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GROWTH, QUALITY AND CAPSAICIN CONCENTRATION OF HOT PEPPER (*CAPSICUM ANNUUM*) UNDER DROUGHT CONDITIONS

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Drought stress is a major production constraint for fruits and vegetable crops in arid and semi-arid regions. This study was conducted to investigate the effect of drought stress on growth, quality and capsaicin concentration of hot pepper. The study was conducted from March 2017 to September 2018 in the greenhouse of Laboratory of Horticultural Science in Tokyo University of Agriculture (TUA). Germinated seeds were grown in a seed tray and were transplanted in plastic pots at 3 to 4-leaf stage. Drought stress treatments initiated after the first anthesis (26 days after transplanting) with moderate (7 days interval irrigation), severe (9 days interval irrigation) and control (daily watering). Results showed that capsaicinoids, sugars accumulation and ethylene production rate in response to drought stress increased significantly compare to daily irrigated. Fruits harvested at 36 and 45 days after anthesis accumulated higher concentration of glucose, fructose and sucrose in response to moderate and severe stress than daily irrigation. Whereas, capsaicin and dihydrocapsaicin content in fruits harvested at 36 and 45 DAA showed a significant increased under severe and moderate stress. The present findings revealed that drought stress will affect capsaicinoids, sugar and organic acid accumulation of hot pepper fruits and might alter the primary and secondary metabolites.

Key words: capsaicinoids, ethylene production rate, organic acid, sugar accumulation

INTRODUCTION

Drought stress is one of the most common abiotic stresses worldwide, which induces major constraints on plant productivity (Turner and Begg 1981; Pedrol et al. 2000). Drought decreases plant growth by stressing several physiological and biochemical processes such as leaf respiration, leaf chlorophyll content, gas exchange, leaf water content, plant relative growth rate, among others. Consequently, drought stress can lead to significant reduction in total yield of crops (Farooq et al. 2012). Over the past decade, studies on plant-water relationship have gained a lot of interest to deepen the understanding of plant growth and production in response to water uptake. Many studies carried out on the impact of drought stress on crops, particularly in field crops, at different stage of plant growth and development. However, no comprehensive attention had paid to vegetable crops such as *Capsicum* spp., crop in which where the production is often associated with abundant water use (Ferrara et al. 2011). *Capsicum* species, popularly known as pepper is popular vegetable in the world. Hot pepper production worldwide in 2016 was 34.5 million tones with 17.79t ha^{-1} average productivity (FAO 2016). China, Turkey, Indonesia, South Korea and India are major hot pepper producers in Asia. Hot pepper is widely utilized as an additive in Afghan dishes while fresh pepper is consumed as well.

Northern provinces in Afghanistan are well known for hot pepper production, mostly exported as a fresh and dried product to Pakistan (Walters et al. 2012). Hot pepper was reported to be very sensitive to both water deficit and excess water condition (Moreno et al. 2003). They are highly sensitive to these stress conditions whereas flower and fruit development stages were regarded as the most critical stage during hot pepper growth (Dagdelen et al. 2004; Phimchan et al. 2012; Techawongstien et al. 1992). Water shortage in hot pepper during these stages may reduce fruit production (Jaimez et al. 2000). However, Afghanistan suffered severely from drought over the past decade, which affected yield and quality of hot pepper as well as other horticultural crops (Beekma and Joel 2011). On the other hand, water-stressed plants typically produce more pungent pods due to what is popularly known as capsaicin. Capsaicin and other related compounds, commonly called capsaicinoids are phenolic compounds abundant in fruits of genus *Capsicum* (Bennett and Kirby 1986). Capsaicin and dihydrocapsaicin, the most abundant capsaicinoids in hot peppers (Iwai et al. 1979) are responsible for 90 percent of their pungency (Govindarajan 1986). These secondary metabolites are of economic importance because of their use in food (preparation of hot sauces), cosmetics (shampoos to prevent hair loss), military (spray repellents against dogs and thieves) and pharmaceuticals (as an additive to pads for relieving muscular pain). However, few researches address the effects of drought stress on ethylene production, sugar and organic acid accumulation. Hence, this research aims to investigate the effect of drought stress on growth, quality and capsaicin concentration of hot pepper.

MATERIALS AND METHODS

Seeds of hot pepper cv. Taka-no-Tsume Togarashi used in this study were provided by Takii Seed Co., Ltd., Kyoto-shi. Seed materials were subjected to two experiments which were conducted in the greenhouse of the Laboratory of Tropical Horticultural Science in Tokyo University of Agriculture (TUA). Seeds were set to germinate in moistened Petri-dishes in the laboratory and were kept in an incubator under 25 °C for 72 hours. Germinated seeds were transferred to trays which were laid on a growing pan half-filled with water to enhance root and shoot growth. Furthermore, OAT House 1 and 2 fertilizers were used with EC values less than 1mS/cm. (OAT Agrio Co., Ltd). Seedlings were transferred into plastic pots with a 20×16 cm height and diameter after it has reached 12-15 cm in height and have grown five pairs of leaves. It was grown in soil media consist of peat moss, vermiculite, organic fertilizer and Akadama soil (Makino Co., Ltd.) with a ratio of 2:1:1:2 by volume. On the other hand, a number of seedlings were placed under controlled conditions in the greenhouse for 120 days and were irrigated regularly until first anthesis.

Drought stress treatments were initiated after the first anthesis (26 days after transplanting). Seedlings were divided into three drought stress treatments: control (daily irrigation), moderate (7-day interval irrigation) and severe (9-day interval irrigation). One-liter water was applied either every seventh (moderate) or ninth (severe) day. Three sets of fifteen plants were used as replicates.

Plant height. The maximum plant height was measured by a ruler placed vertically from the ground to the tip of the longest leaf at 30, 45, 60, 75, 90 and 105 days after transplanting.

Electrolyte leakage. Electrolyte leakage was measured at 25, 36 and 45 days after exposing plants to drought stress. Measurement method was referenced from Jiang et al. (2001) with modification. Ten leaves from five plants in each treatment were collected and excised with a 1-cm diameter stainless steel cork borer. Disks were placed in 1 mL pure water with 25±1°C for 20 minutes with adaxial faces up. The conductivity of the solution (L_1) was measured with an electrical conductivity meter (LAQUATWIN-S070, Horiba Scientific Ltd., Japan). On the other hand, the solution was boiled for 20 min and then immediately cooled to measure the conductivity of the tissues (L_2). The electrolyte leakage was expressed as a percentage of relative ion leakage (%) = (L_1/L_2) ×100.

Ethylene production. Ethylene production was analyzed in hot pepper fruits at 25, 36 and 45 days after exposing to drought stress treatment by gas chromatography (GC) with a flame ionization detector (Shimadzu Gas Chromatograph GC-14B; C-R6A Chromatopac, Japan) using headspace analysis. Six fruits from three replicate plants in each treatment were placed in an 18 mL glass jar and incubated under dark condition for 1 hour at room temperature and 1 mL headspace gas was removed using a plastic syringe for analyzing ethylene production. The GC machine was equipped with a Sunpack A column (3.2 mm i.d. x 2.1 m; Shinwa Chemical Industries, Tokyo, Japan). The parameters were set at injector 180°C, column 80°C detector 200°C. The carrier gas was N_2 and the column pressure was maintained at 6 kg cm^{-2}.

Sugar analysis. Glucose, fructose and sucrose content of hot pepper fruit was quantified by HPLC (Shimadzu 2007) using the method by Ayvaz et al. (2016) with some modifications. Hot pepper fruits were harvested at 25, 36 and 45 days after anthesis and ground into a fine powder using mortar and pestle in the presence of liquid nitrogen. Fruit powder (200 mg) was accurately weighed and placed in 2 mL tube and added to 1.8 mL water containing 5% ethanol. Samples were vortexed, sonicated well and centrifuged at 15000g for 15 min at 4°C. An amount of 900µl supernatant was collected into a new tube where 900µl of HPLC-grade acetonitrile was added and vortexed. The solution was transferred into 2.5 mL syringe equipped with polytetrafluoroethylene (PTFE) 0.20µm syringe filter unit and filtered into 2 mL HPLC vial. Sugars were analyzed by HPLC with an RID-10A refractive index detector and 10µl of sample which were separated using a Shodex sugar KS-801 column (300 mm in length and 8 mm in diameter) with a flow rate of 0.8mL/min of deionized water at 80°C.

Organic acid analysis. Organic acids were measured as per the method described by Vazquez et al. (1993) with some modifications. Frozen fruit powder was weighed to 200 mg and mixed with 1.8 mL ultra-pure water, vortexed and centrifuged at 15000g for 15 min at 4°C. The supernatant was collected in a 2mL tube, and transferred into 2.5mL syringe equipped with 0.45µm DISMIC cellulose acetate syringe. The contents were filtered and transferred into 2mL HPLC vials. Organic acid was analyzed using Shimadzu CDD-10AVP, conductivity detector, column series SCR-102H×2 (length 8mm×300mm×2 at 40°C. An isocratic elution was carried out with 100% 3mM $HClO_4$ at a flow rate of 0.8mL/min.

Extraction and analysis of capsaicin and dihydrocapsaicin. The major capsaicinoid, capsaicin and dihydrocapsaicin in hot pepper fruit during different growth and development stage was extracted and determined as per the method described by Collins et al. (1995). Hot pepper fruits harvested at 25, 36 and 45 days after anthesis were ground into fine powder using mortar and pestle in the presence of liquid nitrogen then dried in a freeze dryer overnight. One (1) g of dried hot pepper powder was extracted by shaking in 10 mL of analytical grade acetonitrile at 80°C for 4 hours. The extract was filtered with a polytetrafluoroethylene (PTFE) 0.20µm syringe filter unit and 10µl of the filtered extract was analyzed by HPLC (Hitachi-Model, D-7000IF, Hitachi, Ltd. Tokyo Japan). The mobile phase was 80:20 methanol: ultrapure water at a flow rate of 1 mL/min with the ODS C-18 column. The detector wavelength was set at 284 nm and the column oven temperature at 30°C. Capsaicin and dihydrocapsain standards (Wako Chemical, Japan) were prepared at 50, 100, 500 and 1000 ppm.

Statistical analysis. The experiment design was complete block design with three replications. Data were subjected to (ANOVA) SPSS 19 software and significance main effects was determined at the $P < 0.05$ probability level. Means were compared using the Tukey test ($P < 0.05$).

RESULTS AND DISCUSSION

Plant height. Drought stress has a significant influence on plant height. Significant difference in plant height were observed when exposed to drought at 30, 45, 60, 75, 90 and 105 days after transplanting. Plants treated with moderate (7-day interval irrigation) and severe (9-day interval irrigation) stress conditions recorded the shortest plant height at 45, 60, 75, 90 and 105 days after transplanting than control (Fig.1). The decrease in plant height might be correlated with photosynthetic activity and leaf water potential. Drought stress decreased plant height in some cultivars (Phimchan et al. 2012). However, plant height showed a varied response with stress level whereas severe drought treated plants were taller than moderate treated plants.

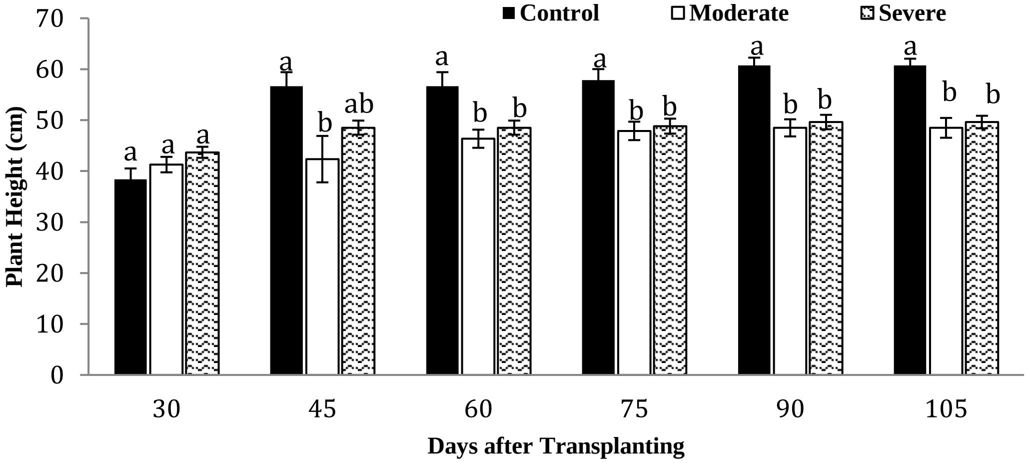

Fig. 1. Plant height of hot pepper seedlings in response to different stress level at 30-105 days after transplant. Results represent means ±SE (n=10); values with different superscripts indicate difference (p<0.05) using Tukey test.

Electrolyte leakage. Exposure of plants to drought stress resulted in a significant increase in electrolyte leakage than control. Hot pepper leaves at 25, 36 and 45 days after moderate (7-day interval irrigation) and severe (9-day interval irrigation) drought treatment recorded higher levels of leaf electrolyte leakage compared to control (Fig. 2). The increased electrolyte leakage in severe and moderate stress might be due to the loss in cell membrane integrity, which resulted in growth impairment in hot pepper plants. It is well known that stress causes leakage in cell membrane and lipid peroxidation. Cell membrane stability has shown to be affected by lipid peroxidation caused by active oxygen species under various stress conditions (Gill and Tetuja 2010).

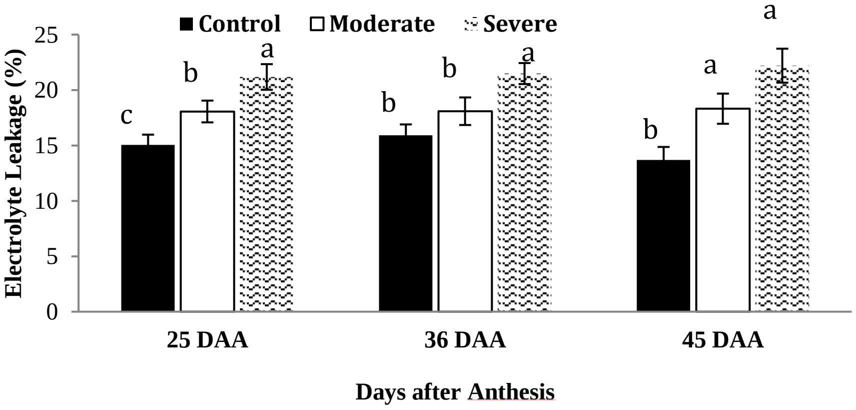

Fig. 2. Effect of different drought stress level on electrolyte leakage of hot pepper leaves at 25, 36 and 45 days after anthesis. Results represent means ±SE (n=10); values with different superscripts indicate difference (p<0.05) using Tukey test.

Ethylene production. Ethylene production in hot pepper fruits significantly increased under drought stress conditions (Fig. 3 and 4). Fruits harvested at 25, 36 45 days after anthesis under moderate (7-day interval irrigation) and severe (9-day interval irrigation) produced higher concentration of ethylene outside and inside pod compared to control (daily irrigation). The increased ethylene production in severe and moderate stress conditions could be attributed to the metabolic reaction between ACC oxidase with its substrates (ACC). This phenomenon is possibly caused by decreased compartmentation due to deterioration of membrane integrity. The membrane lipid content is decreased associated with increased free fatty acid pool under drought conditions. (Zhu et al. 2016; Akhami et al. 2019). Akhami et al (2019) reported that increased ethylene production has caused accelerated senescence as an adaptive measure to decrease the water demand to whole-plant level.

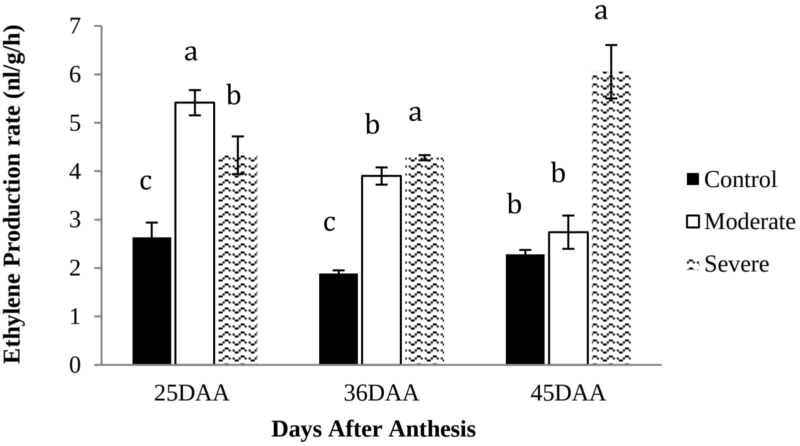

Fig. 3. Effect of different drought stress level on hot pepper fruits ethylene production rate at 25, 36 and 45 days after anthesis Results represent means ±SE (n=6); values with different superscripts indicate difference (p<0.05) using Tukey's test.

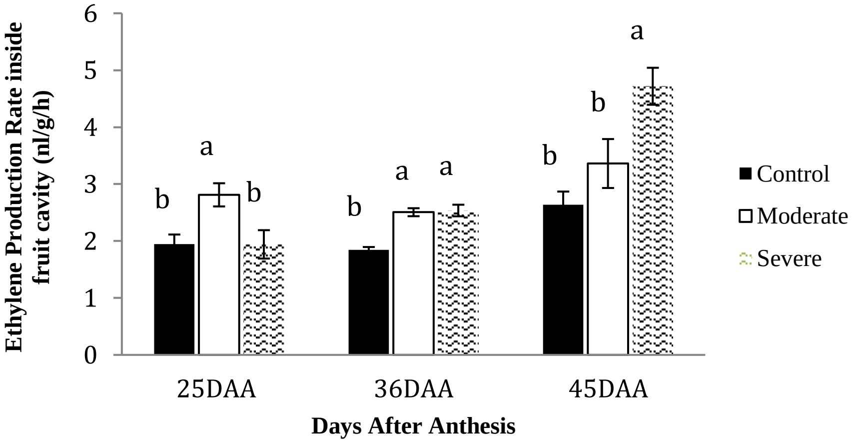

Fig. 4. Effect of different drought stress level hot pepper fruits on ethylene production rate inside pod cavity at 25, 36, 45 days after anthesis. Results represent means ±SE (n=6); values with different superscripts indicate difference (p<0.05) using Tukey test.

Sugars. The sugar accumulation in hot pepper fruits due to drought stress increased significantly than daily irrigation (Fig. 5, 6 and 7). Fruits harvested at 25, 36 and 45 days after anthesis under moderate (7-day interval irrigation) and severe (9-day interval irrigation) accumulated higher glucose and fructose content compared to control (daily irrigation). Meanwhile, sucrose content was significantly affected in fruits harvested at 36 and 45 days after anthesis under moderate and severe stress compared to control.

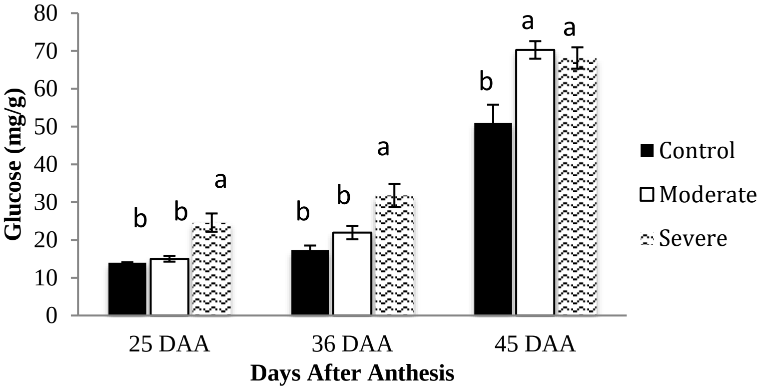

Fig. 2. Effect of different drought stress levels on glucose accumulation of hot pepper fruits at 25, 36, 45 days after anthesis. Results represent means ±SE (n=3); values with different superscripts indicate difference ($p<0.05$) using Tukey test.

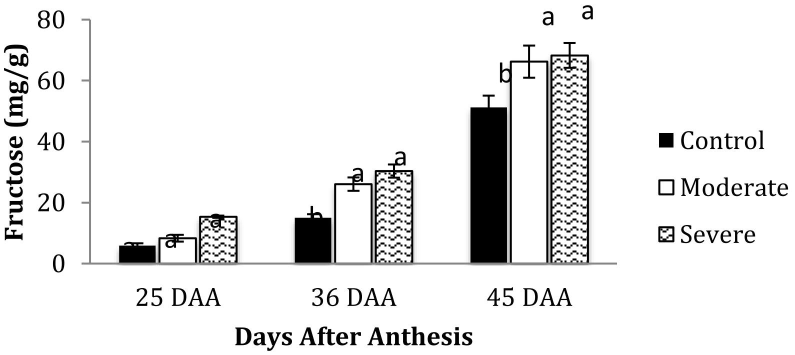

Fig. 6. Effect of different drought stress level on fructose accumulation of hot pepper fruits at 25,36, 45 days after anthesis. Results represent means ±SE (n=3); values with different superscripts indicate difference ($p<0.05$) using Tukey test.

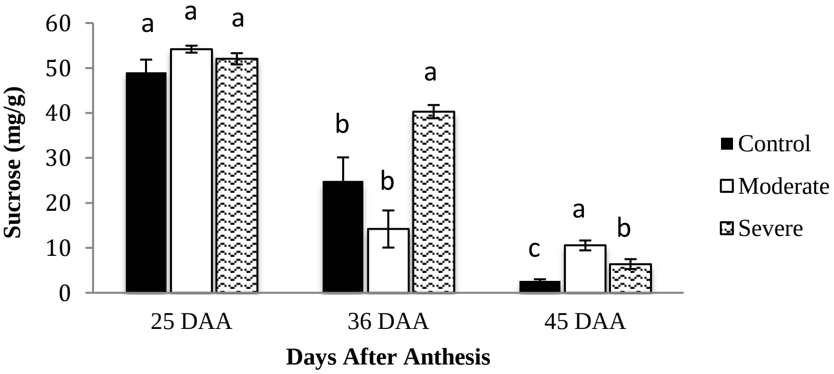

Fig. 7. Effect of different drought stress level on sucrose content of hot pepper fruits at 25, 36, 45 days after anthesis. Results represent means ±SE (n=3); values with different superscripts indicate difference ($p<0.05$) using Tukey test.

The accumulation of sugars under drought stress is a widely accepted fact. The increased sugar concentration of fruits under moderate and severe stress might be maintaining membrane stability or protecting membrane fusion from drought stress. Osmotic potential can be adjusted by increasing the concentration of total soluble sugar, which can decrease water potential of cells without inhibiting enzyme function and does not reduce turgidity of the cell. (Zushi and Matsuzoe 1998; Lipiec et al. 2013; Okunlola et al. 2016)

Organic acid. Organic acid accumulation under drought stress increased slightly. The accumulation of malic acid significantly increased in fruits harvested at 25 and 45 days after anthesis under moderate (7-day interval irrigation) and severe (9-day interval irrigation) than control. However, there was no significant difference in citric acid accumulation in plants subjected treated with drought stress and control when fruits were harvested at 25, 36 and 45 days after anthesis (Fig. 8 and 9). The increased malic acid accumulation might be due to osmoregulation or osmotic pressure and the unchanged organic acids might be due to decreased activity during glycolysis in TCA cycle. Akhami et al. (2019) stated that increased malic acid accumulation is a natural plant response to drought condition which play a role in osmotic adjustment. It likely causes stomatal closure to reduce further water loss through transpiration. Moreover, water deficiency affects the metabolic pathway of organic acid in tomato fruits and grape vine leaves (Zushi and Matsuzoe 1998; Grant et al. 2007).

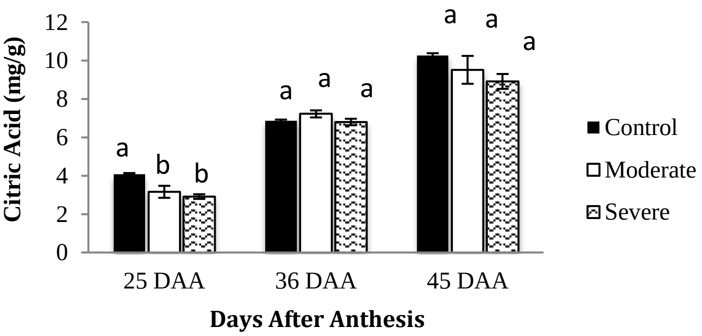

Fig. 8. Effect of different drought stress level on citric acid accumulation of hot pepper in response to different fruit stages. Results represent means ±SE (n=3); values with different superscripts indicate difference ($p<0.05$) using Tukey's test.

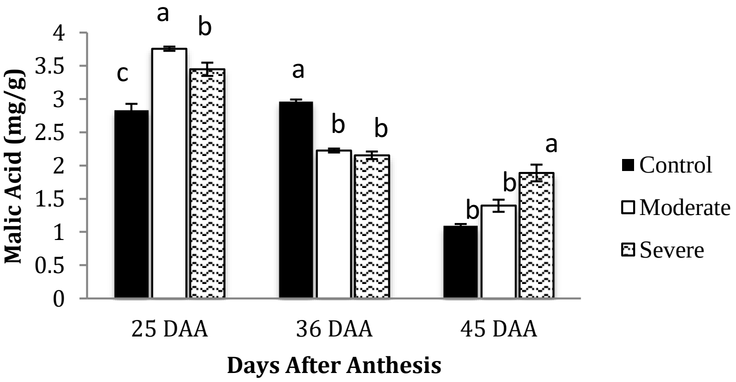

Fig. 9. Effect of different drought stress level on malic acid accumulation of hot pepper in response to different fruit stages. Results represent means ±SE (n=3); values with different superscripts indicate difference ($p<0.05$) using Tukey test.

Capsaicin and dihydrocapsaicin concentration. Capsaicin and dihydrocapsaicin concentration in the drought stressed plants significantly differed from control (Fig. 10 and 11). Fruits treated under moderate (7-day interval irrigation) and severe (9-day interval irrigation) stress that were harvested at 36 and 45 days after anthesis recorded a higher concentration of capsaicin and dihydrocapsaicin than control (daily irrigation). Capaicinoids start accumulating at early fruit development and reach a maximum at 30 to 50 days after flowering, depending on the cultivar (Estrada et al. 2000). Previous studies demonstrated that capsaicin and dihydrocapsaicin content increased under drought stress (Estrada et al.1997; Sung et al. 2005; Ruiz-Lau et al. 2011).

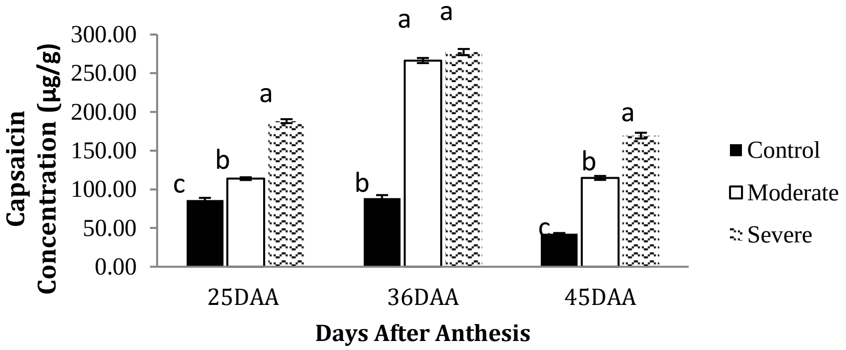

Fig. 10. Effect of different drought stress level on capsaicin content of hot pepper in response to different fruit stages. Results represent means ±SE (n=3); values with different superscripts indicate difference ($p<0.05$) using Tukey test.

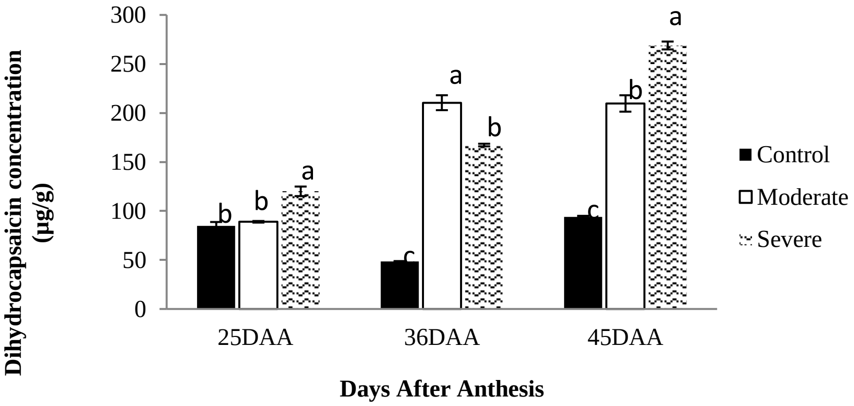

Fig. 11. Effect of different drought stress level on dihydrocapsaicin content of hot pepper in response to different fruit stages. Results represent means ±SE (n=3); values with different superscripts indicate difference ($p<0.05$) using Tukey test.

Among the parameters measured, fruits at 36 days after anthesis have higher concentration of capsaicin and dihydrocapsaicin alongside with some metabolites than fruits at 45 days after anthesis. The experimental results provide basic information on the effect of drought on primary and secondary metabolites including capsaicin and dihydrocapsaicin.

CONCLUSION

Generally, water stress affected the growth and development of hot pepper plant which resulted in some disturbed morphological, physiological and biochemical processes. Moreover, hot pepper plants showed great sensitivity to certain levels of drought stress. Moderate and severe stress conditions affected negatively plant growth, leaf electrolyte leakage, ethylene production and primary and secondary metabolites and as such may results in yield reductions. Additionally, the increased accumulation of capsaicin in fruits harvested at 45 DAA correlated highly with the ethylene produced outside fruit pods. Drought stress treatments led us to conclude that environmental factor and ethylene production were involved in the accumulation of capsaicin and might alter the primary and secondary metabolites. The knowledge obtained in this study may assist farmers selecting drought tolerant varieties or finding a way to alleviate drought stress conditions. Finally, these findings would provide beneficial information that can be useful to ameliorate hot pepper production and related enterprises in southeast Asia and neighboring regions. Different strategies can be set to combat the detrimental impact of drought stress on crop production. In addition, these results can be a valuable tool to regulate growth, yield and quality of hot pepper as water stress encourages the production of secondary metabolites that can increase potentially plant defenses and concentrations of compounds such as capsaicin and dihydrocapsaicin.

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