

## Physio-morphological responses of sweet potato [*Ipomoea batatas* (L.) Lam.] genotypes to water-deficit stress

Suravoot Yooyongwech<sup>1</sup>, Thapanee Samphumphuang<sup>2</sup>, Cattarin Theerawitaya<sup>2</sup>, Suriyan Cha-um<sup>2</sup>

<sup>1</sup>Department of Agricultural Science, Mahidol University, Kanchanaburi Campus, Kanchanaburi 71150, Thailand

<sup>2</sup>National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), 113 Thailand Science Park, Pahonyothin Road, Khlong Nueng, Khlong Luang, Pathum Thani 12120 Thailand

\*Corresponding author: suriyanc@biotec.or.th

### Abstract

Roots of sweet potato [*Ipomoea batatas* (L.) Lam.; Convolvulaceae] are rich source of carbohydrates, vitamins and other nutrients; however, root storage and productivity is very sensitive to water deficit stress. We, therefore, investigated the light harvesting complexes (photosynthetic pigments) and activities (chlorophyll fluorescence), and photosynthetic abilities in three genotypes of sweet potato in response to decreased soil water content (SWC). Single vine cutting was propagated and then water withheld in different soil water contents. Osmotic potential, free proline, chlorophyll pigments, chlorophyll fluorescence, net photosynthetic rate and growth characters were measured. Free proline in the leaf tissues was enriched depending on a degree of water deficit, genotypes and their interaction. Physio-morphological characteristics of water-deficit stressed plants in each genotype of sweet potato were significantly inhibited. Osmotic potential in leaf tissues of water-deficit stressed plants of Tainung 57 sharply declined (-0.044x) when compared to PROC 65-3 (-0.027x) and Japanese Yellow (-0.025x). Chlorophyll a (Chl<sub>a</sub>), chlorophyll b (Chl<sub>b</sub>), total chlorophyll (TC), photon yield of PSII ( $\Phi_{PSII}$ ), stomatal conductance ( $g_s$ ), transpiration rate (E), vine length and number of leaves in cv. PROC 65-3 grown under water deficit were maintained better than those in cvs. Japanese Yellow and Tainung 57. A positive relationship between photosynthetic pigments and photosynthetic abilities was observed ( $R^2 > 0.9$ ) and it correlated directly with net photosynthetic rate ( $P_n$ ). Free proline enrichment may play a key role as osmotic adjustment in sweet potato cv. PROC 65-3, grown under water deficit stress. Photosynthetic pigments, chlorophyll fluorescence activities, net photosynthetic rate and transpiration rate in cv. PROC 65-3 under water deficit condition were retained better than those in cvs. Japanese Yellow and Tainung 57, resulting in maintain growth performance.

**Keywords:** chlorophyll fluorescence; free proline; osmotic adjustment; photosynthetic abilities; photosynthetic pigments.

**Abbreviations:** Chl<sub>a</sub>-Chlorophyll a; Chl<sub>b</sub>-chlorophyll b;  $P_n$ -net photosynthetic rate;  $\Phi_{PSII}$ -photon yield of PSII; SWC-soil water content;  $g_s$ -stomatal conductance, TC-total chlorophyll; E-transpiration rate.

### Introduction

Sweet potato [*Ipomoea batatas* (L.) Lam.; Convolvulaceae] is the seventh most important food crop in the world. A fibrous root of sweet potato can be developed as storage root enriched with carbohydrates, carotenoids and other nutrients (Laurie et al., 2012). However, water-use efficiency, growth performance and above-ground biomass of sweet potato are very sensitive to water deficit stress, leading to loss of storage root's productivity (Gomes and Carr, 2001; Gomes and Carr, 2003a, b; Gomes et al., 2005). In sweet potato, the function of stomatal closure, to limit water loss and reduce CO<sub>2</sub> assimilation, under water deficit stress has been well investigated, especially in the sensitive genotypes (Haimeirong and Kubota, 2003). Roots are the first signal perception organ in plant's response to water limitation in the soil (Kim et al., 2009). During soil dehydration, the water potential in the soil is generally decreased to limit the water absorption and translocation from sink to source, and signals stomatal closure by the function of guard cell, represented by stomatal conductance (van Heerden and Laurie, 2008). However, the information regarding light harvesting complexes and their activities in water-deficit stressed sweet

potato is still lacking. During water deficit stress, plants quickly adapt to water limit by several defense mechanisms. Osmoregulation defense mechanism is one of the most important strategies in higher plants to survive and maintain whole life cycle under water deficit condition. There are many osmolytes, such as soluble sugar, sugar alcohol, proline, polyamine and glycine betaine, that are enriched in the water-deficit stressed cells to function as osmotic adjustment (OA) at the cellular level (Sánchez et al., 1998; Babita et al., 2010; Bandurska et al., 2010). Proline is a small molecule of aromatic amino acid, which has a dual role: protectant and as ROS scavenging and accumulates in plants under a range of abiotic stresses. Proline biosynthesis in plants occurs *via* either glutamate or ornithine routes; however, most of the proline accumulated under abiotic stresses is synthesized from glutamate (Hare and Cress, 1997; Kishor et al., 2005; Trovato et al., 2008; Verslues and Juenger, 2011). Free proline enrichment at the cellular level in drought stressed plants has been reported as the major OA in castor (Babita et al., 2010). In contrast, soluble sugar and carbohydrate in drought stressed pea have played a critical role as OA, while

proline contributes only 1% for osmotic adjustment efficiency (Sánchez et al., 1998). In sweet potato, free proline accumulation and its functional role have not yet been discovered / investigated when plants are subjected to water deficit stress. Previously, six genotypes of sweet potato i.e. Manphuang, Mankorat, PROC 65-3, Banyang 9, Tainung 57 and Japanese yellow were subjected to classify the cluster of water deficit tolerance. Mankorat, PROC 65-3 and Japanese Yellow were classified as water deficit tolerance whereas Manphuang, Banyang 9 and Tainung 57 genotypes were evaluated as water deficit sensitive (Yooyongwech et al., 2013). Recently, shoot growth of sweet potato cv. Evangeline decreased more rapidly than cv. Beauregard with reduce soil moisture (Gajanayake et al., 2014). Thus, the present study investigated the light harvesting complexes (photosynthetic pigments) and activities (chlorophyll fluorescence), and photosynthetic abilities in three genotypes (PROC 65-3, Tainung 57 and Japanese Yellow cultivars) of sweet potato in response to decreased soil water content vis-à-vis free proline accumulation in the leaf tissues.

## Results

### Growth characteristics

Vine length of sweet potato genotypes, PROC 65-3 and Japanese Yellow, grown under low soil water content (SWC) was slightly decreased. In contrast, the vine length of cv. Tainung 57 grown under 15% and 8% SWC decreased by 20.50% and 31.68% (significant at  $p \leq 0.01$ ) over that of control (well watering), respectively (Table 1). Number of leaves in PROC 65-3 and Japanese Yellow under mild water deficit (29% SWC) was maintained; however, it declined in plant grown under extreme water deficit (8% SWC). In Tainung 57 subjected to moderate and extreme water deficit stresses, the number of leaves was significantly declined by 50.00% and 51.61%, respectively, over that in the control (Table 1). Growth performance in terms of vine length and number of leaves in cv. Tainung 57 was sensitive to low SWC when compared with other two cultivars, PROC 65-3 and Japanese Yellow.

### Osmotic potential and free proline determination in the leaf tissues

Osmotic potential ( $\Psi_s$ ) in the leaf tissues of sweet potato genotypes declined significantly in relation to percent SWC reduction (Table 1). The  $\Psi_s$  in the water-deficit stressed plants of cv. Tainung 57 was decreased from  $-1.59$  MPa (at 40% SWC) to  $-3.17$  MPa (at 8% SWC). A positive relationship between SWC and  $\Psi_s$  was observed (Fig. 1). The slope of  $\Psi_s$  reduction in cv. Tainung 57 was  $-0.044x$ , compared to  $-0.025x$  and  $-0.027x$  in cv. PROC 65-3 and Japanese Yellow, respectively. The reduction rate of  $\Psi_s$  in water deficit stressed plants of cv. Tainung 57 was greater than that in cvs. PROC 64-3 and Japanese Yellow (Fig. 1). In contrast to the trend of changes in  $\Psi_s$ , free proline content in the leaf tissues was increased depending on the degree of SWC reduction, sweet potato genotypes and their interaction. Free proline content in the plant grown under well watering and 40% SWC was very low ( $<0.5 \mu\text{mol g}^{-1}$  FW). It rapidly increased to high concentration ( $>1.12 \mu\text{mol g}^{-1}$  FW) in the water deficit stressed plants (Table 1). An accumulation of free proline in the water-deficit stressed plant may play a key role in the osmotic adjustment in sweet potato. The high  $\Psi_s$  in the leaf tissues of water-deficit stressed plants was directly

adjusted by enrichment of free proline in cvs. PROC 64-3 (Fig. 2A) and Japanese Yellow (Fig. 2B). In contrast, the low  $\Psi_s$  in cv. Tainung 57 was related to low concentration of free proline when subjected to water deficit stress (Fig. 2C).

### Photosynthetic abilities

Chlorophyll a ( $\text{Chl}_a$ ), chlorophyll b ( $\text{Chl}_b$ ) and total chlorophyll (TC) contents in the leaf tissues of sweet potato genotypes subjected to 8–15% SWC declined significantly over that in the control (WW). In cv. PROC 65-3,  $\text{Chl}_b$  and TC content in the leaf tissues of sweet potato plants grown under 40% SWC were maintained, whereas  $\text{Chl}_a$  content declined significantly under 29% SWC (Table 2). In contrast, these pigments declined sharply in both Japanese Yellow and Tainung 57 genotypes when exposed to mild water deficit (29% SWC). The  $\text{Chl}_a:\text{Chl}_b$  in cv. PROC 65-3 plants grown under water deficit conditions was increased, whilst the ratio in cvs. Japanese Yellow and Tainung 57 remained unchanged (Table 2). A positive relationship between  $\text{Chl}_a$  and maximum quantum yield of PSII ( $F_v/F_m$ ) was observed (Fig. 3).  $\text{Chl}_a$  degradation in cv. PROC 65-3 was very low in relation to  $F_v/F_m$  diminishing, whereas  $\text{Chl}_a$  content was enriched in cvs. Japanese Yellow and Tainung 57 (Fig. 3). Moreover, TC degradation was also related to photon yield of PSII ( $\Phi_{\text{PSII}}$ ) (Fig. 4). The  $F_v/F_m$  in all sweet potato genotypes grown under mild (29% SWC) to extreme (8% SWC) water deficit condition was significantly diminished. The activity of  $\Phi_{\text{PSII}}$  in cvs. PROC 65-3 and Japanese Yellow grown under 40% SWC was maintained, while it declined in cv. Tainung 57 (Table 3). A positive relationship between  $\Phi_{\text{PSII}}$  diminution and net photosynthetic rate ( $P_n$ ) reduction was observed (Fig. 5). The  $P_n$  was sensitive to water deficit stress, which declined sharply in all sweet potato genotype plants exposed to 40% SWC (Table 3). Under extreme water deficit (8% SWC), the  $P_n$  in cvs. PROC 65-3, Japanese Yellow and Tainung 57 declined by 47.81%, 71.38% and 89.67%, respectively, over that in the control. Stomatal conductance ( $g_s$ ) and transpiration rate (E) in sweet potato genotypes declined significantly when plants were subjected to mild and extreme water deficit stress (Table 3). However, the  $g_s$  and E in water-deficit stressed plants of cv. PROC 65-3 were maintained better than those in Japanese Yellow and Tainung 57.

## Discussion

In the present study, vine length and number of leaves in sweet potato cv. Tainung 57 were sharply dropped under extreme water deficit stress (8% SWC), whereas these were maintained in cv. PROC 65-3. These observations are corroborated by previous findings reporting reduction in growth (length and leaf number) under water stress. For example, over 75% reduction in vine length in sweet potato cv. Resito was reported when grown under 30% field capacity (FC) compared to well watering (100% FC) conditions (van Heeden and Laurie, 2008). The reduction in vine length has been positively correlated to the decline in irrigation rates from 100% full irrigation (221 cm) to 30% irrigation (71.8 cm) over a period of 155 days (Laurie et al., 2009). In *Catharanthus roseus* cvs. Rosea and Alba, shoot height and number of leaves declined under 60% FC water deficit (Jaleel et al., 2008). In contrast, vine length was maintained in cultivar A15 grown under 30% FC water deficit stress (van Heeden and Laurie, 2008). Saraswati et al. (2004) also reported that number of leaves in sweet potato genotype L46 grown under water deficit stress was maintained

**Table 1.** Vine length, number of leaves, leaf osmotic potential ( $\Psi_s$ ) and free proline content in three genotypes of sweet potato grown under water deficit stress in the pot culture.

Genotypes	Treatment	Vine length (cm)	Number of leaves	$\Psi_s$ (MPa)	Free proline ( $\mu\text{mol g}^{-1}$ FW)
PROC 65-3	WW	24.25cd	8.00de	-1.39a	0.15h
	40% SWC	24.50cd	7.75ef	-1.50abc	0.44h
	29% SWC	21.25cd	7.50ef	-1.62c	1.73f
	15% SWC	20.25d	7.00ef	-2.25de	3.45c
	8% SWC	19.00d	5.50f	-2.37fg	6.65a
Japanese	WW	22.25cd	12.50bc	-1.51bc	0.18h
	40% SWC	22.75cd	11.75c	-1.64d	0.33h
Yellow	29% SWC	21.50cd	10.5cd	-1.88e	1.25g
	15% SWC	20.00d	8.75de	-2.21ef	2.34d
	8% SWC	18.00d	7.50ef	-2.55g	4.50b
Tainung 57	WW	40.25a	15.5a	-1.37a	0.17h
	40% SWC	40.00a	14.75ab	-1.59bc	0.22h
	29% SWC	39.25a	12.25bc	-2.05de	1.12g
	15% SWC	32.00b	7.75ef	-2.43g	1.90ef
	8% SWC	27.5bc	7.50ef	-3.17h	2.17de

WW: well watering; SWC: Soil water content. Different letters in each column show significant difference at  $p \leq 0.01$  according to Tukey's HSD.

**Table 2.** Chlorophyll a ( $\text{Chl}_a$ ), chlorophyll b ( $\text{Chl}_b$ ), total chlorophyll (TC) contents and  $\text{Chl}_a:\text{Chl}_b$  ratio in three genotypes of sweet potato grown under water deficit stress in the pot culture.

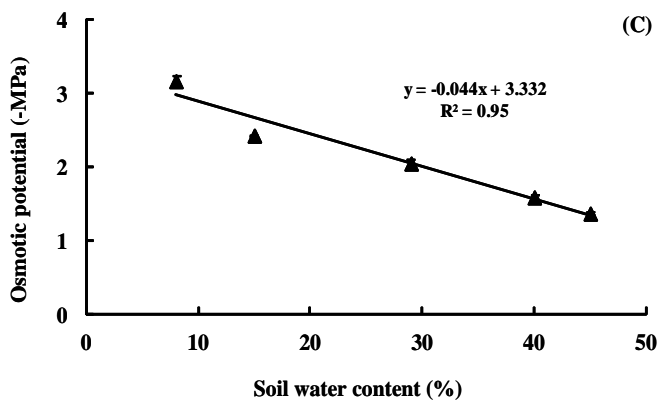
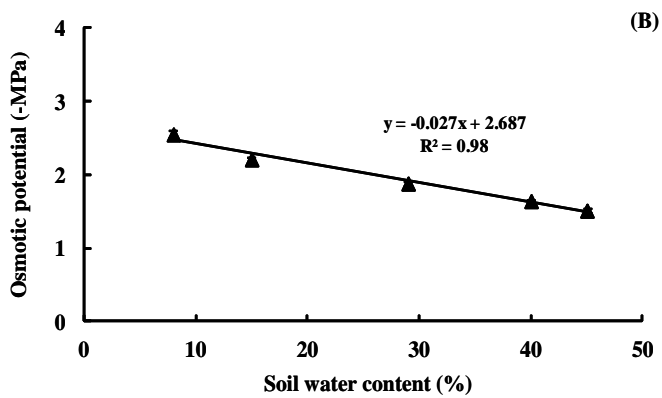
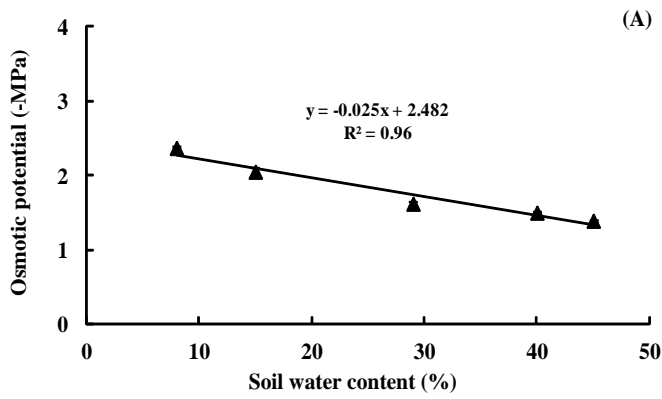
Genotypes	Treatment	$\text{Chl}_a$ ( $\mu\text{g g}^{-1}$ FW)	$\text{Chl}_b$ ( $\mu\text{g g}^{-1}$ FW)	TC ( $\mu\text{g g}^{-1}$ FW)	$\text{Chl}_a:\text{Chl}_b$
PROC 65-3	WW	252.0b	117.5b	369.5b	2.14cde
	40% SWC	256.8b	110.8bc	367.6b	2.32bc
	29% SWC	227.1bc	93.7d	320.9c	2.42bc
	15% SWC	212.4cd	71.1ef	283.5cd	2.99a
	8% SWC	167.2e	60.1fg	227.3e	2.79ab
Japanese	WW	240.7bc	142.3a	383.0b	1.71e
	40% SWC	221.2bc	94.9cd	316.1c	2.35bc
Yellow	29% SWC	184.1de	80.7de	264.8de	2.29bc
	15% SWC	96.6fg	50.2gh	146.8fg	1.92cde
	8% SWC	69.8gh	35.3hi	105.0gh	1.99cde
Tainung 57	WW	334.6a	144.3a	478.9a	2.32bc
	40% SWC	256.8b	116.9b	373.7b	2.19cde
	29% SWC	106.2f	47.2gh	153.4f	2.25cd
	15% SWC	48.3hi	25.1ij	73.4hi	2.19cde
	8% SWC	26.2i	15.3j	41.4i	2.32bc

WW: well watering; SWC: Soil water content. Different letters in each column show significant difference at  $p \leq 0.01$  according to Tukey's HSD.

**Table 3.** Maximum quantum yield of PSII ( $F_v/F_m$ ), photon yield of PSII ( $\Phi_{\text{PSII}}$ ), net photosynthetic rate ( $P_n$ ), stomatal conductance ( $g_s$ ) and transpiration rate (E) in three genotypes of sweet potato grown under water deficit stress in the pot culture.

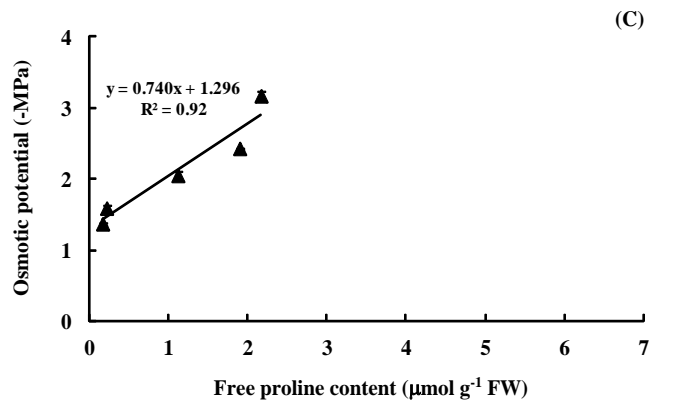
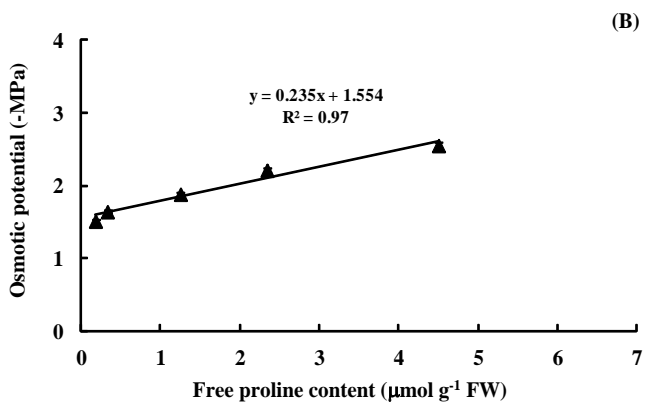
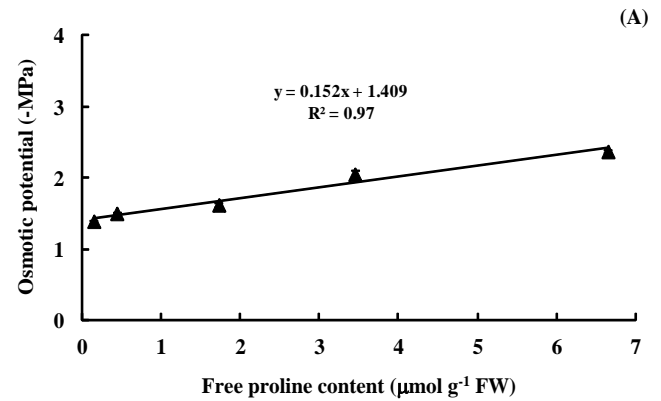
Genotypes	Treatment	$F_v/F_m$	$\Phi_{\text{PSII}}$	$P_n$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	$g_s$ ( $\text{mmol CO}_2 \text{ m}^{-2} \text{s}^{-1}$ )	E ( $\text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$ )
PROC 65-3	WW	0.781ab	0.693a	10.25a	34.00a	1.02ab
	40% SWC	0.758bc	0.662ab	8.40bc	33.03ab	0.76bc
	29% SWC	0.719cd	0.637bc	7.67bc	28.28bc	0.65cd
	15% SWC	0.649d	0.574c	7.31c	23.95cd	0.45ef
	8% SWC	0.564e	0.484d	5.35d	20.28de	0.38fg
Japanese	WW	0.839ab	0.670a	8.81b	32.38ab	0.88ab
	40% SWC	0.772bc	0.661ab	7.57c	24.05cd	0.77bc
Yellow	29% SWC	0.712cd	0.599bc	4.92d	17.93ef	0.54de
	15% SWC	0.554e	0.439d	3.35e	13.03f	0.39fg
	8% SWC	0.323g	0.240f	2.61ef	4.78g	0.23hi
Tainung 57	WW	0.833ab	0.677a	8.42bc	32.67ab	0.59d
	40% SWC	0.804ab	0.639bc	5.47d	20.77de	0.36fg
	29% SWC	0.561e	0.481d	3.33e	13.14f	0.26gh
	15% SWC	0.420f	0.357e	1.68fg	4.30g	0.22hi
	8% SWC	0.335g	0.169g	0.87g	3.03g	0.16i

WW: well watering; SWC: Soil water content. Different letters in each column show significant difference at  $p \leq 0.01$  according to Tukey's HSD.



**Fig 1.** Relationship between soil water content and leaf osmotic potential of sweet potato cvs. PROC 65-3 (A), Japanese Yellow (B) and Tainung 57 (C) grown under water deficit stress. Error bars represent  $\pm$ SE ( $n = 8$ ).

(26.32% reduction) better than in other genotypes, where 43% – 63% reduction was observed). The stem length and number of leaves in parsley cv. Moss Curled were sensitive to water deficit stress and these dropped sharply when plants were subjected to 10% and 30% water supplementation (Najla et al., 2012). The observed decline in leaf osmotic potential with reduction in SWC reduce soil water content by water withholding of pot culture is paralleled by earlier findings of Haimeirong and Kubota (2003), who found that leaf water potential of sweet potato declined from  $-0.51$  MPa to  $-1.32$  MPa upon reduction in soil water potential from  $-0.31$  MPa to  $-3.94$  MPa. Previously, Saraswati et al. (2004) reported a decrease in leaf water potential in 15 cultivars of sweet potato under water deficit stress.



**Fig 2.** Relationship between free proline content and leaf osmotic potential of sweet potato cvs. PROC 65-3 (A), Japanese Yellow (B) and Tainung 57 (C) grown under water deficit stress. Error bars represent  $\pm$ SE ( $n = 8$ ).

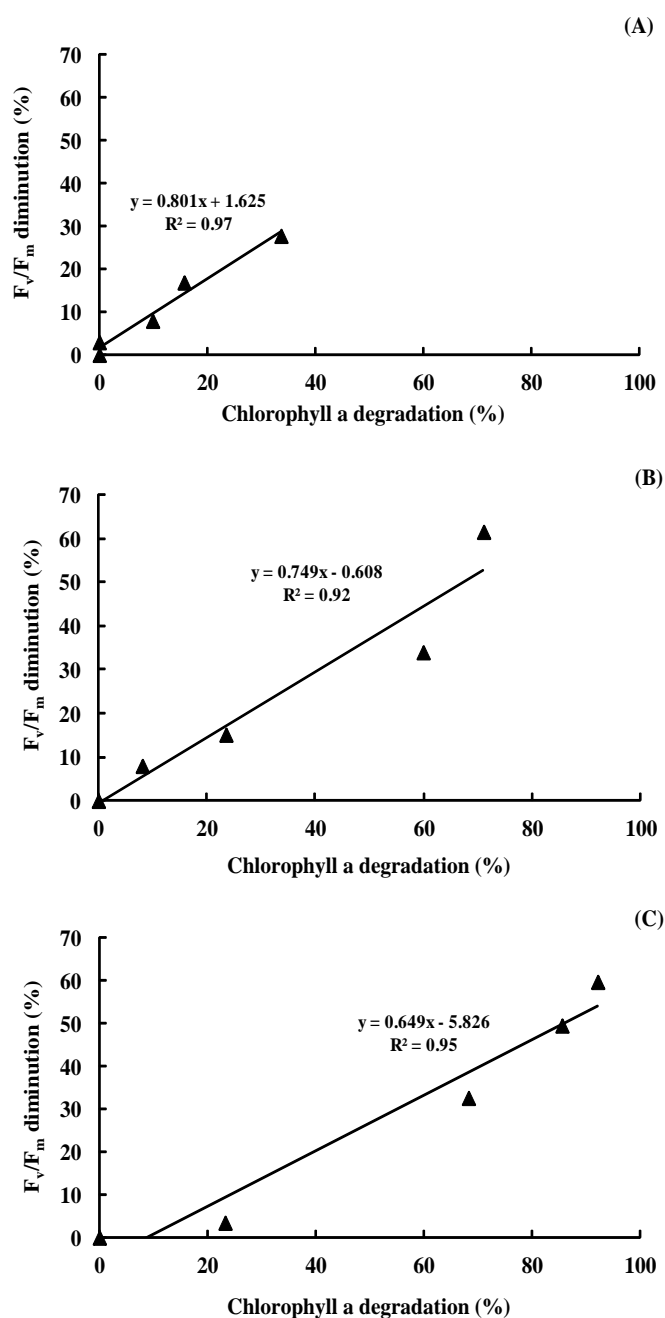
In our study, free proline in water-deficit stressed plants, especially in cv. PROC 65-3, was enriched, possibly to control the osmotic potential of leaves. These observations are in agreement with similar findings in other plants including sweet potato. For example, in sweet potato cvs. Huambachero and Untacip, free proline content increased significantly when plants were subjected to water deficit under hydroponic culture (Rodríguez-Delfín et al., 2012). Free proline content in potato cv. Marfuna subjected to 40% FC enhanced over the control by  $\sim 10$  fold (Farhad et al., 2011). In eggplant, free proline accumulated greatly when plants were exposed to severe soil moisture stress (Sarker et al., 2005). Likewise, in cotton cv. Ca/H 680 (drought tolerant) there was 2.5 fold greater accumulation of free

proline than in cv. Ca/H 148 (drought sensitive) when subjected to water withholding for 10–14 days. These observations are confirmed by increase in the activities of proline biosynthesis enzymes, proline-5-carboxylase synthetase (P5CS) and proline-5-carboxylase reductase (P5CR), and decline in the activity of proline degradation enzyme—proline dehydrogenase (PDH) in (Parida et al., 2008). Babita et al. (2010) observed 4-6 fold increase in proline accumulation in castor plant grown under 10-15% water deficit conditions compared to the control plants. The observed accumulation of free proline in the leaf tissues of sweet potato may possibly play a role as osmotic adjustment in water-deficit stressed plants. In our study, photosynthetic pigment degradation and reduced photosynthetic activities (chlorophyll fluorescence) in water-deficit stressed plants were evidently demonstrated, leading to decreased net photosynthetic rate ( $P_n$ ). These observations are in conformity with previous findings in sweet potato (Rodríguez-Delfin et al., 2012), parsley (Najla et al., 2012), and *Catharanthus roseus* (Jaleel et al., 2008). In sweet potato (cv. Huambachero), total chlorophyll content in leaf tissue declined by 37.37% when plants were subjected to water deficit conditions (Rodríguez-Delfin et al., 2012). In parsley, the total chlorophyll content in cv. Neoplitanum was maintained better than that in cv. Moss curled, when plants were irrigated with only 10% of water requirement (i.e. water stress) (Najla et al., 2012).  $Chl_a$ ,  $Chl_b$  and TC in water deficit stressed leaves of *Catharanthus roseus* cvs. Rosea and Alba declined in the range of 5.45%–31.11% (Jaleel et al., 2008). In our study,  $F_v/F_m$  and  $\Phi_{PSII}$  in all genotypes of sweet potato were diminished especially under severe water deficit (8–15% SWC). In addition, the  $\Phi_{PSII}$  in cv. Tainung 57 was sensitive to water deficit, and declined significantly when subjected to 40% SWC. Previously, it has been reported that  $F_v/F_m$  and  $\Phi_{PSII}$  in sweet potato cv. Koganesengan were maintained better than that in cv. Okinawa-100, when exposed to water deficit stress (Haimeirong and Kubota, 2003). Stomatal conductance ( $g_s$ ) and transpiration rate ( $E$ ) were declined under water stress to limit  $CO_2$  assimilation and water loss through the stomatal pore. The decrease in photosynthetic abilities may not only due to reduced photosynthetic pigments but also due to limited  $CO_2$  assimilation through stomatal pore. For example, the stomatal function of sweet potato cv. Resisto (a sensitive genotype) grown under drought stress (30% FC) was directly inhibited and evidently retarded  $P_n$ , leading to yield loss of > 60% (van Heerden and Laurie, 2008; Laurie et al., 2009). Similarly, the  $g_s$  and  $P_n$  in sweet potato cv. Okinawa-100 grown under water deficit were sharply dropped compared to that in cv. Koganesengan (Haimeirong and Kubota, 2003).

## Materials and Methods

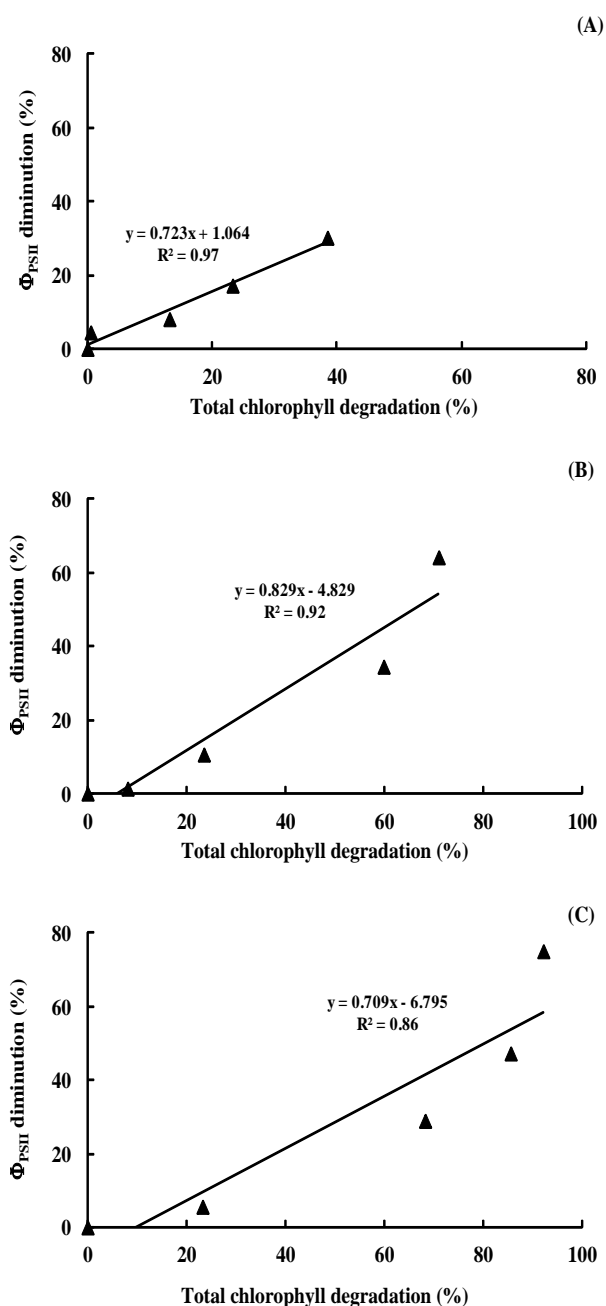
### Plant materials and water deficit treatments

Three genotypes, PROC 65-3, Tainung 57 and Japanese Yellow cultivars, of sweet potato [*Ipomoea batatas* (L.) Lam.] procured from Agricultural Extension Group, Phichit Province, Thailand, were used as master stock material. PROC 65-3 and Japanese Yellow were classified as water deficit tolerance whereas Tainung 57 genotype was evaluated as water deficit sensitive (Yooyongwech et al., 2013). Single vine cutting (12±1 cm in length) without leaf blades was propagated in plastic pots ( $\phi = 20$  cm) containing 2 kg mixed soil (EC = 2.687 dS  $m^{-1}$ ; pH = 5.5; organic matter = 10.36%; total nitrogen = 0.17%; total phosphorus = 0.07%; total potassium = 1.19%). The cutting propagated plants in the pot



**Fig 3.** Relationship between chlorophyll a degradation and diminution of maximum quantum yield of PSII ( $F_v/F_m$ ; %) in sweet potato cvs. PROC 65-3 (A), Japanese Yellow (B) and Tainung 57 (C) grown under water deficit stress.

culture were incubated in a net house under 28±2°C ambient temperature, 80±5% RH and 10 h  $d^{-1}$  photoperiod of 500–1,000  $\mu mol\ m^{-2}\ s^{-1}$  photosynthetic photon flux density (PPFD) for 4 weeks. Thereafter, five groups of plants: well watering (WW; control), 40% soil water content (SWC) by water withholding for 3 days, 29% SWC by water withholding for 6 days, 15% SWC by water withholding for 9 days, and 8% SWC by water withholding for 12 days, were set as the experimental layout. Growth characters (vine length and number of leaves), osmotic potential, free proline content, photosynthetic pigments, chlorophyll fluorescence, net



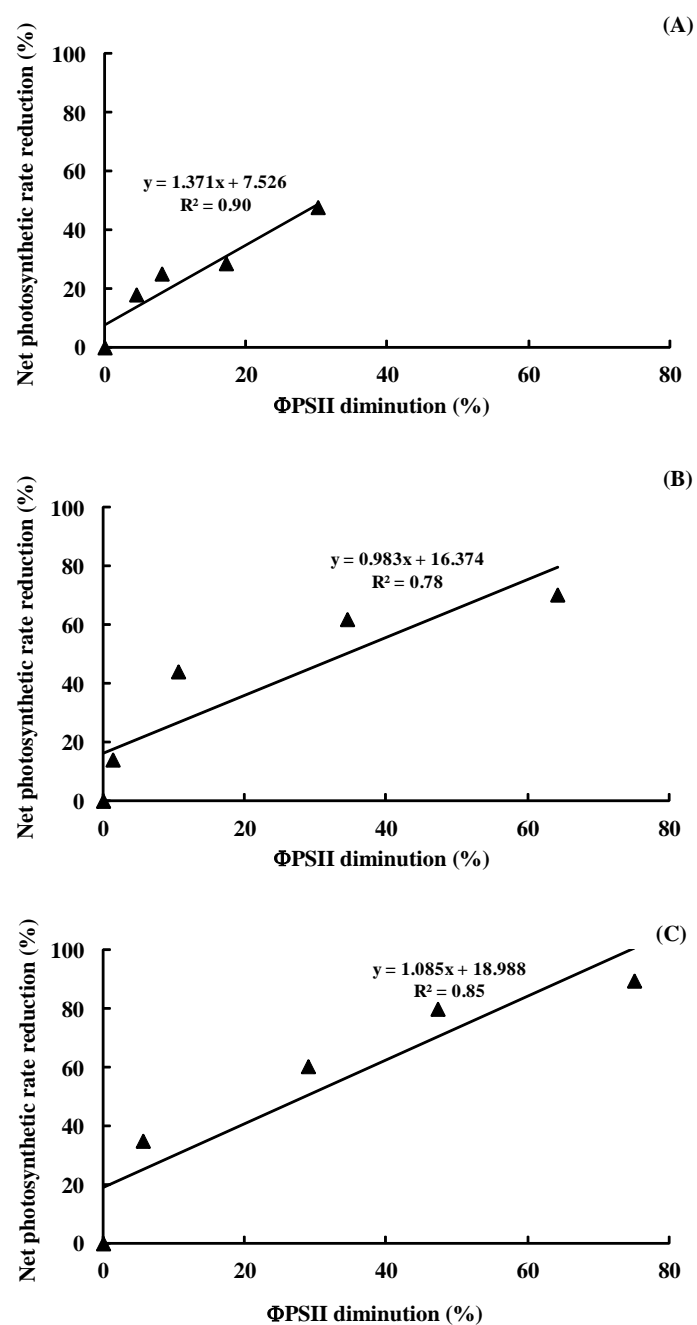
**Fig 4.** Relationship between total chlorophyll degradation and diminution of photon yield of PSII ( $\Phi_{PSII}$ ; %) in sweet potato cvs. PROC 65-3 (A), Japanese Yellow (B) and Tainung 57 (C) grown under water deficit stress.

photosynthetic rate ( $P_n$ ), stomatal conductance ( $g_s$ ) and transpiration rate (E) were measured after 4-weeks.

#### Soil water content (SWC)

Soil samples were collected at 0, 3, 6, 9 and 12 days after water withholding. Soil water content (SWC) was calculated using the weight fraction as:

$$SWC (\%) = [(FW - DW) / DW] \times 100$$



**Fig 5.** Relationship between diminution in photon yield of PSII ( $\Phi_{PSII}$ ; %) and net photosynthetic rate reduction ( $P_n$ ; %) sweet potato cvs. PROC 65-3 (A), Japanese Yellow (B) and Tainung 57 (C) grown under water deficit stress.

Where, FW was the fresh weight of a portion of the soil from the internal area of each pot and DW was the dry weight of the soil portion after drying in a hot air oven at 85°C for 4 days (Coombs et al., 1987).

#### Free proline determination

Free proline in the leaf tissues was assayed according to the method of Bates et al. (1973). In brief, one hundred milligrams of leaves were ground in liquid nitrogen.

The homogenate powder was mixed with 1 mL of aqueous sulfosalicylic acid (3%, w/v) and then filtered through Whatman #1 filter paper (Whatman, England). The extracted solution was reacted with an equal volume of glacial acetic acid and ninhydrin reagent (1.25 mg ninhydrin in 30 mL glacial acetic acid and 20 mL 6 M H<sub>3</sub>PO<sub>4</sub>) and heated at 95°C for 1 h. The reaction was terminated by incubating the reaction tubes in an ice bath. Then, the contents were mixed vigorously with 2 mL toluene followed by cooling. After cooling to 25°C, the chromophore was measured at 520 nm using UV-VIS spectrophotometer (HACH DR/4000; Model 48000, HACH Company, Loveland, Colorado, USA) against a calibration standard of L-proline.

#### Osmotic potential determination

The osmolarity of leaf tissues was measured according to Lanfermeijer et al. (1991). In brief, one hundred milligrams of fresh leaf tissue was cut into small pieces, transferred to 1.5 mL micro tube, and then crushed by stirring with a glass rod. The 20 micro of extracted solution was dropped directly onto a filter paper in an osmometer chamber (5520 Vapro<sup>®</sup>, Wescor, Utah, USA). The osmolarity (mmol kg<sup>-1</sup>) was converted to osmotic potential (MPa) using conversion factor of osmotic potential measurement.

#### Photosynthetic pigment

Chlorophyll a (Chl<sub>a</sub>), chlorophyll b (Chl<sub>b</sub>) and total chlorophyll (TC) concentrations were analyzed as per the method given by Shabala et al. (1998). In brief, one hundred milligrams of leaf material was collected from the second and third nodes of the shoot tip. The leaf samples were placed in a 25 mL glass vial (Opticlear<sup>®</sup>; KIMBLE, Vineland, NJ, USA), along with 10 mL of 95.5% acetone, and blended with a homogenizer (T25 basic Ultra-Turrax<sup>®</sup>; IKA, Kuala Lumpur, Malaysia). The glass vials were sealed with Parafilm<sup>®</sup> to prevent evaporation and then stored at 4°C for 48 h. Chl<sub>a</sub>, Chl<sub>b</sub> and TC concentrations were measured at 662, 644 and 470 nm, respectively, using a UV-visible spectrophotometer (DR/4000; Hach, Loveland, CO, USA). A solution of 95.5% acetone was used as a blank.

#### Chlorophyll fluorescence

Chlorophyll fluorescence emission from the adaxial surface of the leaf was measured using a fluorescence monitoring system (model FMS 2; Hansatech Instruments Ltd., Norfolk, UK) in the pulse amplitude modulation mode, as previously described by Loggini et al. (1999) and Maxwell and Johnson (2000).

#### Net photosynthetic rate (P<sub>n</sub>) and transpiration rate (E)

Net photosynthetic rate (P<sub>n</sub>; μmol m<sup>-2</sup> s<sup>-1</sup>), stomatal conductance (g<sub>s</sub>; mmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>) and transpiration rate (E; mmol m<sup>-2</sup> s<sup>-1</sup>) were measured using a Portable Photosynthesis System fitted with an Infra-red Gas Analyzer (IRGA) (Model: LI 6400, LI-COR Inc., Lincoln, Nebraska, USA). E was measured continuously by monitoring the content of the air entering and existing in the IRGA headspace chamber according to Cha-um et al. (2007).

#### Experimental design and statistical analysis

The experiment was arranged as 3 × 5 factorial in Completely Randomized Block Design (CRBD) with eight replicates (n =

8). The mean values obtained were compared using Tukey's HSD and analyzed with SPSS software.

#### Conclusion

Osmotic potential in the leaf tissues of sweet potato declined depending on reduced SWC, genotypes and their interaction. Free proline accumulation played a key role as osmotic adjustment in sweet potato cultivar, especially cv. PROC 65-3, grown under water deficit stress. Under water deficit conditions, photosynthetic pigments, chlorophyll fluorescence activities, net photosynthetic rate and transpiration rate in cv. PROC 65-3 were retained better than those in cvs. Japanese Yellow and Tainung 57, thereby resulting in better growth performance.

#### Acknowledgements

The authors would like to thank National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA) for funding support.

#### References

- Babita M, Maheswari M, Rao LM, Shanker AK, Rao DG (2010) Osmotic adjustment, drought tolerance and yield in castor (*Ricinus communis* L.) hybrids. *Environ Exp Bot*. 69:243–249.
- Bandurska H, Józwiak W (2010) A comparison of the effects of drought on proline accumulation and peroxidases activity in leaves of *Festuca rubra* L. and *Lolium perenne* L. *Acta Soc Bot Pol*. 79:111–116.
- Bates LS, Waldren RP, Teare ID (1973) Rapid determination of free proline for water-stress studies. *Plant Soil*. 39:205–207.
- Cha-um S, Supaibulwatana K, Kirdmanee C (2007) Glycinebetaine accumulation, physiological characterizations and growth efficiency in salt-tolerant and salt-sensitive lines of indica rice (*Oryza sativa* L. ssp. *indica*) in response to salt stress. *J Agron Crop Sci*. 193:157–166.
- Coombs J, Hall DO, Long SP, Scurlock JMO (1987) *Techniques in Bioproductivity and Photosynthesis*. Pergamon, Oxford, UK.
- Farhad MS, Babak AM, Reza ZM, Hassan RSM, Afshin T (2011) Response of proline, soluble sugars, photosynthetic pigments and antioxidant enzymes in potato (*Solanum tuberosum* L.) to different irrigation regimes in greenhouse condition. *Aust J Crop Sci*. 5:55–60.
- Gajanayake B, Reddy KR, Shankle MW, Arancibia RA (2014) Growth, developmental, and physiological responses of two sweet potato (*Ipomoea batatas* L. [Lam]) cultivars to early season soil moisture deficit. *Sci Horti*. 168:218–228.
- Gomes F, Carr MKV (2001) Effects of water availability and vine harvesting frequency on the productivity of sweet potato in Southern Mozambique. I. Storage root and vine yields. *Exp Agric*. 37:523–537.
- Gomes F, Carr MKV (2003a) Effects of water availability and vine harvesting frequency on the productivity of sweet potato in Southern Mozambique. II. Crop water use. *Exp Agric*. 39:39–54.
- Gomes F, Carr MKV (2003b) Effects of water availability and vine harvesting frequency on the productivity of sweet potato in Southern Mozambique. III. Crop yield and water use response functions. *Exp Agric*. 39:409–421.

- Gomes F, Carr MKV, Squire GR (2005) Effects of water availability and vine harvesting frequency on the productivity of sweet potato in Southern Mozambique. IV. Radiation interception, dry matter production and partitioning. *Exp Agric*. 41:93–108.
- Haimeirong Kubota F (2003) The effects of drought stress and leaf ageing on leaf photosynthesis and electron transport in photosystem 2 in sweet potato (*Ipomoea batatas* Lam.) cultivars. *Photosynthetica*. 41:253–258.
- Hare PD, Cress WA (1997) Metabolic implications of stress-induced proline accumulation in plants. *Plant Growth Regul*. 21:77–102.
- Jaleel CA, Manivannan P, Lakshmanan GMA, Gomathinayagam M, Panneerselvam R (2008) Alterations in morphological parameters and photosynthetic pigment responses of *Catharanthus roseus* under soil water deficits. *Coll Surf B: Biointer*. 61:298–303.
- Kim SH, Song WK, Kim YH, Kwon SY, Lee HS, Lee IC, Kwak SS (2009) Characterization of full-length enriched expressed sequence tags of dehydration-treated white fibrous roots of sweet potato. *BMB Rep*. 42:271–276.
- Kishor PBK, Sangam S, Amrutha RN, Laxmi PS, Naidu KR, Rao KRSS, Rao S, Reddy KJ, Theriappan P, Sreenivasulu N (2005) Regulation of proline biosynthesis, degradation uptake and transport in higher plants: Its implications in plant growth and abiotic stress tolerance. *Curr Sci*. 88:424–438.
- Lanfermeijer FC, Koerselman-Kooij JW, Borstlap AC (1991) Osmosensitivity of sucrose uptake by immature pea cotyledons disappears during development. *Plant Physiol*. 95:832–838.
- Laurie RN, du Plooy CP, Laurie SM (2009) Effect of moisture stress on growth and performance of orange fleshed sweet potato varieties. *Afri Crop Sci Conf Proc*. 9:235–239.
- Laurie SM, Faber M, van Jaarsveld PJ, Laurie RN, du Plooy CP, Modisane PC (2012)  $\beta$ -Carotene yield and productivity of orange-fleshed sweet potato (*Ipomoea batatas* (L.) Lam) as influenced by irrigation and fertilizer application treatments. *Sci Hortic*. 142:180–184.
- Loggini B, Scartazza A, Brugnoli E, Navari-Izzo F (1999) Antioxidant defense system, pigment composition, and photosynthetic efficiency in two wheat cultivars subjected to drought. *Plant Physiol*. 119:1091–1099.
- Maxwell K, Johnson GN (2000) Chlorophyll fluorescence—a practical guide. *J Exp Bot*. 51:659–668.
- Najla S, Sanoubar R, Murshed R (2012) Morphological and biochemical changes in two parsley varieties upon water stress. *Physiol Mol Biol Plant*. 18:133–139.
- Parida AK, Dagaonkar VS, Phalak MS, Aurangabadkar LP (2008) Differential responses of the enzymes involved in proline biosynthesis and degradation in drought tolerant and sensitive cotton genotypes during drought stress and recovery. *Acta Physiol Plant*. 30:619–627.
- Rodríguez-Delfín A, Posadas A, León-Valarde C, Mares V, Quiroz R (2012) Effect of salt and water stress on the proline and total chlorophyll content and nutrients uptake on two sweet potato cultivars grown on soilless culture. *Acta Hortic*. 947:55–62.
- Sánchez FJ, Manzanares M, de Andres EF, Tenorio JL, Ayerbe L (1998) Turgor maintenance, osmotic adjustment and soluble sugar and proline accumulation in 49 pea cultivars in response to water stress. *Field Crops Res*. 59:225–235.
- Saraswati P, Johnston M, Coventry R, Holtum J (2004) Identification of drought tolerant sweet potato (*Ipomoea batatas* (L.) Lam) cultivars. New direction for adverse planet: Proceedings of the 4<sup>th</sup> International Crop Science Congress, 26 September – 1 October 2004, Brisbane, Australia.
- Sarker BC, Hara M, Uemura M (2005) Proline synthesis, physiological responses and biomass yield of eggplants during and after repetitive soil moisture stress. *Sci Hortic*. 103:387–402.
- Shabala SN, Shabala SI, Martynenko AI, Babourina O, Newman IA (1998) Salinity effect on bioelectric activity, growth, Na<sup>+</sup> accumulation and chlorophyll fluorescence of maize leaves: a comparative survey and prospects for screening. *Aust J Plant Physiol*. 25:609–616.
- Trovata M, Mattioli R, Costantino P (2008) Multiple roles of proline in plant stress tolerance and development. *Rend Lin*. 19:325–346.
- van Heerden PDR, Laurie R (2008) Effects of prolonged restriction in water supply on photosynthesis, shoot development and storage root yield in sweet potato. *Physiol Planta*. 134:99–109.
- Verslues PE, Juenger TE (2011) Drought, metabolites, and Arabidopsis natural variation: a promising combination for understanding adaptation to water-limited environments. *Curr Opin Plant Biol*. 14:240–245.
- Yooyongwech S, Theerawitaya C, Samphumphuang T, Cham S (2013) Water-deficit tolerant identification in sweet potato genotypes [*Ipomoea batatas* (L.) Lam.] in vegetative developmental stage using multivariate physiological indices. *Sci Hortic*. 162:242–251.