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Influence of water stresses on capsaicinoid production in hot pepper (*Capsicum chinense* Jacq.) cultivars with different pungency levels

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A B S T R A C T

Although water stress reduces fruit yield, it also increases capsaicinoid accumulation in hot pepper. The aim of this study was to investigate the effect of different water regimes on capsaicinoid production in *Capsicum chinense* Jacq. having different pungency levels. Four hot pepper (*C. chinense*) cultivars were planted with four water regimes after anthesis: daily irrigation (control; S1), every 2 days (S2), every 3 days (S3) and every 4 days (S4). The results found that Akanee Pirote with the S2 treatment gave the highest capsaicinoid yield, and the increase of capsaicinoid yield was attributed from increasing the absolute capsaicinoid content and reducing the dry fruit yield as compared to the control. Capsaicinoid yield of Bhut Jolokia, Orange Habanero, and BGH1719 responded to the water stresses, but produced less capsaicinoid yield as compared to the control. This study reveals that appropriate water stress could increase capsaicinoid yield in some, but not all, hot pepper cultivars.

1. Introduction

Chili pepper production worldwide in 2013 was 3.91 million hectares, with 1.78 t/ha average productivity of dry chili pepper pods (FAO, 2013). China, India, Indonesia and Thailand are major chili pepper producers in Asia. Chili peppers cultivated in tropical and subtropical areas are often subjected to water stress from flowering to fruit setting stages (Phimchan, Chanthai, Bosland, & Techawongstien, 2012) and results in flower drop, as well as lower fruit yield and quality (Techawongstien, Nawata, & Shigenaga, 1992).

The *Capsicum* genus contains five domesticated species and *C. annuum* is the most commonly cultivated. However, the world's hottest hot peppers are identified within the *Capsicum chinense* Jacq., such as 'Bhut Jolokia' (about 10,00,000 SHU) (Bosland & Baral, 2007). The pungency of hot peppers is related to capsaicinoid, unique alkaloids that accumulate only in *Capsicum* fruit. Capsaicin and dihydrocapsaicin are normally the two main capsaicinoids accounting for approximately 90% of pungent flavour (Govindarajan, 1986; Iwai, Suzuki, & Fujiwake, 1979; Kawada, Watanare, Katsura, Takami, & Iwai, 1985; Kosuge & Furata, 1970). The capsaicinoids in hot pepper fruit are becoming increasingly important to many industries, including food (preparing hot sauce), cosmetic (preventing hair loss shampoo) and pharmaceutical industries (muscle pain relief creams). Consequently, there is a need for identifying production management practices, as well as hot pepper cultivars to maximize capsaicinoid production.

An increase in capsaicinoid production in hot peppers can be accomplished by selecting varieties with high capsaicinoid content, and improving cultural production practices. Water stress is an environmental stress that increases capsaicinoid accumulation in *C. annuum* (Estrada, Pomar, Díaz, Merino, & Bernal, 1999). Capsaicinoid content under water stress was increased up to 2.56-fold higher than plants under non-water stress (Estrada et al., 1999; Sung, Chang, & Ting, 2005). An irrigation frequency every 7 or 9 day interval increased capsaicinoid accumulation of the high pungent Habanero cultivar when compared to daily irrigation frequency due to water status changes (Ruiz-Lau et al., 2011). Moreover, the increase in capsaicinoid under different irrigation frequencies might not be dependent on total volume of water per hot pepper plant (Valiente-Banuet & Gutiérrez-Ochoa, 2016). However, increased capsaicinoid level under water stress is dependent on cultivar, species and drought stress level (Phimchan et al., 2012; Sung et al., 2005). Hot pepper with high pungency and small fruit size are less affected by water stress on total capsaicinoid content (Gurung, Techawongstien, Suriharn, & Techawongstien, 2011; Phimchan et al., 2012), but capsaicinoid content of the Habanero cultivar, which is a high pungency cultivar, was increased under severe water stress (Ruiz-Lau et al., 2011). These results suggest that it may be possible to select hot pepper cultivars that will produce more capsaicinoids with a specific water regime. The information obtained would

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be a benefit to the extraction industry, and would be an advantage to growers when paid on the amount of capsaicinoid produced.

Capsaicinoid yield is an important trait for the extraction industry, and is defined as the combination of total capsaicinoid content and weight of dry powder yield of a hot pepper cultivar. Although, the increase of capsaicinoids under water stress is documented, severe water stress causes flower drop and low fruit setting producing low dry fruit yield in hot pepper (Dorji, Behboudian, & Zegbe-Dominguez, 2005; Phimchan et al., 2012; Ruiz-Lau et al., 2011). Many studies have focussed only on capsaicinoid content under severe water stress with only a few hot pepper cultivars (Ruiz-Lau et al., 2011; Valiente-Banuet & Gutiérrez-Ochoa, 2016). Thus, for high capsaicinoid production, water stress that was less or did not negatively affect crop productivity and increased capsaicinoid content is necessary. Moreover, physiological and morphological responses of a plant under water regime that corresponded with fruit and capsaicinoid yield should be examined to select criteria for evaluating hot peppers under different water stresses.

Information about the water regime in hot pepper (*C. chinense*) for high capsaicinoid yield is limited, especially in hot pepper cultivars that differ in pungency levels. Therefore, this study investigated the effect of different water stresses on capsaicinoid production of hot pepper cultivars having different levels of pungency. The results will help growers to select hot pepper cultivars and an appropriate water stress level that can result in high capsaicinoid production.

2. Materials and methods

For this study, four cultivars of *C. chinense,* Bhut Jolokia, Akanee Pirote, Orange Habanero and BGH1719 were selected. Bhut Jolokia is acknowledged as one of the hottest peppers in the world (Bosland & Baral, 2007). Bhut Jolokia has large fruit size and a heat level of 8,00,000–12,00,000 SHU. Akanee Pirote is a hot pepper hybrid developed at Khon Kaen University that is currently used for capsaicinoid extraction by the pharmaceutical industry in Thailand and has large fruit size and a mean heat level of 4,00,000–6,00,000 Scoville Heat Units (SHU). Orange Habanero is increasing in popularity in the industrial extraction of capsaicinoids (Sanatombi & Sharma, 2008), and BGH1719 has suitable fruit yield and produces acceptable capsaicinoid levels under drought stress (Phimchan et al., 2012). The cultivars Orange Habanero and BGH 1719 both have a heat level of 1,00,000–2,50,000 SHU.

The study was carried out in a plastic-net house at the experimental farm of Khon Kaen University (latitude 16°28′ N, longitude 102°48′ E, 200 m MASL), Khon Kaen Province, Thailand during the May to November 2013 growing season. In this study, hot pepper cultivars seeds were sown between 15 and 30 April 2013 because they varied in plant maturity, and thus the different sowing dates helped to synchronize flowering date. At the 5-true leaf stage, seedlings were transplanted into 12-l (0.28 m × 0.20 m; $W \times H$) plastic pots that were filled with a mixed media, consisting of rice husk, charcoal rice husk, peat moss and cow manure in a ratio of 2:1:1:0.5 (v:v), respectively. The pots were placed 80 *cm* apart in all directions. All plants received the same amount of fertilizer by fertigation throughout the experiment (adapted from Patricia, 1999). Pots were irrigated uniformly to field capacity for four weeks after which time the different water treatments were assigned. Average maximum air temperature was 40 °C and the average minimum air temperature was 25 °C. The monthly maximum average relative humidity (RH) was quite high, averaging 80% throughout the experiment. In addition, the minimum rainfall was 40 mm in October and the maximum was 249 mm in July.

A split-plot design with three replications evaluated capsaicinoid production and its components. Main plots were four water regime treatments, and sub-plots were four hot pepper cultivars (Bhut Jolokia, Akanee Pirote, Orange Habanero and BGH1719). Water was applied to the hot pepper plants by a drip irrigation system. For the control treatment (S1), hot pepper plants were watered daily at field capacity for each cultivar throughout the experiment. The different water stress treatments were started at first anthesis. For the other treatements, plants were watered every two days (S2), every third day (S3) or every 4 days (S4) to field capacity. Measurements were recorded on the last day of the deficit period for each treatment. Substrate media water potential (SWP) was determined by using a tensiometer at 0.15 m away from the stem and a depth of 30 *cm* at 28, 42, and 56 days after flowering (DAF). Midday leaf water potential (LWP) was determined from two fully exposed mature leaves per plant from the middle of the canopy. Measurements were made every two weeks on the last day of no watering (28, 42, and 56 DAF) at 10:00 and 14:00 h using a pressure chamber model DIK-700 (Daiki Rika Kogyo Co. Ltd., Japan).

Air temperatures and RH were recorded daily using a data logger (HOBO U12 Data Loggers, Onset Computer Corporation, Massachusetts, U.S.A). Plant height and canopy width were determined by measuring the tallest and widest portion of the plant. After the last fruit harvest, shoots were removed and shoot dry weight determined by drying the material at 80 °C for at least 48 h. The roots were washed carefully for each plant, dried and weighed as described above and total dry mass calculated as a sum of shoot and root dry mass. Four harvests determined the fruit number and fresh fruit yield. Fruit was oven dried at 70 °C for 48-h to obtain the fruit dry yield. Capsaicinoids were extracted and quantified according to the 'short run' method with high-performance liquid chromatography (HPLC) (Collins, Wasmund, & Bosland, 1995). For capsaicinoid extraction, 1 g of ground hot pepper powder was extracted with 10 ml of acetonitrile at 80 °C for four hours. The extract solution was filtered and 10 µl was injected into a Shimadzu-Model, 10AT-VP HPLC series (Shimadzu Company, Japan) for analysis. The mobile phase was methanol and deionized water at a ratio of 80:20 at a flow rate of 1.5 $ml{\cdot}min^{-1}$ with the ODS C-18 column. The detector's wavelength was set at 284 nm. The standards of capsaicin and dihydrocapsaicin (Fluka #37,274; Fluka Chemie, Buchs, Switzerland) were prepared in 0, 50, 100, 500 and 1000 ppm solutions. The capsaicin and dihydrocapsaicin data were converted to Scoville Heat Units (SHU), as described by Collins et al. (1995). The capsaicinoid yield was calculated using the formula: [capsaicinoid (mg) × fruit dry weight]/sample weight].

Analysis of variance was performed for each data set based on a split-plot design. The means of the treatments were compared with least significant difference test ($P \le .05$) (Gomez & Gomez, 1976).

3. Results

Substrate media water potential (SWP) was significantly decreased with increasing water stress levels when compared to the control (S1) treatment (Fig. 1a). Before watering, SWP values were −9 to −11 kPa, −14 to −18 kPa and −20 to −25 kPa for the S2, S3, and S4 treatments, respectively. After re-watering, SWP values reached field capacity immediately (−3 to −5 kPa).

A decrease in leaf water potential (LWP) within the water stress treatments was observed when compared to the control treatment (Fig. 1b). In addition, different responses to water stress were observed among the cultivars. Under the S2 treatment, the LWP was significantly decreased in Akanee Pirote and Orange Habanero (−0.37 and −0.36 MPa, respectively) as compared to the control treatment (0.29 and 0.27 MPa, respectively). Most cultivars showed a significant reduction in LWP under the S3 and S4 treatments as compared to control treatment. However, Akanee Pirote and Orange Habanero exhibited a greater reduction in LWP than the other cultivars.

In general, Akanee Pirote had the highest canopy width and plant height followed by Bhut Jolokia (Table 1). Although, water stress reduced plant height and canopy width, the reductions were varied by hot pepper cultivar. Plant height and canopy width of Bhut Jolokia and Akanee Pirote were not significantly different between the control and the S2 treatments, and were significantly decreased under S3 and S4 treatments. A slight reduction in plant height and canopy width was