

Changes in leaf gas exchange, biochemical properties, growth and yield of chilli grown under soilless culture subjected to deficit fertigation

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Abstract

Deficit fertigation (DF) may control transpiration and improve crop water-use efficiency (WUE) without much yield reduction. This study was conducted to assess and compare the effects of deficit fertigation (DF) with standard local grower's practices on growth, yield, leaf gas exchanges and biochemical changes on chilli plant grown in soilless culture. Five treatments including (1) standard local grower's practice (control, T0), (2) 100% fertigation of crop evapotranspiration (ET_c, T1), (3) 100% ET_c and 2 days fertigation interval (T2), (4) 75% ET_c and 2 days fertigation interval (T3) and (5) 50% ET_c and 2 days fertigation interval (T4) based on pan evaporation method were evaluated in this experiment. DF was found to affect the production of biomass allocation into leaf, stem, root and yield of chilli. Increase the degree of DF significantly reduced chilli yield from 1060 g plant⁻¹ to 28 g plant⁻¹. All treatments under DF also decreased photosynthesis rate (P_n), stomatal conductance (g_s), maximum quantum of yield PSII (F_v/F_m) and relative chlorophyll content of chilli plants. A higher P_n and g_s obtained at all growth stages in control plants. Higher fertigation use efficiency (FUE) was found in 100 % ET with 2 days fertigation intervals. Catalase (CAT) and ascorbate peroxidase (APX) were increased from bloom to fruit set and then gradually decreased until fruit ripening stage at all levels of DF treatments. In contrast, guaiacol peroxidase (GPX) was gradually increased from bloom and fruit set until fruit ripening stage at all levels of DF. Thus, the study indicated that growth, leaf gas exchange and yield of chilli grown in soilless culture were significantly reduced under DF than that of local grower's practice.

Keywords: *Capsicum annum*; Deficit fertigation; Catalase; Ascorbate peroxidase; Guaiacol peroxidase; Plant biomass.

Abbreviations: APX_ascorbate peroxidase; CAT_catalase; DAT_days after transplanting; DF_deficit fertigation; DI_deficit irrigation; ET_c_crop evapotranspiration; FUE_fertigation use efficiency; F_v/F_m _maximum quantum of yield PSII; g_s _stomatal conductance; GPX_guaiacol peroxidase; IWUE_irrigation water use efficiency; P_n _photosynthesis rate; SMC_substrate moisture content.

Introduction

Water is an important natural resource, which is becoming scarce with time. Declining availability and high cost of water threatens irrigated farming system across the world. Agriculture in the future is needed to increase its production to feed the growing world population with limited fresh water (Howell, 2001). Therefore it is necessary to optimize water management especially in irrigated farming system. Irrigation is important to avoid water deficit in crop production. However, to minimize the water use in plants, an efficient irrigation method and appropriate management should be applied. Chilli (*Capsicum annum* L.) is an important cash crop in the world. Vegetable growers prefer chilli due to its high demand and stable market price. In Malaysia, chilli is commonly grown under soilless culture by using fertigation system. However, limited fresh water, fluctuated fertilizer prices and tendency of overwater to plants by local growers are the major constraints of chilli cultivation. Deficit irrigation (DI) is a water-saving strategy where the whole root zone is irrigated with less than evapotranspiration

requirements. DI increased irrigation water use efficiency (IWUE) of chilli (Dorji et al., 2005). However, previous studies also reported that DI reduced fruit weights of chilli (Delfine et al., 2001) and tomato grown in both glasshouse and field conditions (Zegbe-Domínguez et al., 2003). The synthesis of reactive oxygen species (ROS) is an important biochemical changes occurred during biotic or abiotic stress in plants. They are highly reactive and can seriously disrupt the normal metabolism of plant in the absence of protective mechanisms. Increased levels of ROS such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (HO^\cdot) and singlet oxygen (1O_2) are associated with limited water condition (Smirnoff, 1993). It was documented that oxidative damage could be avoided by the production of antioxidant enzymes as well as catalase (CAT), ascorbate peroxidase (APX) and guaiacol peroxidase (GPX). Many studies have been conducted on the effect of DI on growth and yield of vegetable crops under glasshouse and field conditions. However, effect of DF under soilless culture on plant growth,

Table 1. Effect of deficit fertigation on leaf dry weight, stem dry weight, root dry weight, total dry mass and root: shoot ratio in chilli plants.

Treatment	Leaf dry weight (g plant ⁻¹)	Stem dry weight (g plant ⁻¹)	Root dry weight (g plant ⁻¹)	Total dry mass (g plant ⁻¹)	Root: Shoot Ratio
T0 (Control)	111.68 a	269.61a	33.65 a	414.93 a	0.09 b
T1	42.88 b	88.96 b	12.23 b	144.06 b	0.10 b
T2	17.68 c	45.31 bc	14.48 b	77.47 c	0.22 a
T3	13.07 cd	35.46 c	11.61 bc	60.13 c	0.23 a
T4	11.43 d	31.03 c	9.28 c	51.73 c	0.24 a
Significant	**	**	**	**	**
C.V	8.56	21.48	8.40	13.06	4.09

0= standard local growers' practices (control), T1= 100% ET_c with daily fertigation, T2= 100% ET_c and 2 days fertigation interval, T3= 75% ET_c and 2 days fertigation interval, T4= 50% ET_c and 2 days fertigation interval. Means followed by the same letters within a column are not significantly different from each other according to the Least significant difference (LSD) test. ** significant at P≤0.01.

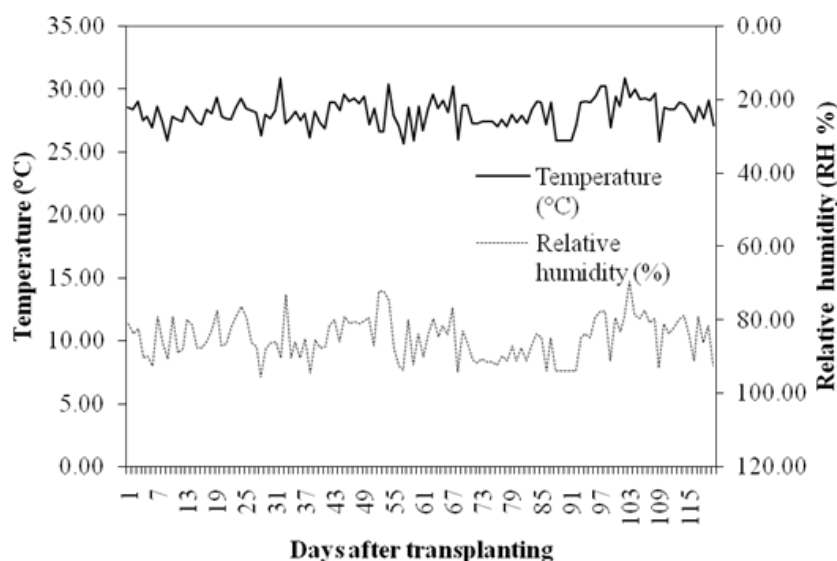


Fig 1. Mean temperature and relative humidity under rain shelter condition throughout the experimental period.

yield and physiological and biochemical responses are not well documented. Hence, the study was undertaken to evaluate the effect of different levels of DF on yield, plant growth, leaf gas exchange and biochemical changes of chilli plants grown in soilless culture.

Results

Deficit fertigation on substrate moisture content

Different levels of DF with 2 days interval significantly ($p \leq 0.05$) affected substrate moisture content (SMC) at different measurement times. In contrary, no significant ($p \leq 0.05$) differences was observed in SMC among treatments at 14, 56 and 108 DAT. Control plant (T0) had a highest SMC, while T1 had the lowest SMC throughout the experiment (Fig. 2).

Deficit fertigation on plant height, stem diameter and total leaf area

The differences in plant height were significant ($p \leq 0.05$) among treatments at all growth stages except for seedling and vegetative stages. Plant height was decreased after bloom and fruit set stage at all levels of DF with 2 days fertigation intervals (Fig. 3A). Similarly to the plant height, stem diameter was significantly ($p \leq 0.01$) different between all

treatments at all growth stages. However, no significant difference was observed in stem diameter among treatments at seedling and vegetative stages. Stem diameter was also reduced after bloom and fruit set stage at all levels of DF with 2 days fertigation intervals (Fig. 3B). Reduction in stem diameter in T1, T2, T3 and T4 were 25, 31, 24 and 42%, respectively, compared to the control plants at bloom and fruit set stage. Total leaf area was significantly ($P \leq 0.05$) different between all treatments at all growth stages. The difference in total leaf area was not significant among treatments at seedlings and vegetative stages. Total leaf area increased with age in all the treatments until bloom and fruit set stage and followed a decline due to leaf shading (Fig. 3C). At later fruit bearing stage, total leaf area was significantly inhibited by 83% in T4 treatment compared to the control.

Biomass production and dry matter partitioning

There were highly significant ($P \leq 0.01$) differences in the dry matter at all plant parts of chilli in all treatments. DF with 2 days fertigation intervals significantly reduced leaf, stem and root dry weights and total dry mass (Table 1). A greatest reduction in the dry mass of plant parts was detected in T4 (90, 88, 72 and 88%, respectively) followed by T3 (88, 87, 65 and 86%, respectively), T2 (84, 83, 57 and 81%, respectively) and T1 (62, 67, 64 and 65%, respectively).

Table 2. Fresh fruit yield, total volume of nutrient solution and fertigation use efficiency in chilli plants under different levels of deficit fertigation.

Treatment	Fresh fruit weight (g plant ⁻¹)	Total volume of nutrient solution applied (L plant ⁻¹)	Fertigation use efficiency (g L ⁻¹)
T0 (Control)	1060 a	288.7	3.69b
T1	233 b	52.3	4.46ab
T2	99 c	18.36	5.39a
T3	64 c	14.01	4.55ab
T4	28 d	7.6	3.63b
Significant	**		**
C.V	12.17		10.79

T0= standard local growers' practices (control), T1= 100% ET_c with daily fertigation, T2= 100% ET_c and 2 days fertigation interval, T3= 75% ET_c and 2 days fertigation interval, T4= 50% ET_c and 2 days fertigation interval. Means followed by the same letters within a column are not significantly different from each other according to the Least significant difference (LSD) test. ** significant at P≤0.01.

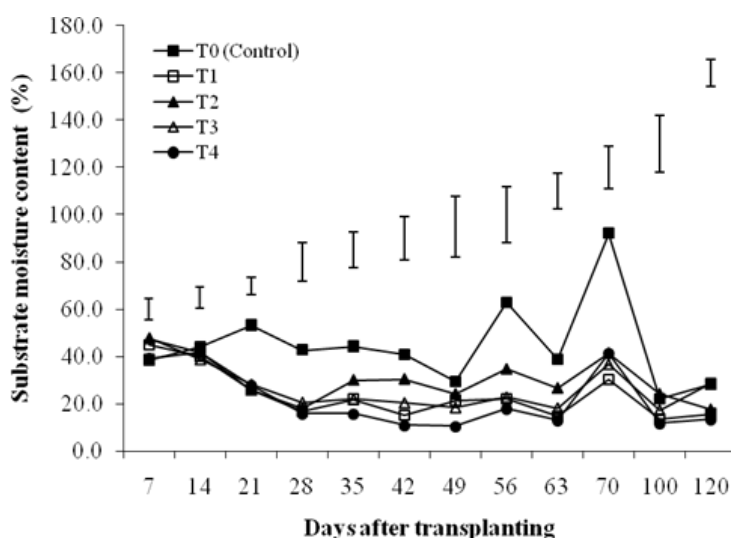


Fig 2. Effect of deficit fertigation on substrate moisture content during chilli cultivation. T0= standard local growers' practices (control), T1= 100% ET_c with daily fertigation, T2= 100% ET_c and 2 days fertigation interval, T3= 75% ET_c and 2 days fertigation interval, T4= 50% ET_c and 2 days fertigation interval. Vertical bar represents least significant difference (LSD) at (P≤0.05).

In contrast, root to shoot ratio was significantly higher in T4 treatment (0.24) than in control plants (0.09).

Responses of leaf gas exchanges, chlorophyll fluorescence and relative chlorophyll content under deficit fertigation

Significant (P≤0.05) differences were observed in P_n among treatments at all growth stages (Fig. 4A). However, no significant differences in P_n were recorded at seedling and vegetative stage. Control plants had 12 and 25% higher P_n than in the T3 and T2 treatments, respectively, at bloom and fruit set and vigorous fruit bearing stages. In general, T4 treatment produced the lowest P_n at all growth stages. Stomatal conductance was observed highly significantly (P≤0.01) different in all treatments at all growth stage. Similarly, the differences in g_s were no significant at seedling and vegetative stage. Stomatal conductance in all levels of DF with 2 days fertigation intervals showed a similar trend to that P_n in contrast to control plants (Fig. 4B). However, plants subjected to T1 treatment had significantly higher g_s (21%) than control plants at bloom and fruit set stage, which decreased at the following stages. There was significant (P≤0.05) difference in maximum yield of PSII (F_v/F_m) between treatments at all growth stages. Maximum quantum yield of PSII was not significant among treatments at seedling and later fruit bearing stage. Maximum quantum yield of PSII slightly reduced at bloom and fruit set stage

until the end of the growing stage for all treatments. On the other hand, F_v/F_m value at the end of growing stage was increased with highest value recorded on control followed by T1, T3, T2 and T4, which slightly reduced by 1, 4, 5 and 9%, respectively, compared to control plant. Relative chlorophyll content was highly significantly (P≤0.01) decreased under all levels of DF with 2 day fertigation intervals compared to control plants (Fig. 4D). Relative chlorophyll content was higher in control and T1 treatment at all growth stages and the difference between control and T1 was not significant. Relative chlorophyll content at the end of the growing stage decreased with greater value observed in T3 (29%) followed by T2 (23%) and T4 (20%) compared to that in control plants. However, no significant (P>0.05) difference was observed in relative chlorophyll content among T3, T2 and T4 at that growth stage.

Changes in the enzyme activities

Leaf CAT activity decreased slightly with age in control plants (Fig.5A). However, significantly (P≤0.05) higher CAT activity was remarkable in plants subjected to all levels of DF with 2 days fertigation treatments at bloom and fruit setting stage and onwards, compared to control plants. However, gradual decrease in CAT activity was observed in plants subjected to all levels of DF with 2 days fertigation intervals by the age. In contrary, there was no significant

difference among the treatments at the vigorous fruit bearing stage. Ascorbate peroxidase activity (APX) was significantly ($P \leq 0.05$) slightly decreased by age in plants subjected to all levels of DF with 2 days fertigation intervals (Fig. 5B). However, plants subjected to all levels of DF with 2 days fertigation intervals had higher APX activity than in control plants. Plants subjected to T4 treatments showed a greater (158%) increase at the end of experimental period, compared to the control plants. In contrast, GPX activity significantly ($P \leq 0.05$) increased after bloom and fruit set stage until the end of the experiment period in plants imposed to all levels of DF with 2 days fertigation intervals compared to control plants (Fig. 5C). Gradual reduction was observed in control plants from vigorous fruit bearing to the fruit ripening stage. Treatment T4 had higher GPX activity at fruit ripening stage followed by T3, T2 and T1 treatments.

Deficit fertigation on fresh fruit weight, irrigation amount and fertigation use efficiency

Fresh fruit weight was highly significant ($P \leq 0.01$) among the treatments (Table 2). Total fresh weight of fruit per plant in T1 was 134 g higher than T2 treatment, but it was 827 g lower than that in control plants. The highest reduction (97%) was recorded in T4 than in control plants. Highest amount of total nutrient solution consumed by the control plants was 288.7 L plant⁻¹ while the lowest was found in treatment T4 with 7.6 L plant⁻¹ (Table 2). FUE was significantly affected by all levels of DF with 2 days fertigation intervals (Table 2). The highest FUE value of 5.39 g L⁻¹ was obtained in T2 treatment and the lowest value of 3.63 g L⁻¹ was observed in T4 treatment.

Discussion

Substrate moisture content (SMC) was always lower under DF treatments than in control at all measurement day. The reduction in SMC was attributed to the lower amount of water and nutrients applied. The result was consistent with the findings on greenhouse grown tomato (Zegbe et al., 2004), where full irrigation had resulted higher SMC compared to DI throughout the growing season. They claimed that a reduction in SMC in DI was high enough to cause a reduction in plant water potential, which in turn, resulted from water losses and reduction of cell turgor. Plant height, stem diameter and total leaf area were reduced with DF. The reduction in these growth parameters suggests that DF is one of the major factors which determine growth and development in chilli plants. The reduction in the growth parameters might be associated with the reduction in SMC (Tesfaye et al., 2008). The reduced plant height and stem diameter under all levels of DF might be attributed to the reduction in cell turgor which would result in inhibition of cell division and cell expansion. Reduction in cell turgor was attributed to the reduction in cell water potential resulted to the dehydration (Luvaha et al., 2008). The reduction in total leaf area presumably attributed to the reduction in leaf number, leaf senescence and reduction in rate of leaf emergence (Zewdie et al., 2007). Furthermore, reduction in leaf area in plant subjected to water stress might be attributed to the changes in leaf angles and resulted in reduced gas exchange and carbon assimilation in a longer period (Turner and Begg, 1981).

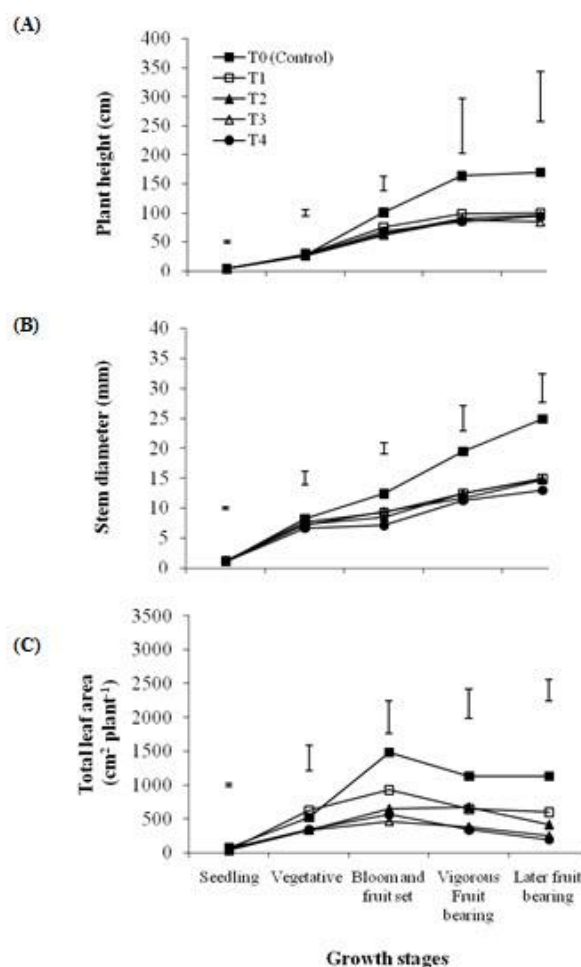


Fig 3. Effect of deficit fertigation on plant height (A) stem diameter (B) total leaf area (C) on chilli plants at different growth stages. T0= standard local growers' practices (control), T1= 100% ET_c with daily fertigation, T2= 100% ET_c and 2 days fertigation interval, T3= 75% ET_c and 2 days fertigation interval, T4= 50% ET_c and 2 days fertigation interval. Vertical bar represents Least significant difference (LSD) at ($P \leq 0.05$).

Productivity of plants is determined in part by the allocation of photosynthates among organs. Water stress can affect the growth of each plant organ differently and alter the pattern of dry mass accumulation within plants (Cox and Conran, 1996). In the present study, DF has increased the root: shoot ratio. These results indicated that a large proportion of photosynthates were allocated to underground plant parts under DF, which means plants preferably increased allocation of assimilates to roots rather than in leaves and stems (Rodrigues et al., 1995). Under lower substrate water availability, the plants invested more dry mass in root growth, in order to absorb more water and ensure higher survival competitiveness. Chartzoulakis et al. (1993) suggested that higher root: shoot ratio under water stress could be attributed to the higher osmotic adjustment in roots than in leaf cells. Deficit fertigation greatly affected P_n and g_s in chilli plants. In the present study, DF decreased P_n and g_s progressively with growth stages. The results suggested that the decline in net photosynthesis was closely associated with the consequence of stomatal limitation. The earliest response of plant to water stress is stomatal closure because stomatal closure decrease CO₂ diffusion into leaf perturbing photosynthesis.

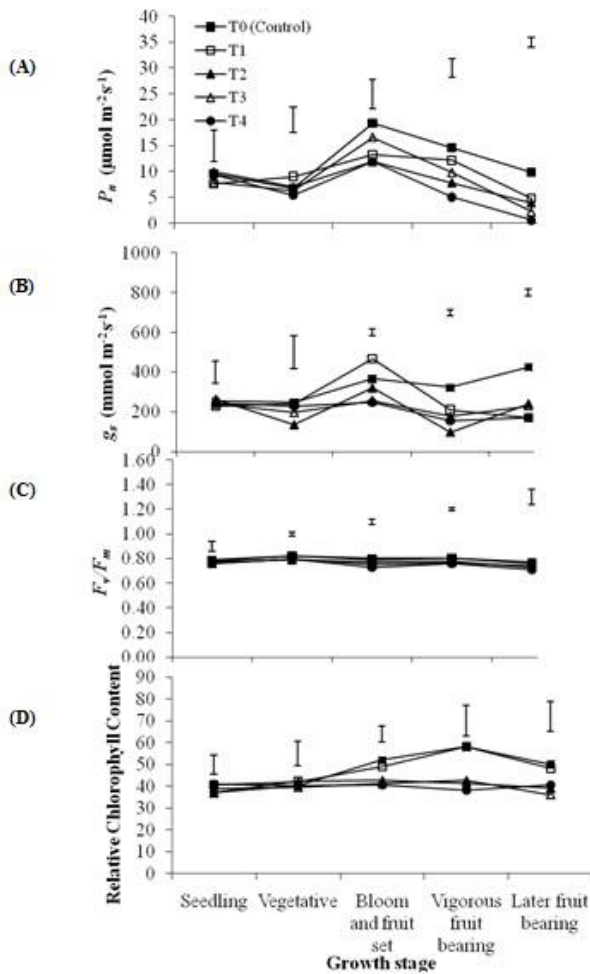


Fig 4. Effect of deficit fertigation on photosynthesis rate (P_n) (A), stomatal conductance (g_s) (B), maximum quantum of yield PSII (F_v/F_m) (C) and relative chlorophyll content (D) response of chilli at different growth stages. T0= standard local growers' practices (control), T1= 100% ET_c with daily fertigation, T2= 100% ET_c and 2 days fertigation interval, T3= 75% ET_c and 2 days fertigation interval, T4= 50% ET_c and 2 days fertigation interval. Vertical bar represents Least significant difference (LSD) at $P \leq 0.01$.

This result was similar with the previous studies, where positive relationship between g_s and P_n were observed in neem plants (Zheng et al., 2010). A chlorophyll fluorescence parameter is maximum quantum of yield. PSII (F_v/F_m) reduced under all levels of DF. The decrease in F_v/F_m was presumably attributed to the reduction in F_m than an increase in F_o (Guang-Cheng et al., 2011). Increase in photoprotective energy dissipation or decreases in photochemistry, photo inhibition are associated with an over-reduction of PSII (Maxwell and Johnson, 2000) and; thus, would lead to damage of PSII photochemistry and disrupt the functioning of PSII system. The ability to maintain high F_v/F_m under water deficit; thus, indicates a high efficiency of radiation use possibly for photochemistry and carbon assimilation (Guang-Cheng et al., 2011). Deficit fertigation reduced relative chlorophyll content of chilli plants. The reduction in relative chlorophyll content could be associated with the oxidative damage in chloroplast (Munne-Bosch et al., 2001). Development in oxidative damage by reduction in chlorophyll content also resulted in induced lipid membrane peroxidation of the tylakoides and; thus, chlorophyll degradation (Osmond et al., 1997).

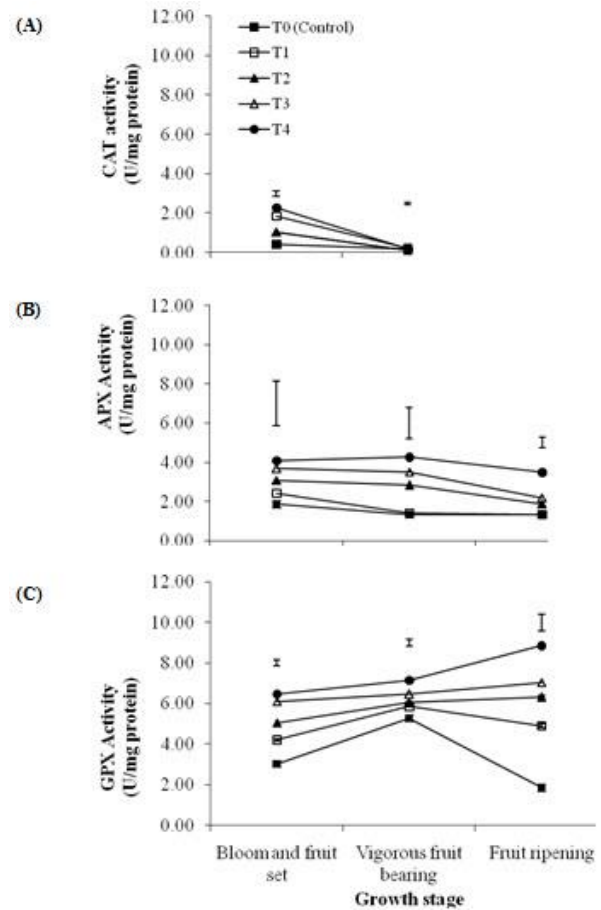


Fig 5. Effect of deficit fertigation on catalase (CAT) (A), ascorbate peroxidase (APX) (B) and guaiacol peroxidase (GPX) (C) activities in leaves of chilli plants. T0= standard local growers' practices (control), T1= 100% ET_c with daily fertigation, T2= 100% ET_c and 2 days fertigation interval, T3= 75% ET_c and 2 days fertigation interval, T4= 50% ET_c and 2 days fertigation interval. Vertical bar represents Least significant difference (LSD) at $P \leq 0.05$.

Loss of chlorophyll is always associated with the impairment of photosynthesis (Aruyanark et al., 2008). There was a reduction in photosynthesis rate under DF. The beginning of possible impairment in photosynthesis process was occurred when the relative chlorophyll content was reduced (Ladjal et al., 2000). In the present study, DF increased the catalase (CAT) activity in leaf tissues of chilli plants at bloom and fruit set stages. Increased CAT activity in leaves is an adaptation for scavenging photorespiratory H_2O_2 produced during water stress. However, DF decreased CAT activity progressively by growth stages. A decrease in CAT is a general response to many stresses (Liu et al., 2008). The reduction in CAT activity is possibly due to the inhibition of enzyme synthesis or the assembly of enzyme sub-units changing under water deficit conditions. This might be associated with the degradation of peroxisomal proteases or continuous enzyme photoinactivation, especially under severe photooxidative conditions (Tayebeh and Hassan, 2010). This result was supported by other reports that increased antioxidant activities at the beginning of water deficit and decreased progressively indicating prolonged

deficit water stress might result in decreased antioxidant activities (Anjum et al., 2012).

There was an increase in ascorbate peroxidase (APX) activity in leaves of chilli plants exposed to DF. APX are enzymes that detoxify peroxides such as hydrogen peroxide using ascorbate as a substrate. The reaction they catalyse is the transfer of electrons from ascorbate to peroxide producing dehydroascorbate and water as product (Raven, 2000). DF reduced APX activity progressively by growth stages. This condition was almost similar in CAT activity in the present study. DaCosta and Huang, (2007) explained that decreasing the level of irrigation with increasing stress duration in pepper plants resulted in extensive membrane lipid peroxidation and decreased the protective enzyme activities. It was reported that guaiacol peroxidase (GPX) is able to decrease H_2O_2 accumulation, eliminating malondialdehyde (MDA) and resulted in cell peroxidation of membrane lipids and maintained cell membrane integrity (Jaleel et al., 2008). DF increased activity of guaiacol peroxidase (GPX) progressively compared to control plants. The present study suggests that increased GPX activity might be a key point for decomposition of H_2O_2 especially under CAT inactivation (Tayebeh and Hassan, 2010). Fresh fruit yield decreased proportionally to the levels of DF. Fresh fruit yield was reduced as a result of lower amount of water and nutrients supplied. The lower amount of nutrient solution applied to plants resulted to the changes in SMC. In the present study, SMC was approximately 13% higher in control treatment than in DF treatments throughout the growing season. The reduction in fresh fruit yield might be attributed to the reduction in fruit number per plants as a result of low SMC during flowering and fruit development stage. Water shortage just prior and during early flowering reduced the number of fruits and resulted in decreased final fruit production (Jaimez et al., 2000). Furthermore, increased soil water tension during fruit development and maturity stage would increase abortion of small green fruits which results in reduction of fruit number per plant (Maroullie and Silva, 2007). Decreased fresh fruit yield was also associated with the reduction in leaf area, lower photosynthetic rates and high evaporation demand (Jaimez et al., 1999). The highest value corresponded to treatment T2 in terms of FUE. The maximum value of fresh fruit yield corresponded to the higher amount of nutrient solution supply (control), while the minimum value of fresh fruit yield received by minimum nutrient solution supply (T4). The results suggest that DF induce production losses and the crops do not sustain reasonable yield from the supplied water and nutrient compared to control plants. Therefore, it is clear that to save water and nutrient by improving its use efficiency in chilli plants is not reliable by using DF.

Materials and methods

Experimental site and treatments

This experiment was conducted at a Rain Shelter, Universiti Putra Malaysia. Mean daily temperature and relative humidity ranged from 25 - 31°C and 69 - 95%, respectively (Fig. 1) 3.47 mm evaporation and 0.3 - 0.73 ms^{-1} of wind speed. The research was carried out with five different levels of fertigation comprised of (1) standard local grower's practice, control (T0), (2) 100% ET_c with daily fertigation, (3) 100% ET_c with 2 days fertigation interval (4) 75% ET_c with 2 days fertigation interval and (5) 50% ET_c with 2 days fertigation interval.

Plant materials and media preparation

Chilli plants (*Capsicum annum* var. Kulai) were raised in a glasshouse in 25 mm seed trays filled with peat soil. Four weeks after germination, young plants with 4 to 5 leaves were selected and transplanted into 20 × 20 cm white polybags filled with rooting media comprising of coconut dust and empty fruit bunches (3:1, v/v).

Treatments application

Evaporation (ET_0) was measured daily and fertigation requirements of the chilli plants were calculated following Allen et al. (1998). All fertigation treatments were based on the crop evapotranspiration (ET_c) using Class A Pan evaporation. Fertigation treatments were slightly modified with Cooper nutrient formulation with electrical conductivity (EC) of 1.5 - 2.5 dSm^{-1} (Cooper, 1976). Fertigation amounts applied to other treatments were decreased stepwise to 75 and 50% of the treatment 100% ET_c .

Substrate moisture content

Substrate moisture content was determined using a HH2 Soil moisture meter with Theta Probe ML-2x (Delta T Devices, Cambridge, England). Four readings were recorded for each treatment at 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 100 and 120 days after transplanting.

Plant growth, total leaf area and plant biomass determination

The plant height was measured from ground level to shoot tip using a measuring tape, stem diameter with a pair of caliper and total leaf area were obtained using leaf area meter (model, L1-3100; LI-COR. Inc. Lincoln, Nebraska, USA). All measurements were taken at several different growth stages including seedling, vegetative, bloom and fruit set, vigorous fruit bearing and fruit ripening stages.

Plants were harvested at fruit ripening stage and segmented into leaves, stems, and roots before oven dried at 80⁰ C temperature for 48 h for dry biomass determination. Root to shoot ratios was calculated using following formula (Hunt, 1978).

Determination of photosynthesis rate, stomatal conductance, chlorophyll fluorescence and relative chlorophyll content

Assessment of photosynthesis rate (P_n), stomatal conductance (g_s), chlorophyll fluorescence and relative chlorophyll content were performed at seedling, vegetative, bloom and fruit setting, vigorous fruit bearing and late fruit bearing on the most recently emerged and fully expanded leaves. P_n was determined using a portable gas exchange measuring system (model LI 6400xt, LI-COR, Lincoln, Nebraska, USA) in the morning between 9:00 and 10:00 h. Chlorophyll fluorescence parameter, the maximum quantum of yield PSII (F_v/F_m) was obtained using a portable photosynthetic efficiency analyzer (PEA) (model Handy-PEA, Hansatech Instrument Ltd., Norfolk, UK) and taken between 10:00 and 11:00 h. Relative chlorophyll content was collected using a SPAD-502 meter (model Minolta chlorophyll meter SPAD-502, Spectrum Technologies, Inc., Plainfield, IL).

Determination of protein content and enzymatic activities

Protein concentration of the enzyme extracts was determined following the method of Bradford (1976) using bovine serum albumin. Catalase (CAT, EC 1.11.1.6) activity was determined following the method of Aebi (1983). The APX activity was measured following the method of Rao et al. (1997) with slight modification at 290 nm. The activity of GPX was performed according to the procedure described in Nakano and Asada (1981) at 470 nm.

Yield, total amount of nutrient solution applied and fertigation use efficiency

Fresh fruits of chilli were harvested when the fruits were at least 90% red. Fruits were harvested at every three days until the end of the experiment. Harvested fruits were weighed using an electronic balance and total fresh weight of chilli fruits in each treatment was determined at the end of the study. Total amount of nutrient solution applied were obtained from total amount of water requirement based on ET_c for whole planting period. FUE was estimated according to formula of Zegbe-Dominguez et al. (2003).

Statistical analysis

The experiment was conducted following a randomized completely block design with four replications. Data were analyzed using SAS statistical program version 9.1. The treatments means were separated using Least Significance Difference test at the 5 % level of probability.

Conclusion

Deficit fertigation with 2 days interval affects growth, physiological and biochemical changes as well as yield of chilli plants. Antioxidant enzymes such as CAT, APX and GPX decreased progressively by the growth stages indicated that antioxidant enzymes were not able to protect plant cells from the oxidative damage. A significant reduction in chilli yield obtained under DF with 2 days interval indicating that other approaches are need to be find out in order to improve water and fertilizer use efficiency without much reduction in yield.

Acknowledgements

The authors acknowledged the support of Ministry of Higher Education, Malaysia for Graduate Research fellowship and Universiti Putra Malaysia for their financial support without which this research would have been impossible.

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