Agr. Nat. Resour. 53 (2019) 581–589

AGRICULTURE AND
NATURAL RESOURCES

Journal homepage: http://anres.kasetsart.org

Research article

Effect of water stress on the growth, physiological response and antioxidative gene expression of grafted sweet pepper plants

Wannida Sae-Tang, Eiji Nawata*

Division of Environmental Science and Technology, Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan.

Article Info

Article history:
Received 23 March 2018
Revised 3 July 2018
Accepted 5 July 2018
Available online 25 December 2019

Keywords:
Capsicum,
Drought,
Photosynthesis,
Rootstock,
ROS

Abstract

Grafting has been introduced as an alternative technique to alleviate damage to horticultural crops from water stress and to improve tolerance. In this study, non-grafted (FT) and grafted sweet pepper 'Fushimiamanaga togarashi' onto chili pepper 'Huaysiiton' (FT/HS) and 'Shima togarashi' (FT/ST) plants were grown under drought conditions. The results indicated that water stress had a considerable influence on both non-grafted and grafted plants. The growth of sweet peppers decreased due to water stress, with the FT/ST plants having a reduced decrease compared to plants with sufficient watering. Consequently, these grafted plants had the smallest yield reduction caused by water deficit. However, under well-watered conditions, non-grafted plants had greater total yields than grafted plants. The photosynthetic rates, transpiration rates and carbon dioxide intercellular space concentrations also declined significantly due to drought. The grafted plants were able to maintain photosynthesis under water stress conditions to some extent. The leaf water and leaf osmotic potentials declined substantially under water stress conditions, with the FT/ST plants having the lowest leaf osmotic potential, even though the leaf water potential remained rather high. The expression of antioxidative genes relating to *CuZnSOD*, *MnSOD*, *CAT* and *APX* were upregulated at transcriptional levels under water stress conditions, especially in FT/ST. The Shima togarashi chili pepper may have potential to be used as a vigorous rootstock under water stress conditions based on physiological adaptation and antioxidative gene expression, although it produced a lower yield than non-grafted plants under non-stressed conditions.

Introduction

Drought has become an important issue due to climate changes, with the frequency and intensity of extreme drought conditions having increased in many areas (Intergovernmental Panel on Climate Change, 2013), having a great impact on crop production (Farooq et al., 2012).

Sweet pepper, (*Capsicum annuum* L.), is one of horticultural crops belonging to Solanaceae family and is an important cash crop in many countries as it is widely grown around the world (Lin and Saltveit, 2012). Unfortunately, among horticultural crops it is considered as one of the most susceptible to water stress (Alvino et al., 1994). The photosynthetic limitations occur due to decreasing stomatal conductance, which is sufficient to reduce plant growth, fruit yield and fruit marketability (Delfine et al., 2002). Improvement of crop management is needed to alleviate the impact of water stress in sweet pepper.

Grafting, as a horticultural practice, has been widely introduced in many countries especially in Asia since the late 1920s (King et al., 2010). The main purposes of grafting include the protection of plants from soil-borne diseases and the improvement of yield and fruit quality, while additionally, it may increase abiotic stress tolerance to

---

* Corresponding author.
E-mail address: nawata@kais.kyoto-u.ac.jp (E. Nawata).

https://doi.org/10.34044/j.anres.2019.53.6.04

heat, salinity and drought (Lee et al., 2010). Drought stress tolerance was increased by grafting with appropriate rootstocks in tomato (Wahb-Allah et al., 2011). In bell pepper, marketable yields were higher for some specific combinations of grafted plants under water stress conditions compared to non-grafted plants (Penella et al., 2014).

In order to understand the effect of drought in plant, several physiological mechanisms can be monitored and considered as drought stress indicators. Under water stress conditions, plants adapt themselves by minimizing water loss and maximizing water uptake, with stomatal closure being the quickest response to reduce water loss in plants (De Oliveira et al., 2013). While transpiration is decreasing, carbon dioxide assimilation is also reducing as a result of the decreased photosynthesis caused by stomatal closure and many activities in plants will alter as a result of metabolic adjustments. These responses lead to growth adjustments and adaptations. In addition, plants have to increase the uptake of water by some possible mechanisms, such as osmotic adjustment, since when the osmotic potential in plant tissue is less than in the soil, water absorption will be improved (Sanders and Arndt, 2012).

When plants are exposed to a water deficit and excess light conditions, oxidative stress is easily induced by over generation of reactive oxygen species (ROS)which demolish plant structure and metabolisms by oxidative damages to lipids, proteins and other macromolecules (Hernández et al., 2012). Plants have protective mechanisms to protect against such damage which are activated by ROS scavengers, including enzymatic and non-enzymatic components with enzymatic antioxidants, including superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) being the major components that play an important role in plant defense mechanisms (Møller et al., 2007). An increase in these enzyme activities may be related to drought tolerance and it could be observed through their encoded genes expression (You and Chan, 2015).

Nevertheless, grafting in pepper plant for this purpose has not been practiced commercially to date and general information is lacking. In addition, the details of grafting for the genus *Capsicum* under water stress conditions have not been yet clarified in terms of the production and physiological and molecular responses. Therefore, this study was conducted to examine the grafting technique in Japanese sweet pepper, to improve plant tolerance to water stress and to investigate adequate rootstock using chili peppers. The results were discussed based on growth, physiological responses and antioxidative genes expression under water stress conditions.

Materials and Methods

Plant materials

The seedlings were prepared at several stages to obtain similar stem diameters in grafting. The 6–8 leaves state of commercial Japanese sweet pepper ‘Fushimiamanaga togarashi’ (*Capsicum annuum* L.: FT) seedlings was used as scions. The rootstocks consisted of the 8–10 leaves stage of Thai chili pepper ‘Huaysiiton’ (*C. annuum* L.: HS) and the 16–18 leaves stage of Japanese chili pepper ‘Shima togarashi’ (*C. frutescens*: ST) seedlings, having a high possibility to tolerate drought. The FT scions were grafted using the cleft method onto two rootstocks, HS (FT/HS) and ST (FT/ST). The non-grafted plant (FT) was used as a control. All seedlings were maintained under shade and high humidity conditions to accelerate grafting union formation for 14 d. They were transplanted to plastic pots (diameter 21 cm) filled with mixture of new super soil (Akimoto Tensanbutsu Co. Ltd., Japan) and sand at the ratio of 1:1 on 30 d after grafting. All seedlings were irrigated appropriately until the start of the treatment and supplied with nutrient solution (0.2% Hyponex; 60 g/L N, 100 g/L P, 50 g/L K; Hyponex Co. Ltd., Japan) and 15-15-15 water soluble fertilizer ($N-P_2O_5-K_2O-MgO-CaO$:15-15-15-1.5-5%, OAT Agrio Co. Ltd., Japan) alternatively once a week.

The experiment was conducted in a glass greenhouse during from May to October 2016 at the Graduate School of Agriculture, Kyoto University, Kyoto, Japan (35.0°N, 135.8°E).

Water stress conditions

In a preliminary experiment, FT sweet pepper plants were grown under different water conditions, consisting of 100%, 60% and 30% of field capacity (FC). The results showed that growth parameters were significantly decreased at 30% FC (data not published). In this experiment, water stress was represented at 30% FC.

From 10 d after flowering started until the end of the crop, all plants were divided into two groups, and soil moistures were differently maintained at 100% FC (control) and 30% FC (water stressed). The soil moisture in individual pots was monitored based on weighing and the soil moisture conditions were adjusted using tap water. Control plants were watered daily to maintain 100% FC, whereas water stressed plants were watered at 30% FC when wilting symptoms appeared.

The plant growth, physiological parameters and genes expression were determined at 2 wk after the initiation of the drought treatments. The fruits were harvested three times at 10-day intervals at the commercial maturity stage.

Plant growth measurements

The stem height and the number of lateral branches were recorded. The area of the third leaf as the youngest, fully expanded leaf was also measured from photographs, using the Easy Leaf Area software (Easlon and Bloom, 2014).

Yield and fruit quality

The total fruit numbers were counted and all fruits were weighed to determine the total fresh weight per plant. The fruit length and head diameter were measured. The juice was extracted from the pericarp and the contents of total soluble solids (TSS) were evaluated using a hand refractometer.

Photosynthetic parameters

Gas exchange parameters were measured using a LI-6400 portable photosynthesis system (LI-COR Inc.; USA) in the youngest, fully expanded leaves. The net CO_2 assimilation rate (P_n), transpiration rate (E) and CO_2 intercellular concentration (C_i) were measured under steady-state conditions of saturation light intensity (1,000 µmol/m^2·s) and at 380 parts per million CO_2 concentration. The mean leaf temperature was maintained at 29°C. The evaluation was performed from 0900–1400 hours.

The chlorophyll fluorescence (F_v/F_m ratio) was determined using an OS-30p chlorophyll fluorometer (Opti-Sciences; USA), using the same leaf that was used for the gas exchange measurements. The evaluation was performed at 30 min after sunset (2000–2100 hours). Leaf greenness was measured using a Soil and Plant Analytical Development (SPAD) meter (Minolta SPAD502; Spectrum Technologies, Inc., USA), using the same leaf that was used for the measurement of the gas exchange.

Leaf water potential and osmotic potential

The leaf water potential (Ψ_w) was measured at 1200 hours using a pressure chamber (Soil Moisture Equipment Corp., USA), using the youngest fully expanded leaves. The same leaves were used to extract the sap and to measure the osmotic potential (Ψ_s) using a WP4C dew point psychrometer (Decagon Devices, Inc., USA).

The relative water content (RWC) was determined using Equation 1:

$$RWC = (FW - DW) / (TW - DW) \quad (1)$$

where the leaves were collected and immediately weighed for fresh weight (FW); turgid weight (TW) was determined using leaves kept in distilled water in darkness at room temperature for 24 hr and dry weight (DW) was obtained by drying leaves in a hot air oven at 70°C for 72 hr.

Antioxidative genes expression analysis

In the second week of the water stress treatments, the third leaf from the top was sampled and frozen immediately in liquid nitrogen and kept at -80°C. Total RNA was extracted from sample leaves using a Sepasol-RNA I Super G (Nacalai Tesque, Japan) and purified using LiCl and DNaseI (TOYOBO, Japan). Reverse transcription was performed to synthetize first-strand complementary DNA (cDNA) from the total RNA using ReverTraAce (TOYOBO, Japan) activity. Semi-quantitative real-time polymerase chain reaction (PCR) was done to amplify the cDNA using primers designed for the *CAT, MnSOD, CuZnSOD, APX* and *Actin* genes shown in Table 1. The PCR was performed using KODFxNeo DNA polymerase (TOYOBO, Japan) with 0.5 µL cDNA in a total volume of 25 µL for 33 cycles under the following conditions: 2 min at 94°C, 10 s at 98°C, 30 s at 60°C, 10 s at 68°C and a final 7 min at 68°C for extension. Then, 5 µL of PCR products were run for electrophoresis on 1.2% agarose gel at 100 V for 25 min. Gel-image analysis using the ImageJ software (National Institutes of Health, USA) was performed to quantify the DNA fragment intensities. The relative expression levels of target genes were normalized to the expression level of *Actin* as a reference gene.

Statistical analysis

The experimental plots were arranged in a complete randomized design. The collected data were analyzed using analysis of variance. Difference of means was tested using Duncan's multiple range test at a significance level of $p < 0.05$.

Results

Effect of water stress on the growth and yield

The growth of sweet pepper was limited under drought conditions as shown in Table 2. The stem height of water-stressed plants was significantly shorter than for well-watered plants in FT (-12.4 cm) and FT/HS (-11.0 cm). Interestingly, compared to the well-watered treatment, no significant decrease of stem height was observed in FT/ST (-3.0 cm) under stress conditions. Non-grafted plants produced the highest number of lateral branches at 100% FC and this was decreased by water stress. In contrast, the number of lateral branches slightly increased in FT/HS plants under low FC conditions. The FT/ST stressed plants did not have any statistical differences from the well-watered plants for the stem height and number of lateral branches. There were no significant effects of water stress and grafting on the area of the third leaf.

Table 1 Oligonucleotide sequence of primers used in semi-quantitative real-time polymerase chain reaction analysis

Gene	ROS scavenging enzyme	Accession No.	Oligonucleotide sequences 5' to 3'	Product size (bp)
CAT	Catalase	AF227952	Fw : GATTTCTTCTCTTTCCTCC Rv : CGATGTTCCTATTCAATACC	418
MnSOD	Mn-superoxide dismutase	AY572427	Fw : CATGCAGCTTCATCACCAGA Rv : ATAACAAGGCGCTTCAGCTC	354
CuZnSOD	CuZn-superoxide dismutase	AF009734	Fw : TGTTAGTGGCACCATCCTCT Rv : GGCCGATAATACCACAAGCA	410
APX	Ascorbate peroxidase (cyt.)	X81376	Fw : TGTGCTCCTCTTATGCTCC Rv : CTCAAAACCAGAACGCTCC	435
Actin	Actin	AY572427	Fw : ACTCTTAATCAATCCCTCC Rv : GCACTGTATGACTGACACC	573

ROS = reactive oxygen species.

Table 2 Growth parameters of non-grafted and grafted sweet pepper under different field capacity levels at 2 wk after the initiation of drought treatments.

Grafting	% FC	Stem height (cm)	Number of branches	3^{rd} Leaf area (cm^2)
FT	100	75.8 ± 1.4^{a}	30.0 ± 2.5^{a}	8.9 ± 0.8
	30	63.4 ± 1.9^{b}	21.2 ± 1.4^{b}	6.3 ± 0.6
FT/HS	100	79.2 ± 1.9^{a}	23.6 ± 2.4^{b}	8.0 ± 0.4
	30	68.2 ± 2.1^{b}	25.6 ± 0.7^{ab}	7.7 ± 0.8
FT/ST	100	69.4 ± 2.9^{b}	21.0 ± 1.4^{b}	6.6 ± 0.4
	30	66.4 ± 1.9^{b}	21.0 ± 1.1^{b}	7.1 ± 0.7
F test		**	*	ns

FT = non-grafted, and FT/HS = grafted sweet pepper 'Fushimiamanaga togarashi' onto chili pepper 'Huaysiiton' and 'Shima togarashi' (FT/ST); FC = field capacity. Means±SE in the same column superscripted with the same letter indicate non-significant difference at $p < 0.05$; * and ** = significant differences at $p < 0.05$ and $p < 0.01$, respectively.

Sweet pepper fruits were harvested three times and the yields and related traits were determined (Table 3). Under well-irrigated conditions, FT and FT/HS plants produced greater total yields and numbers of fruit than FT/ST plants. When plants were exposed to water stress, their total yields reduced significantly in FT, FT/HS and FT/ST by 44.8%, 45.5% and 32.2%, respectively. The number of fruits was also reduced by water stress, especially in FT/HS. FT/ST had the highest fruit weight and size among treatments. Water stress significantly reduced the fruit diameter of FT and also the fruit length of FT and FT/HS plants. The fruits contained significantly higher TSS contents under water stress conditions than under well-watered conditions. The highest TSS content was approximately 6.9 °Brix in the 30% FC treatments and there were no significant differences among grafting treatments. In contrast, the lowest TSS content was recorded in plants under well-watered conditions, (4.9°Brix, 5.1°Brix and 5.6°Brix in FT, FT/HS and FT/ST, respectively). The FT/ST had the smallest increase in TSS content under water stress conditions.

Effect of water stress on photosynthesis

There was a large negative impact of water deficit on net photosynthetic rates (P_n), transpiration rates (E) and carbon dioxide intercellular space concentrations (C_i) for both non-grafted and grafted plants (Fig. 1). On average, P_n was 16–18 µmol $CO_2/m^2{\cdot}s$ under well-watered conditions, which was not significantly different from other grafting treatments. Under 30% FC, P_n values deceased significantly by 71.68%, 85.33% and 54.25% in FT, FT/HS and FT/ST, respectively. The P_n values for FT/ST were in most cases the maximum under water stress conditions. Values for E and C_i also were reduced by water stress, similarly to P_n. Under well-irrigated conditions, FT/ST had the lowest E and C_i values but there was no significant difference among grafting treatments. However, under water deficit conditions, the FT/ST combination had higher values than FT and FT/HS combinations. There were slight changes in the chlorophyll fluorescence of plants exposed to water stress, although the FT/HS stressed plants had a significantly smaller (0.77) value. The mean F_v/F_m values of all plants were in the range 0.77–0.80. Leaf greenness in terms of SPAD units was reduced by the effects of water stress, although a significant reduction was only observed in non-grafted plants.

Effect of water stress on water relation

The water relation parameters were examined in sweet pepper leaves (Fig. 2). Water stress significantly reduced the midday leaf water potential (Ψ_w). Under drought conditions, an effect of grafting was found in FT leaves which had the lowest Ψ_w (-1.33 MPa), but FT/ST leaves had the highest Ψ_w (-1.02 MPa). There were no effects of grafting on well-watered plants with regard to Ψ_w. The sap of leaves was extracted and measured for osmotic potential (Ψ_s) and this was significantly different among drought and grafting treatments. Under 100% FC, high Ψ_s values were recorded for FT/HS (-0.92 MPa), FT (-0.95 MPa) and FT/ST (-1.14 MPa) plants. The value of Ψ_s also decreased by approximately -0.14 in FT/HS plants and by approximately -0.08 in FT and in FT/ST plants under 30% FC treatments. The leaf water content was calculated as a relative water content (RWC). There were no differences among the grafting treatments for the well-watered treatments. Only FT/HS leaves maintained a high RWC under drought conditions. However, water stress reduced the RWC of FT and FT/ST leaves by an average of 5.53.

Table 3 Total yield, fruit size and total soluble solid content of non-grafted and grafted sweet pepper under different field capacity over 3 harvesting times.

Grafting	% FC	Total yield (g)	Number of fruits	Weight/fruit (g)	Fruit diameter (mm)	Fruit length (mm)	TSS (°Brix)
FT	100	200.1 ± 8.7^{a}	31.6 ± 1.4^{a}	6.2 ± 0.3^{a}	12.3 ± 0.2^{a}	102.4 ± 3.7^{a}	4.9 ± 0.1^{c}
	30	110.5 ± 7.8^{c}	27.0 ± 2.7^{ab}	4.1 ± 0.2^{c}	10.9 ± 0.2^{c}	84.8 ± 3.3^{b}	6.9 ± 0.2^{a}
FT/HS	100	192.0 ± 11.5^{a}	30.2 ± 2.2^{a}	6.4 ± 0.4^{a}	12.6 ± 0.3^{a}	103.3 ± 3.8^{a}	5.1 ± 0.1^{bc}
	30	104.7 ± 5.9^{c}	21.6 ± 1.2^{c}	4.7 ± 0.3^{bc}	11.7 ± 0.4^{abc}	87.5 ± 3.7^{b}	6.9 ± 0.2^{a}
FT/ST	100	151.7 ± 11.3^{b}	26.8 ± 1.6^{ab}	5.5 ± 0.3^{ab}	11.9 ± 0.4^{ab}	96.2 ± 3.6^{ab}	5.6 ± 0.2^{b}
	30	102.8 ± 3.6^{c}	22.0 ± 2.3^{c}	4.9 ± 0.3^{bc}	11.2 ± 0.4^{bc}	88.9 ± 4.3^{b}	6.7 ± 0.2^{a}
F test		**	*	**	*	**	**

FT = non-grafted, and FT/HS = grafted sweet pepper 'Fushimiamanaga togarashi' onto chili pepper 'Huaysiiton' and 'Shima togarashi' (FT/ST); FC = field capacity; TSS = total soluble solids.

Means±SE in the same column superscripted with the same letter indicate non-significant difference at $p < 0.05$; * and ** = significant differences at $p < 0.05$ and $p < 0.01$, respectively.

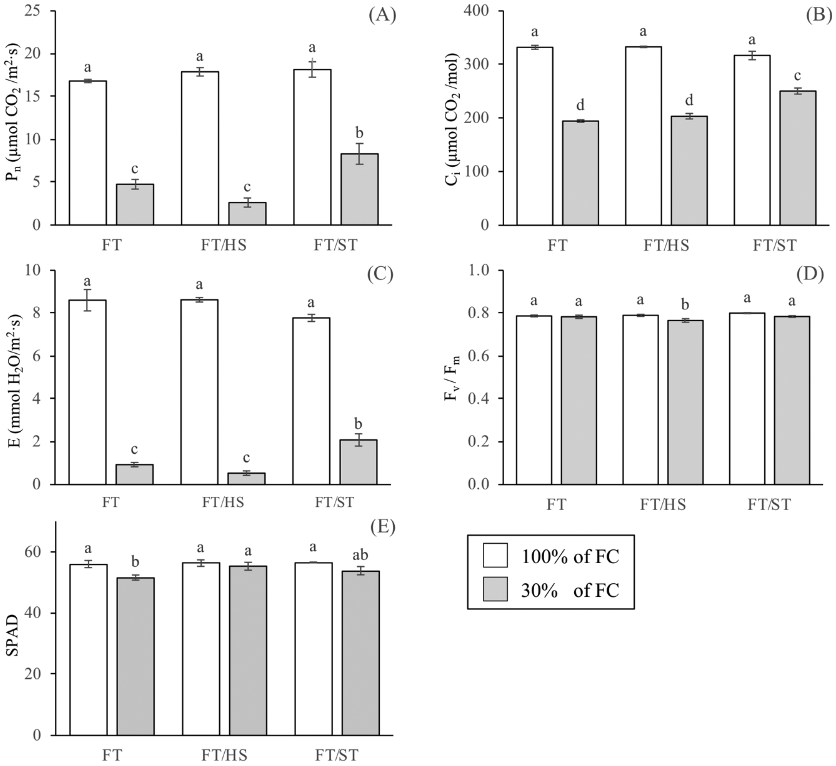

Fig. 1 (A) Net photosynthetic rate (P_n); (B) intercellular space carbon dioxide concentration (C_i); (C) transpiration rate (E); (D) chlorophyll fluorescence (F_v/F_m); (E) leaf greenness (SPAD) of non-grafted and grafted sweet pepper under 100% and 30% field capacity (FC) at 2 wk after initiation of drought treatments, where the error bar = ±SE; the same letters in above histograms denote non-significant difference at $p < 0.05$; FT = non-grafted, and FT/HS = grafted sweet pepper 'Fushimiamanaga togarashi' onto chili pepper 'Huaysiiton' and 'Shima togarashi' (FT/ST)

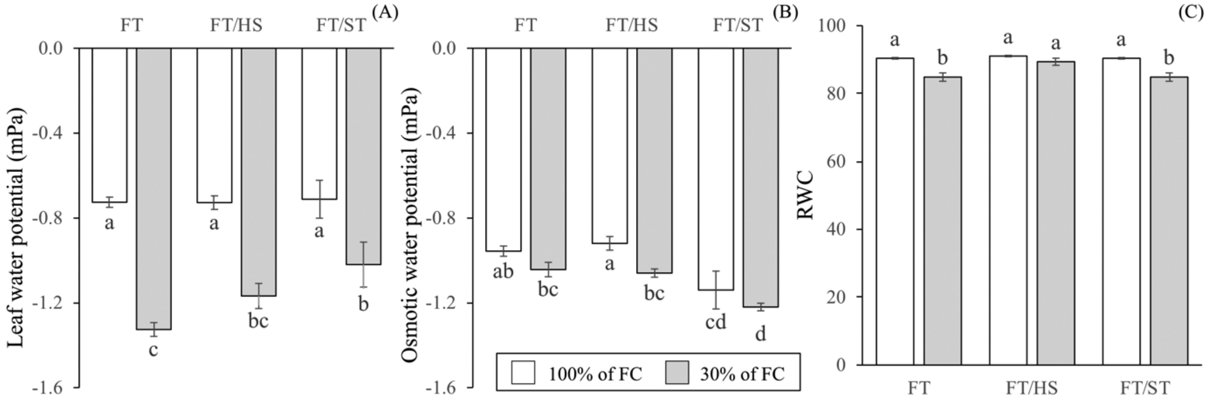

Fig. 2 (A) Leaf water potential; (B) osmotic water potential; (C) relative water content (RWC) of non-grafted and grafted sweet pepper under 100% and 30% of FC at 2 weeks after the initiation of drought treatments, where the error bar = ±SE; the same letters below histograms denote non-significant difference at $p < 0.05$; FT = non-grafted, and FT/HS = grafted sweet pepper 'Fushimiamanaga togarashi' onto chili pepper 'Huaysiiton' and 'Shima togarashi' (FT/ST)

Effect of water stress on gene expression

After 2 wk of water stress, leaves were used for transcriptional expression analysis focusing on the antioxidative genes: copper/zinc-superoxide dismutase (*CuZnSOD*)*,* manganese-superoxide dismutase (*MnSOD*)*,* catalase (*CAT*) and cytosolic ascorbate peroxidase (*APX*). The results showed that these genes expressed differently among grafting treatments under well-watered conditions. The expression responses of plants to water stress were also affected by grafting as shown in Fig. 3. Water stress induced the expression of *CuZnSOD* and *MnSOD* in FT and FT/ST plants, but inhibited their expression in FT/HS plants. Under sufficient irrigation, FT/HS had the highest expression of both genes. The cytosolic *APX* gene also showed similar trends to those of the *CuZnSOD* and *MnSOD* genes. Furthermore, the transcript abundance of *CAT* decreased with water stress severity in FT and FT/HS but increased in FT/ST plants.

Discussion

A number of physiological changes are induced while plants are exposed to drought conditions, resulting in plant growth and development limitations (Farooq et al., 2012). A grafting technique has been utilized as a measure for crop tolerance improvement under abiotic stress conditions, especially water stress, including cucumber (Huang et al., 2009), mini-watermelon (Rouphael et al., 2008), tomato (Sanchez-Rodriguez et al., 2012) and tobacco (Liu et al., 2014). However, studies in the genus *Capsicum* under water stress conditions are still limited. In the present study, 'Fushimiamanaga togarashi', long green sweet peppers (*Capsicum annuum* L.) were selected as scions grafted onto two chili peppers rootstocks having potential to increase the tolerance to water stress, consisting of 'Huaysiiton' (*C. annuum* L.) being the same species and 'Shima togarashi' (*C. frutescens*) being the same genus but a different species. All plants were evaluated on the influence of water stress under 30% FC conditions compared to 100% FC conditions, subjected during the flowering stages, the most sensitive period to drought in *Capsicum* (Dagdelen et al., 2004).

The results demonstrated that the growth and yield of sweet peppers were inhibited substantially under water deficit conditions. Similar inhibition in sweet pepper was also observed in many studies (Delfine et al., 2001; Yildirim et al., 2012; Cosic et al., 2015). Under well-watered conditions, non-grafted plants and those grafted onto HS plants thrived better based on stem height than those grafted onto ST plants, which may have been caused by greater compatibility between FT and HS, both of which belong to the same species. Nevertheless, Penella et al. (2013) reported good grafting affinities between *C. annuum* L. and *C. chinense* Jacq. and between *C. annuum* L. and *C. baccatum* L. Otherwise, grafting incompatibility may have appeared during fruiting stages, at which stage plants have high demands for water and nutrients (Martinez-Ballesta et al., 2010). However, in the current experiment, ST, *C. frutescens*, was grafted as a rootstock at an older stage than others. The growth of grafted sweet peppers at old stages is inhibited by poor callus formation at the grafting interface and poor xylem development (Johkan et al., 2009). Furthermore, ST rootstock grafted plants had the smallest reductions in growth and yield under water stress compared to their corresponding controls, which exhibited the characters of the vigorous

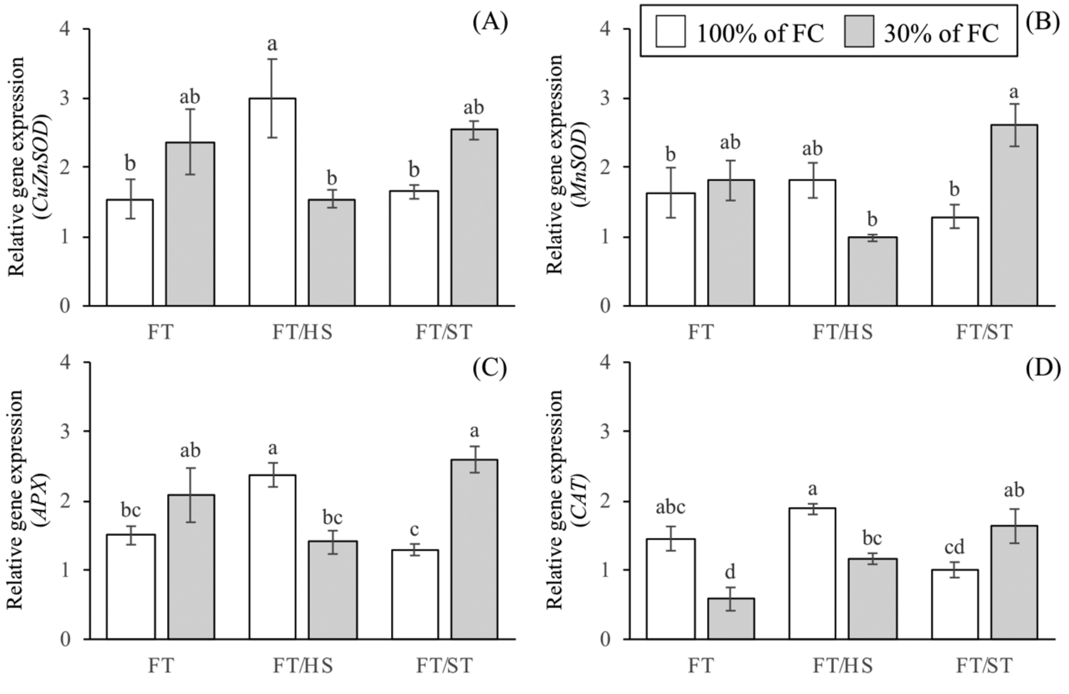

Fig. 3 Relative expression of antioxidative genes for (A) *CuZnSod*; (B) *MnSOD*; (C) *APX*; (D) *CAT* of non-grafted and grafted sweet pepper under 100% and 30% field capacity (FC) at 2 wk after initiation of drought treatments, where the error bar = ±SE; the same letters above histograms denote non-significant difference at $p < 0.05$; FT = non-grafted, and FT/HS = grafted sweet pepper 'Fushimiamanaga togarashi' onto chili pepper 'Huaysiiton' and 'Shima togarashi' (FT/ST)

rootstock and this combination could have alleviated the impact of water stress by maintaining their growth (Schwarz et al., 2010). In addition, water stress increased the TSS content in the pericarp which may have been influenced by a fruit water content reduction.

Under water deficit conditions, plants close the stomata as a quick response to limit water loss due to transpiration, accompanied by the restriction of carbon dioxide absorption and due to smaller carbon dioxide concentrations, photosynthetic assimilation is reduced, resulting in decreases in vegetative growth and yield (Grant, 2012). Based on the current study, the involved photosynthetic parameters also declined substantially due to water stress in both non-grafted and grafted sweet pepper plants. Interestingly, plants grafted onto ST had the highest photosynthetic rate, intercellular space carbon dioxide concentration and transpiration compared to the other stressed plants, which indicated the adaptation to drought (Pinheiro and Chaves, 2011). The appropriate graft combinations performance better regarding photosynthetic activity in tomato (Nilsen et al., 2014). The chlorophyll fluorescence (F_v/F_m) did not indicate clear influences of drought in either non-grafted and grafted plants. Some authors have claimed that PSII photochemical systems cannot be impaired by relatively severe water stress even when photosynthetic rates change substantially (Penella et al., 2015). Previous experiments also reported that chlorophyll fluorescence was not affected significantly by water stress in bell pepper (Delfine et al., 2002) and potato (Schafleitner et al., 2007). In the current study, photosynthesis was inhibited under drought conditions that may not have been caused by factors related to chlorophyll fluorescence.

The reduced water potential occurred a result of water loss through transpiration and also decreased water uptake. In this regard, drought-tolerant cultivars can maintain high leaf water potential under water deficit conditions (Farooq et al., 2009). On the other hand, water absorption by the roots becomes weaker due to decreased soil water potential and so to maximize the uptake of water, some plants generate low osmotic potential conditions through osmotic adjustment by raising solute concentrations (Sanders and Arndt, 2012). The plants grafted onto ST maintained the lowest leaf osmotic potential, even though their leaf water potentials were rather high. This combination may have performed some osmotic adjustment to uptake more water under drought conditions. Furthermore, all stressed plants seemed to be able to maintain the relative water content of leaves, especially plants grafted onto HS. It is possible that in some plants, the ability of photosynthesis declines dramatically under water deficit conditions even though the RWC is still maintained (Flexas et al., 2004).

Reactive oxygen species (ROS) are generated continuously even under normal conditions in plants (You and Chan, 2015), but large ROS production is a result of oxidative stress during the drought episode (Impa et al., 2012). ROS accumulation damages the cell membrane, DNA structure and metabolic processes (Hernández et al., 2012). ROS also play an important role in cellular signaling systems in plant responses to environmental stresses, including drought (Møller et al., 2007). Parts of ROS scavenging processes are performed by enzymatic antioxidants activities, including those by SOD, CAT and APX (Cruz de Carvalho, 2008). In the current study, the encoded genes of the three enzymes *CAT, SOD* (*MnSOD* and *CuZnSOD*) and *APX* (cyt.) were investigated for their expressions at the transcription level. The *CuZnSOD, MnSOD* and *APX* expressions increased significantly under water stress conditions in non-grafted and ST-rootstock grafted plants. On the other hand, *CAT* expressions were suppressed under drought conditions differently in non-grafted and HS rootstock grafted plants. Similar to the findings of the current study, Bian and Jiang (2009) discussed that antioxidant genes were expressed variously under drought stress conditions in several crops. SOD plays a major role in changing the superoxide radical to oxygen and hydrogen peroxide. Next, the hydrogen peroxide is scavenged to generate water by APX in the water-water cycle (Cruz de Carvalho, 2008). In the current study, the CAT activity was mostly stable despite the water deficit. Interestingly, in grafted ST rootstock plants, all the studied genes were upregulated under severe water stress conditions. The significant increase in the expression of *APX* (cyt.) in ST-grafted plants may protect chloroplast protein by ROS scavenging activities under water stress (Osakabe et al., 2014). It is possible that a high ROS detoxification level may have occurred in the current study, leading to high gene expression of *SOD, APX* and *CAT* (Zheng et al., 2008). In addition, the three antioxidative genes were inhibited by water stress in HS-rootstock grafted plants. The overproduction of ROS results in photoinhibition (Farooq et al., 2012), which may have resulted in this treatment having the lowest F_v/F_m value under water stress conditions.

A small yield was observed in the ST-rootstock grafted plants, and Penella et al. (2014) found that although tolerant *Capsicum* cultivars were proofed in terms of photosynthesis under water stress conditions, grafting sweet pepper onto those tolerant cultivars produced lower yields under well irrigated conditions. According to Paongpetch and Techawongstien (2008), highly pungent peppers had higher drought tolerance than low pungent cultivars. Thus, 'Huaysiiton' is regarded as one of drought tolerant cultivars, due to its small reduction in leaf area, shoot-to-root ratio and fruit yield (Paongpetch et al., 2012). The examination of water relations in peppers showed that *C. frutescens* had greater osmotic adjustment than *C. annuum* L. (Ismail and Davies, 1997). In addition, bell pepper was more sensitive to drought.

In summary, water stress caused several physiological changes, and consequently, growth and yield production were also limited. Grafting to ST rootstocks improved sweet pepper scion vigor by increasing the photosynthetic rate and inducing antioxidative genes expression.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Acknowledgements

This study was supported by MEXT. The authors thank Associate Prof. Masahiro Sakamoto, Laboratory of Forest Biochemistry, Graduate School of Agriculture, Kyoto University, for collaboration in the molecular study.

References

Alvino, A., Centritto, M., DeLorenzi, F. 1994. Photosynthesis response of sunlit and shade pepper (*Capsicum annuum*) leaves at different positions in the canopy under two water regimes. Aust. J. Plant Physiol. 21: 377–391.

Bian, S., Jiang, Y. 2009. Reactive oxygen species, antioxidant enzyme activities and gene expression patterns in leaves and roots of Kentucky bluegrass in response to drought stress and recovery. Sci. Hortic. 120: 264–270.

Cosic, M., Djurovic, N., Todorovic, M., Maletic, R., Zecevic, B., Stricevic, R. 2015. Effect of irrigation regime and application of kaolin on yield, quality and water use efficiency of sweet pepper. Agric. Water Manag. 159: 139–147.

Cruz de Carvalho, M.H. 2008. Drought stress and reactive oxygen species: production, scavenging and signaling. Plant Signal Behav. 3: 156–165.

Dagdelen, N., Yilmaz, E., Sezgin, F., Gurbuz, T. 2004. Effects of water stress at different growth stages on processing pepper (*Capsicum annuum* cv. *Kapija*) yield water use and quality characteristics. Pak. J. Biol. Sci. 7: 2167–2172.

De Oliveira, A.B., Alencar, N.L.M., Gomes-Filho, E. 2013. Comparison between the water and salt stress effects on plant growth and development. In: Akinci, S. (Ed.). Responses of Organisms to Water Stress. InTech. Rijeka, Croatia, pp. 67–94.

Delfine, S., Loreto, F., Alvino, A. 2001. Drought-stress effects on physiology, growth and biomass production of rainfed and irrigated bell pepper plants in the Mediterranean region. J. Amer. Soc. Hort. Sci. 126: 297–304.

Delfine, S., Tognetti, R., Loreto, F., Alvino, A. 2002. Physiological and growth responses to water stress in field-grown bell pepper (*Capsicum annuum* L.). J. Hortic. Sci. Biotech. 77: 697–704.

Easlon, H.M., Bloom, A.J. 2014. Easy leaf area: Automated digital image analysis for rapid and accurate measurement of leaf area. Appl. Plant Sci. 2: 1400033.

Farooq, M., Hussain, M., Wahid, A., Siddique, K. 2012. Drought stress in plants: an overview.. In: Aroca, R. (Ed). Plant Responses to Drought Stress. Springer. Berlin, Germany, pp. 1–33.

Farooq, M., Wahid, A., Kobayashi, N., Fujita, D., Basra S.M.A. 2009. Plant drought stress: effects, mechanisms and management. Agron. Sustain. Dev. 29: 185–212.

Flexas, J., Bota, J., Cifre, J., Mariano Escalona, J., Galmés, J., Gulías, J., Riera, D. 2004. Understanding down-regulation of photosynthesis under water stress: future prospects and searching for physiological tools for irrigation management. Ann. Appl. Biol. 144: 273–283.

Grant, O.M. 2012. Understanding and exploiting the impact of drought stress on plant physiology. In: Ahmad, P., Prasad, M.N.V. (Eds.). Abiotic Stress Responses in Plants: Metabolism, Productivity and Sustainability. Springer. New York, NY, USA, pp. 89–104.

Hernández, I., Cela, J., Alegre, L., Munné-Bosch, S. 2012. Antioxidant defenses against drought stress. In: Aroca, R. (Ed.). Plant Responses to Drought Stress. Springer. Berlin, Germany, pp. 231–258.

Huang, Y., Tang, R., Cao, Q., Bie, Z. 2009. Improving the fruit yield and quality of cucumber by grafting onto the salt tolerant rootstock under NaCl stress. Sci. Hortic. 122: 26–31.

Impa, S., Nadaradjan, S., Jagadish, S. 2012. Drought stress induced reactive oxygen species and anti-oxidants in plants. In: Ahmad, P., Prasad, M.N.V. (Eds.). Abiotic Stress Responses in Plants: Metabolism, Productivity and Sustainability. Springer. New York, NY, USA, pp. 131–147.

Intergovernmental Panel on Climate Change. 2013. Climate Change 2013: The Physical Science Basis. Cambridge University Press. Cambridge, UK.

Ismail, M.R., Davies, W. 1997. Water relations of *Capsicum* genotypes under water stress. Biol. Plant. 39: 293–297.

Johkan, M., Mitukuri, K., Yamasaki, S., Mori, G., Oda, M. 2009. Causes of defoliation and low survival rate of grafted sweet pepper plants. Sci. Hortic. 119: 103–107.

King, S.R., Davis, A.R., Zhang, X., Crosby, K. 2010. Genetics, breeding and selection of rootstocks for Solanaceae and Cucurbitaceae. Sci. Hortic. 127: 106–111.

Lee, J.M., Kubota, C., Tsao, S.J., Bie, Z., Echevarria, P.H., Morra, L., Oda, M. 2010. Current status of vegetable grafting: Diffusion, grafting techniques, automation. Sci. Hortic. 127: 93–105.

Liu, J., Li, J., Su, X., Xia, Z. 2014. Grafting improves drought tolerance by regulating antioxidant enzyme activities and stress-responsive gene expression in tobacco. Environ. Exp. Bot. 107: 173–179.

Martinez-Ballesta, M.C., Alcaraz-Lopez, C., Muries, B., Mota-Cadenas, C., Carvajal, M. 2010. Physiological aspects of rootstock-scion interactions. Sci. Hortic. 127: 112–118.

Møller, I.M., Jensen, P.E., Hansson, A. 2007. Oxidative modifications to cellular components in plants. Annu. Rev. Plant Biol. 58: 459–481.

Nilsen, E.T., Freeman, J., Grene, R., Tokuhisa, J. 2014. A rootstock provides water conservation for a grafted commercial tomato (*Solanum lycopersicum* L.) line in response to mild-drought conditions: a focus on vegetative growth and photosynthetic parameters. PLoS ONE 9: e115380. doi:10.1371/journal.pone.0115380

Osakabe, Y., Osakabe, K., Shinozaki, K. 2014. Response of plants to water stress. Front. Plant Sci. 5: 1–8.

Paongpetch, P., Techawongstien, S., 2008. Response of chilli pepper to water deficit. Agricultural Sci. J. 39: 273–276.

Paongpetch, P., Techawongstien, S., Chanthai, S., Bosland, P.W. 2012. Impact of drought stress on the accumulation of capsaicinoids in *Capsicum* cultivars with different initial capsaicinoid levels. Hort. Sci. 47: 1204–1209.

Penella, C., González Nebauer, S., López Galarza, S. V., San Bautista Primo, A., Rodríguez Burruezo, A., Calatayud, A. 2014. Evaluation of some pepper genotypes as rootstocks in water stress conditions. Hort. Sci. (Prague). 41: 192–200.

Penella, C., Nebauer, S.G., Bautista, A.S., López-Galarza, S., Calatayud, A. 2015. Strategies to avoid salinity and hydric stress of pepper grafted plants. Procedia Environ. Sci. 29: 211–212.

Penella, C., Nebauer, S.G., Lopéz-galarza, S., Sanbautista, A., Gorbe, E. 2013. Evaluation for salt stress tolerance of pepper genotypes to be used as rootstocks. J. Food Agric. Environ. 11: 1101–1107.

Pinheiro, C., Chaves, M.M. 2011. Photosynthesis and drought: Can we make metabolic connections from available data. J. Exp. Bot. 62: 869–882.

Rouphael, Y., Cardarelli, M., Colla, G., Rea, E. 2008. Yield, mineral composition, water relations, and water use efficiency of grafted mini-watermelon plants under deficit irrigation. Hort. Sci. 43: 730–736.

Sanchez-Rodriguez, E., Rubio-Wilhelmi, M.M., Blasco, B., Leyva, R., Romero, L., Ruiz, J.M. 2012. Antioxidant response resides in the shoot in reciprocal grafts of drought-tolerant and drought-sensitive cultivars in tomato under water stress. Plant Sci. 188–189: 89–96.

Sanders, G.J., Arndt, S.K. 2012. Osmotic adjustment under drought conditions. In: Aroca, R. (Ed.). Plant Responses to Drought Stress. Springer. Berlin, Germany, pp. 199–229.

Schafleitner, R., Gutierrez, R., Espino, R., et al. 2007. Field screening for variation of drought tolerance in *Solanum tuberosum* L. by agronomical, physiological and genetic analysis. Potato Res. 50: 71–85.

Schwarz, D., Rouphael, Y., Colla, G., Venema, J.H. 2010. Grafting as a tool to improve tolerance of vegetables to abiotic stresses: Thermal stress, water stress and organic pollutants. Sci. Hortic. 127: 162–171.

Wahb-Allah, M.A., Alsadon, A.A., Ibrahim, A.A. 2011. Drought tolerance of several tomato genotypes under greenhouse conditions. World Appl. Sci. J. 15: 933–940.

Lin, W.C., Saltveit, M. 2012. Greenhouse production. In: Russo, V.M. (Ed.). Peppers: Botany, Production and Uses. CABI. Wallingford, UK, pp. 57–86.

Yildirim, M., Demirel, K., Bahar, E. 2012. Effect of restricted water supply and stress development on growth of bell pepper (*Capsicum annuum* L.) under drought conditions. J. Agron. Crop Sci. 3: 1–9.

You, J., Chan, Z. 2015. ROS regulation during abiotic stress responses in crop plants. Front. Plant Sci. 6: 1092.

Zheng, Y., Fung, R.W., Wang, S.Y., Wang, C.Y. 2008. Transcript levels of antioxidative genes and oxygen radical scavenging enzyme activities in chilled zucchini squash in response to superatmospheric oxygen. Postharvest Biol. Technol. 47: 151–158.