80% MWHC, whereas the leaf greenness of the 40% MWHC was not statistically different from the control. Super-Hot 2 received 60 and 80% MWHC resulting in the highest leaf greenness while Hueay-Sii-Ton received 40 and 100% MWHC treatments yielding the lowest leaf greenness.

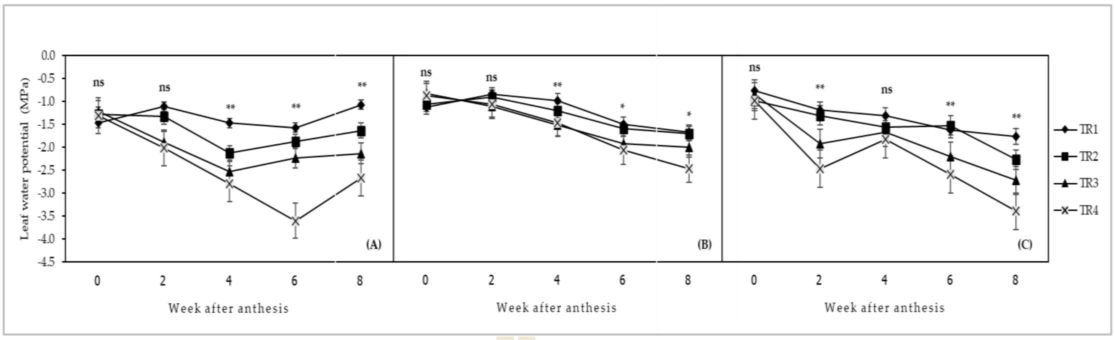

Figure 4.1 Changes in leaf water potential of *C. annuum* Super-Hot 2 (A) and Hueay-Sii-Ton (B) and *C. frutescens* Kee-Nu-Suen (C) responded to different watering regimes (% maximum water holding capacity; MWHC) at anthesis (0 WAA) to 8 weeks after anthesis (WAA). The ** and * indicate a significant difference between means by DMRT ($p < 0.01$ and 0.05, respectively) while ns means a non-significant difference ($p > 0.05$).

In Table 4.2, the variation in leaf greenness of three chili cultivars from 0 to 8 WAA is shown. Significant differences were found in some studied weeks, in general, the leaf greenness of plants grown with water restriction was higher than those grown at 100% MWHC. However, Super-Hot 2 may be more sensitive to severe water restriction than others since its leaf greenness was dropping when receiving 40% MWHC watering. Leaf greenness is usually reduced by the effects of water stress (Sae-Tang *et al*., 2019), and to a certain degree, the result found in this study was contrasted with the previous findings. In this study, the leaf greenness of water-restricted plants was higher or remained close to the control. However, a similar trend was also found in water-stressed Karen KPS chili (Arom and Jinagool, 2021). Under the water restriction, decrement of LWP_{md} induced leaf greenness, which 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 (Borrell *et al*., 2000; Yan *et al*., 2004). Maintenance of leaf greenness under drought stress is generally considered a positive trait as it indicates reduced chlorophyll degradation (Sikuku *et al*., 2010).

Table 4.2 Leaf greenness of *C. annuum* (Super-Hot 2, Hueay-Sii-Ton) and *C. frutescens* (Kee-Nu-Suen) responded to different watering regimes (% maximum water holding capacity; MWHC) at anthesis (0 WAA) to 8 weeks after anthesis (8 WAA).

Chili cultivar	Watering regimes (MWHC) [(1)]	Leaf greenness (SPAD) [(2)]				
		0 WAA	2 WAA	4 WAA	6 WAA	8 WAA
Super-Hot 2	100%	54.02±0.61	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)] Maximum water holding capacity

[(2)] Values in columns are means with SE, the different letters indicate significant differences by DMRT (**, $p < 0.01$; * $p < 0.05$; ns, $p > 0.05$).

The F_v/F_m of three chili cultivars were similar and ranged between 0.75 – 0.76 whereas, the watering regimes caused significant changes in F_v/F_m. At 100% MWHC, the F_v/F_m value was the highest and it was reduced with the decreased MWHC. This trend was also observed when comparing the combined effects of chili cultivars and watering regimes (Table 4.1). 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 has already fluctuated even before the application of watering regimes (Table 4.3). After the application of watering regimes, the F_v/F_m decreased under moderate to severe water deficit. Different watering regimes can cause different levels of drought stress on chili and can alter the water status inside the plant. In this experiment, despite the obvious changes in LWP_{md}, the F_v/F_m was rather stable. However, we observed that the reduction of LWP_{md} was reflected with slight decrements in F_v/F_m as shown in Table 10. The reasons behind this contrast between LWP_{md} and F_v/F_m can be due to the impact of moderate drought is low on the F_v/F_m (Christen *et al*., 2007; Genty *et al*., 1987; Oukarroum *et al*., 2007; Tezara *et al*., 1999) and the small decreases can be interpreted as a photo-protection mechanism and photoinhibition (reversible) in photosystem II (PSII, Adams *et al*., 2006; Takahashi and Badger, 2011).

Table 4.3 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 (% maximum water holding capacity; MWHC) at anthesis (0 WAA) to 8 weeks after anthesis (8 WAA).

Chili cultivar	Watering regimes (MWHC) [(1)]	Maximum quantum yield of PSII (F_v/F_m) [(2)]				
		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}	0.78±0.01	0.79±0.01^{a}	0.78±0.00^{a}	0.78±0.01^{a}
	80%	0.77±0.00^{b}	0.77±0.01	0.78±0.01^{a}	0.75±0.01^{b}	0.69±0.02^{b}
	60%	0.77±0.01^{b}	0.78±0.01	0.77±0.01^{ab}	0.76±0.01^{b}	0.71±0.02^{b}
	40%	0.72±0.01^{c}	0.77±0.01	0.75±0.01^{b}	0.75±0.01^{b}	0.71±0.02^{b}
F-test		**	ns	*	**	**
Kee-Nu-Suen	100%	0.79±0.00^{a}	0.77±0.01	0.77±0.01^{a}	0.79±0.00^{a}	0.76±0.01^{a}
	80%	0.77±0.00^{b}	0.76±0.01	0.78±0.01^{a}	0.77±0.01^{a}	0.71±0.01^{b}
	60%	0.74±0.01^{c}	0.78±0.00	0.76±0.00^{a}	0.76±0.01^{a}	0.74±0.01^{ab}
	40%	0.73±0.01^{c}	0.77±0.01	0.74±0.01^{b}	0.72±0.02^{b}	0.66±0.03^{c}
F-test		**	ns	**	**	**

(1) Maximum water holding capacity

(2) Values in columns are means with SE, the different letters indicate significant differences by DMRT (**, $p < 0.01$; * $p < 0.05$; ns, $p > 0.05$).

4.1.2 Growth and development

Canopy height and width during the anthesis to 8 WAA were used to calculate RGR_{height} and RGR_{width} and the results are shown in Table 4.4. It appeared that RGR_{height} of Super-Hot 2 was significantly lower than Hueay-Sii-Ton while the watering regimes and the interaction effects between chili cultivars and watering regimes were not affecting RGR_{height}. On the other hand, the RGR_{width} was only affected by watering regimes. The 60 and 40% MWHC resulted in a significantly lower RGR_{width} when compared with the 100 and 80% MWHC.

Table 4.4 Changes in relative growth rate in terms of plant height and canopy width (RGR_{height} and RGR_{width}, respectively) and leaf area index (LAI) of *C. annuum* (Super-Hot 2 and Hueay-Sii-Ton) and *C. frutescens* (Kee-Nu-Suen) responded to different watering regimes (% maximum water holding capacity; MWHC) and the interaction effects.

Studied factor		RGR_{height} [(2)]	RGR_{width}	LAI
Chili cultivar (A)	Super-Hot 2	0.003±0.001^{b1}	0.003±0.001	0.35±0.08^{a}
	Hueay-Sii-Ton	0.007±0.001^{a}	0.005±0.001	0.30±0.05^{a}
	Kee-Nu-Suan	0.005±0.001^{ab}	0.004±0.001	0.11±0.02^{b}
	F-test	*	ns	**
Watering regimes (B)	100% MWHC[(1)]	0.005±0.001	0.006±0.001^{a}	0.49±0.10^{a}
	80% MWHC	0.006±0.001	0.006±0.001^{a}	0.27±0.05^{b}
	60% MWHC	0.006±0.001	0.003±0.001^{b}	0.18±0.03^{bc}
	40% MWHC	0.004±0.001	0.001±0.001^{b}	0.09±0.02^{c}
	F-test	ns	**	**
AxB	Super-Hot 2 100% MWHC	0.004±0.001	0.005±0.002	0.74±0.17
	Super-Hot 2 80% MWHC	0.004±0.001	0.004±0.002	0.31±0.05
	Super-Hot 2 60% MWHC	0.004±0.001	0.002±0.002	0.25±0.03
	Super-Hot 2 40% MWHC	0.002±0.001	0.001±0.001	0.11±0.04
	Hueay-Sii-Ton 100% MWHC	0.007±0.002	0.007±0.002	0.54±0.04
	Hueay-Sii-Ton 80% MWHC	0.007±0.001	0.008±0.001	0.36±0.11
	Hueay-Sii-Ton 60% MWHC	0.007±0.002	0.004±0.003	0.19±0.04
	Hueay-Sii-Ton 40% MWHC	0.005±0.001	0.001±0.002	0.14±0.04
	Kee-Nu-Suan 100% MWHC	0.003±0.001	0.006±0.001	0.19±0.07
	Kee-Nu-Suan 80% MWHC	0.007±0.002	0.006±0.002	0.12±0.02
	Kee-Nu-Suan 60% MWHC	0.006±0.002	0.003±0.002	0.09±0.03
	Kee-Nu-Suan 40% MWHC	0.004±0.001	0.001±0.002	0.04±0.02
	F-test	ns	ns	ns

[(1)] Maximum water holding capacity

[(2)] Values in columns are means with SE, the different letters indicate significant differences by DMRT (**, $p < 0.01$; * $p < 0.05$; ns, $p > 0.05$).

It appeared that during the first two WAA, chili plants still grew in height and canopy width, and they were affected by reduced water supply. Though, the effects were not seen in RGR_{height} of Hueay-Sii-Ton. After 2 weeks of anthesis, the growth of the chili plant was mostly stopped as can be seen from RGR_{height} and RGR_{width} was closed to 0 (Table 4.5). This result is consistent with another study which found that vegetative growth after anthesis was minimum and it was not a drought-sensitive period for vegetative development (Arom and Jinagool, 2021). In some studied periods, declines in RGR_{height} and RGR_{width} were also observed (Table 4.5). In our experiment, we also observed that the water deficit caused a reduction in LWP_{md}, this reduction was correlated with the F_v/F_m and led to the reduction of the growth rate. It can also be the result of a limitation in water supply, which caused a reduction in turgor pressure, resulted in wilting, and led to decreases in vegetative growth (Ahmed *et al.*, 2014).

For the changes in LAI after the anthesis, the chili cultivars and watering regimes cause affected LAI, while the interaction effects between chili cultivars and watering regimes were not affecting LAI (Table 4.4). The chili cultivars, Super-Hot 2 and Hueay-Sii-Ton had a higher LAI than Kee-Nu-Suan. In terms of the watering regimes, the 60 and 40% MWHC resulted in a significantly lower LAI when compared with the 100% MWHC. A correlation between reduced F_v/F_m and LAI was observed. In general, plants with a water deficit will reduce the water absorption rate by plant roots. Its effect is growth disorders, especially in meristematic tissue (Prihastanti, 2010). Moreover, drought stress will decrease the formation and expansion of leaves, accelerated aging and leaf shedding, or both. Accelerated leaf aging due to limited watering conditions tends to occur in the lower leaves, which part less active in photosynthesis and assimilates supply, thus they had less contribution effect to yield (Yusniwati, 2008). Previous studies suggested that the combined drought and temperature stresses caused disproportionate damage to plant growth compared with the individual stress. These stresses noticeably reduced physiological traits; photosynthetic activity, affected stomatal conductance and decreased the leaf area (Rajeswari *et. al*., 2020; Shah and Paulsen, 2003).

Table 4.5 Relative growth rate of height and relative growth rate of canopy width of *C. annuum* (Super-Hot 2, Hueay-Sii-Ton) and *C. frutescens* (Kee-Nu-Suen) responded to different watering regimes (% maximum water holding capacity; MWHC) at anthesis (0 WAA) to 8 weeks after anthesis (8 WAA).

Chili cultivar	Watering regimes (MWHC) [(1)]	RGR_{height} (cm/day) [(2)]				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
	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)] Maximum water holding capacity

[(2)] Values in columns are means with SE, the different letters indicate significant differences by DMRT (**, $p < 0.01$; * $p < 0.05$; ns, $p > 0.05$)

4.1.3 Yield and capsaicin content

Ripened chili fruits were harvested and measured for fruit characteristics and yield. The analysis found effects of cultivars and watering regimes on all studied traits, however, the significant combination effects were only found on fruit size (Table 4.6). For the chili cultivars, Super-Hot 2 was the one with the greatest fruit characteristics and yield except for fruit width which was smaller than Hueay-Sii-Ton but similar to Kee-Nu-Suan. Watering regimes caused a significant reduction of all fruit characteristics and yield while the interaction effects indicated that all cultivars had smaller fruit sizes when growing under limiting water supply. These findings affirm the effects of water supply on crop production (Jeeatid *et al*., 2014; Yang *et al*., 2018). The lower watering regimes affected leaf water potential and caused the reduction of F_v/F_m and LAI, and finally, they caused the decrements in the number of fruits, fruit weight, and size. If focusing on yield, the reduction of water supply is not recommended for the production of chili since it can drop the yield and yield quality just by restricted irrigation to 80% of MWHC. The results were different from a previous study which suggested that 80% MWHC can be used to produce *C. frutescens* cv. Karen KPS because it did not affect the yield of studied chili (Arom and Jinagool, 2021). The contrasted result may be due to the sensitivity of chili cultivars to water stress. In our experiment, greenhouse conditions had an extremely high temperature and low RH. When these conditions combined with limiting water supply, they increased stress intensity in studied plants and impacted yield and fruit characteristics. High-temperature and drought stress has been reported as the major environmental factors that can markedly affect plant productivity and the quality of many cultivated crops (El-Haddad *et al*., 2021). Both high-temperature and drought stress hamper plant growth by disturbing the normal physiology and morphology, thereby influencing an array of processes including growth, floral development, and carbohydrates, which ultimately affect yield and quality (Johansson *et al.*, 2020; Maqbool *et al*., 2020). During anthesis and flowering periods, high-temperature and drought stress led to fertilization failures because of reduced pollen and ovule function and inhibited pollen development (Sinha *et al.,* 2021).

However, the HI of the chili cultivars and watering regimes were significantly different, Super-Hot 2 and Kee-Nu-Suan were the cultivars with a higher HI, while Hueay-Sii-Ton was the lowest. The differences between both chili cultivars were that Super-Hot 2 is hybrid chili, derived from F1 hybrid seed, while Hueay-Sii-Ton and Kee-Nu-Suen were seed selections from an opened breed (OP). Normally, F1 hybrid plants provide a higher yield, and they provide greater genetic homogeneity than the OP line.

Therefore, the HI of the two chili cultivars was different, this is partly due to the difference in seed selection.

In our experiment, watering regimes at 60 and 40% MWHC caused a significant reduction in HI. Moreover, no significant difference was observed in interaction effects (Table 4.6). Reduction of HI corresponds to the loss of yield under water deficit conditions. Beese *et al*. (1982) reported a reduction in final yields of above and below-ground plant parts in chili due to the drought stress effect.

Table 4.6 Changes in fruit characteristics and harvest index (HI) of *C. annuum* (Super-Hot 2 and Hueay-Sii-Ton) and *C. frutescens* (Kee Nu Suen) responded to different watering regimes (% maximum water holding capacity; MWHC) and the interaction effects.

Studied factor		Number of fruits/plant[(2)]	Fruit fresh weight (g/plant)	Fruit dry weight (g/plant)	Fruit length (cm)	Fruit width (mm)	HI (%)
Chili cultivar (A)	Super-Hot 2	17.00±3.06^{a1}	5.84±3.09^{a}	4.68±0.90^{a}	4.45±0.07^{a}	6.02±0.07^{b}	14.93±1.43^{a}
	Hueay-Sii-Ton	5.07±1.68^{b}	4.05±1.54^{c}	1.45±0.53^{c}	3.52±0.09^{c}	6.49±0.10^{a}	5.36±1.26^{b}
	Kee-Nu-Suan	8.52±2.61^{b}	8.14±2.43^{b}	2.77±0.81^{b}	4.14±0.09^{b}	5.97±0.08^{b}	17.67±2.53^{a}
	F-test	**	**	**	**	**	**
Watering regimes (B)	100% MWHC[(1)]	22.27±3.38^{a}	20.92±3.54^{a}	6.61±1.00^{a}	4.72±0.08^{a}	6.84±0.06^{a}	18.74±2.56^{a}
	80% MWHC	9.60±2.55^{b}	8.93±2.28^{b}	2.85±0.69^{b}	4.16±0.09^{b}	6.19±0.08^{b}	14.51±3.17^{ab}
	60% MWHC	5.80±1.31^{bc}	4.58±1.23^{c}	1.46±0.34^{c}	3.68±0.09^{c}	5.63±0.08^{c}	10.30±1.86^{bc}
	40% MWHC	3.11±1.02^{c}	2.93±0.85^{c}	0.94±0.23^{c}	3.54±0.10^{c}	5.72±0.10^{c}	7.08±1.61^{c}
	F-test	**	**	**	**	**	**
AxB	Super-Hot 2 100% MWHC	32.00±3.14	31.53±2.50	9.16±0.68	4.94±0.09^{ab}	6.71±0.10^{b}	19.74±1.23
	Super-Hot 2 80% MWHC	19.33±1.68	17.26±1.14	5.31±0.38	4.66±0.12^{bc}	6.18±0.13^{c}	17.31±0.60
	Super-Hot 2 60% MWHC	10.33±0.37	9.18±0.36	2.68±0.13	4.00±0.12^{de}	5.45±0.08^{d}	14.02±1.00
	Super-Hot 2 40% MWHC	6.33±1.99	5.39±1.36	1.57±0.35	4.12±0.12^{df}	5.66±0.14^{d}	8.67±2.57
	Hueay-Sii-Ton 100% MWHC	14.40±1.22	12.60±1.50	4.40±0.50	4.11±0.12^{de}	7.12±0.13^{a}	12.30±0.36
	Hueay-Sii-Ton 80% MWHC	3.40±0.76	2.27±0.49	0.80±0.17	3.38±0.14^{f}	6.18±0.16^{c}	3.74±0.80
	Hueay-Sii-Ton 60% MWHC	1.47±0.35	0.87±0.14	0.37±0.07	3.05±0.18^{f}	6.05±0.21^{c}	3.64±0.88
	Hueay-Sii-Ton 40% MWHC	1.00±0.50	0.47±0.24	0.23±0.13	3.23±0.20^{f}	6.40±0.20^{bc}	1.76±0.56
	Kee-Nu-Suan 100% MWHC	20.40±6.97	18.64±6.93	6.27±2.33	5.12±0.22^{a}	6.71±0.23^{b}	24.17±6.41
	Kee-Nu-Suan 80% MWHC	6.07±1.44	7.26±1.62	2.45±0.55	4.39±0.12^{cd}	6.20±0.14^{c}	22.48±5.08
	Kee-Nu-Suan 60% MWHC	5.60±0.42	3.71±0.07	1.34±0.14	3.78±0.14^{e}	5.54±0.10^{d}	13.23±2.47
	Kee-Nu-Suan 40% MWHC	2.00±0.40	2.95±0.84	1.02±0.18	3.28±0.13^{f}	5.42±0.14^{d}	10.81±1.27
	F-test	ns	ns	ns	**	*	ns

[(1)] Maximum water holding capacity

[(2)] Values in columns are means with SE, the different letters indicate significant differences by DMRT (**, $p < 0.01$; * $p < 0.05$; ns, $p > 0.05$).

The 40% MWHC gave a very small yield production from the three studied chili cultivars, it was impossible to prepare the dry chili powder for the analysis of capsaicin content. Thus, Table 4.7 only showed the results of capsaicin content, pungency level, and capsaicin yield 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 and only capsaicin yield was affected by the combined effects of the chili cultivar and watering regime. The Super-Hot 2 was a cultivar that produced the highest capsaicin yield followed by Kee-Nu-Suan and Hueay-Sii-Ton, respectively. This was a result of different fruit dry weights obtained from different chili cultivars as shown in Table 4.6. A similar reason can be applied to the effect of watering regimes on capsaicin yield.

Table 4.7 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 (% maximum water holding capacity; MWHC) and the interaction effects.

Studied factor		Capsaicin content (ug/g) [(2)]	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% MWHC[(1)]	471.85±4.45	7,423.11±71.21	3,072.83±470.08^{a}
	80% MWHC	454.63±7.56	7,476.76±120.96	1,341.13±317.79^{b}
	60% MWHC	469.15±11.35	7,546.36±181.57	694.17±164.82^{b}
	F-test	ns	ns	**
AxB	Super-Hot 2 100% MWHC	463.03±14.14	7,447.20±226.19	4,268.72±393.65
	Super-Hot 2 80% MWHC	470.28±24.58	7,563.20±393.26	2,492.31±58.72
	Super-Hot 2 60% MWHC	482.23±36.74	7,754.40±587.89	1,290.06±32.10
	Hueay-Sii-Ton 100% MWHC	454.91±0.65	7,317.20±10.39	2,014.07±228.95
	Hueay-Sii-Ton 80% MWHC	454.46±0.41	7,310.00±6.58	374.55±76.50
	Hueay-Sii-Ton 60% MWHC	454.54±1.13	7,311.33±18.08	158.20±34.10
	Kee-Nu-Suan 100% MWHC	466.63±1.17	7,504.93±18.74	2,935.69±1,082.83
	Kee-Nu-Suan 80% MWHC	469.90±0.28	7,557.07±4.43	1,156.53±259.90
	Kee-Nu-Suan 60% MWHC	470.92±0.22	7,573.33±3.45	634.24±63.50
	F-test	ns	ns	ns

[(1)] Maximum water holding capacity

[(2)] Values in columns are means with SE, the different letters indicate significant differences by DMRT (**, $p < 0.01$; * $p < 0.05$; ns, $p > 0.05$).

The findings were not inconsistent with a previous study which found that the restriction of water supply to 40% of field capacity resulted in the highest capsaicin yield (Arum and Seto-Sugianto, 2017) while another study showed no benefit of restricted water supply on capsaicin content of *C. annuum* (Khan *et al*., 2014). Thus, the restricted watering regime can differently affect capsaicinoid production in chili cultivars. 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 (Phimchan *et al*., 2012). 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 was not significantly differed between watering regimes (Arom and Jinagool, 2022). Similarly, a study also found that lower water availability can increase capsaicin levels of *C. frutescens* cayenne pepper plants (Lathifah and Siswanti, 2021). In addition, the effect of drought stress on the pungency level in chili still is a debatable topic, ether it increases or decreases. Moreover, there is evidence that capsaicin is upregulated and in some cases downregulated under drought stress conditions; this depends on the genetic makeup of cultivars and the stress levels (Mahmood *et al*., 2021).

In this experiment, the capsaicin content and pungency level of three chili cultivars were lower when compared with other studies. These results may be due to the content and composition of secondary metabolites in plants being affected by the plant's genetic structure, soil characteristics, environmental factors, and agricultural practices as well as post-harvest practices (Yaldiz *et al*., 2010) which were different in each study. Therefore, capsaicin biosynthesis and accumulation were different.

4.2 The effects of water regimes on agronomic traits and capsaicinoid content of chili under field conditions

4.2.1 Effects on growth and development

Changes in canopy height and width during the watering regimes were used to calculate RGR_{height} and RGR_{width} and the results are shown in Table 4.8. The analysis shows significant differences in RGR_{height} and RGR_{width} when compared between chili cultivars in which Kee-Nu-Suan had a greater growth rate than the Super-Hot 2. Watering regimes did not significantly affect the studied growth parameters while the interaction effects between chili cultivars and watering regimes were significantly differed for the RGR_{height} but not found in RGR_{width}. It appeared that the reduction of watering regimes from 100 to 60% MWHC did not cause a reduction in RGR_{height} of Kee-

Nu-Suan. Surprisingly, the RGR_{height} of Super-Hot 2 was increased when receiving 60% MWHC (Table 4.8).

Table 4.8 Changes in relative growth rate in terms of plant height and canopy width (RGR_{height} and RGR_{width}), and leaf area index (LAI) of *C. annuum* (Super-Hot 2) and *C. frutescens* (Kee-Nu-Suen) responded to different watering regimes (% maximum water holding capacity; MWHC) and the interactions.

	Studied factors	RGR_{height} $^{(2)}$	RGR_{width}	LAI
Chili cultivar (A)	Super-Hot 2	0.005±0.000^{b1}	0.006±0.000^{b}	3.46±0.12^{a}
	Kee-Nu-Suan	0.008±0.000^{a}	0.010±0.001^{a}	1.97±0.13^{b}
	F-test	*	**	**
Watering regimes (B)	100% MWHC$^{(1)}$	0.006±0.000	0.008±0.001	2.81±0.15
	60% MWHC	0.007±0.000	0.008±0.001	2.61±0.13
	F-test	ns	ns	ns
AxB	Super-Hot 2 100% MWHC	0.004±0.000^{c}	0.006±0.001	3.72±0.19
	Super-Hot 2 60% MWHC	0.006±0.001^{b}	0.006±0.001	3.21±0.15
	Kee-Nu-Suan 100% MWHC	0.008±0.001^{a}	0.010±0.001	1.90±0.16
	Kee-Nu-Suan 60% MWHC	0.007±0.001^{a}	0.010±0.001	2.03±0.20
	F-test	*	ns	ns

(1) Maximum water holding capacity

(2) Values in columns are means with SE, the different letters indicate significant differences by DMRT (**, $p < 0.01$; * $p < 0.05$; ns, $p > 0.05$).

It appeared that during the first two weeks after anthesis, chili plants still grew in height and canopy width. In Super-Hot 2, the RGR_{height} of chili plants received 60% MWHC was greater than the 100% MWHC during 2-4 WAA. However, the RGR_{width} was not significantly differed between the water regimes at 0-2 and 6-8 WAA. In Kee-Nu-Suan the RGR_{height} at 0-2 WAA, and RGR_{width} at 2-4 WAA of the 60% MWHC were lower when compared with 100% MWHC. The growth of the chili plant was mostly stopped as can be seen from RGR_{height} was close to 0 at 6-8 WAA, while RGR_{width} was still gradually increased (Table 4.9). Therefore, the effects of watering regimes on RGR_{height} and RGR_{width} at the reproductive stage were minimum. These results are consistent with a study by Arom and Jinagool (2021) which found that vegetative growth after anthesis was minimum, and it was not a drought-sensitive period for vegetative development. Khan *et al*. (2008) observed that in plant height, the drought stress developed during the vegetative stage markedly suppressed the vegetative growth and the plant became stunted, while the flowering stage and fruiting stage were less affected.

Table 4.9 Relative growth rate of height and relative growth rate of canopy width of *C. annuum* (Super-Hot 2) and *C. frutescens* (Kee-Nu-Suen) responded to different watering regimes (% maximum water holding capacity; MWHC) at anthesis (0 WAA) to 8 weeks after anthesis (8 WAA).

Chili cultivars	Watering regimes (MWHC)[1]	RGR_{height} (cm/day)[2]				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%	0.003±0.001	0.005±0.001[b]	0.006±0.001	0.002±0.001	0.002±0.001	0.006±0.001	0.007±0.001	0.008±0.001
	60%	0.005±0.001	0.009±0.001[a]	0.006±0.001	0.003±0.001	0.002±0.001	0.008±0.002	0.008±0.001	0.008±0.001
F-value		ns	**	ns	ns	ns	ns	ns	ns
Kee-Nu-Suen	100%	0.011±0.001[a]	0.012±0.001	0.006±0.001	0.004±0.001	0.004±0.001	0.020±0.001[a]	0.009±0.001	0.010±0.001
	60%	0.008±0.001[b]	0.011±0.001	0.006±0.001	0.004±0.001	0.006±0.001	0.011±0.002[b]	0.011±0.001	0.012±0.001
F-value		*	ns	ns	ns	ns	**	ns	ns

[1] Maximum water holding capacity

[2] Values in columns are means with SE, the different letters indicate significant differences by DMRT (**, $p < 0.01$; * $p < 0.05$; ns, $p > 0.05$).

For the changes in LAI after the anthesis, it can be seen that only the chili cultivars affected the LAI (Table 4.8) in which Super-Hot 2 had a higher LAI than Kee-Nu-Suan. However, when considering the growth in LAI between the studied weeks, variations can be observed in the LAI of each cultivar in response to the watering regimes (Table 4.10). In Super-Hot 2, the LAI of chili plants at anthesis (0 WAA) to 8 WAA was not significantly different. However, after 2 WAA onward, the development in LAI of Super-Hot 2 from both watering regimes was constantly increased and at 8 WAA, the values were maximized. For Kee-Nu-Suan, the LAI at anthesis was similar for both watering regimes, however, it statistically differed for most of the later weeks. At 2 and 4 WAA, the LAI of 100% MWHC treatment had higher LAI compared with the plants under 60% MWHC. The LAI of plants from the two watering treatments became similar again at 6 WAA while at 8 WAA, the LAI of 60% MWHC exceeded the 100% MWHC.

Table 4.10 Leaf area index of *C. annuum* (Super-Hot 2) and *C. frutescens* (Kee-Nu-Suen) responded to different watering regimes (% maximum water holding capacity; MWHC) at anthesis (0 WAA) to 8 weeks after anthesis (8 WAA).

Chili cultivars	Watering regimes (MWHC)[1]	LAI[2]				
		0 WAA	2 WAA	4 WAA	6 WAA	8 WAA
Super-Hot 2	100%	2.85±0.29	3.22±0.34	3.59±0.46	3.60±0.31	5.31±0.49
	60%	2.57±0.29	3.07±0.26	2.95±0.26	3.07±0.30	4.40±0.45
F-value		ns	ns	ns	ns	ns
Kee-Nu-Suen	100%	0.00±0.00	1.30±0.14[a]	2.02±0.23[a]	2.56±0.23	3.63±0.20[b]
	60%	0.00±0.00	1.15±0.15[b]	2.00±0.30[b]	3.09±0.31	3.92±0.43[a]
F-value		ns	*	*	ns	*

(1) Maximum water holding capacity

(2) Values in columns are means with SE, the different letters indicate significant differences by DMRT (**, $p < 0.01$; * $p < 0.05$; ns, $p > 0.05$).

4.2.2 Effect of different water regimes on yield and yield quality

Ripened chili fruits were measured for fruit characteristics and yield. The analysis found the effects of cultivars and watering regimes on some studied traits. However, significant combination effects were found only on fruit length (Table 4.11). For the chili cultivar, Kee-Nu-Suan was the one with the greatest number of fruits/plant and fruit dry yield, while Super-Hot 2 was higher in fruit length and fruit width. The differences in chili fruit size were the characteristics of chili cultivars. Kee-Nu-Suan had a small chili fruit, shorter and slimmer than the Super-Hot 2 which had a larger chili fruit size (Chanthai and Bosland, 2012). In addition, the reduction of watering regimes caused a significant reduction in fruit length and fruit width. The 60% MWHC caused a significant increase in fruit dry weight. However, the interaction effects indicated that all cultivars had a smaller fruit length when growing under a limiting water supply.

In this experiment, a limited watering regime reduced fruit size in both length and width. Corresponded with Khan *et al*. (2008) who observed that fruit length and diameter were affected by the water stress. Techawongstein *et al*. (1992) also reported that fruit length and diameter were reduced in water-stressed plants. Fruit diameter was also reduced in pears due to water stress compared to control plants, because of osmotic adjustment in fruit (Behboudian *et al*., 1994). However, the expansion of chili fruit requires several factors, an adequate flow of water to the organ and sufficient turgor to drive cell enlargement. Diurnal fluctuations in fruit size are related to the difference in water potential between the stem and fruit. While plants received water deficit, the expansion of fruit was restricted due to cell volume being decreased, slowing the rate of turgor loss with decreasing water potential (Pomper and Breeen, 1997) for maintaining water in the plant.

In contrast, fruit dry weight was slightly increased by the reduction of water supply (60% MWHC; watering at 3 days intervals). While watering regimes at 100% MWHC (daily watering) produced lower values of fruit dry weight. In our experiment, watering regimes under field conditions may also impact by rainfall in the period of study, which can affect stress intensity. The study by Khan *et al*. (2009) reported that watering daily once to twice gave excess water, and watering at 16 days interval to no watering was deficit water, and these caused the decrease in yield characteristics. On the other hand, watering at 4- to 8-day intervals produced better yield characters. In our experiment, this may indicate that 100% MWHC causes excess soil moisture and affect fruit dry weight. Over-irrigation leads disturb of oxygen balance of the root zone, drowns roots, reduces plant water uptake, and thus stresses plant (Irmak and Rathje, 2008), in case of water deficit, limiting soil water, lead to reduces plant water uptake and this cause decreases biomass accumulation, tissue expansion, and reduces cell number (Tardieu *et al*., 2011). The result of our experiment is in agreement with a study by Hedge (1989) who observed the adverse effect of both excess and deficit soil moisture on the fruit yield of chili. Lower fruit yield in chili was also reported by Ayob (1986) with excess and deficit soil moisture. The growth and yield of chili showed a declining trend with higher levels of irrigation (Sadykov and Mikhoet, 1981). However, Yang et al. (2017) reported that deficit irrigation during the vegetative, flowering, and fruit-setting stages did not affect the hot pepper yield.

Table 4.11 Changes in fruit characteristics and harvest index (HI) of Super-Hot 2 (*C. annuum*) and Kee-Nu-Suen (*C. frutescens*) responded to different watering regimes (% maximum water holding capacity; MWHC) and interactions.

	Studied factors	Number of fruits/plant[2]	Fruit fresh weight (g/plant)	Fruit dry weight (g/plant)	Fruit dry yield (kg/rai)	Fruit length (cm)	Fruit width (mm)	HI (%)
Chili cultivar (A)	Super-Hot 2	228.37±11.47^{b}	300.75±14.54	39.64±2.52	253.68±16.15^{b}	5.12±4.58^{a}	6.41±4.57^{a}	21.32±1.95^{a}
	Kee-Nu-Suan	610.23±30.88^{a}	267.65±12.45	42.95±2.63	274.87±16.81^{a}	2.72±0.13^{b}	5.49±0.13^{b}	15.35±1.02^{b}
	F-test	**	ns	ns	*	**	**	**
Watering regimes (B)	100% MWHC[1]	399.33±37.42	274.12±13.11	37.36±2.26^{b}	239.09±14.46	4.03±4.57^{a}	6.10±4.57^{a}	17.60±1.67
	60% MWHC	439.27±46.60	294.29±14.36	45.23±2.70^{a}	289.46±17.30	3.91±0.13^{b}	5.81±0.14^{b}	19.07±1.59
	F-test	ns	ns	*	ns	**	**	ns
AxB	Super-Hot 2 100% MWHC	228.13±18.00	301.95±20.62	35.87±3.37	229.59±21.56	5.28±0.05^{a}	6.54±0.04	20.27±2.94
	Super-Hot 2 60% MWHC	228.60±14.87	299.56±21.22	43.40±3.60	277.78±21.66	5.06±0.04^{b}	6.28±0.06	22.38±2.61
	Kee-Nu-Suan 100% MWHC	570.53±35.91	246.28±13.29	38.84±3.08	248.59±18.51	2.79±0.02^{c}	5.65±0.04	14.94±1.41
	Kee-Nu-Suan 60% MWHC	649.93±49.36	289.02±20.01	47.05±4.10	301.14±24.65	2.75±0.03^{c}	5.34±0.10	15.75±1.50
	F-test	ns	ns	ns	ns	**	ns	ns

[1] Maximum water holding capacity

[2] Values in columns are means with SE, the different letters indicate significant differences by DMRT (**, $p < 0.01$; * $p < 0.05$; ns, $p > 0.05$).

The HI of the two chili cultivars were significantly different, Super-Hot 2 was the cultivar with a higher HI. No significant difference was observed between watering regimes and interaction effects (Table 4.11). In addition, the HI at 6 and 8 WAA were similar (Table 4.12). The HI or harvest index is the ratio of commercial yield to total plant biomass (shoots plus roots) and is a measure of reproductive efficiency, it is determined by interactions between genotypes, environment, and crop management (Porker *et al*., 2020). In this study, the effect of genotype is evident, on the other hand, the watering regimes after anthesis did not affect the HI. The differences between both chili cultivars were that Super-Hot 2 is hybrid chili, derived from F1 hybrid seed, while Kee-Nu-Suen was a seed selection from an opened breed (OP). Normally, F1 hybrid plants can give higher yields than the open breed lines plant, due to using hybrid cultivars can improve the yield limitation in pure line cultivars (Soehendi and Srinives, 2005), and they provide greater genetic homogeneity than OP line. Therefore, the HI of the two chili cultivars was different, this is partly due to the difference in seed selection.

Table 4.12 Harvest index (HI) of *C. annuum* (Super-Hot 2) and *C. frutescens* (Kee-Nu-Suen) responded to different watering regimes (% maximum water holding capacity; MWHC) at 6 weeks after anthesis (6 WAA) to 8 weeks after anthesis (8 WAA).

Chili cultivars	Watering regimes (MWHC)[1]	HI (%)	
		6 WAA	8 WAA
Super-Hot 2	100%	8.54±1.37	31.99±0.75
	60%	12.07±1.35	32.69±0.78
F-value		ns	ns
Kee-Nu-Suen	100%	11.56±1.12	18.31±2.08
	60%	11.42±1.18	20.09±1.87
F-value		ns	ns

[1] Maximum water holding capacity

[2] Values in columns are means with SE, the different letters indicate significant differences by DMRT (**, $p < 0.01$; * $p < 0.05$; ns, $p > 0.05$).

4.2.3 Effect of different water regimes on capsaicinoids

Studied chili cultivars differed in capsaicinoid contents, pungency, and capsaicinoid yield. A lower watering regime also can induce these traits, however, the combined effects of chili cultivars and watering regimes were not different (Table 4.13). Kee-Nu-Suan had a greater performance in terms of capsaicin and dihydrocapsaicin contents, pungency level, and capsaicinoids yield. Capsaicinoid contents can vary among chili genotypes (Mahmood *et al*., 2021). Several studies reported that *C. frutescens* has been reported as a highly pungent chili (Greenleaf, 1986; Bosland and Votava, 1999). While *C. annuum* had a greater variation in terms of capsaicinoids (Li *et al*., 2022). However, the study of Kraikruan *et al.* (2008), found that *C. annuum* cultivars (Huayseeton-SK1) and one of the *C. frutescens* cultivars (K07), had particularly high total capsaicinoid contents, this can indicate that the pungency level depended on the cultivar and fruit morphology, not the species of chili.

Despite, the capsaicinoid content in chili being controlled by genetics, its variation is significantly affected by growing conditions (Harvell and Bosland, 1997). In this study, the limited water supply (60% MWHC) gave higher capsaicin, dihydrocapsaicin, pungency, and capsaicinoid yield. Corresponding with the study by Sung *et al*. (2005), capsaicin concentration was greatest in “Beauty Zest” chilies (*C. annuum* L.) in the water deficit treatment. “Padron” and “Karayatsubusa” chili plants subjected to water deficit also have higher capsaicin and dihydrocapsaicin concentration than control plants (Estrada *et al*., 1999). Drought stress increases the activity of enzymes necessary for capsaicin biosynthesis (PAL, phenylalanine ammonia-lyase; C4H, cinnamate 4-hydroxylase; and CS) and reduces capsaicin degradation by peroxidases (Contreras-Padilla and Yahia, 1998; Sung *et al*., 2005). Another study gave opposite views, capsaicin synthase activity was reduced in response to water stress in Habanero pepper (*C. chinense* Jacq.), and this effect depended on both stress severity and fruit age. (Lau *et al*., 2011). Nevertheless, drought stress effects were high in the low- and medium-pungent cultivars but not in the high-pungent cultivars (Phimchan *et al*., 2012).

Table 4.13. Capsaicin and dihydrocapsaicin content, pungency level, and capsaicinoids yield of Super-Hot 2 (*C. annuum*) and Kee-Nu-Suen (*C. frutescens*) responded to different watering regimes (% maximum water holding capacity; MWHC) and interactions.

	Studied factors	Capsaicin (ug/g) [2]	Dihydrocapsaicin (ug/g)	Pungency (SHU)	Capsaicinoids yield (ug/plant)	Capsaicinoids yield (g/rai)
Chili cultivar (A)	Super-Hot 2	228.65±10.05^{b}	105.19±4.01^{b}	6301.47±221.58^{b}	16125.68±1422.23^{b}	24.45±2.39^{b}
	Kee-Nu-Suan	345.17±23.37^{a}	136.38±6.98^{a}	7848.75±482.20^{a}	21483.61±2195.66^{a}	37.80±3.30^{a}
	F-test	**	**	**	*	**
Watering regimes (B)	100% MWHC[1]	276.47±14.33^{b}	106.81±5.49^{b}	6132.58±312.49^{b}	14218.68±1078.30^{b}	25.38±1.89^{b}
	60% MWHC	366.35±19.40^{a}	134.75±6.16^{a}	8017.63±404.93^{a}	23390.61±2173.82^{a}	39.87±3.36^{a}
	F-test	**	**	**	**	**
AxB	Super-Hot 2 100% MWHC	254.74±10.96	91.73±4.18	5543.50±236.18	12670.96±1503.82	21.30±2.42
	Super-Hot 2 60% MWHC	322.56±11.56	118.65±4.81	7059.44±256.02	19580.40±2100.80	33.60±3.32
	Kee-Nu-Suan 100% MWHC	298.21±25.76	121.90±8.64	6721.67±546.99	15766.41±1487.72	29.46±2.41
	Kee-Nu-Suan 60% MWHC	410.14±33.93	150.85±9.85	8975.82±694.70	27200.00±3617.96	46.14±5.02
	F-test	ns	ns	ns	ns	ns

[1] Maximum water holding capacity

[2] Values in columns are means with SE, the different letters indicate significant differences by DMRT (**, $p < 0.01$; * $p < 0.05$; ns, $p > 0.05$).

CHAPTER V
CONCLUSION AND RECOMMENDATION

5.1 The effects of water regimes on physiological traits, growth, yield, and capsaicin content of chili under greenhouse conditions

Restricted water supply affected 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 terms of plant height but the 60 and 40% MWHC can reduce the relative growth rate of canopy width. The reduced 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 the capsaicin yield was greatly reduced by severe reduction of fruit dry yield. Therefore, for the three chili cultivars, a reduced water supply during the anthesis through fruit development should be avoided under greenhouse conditions. In addition, the restricted water supply may not be 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.

5.2 The effects of water regimes on agronomic traits and capsaicinoids content of chili under field conditions

Restricted watering during the anthesis to fruit ripening unaffected the growth and development traits of the chili plants in terms of height and canopy width, leaf area index, and harvest index. The reduced water supply (60% MWHC) also decreased fruit size in both of Super-Hot 2 and Kee-Nu-Suen, but did not impact yield while the fruit's dry weight was increased. In terms of capsaicinoids, restriction of water after the anthesis can induce capsaicinoid contents, pungency level, and capsaicinoid yield under field conditions. This increment in the capsaicinoid yield was increased by an increase in fruit dry yield.

Among the two studied chili cultivars, Kee-Nu-Suan was a cultivar that produced the highest capsaicin and dihydrocapsaicin contents, resulting in higher pungency and capsaicinoid yield. In addition, the restricted water supply at medium stress level may be suitable management for capsaicinoid production in chili under field conditions. However, further study is required to relate environmental factors for enhanced yield and yield quality as well as production efficiency of capsaicinoids under field conditions. Selection of the chili cultivar from the groups of medium to high capsaicinoids content, high yield, and well-adapt to environmental conditions, these characteristics are important in the evaluation for cultivar potential combined with water management for suitability for capsaicin production.

In our study, different chili responses were found between the two experiments. All the responses of chili cultivars to water deficit in the pot under greenhouse conditions were extremely decreased. The limiting water supply combined with extremely high temperatures and low relative humidity could be more severe on the plant than the individual stress impact. The reductions found in physiological traits greatly reduced crop yield, while capsaicin content was not responded to different watering regimes. On the other hand, the evaluation under field conditions found the water deficit caused a slight decrease in fruit size but did not impact yield, while capsaicin content can be increased by water deficit. However, watering regimes may also impact by rainfall under field conditions, which decreased stress intensity, while plants under greenhouse conditions received higher stress of water deficit and high temperature. This suggests that the responses of chili cultivars to water deficit in field conditions were slightly decreased when compared with the pot under greenhouse conditions. In greenhouse conditions, high temperatures and low relative humidity are stressors in addition to the water limitation in this experiment. Management to avoid these additional stresses may be achieved by avoiding planting in seasons of high heat and low relative humidity, such as winter and summer, as well as by choosing well-ventilated greenhouses or greenhouses with ventilation systems to reduce temperatures inside. In terms of chili cultivars, Kee-Nu-Suen had a great yield performance and provide the highest capsaicinoids content and capsaicinoids yield. The 60% MWHC irrigation is appropriate to use for increasing capsaicinoids under field conditions.

From these results, water management by limiting irrigation under field conditions for high capsaicinoid yield in chili shows a promising potential that can be managed by the chili growers. Even though, the water restriction alone may not be sufficient for capsaicinoid production for the industrial sectors due to capsaicinoid content and pungent which were still lower than the demand of the industry sector. It is interesting to further study the combinations of management such as cultivar selection, fertilizer management, and plant growth regulators as they are other factors that studies have also shown their potential in capsaicinoids induction.

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